

*UCSB LABORATORY
SAFETY MANUAL
and
CHEMICAL HYGIENE PLAN*

**Prepared by
UCSB Environmental Health & Safety**

**Editors:
Dave Vandenberg
Alex Moretto**



Acknowledgements: thanks to the following for their assistance and expertise in compiling this manual:
Hector Acuna, UCSB Environmental Health & Safety
UCSB Laboratory Safety Committee
UCLA Environmental Health & Safety

MANUAL DESCRIPTION

MANUAL PURPOSE - this manual serves two basic purposes:

1. It is the basic laboratory safety manual for UCSB
2. Serves as the campus **Chemical Hygiene Plan (CHP)** as required by the *California Occupational Safety and Health Administration (Cal-OSHA)*. In short, OSHA requires that a written chemical safety plan address the policies and procedures that an employer has in place to minimize the exposure of its lab employees to chemicals.

For **lab supervisors**, the most important portion of this manual (Section I) contains the templates for developing their **required Lab-specific Chemical Hygiene Plan**. The templates allow the supervisor to **customize** this generic CHP to the specific hazards and control measures of their operations – generally with a focus on **Particularly Hazardous Substances** (human carcinogens, acutely toxic chemicals and reproductive toxins).

To put this requirement in more practical terms – if there is a significant chemical incident, OSHA will ask the PI/supervisor to provide their CHP and the documentation that their workers have been trained on it (a training documentation form is included at the end of Section I).

MANUAL STRUCTURE

- **Section I: Lab-specific Chemical Hygiene Plan.** Guidelines and templates for customizing your CHP. Templates available via the EH&S website as a file which is down-loadable and editable, with embedded web links to information useful in completing the templates.
- **Section II: Campus policies, procedures and resources.** Summaries of key/core lab safety issues that apply to most/all laboratories.
- **Section III: Regulatory Framework.** An overview of the Cal-OSHA Laboratory Safety Standard and its relationship to this manual; specific University policies relative to the Lab Standard; the liability, roles and responsibilities of University personnel in the program.

How to Use This Laboratory Safety Manual

Principal Investigators and Laboratory Supervisors

1. Review this Manual, particularly those sections that apply to your research operations. Sections of particular importance to all PIs include:
 - a. Sec. I (lab-specific Chemical Hygiene Plan)
 - b. Sec. II: Personal Protective Equipment; Laboratory Safety Training
 - c. Sec. III: subparts B.2b (overall PI responsibilities), B.3, C.1, C.5, C.7
2. Customize the *Chemical Hygiene Plan* to your operations by completing the templates in Sec. I
3. Review, modify as necessary, and sign-off on the Standard Operating Procedures (SOPs) that are provided to you by Environmental Health & Safety. Incorporate these into your CHP – electronically and/or hard copies.
4. Ensure that your workers have reviewed the manual, particularly the lab-specific portions, and have also signed-off on your SOPs
5. Ensure that all your workers have ready-access to your CHP (bookmark and/or place copy on group's website)
6. Document safety training provided on this CHP and any other safety training (EH&S documents the training that they perform).

Laboratory Personnel

1. Understand the department's **Injury & Illness Prevention Program** (OSHA requirement). On [this page](#) of the department's website are located:
 - a. Summary of the IIPP regulation/program (read this page)
 - b. Link to the full department written planYou should also know:
 - a. The Department Safety Representative (currently Alex Moretto)
 - b. Know how to report an unmitigated hazard in your laboratory (inform your supervisor, or Alex, or anonymously report [here](#))

- c. Know how to report injuries (report these to Alex and use *UCSB Accident Report Form* in department office)
2. Review this **UCSB Laboratory Safety Program and Chemical Hygiene Plan**, with particular attention to
 - your responsibilities listed in Sec. III.B.2.g
 - pages in Sec. II on: use of personal protective equipment, fume hoods and emergency response (find the *Emergency Information Flipchart* in your lab)
3. Review the laboratory-specific **Standard Operating Procedures** with your PI and document your training. All training, whether formal or on-the-job, should be documented and filed per your lab group's procedures.
4. Attend the EH&S **Fundamentals of Laboratory Safety training**
5. Ask for clarification if there are any questions related to your lab work before you begin a new task.

Other UCSB Research Safety Programs/Regulations

Given the great breadth and diversity of research at UCSB, there are other campus safety programs/regulations that can apply to a given research operation. So, in the interests of keeping this manual to a usable size, some more specialized programs and their associated manuals, are not included herein. Instead, links to these programs are provided here and affected PIs and individuals should contact these program managers for further information: The programs toward the bottom of this list rarely apply to UCSB lab operations.

[Biological Safety Program](#)

Biological Use Authorizations; Aerosol Transmittable Diseases; Bloodborne Pathogens; Medical Waste Management

[Radiation Safety Program](#)

Radioactive Materials; Radiation-producing Machines; Lasers

[Hazard Communication Standard Program](#)

Safety Data Sheets (formerly MSDS); chemical labeling
(much of the HazCom program is superseded by the Laboratory Safety Standard/CHP program)

[Research Diving and Boating Safety Program](#)

[Controlled Substance Program](#)

[Animal Care and Use](#)

[Confined Space Program](#)

[Indoor Air Quality Program](#)

[Hearing Conservation Program](#)

[Heat Illness Program](#)

CHEMICAL HYGIENE PLAN

SECTION I

A.	SOP Binder & MSDS Access	2
B.	Stucky Group Procedures and Manuals	
	Safety Check-In Procedure	3
	Check-Out procedure	5
	Working with Biohazards and Biohazard Area User Guide	7
	TEG User Guide	8
	Lyophilizer User Guide	9
	UV-Vis User Guide	10
	GC User Guide	11
	Solar Simulator User Guide	14
	Glovebox User Guide	15
	Schlenk Line Operation and Maintenance	17
	Zetasizer User Guide and Manual	20

SOP Binder and MSDS Access

Standard Operating Procedures (SOPs) for chemicals, processes or physical hazards are included in a separate "SOP Binder" located in PSBN 3622, in Section IB of this Chemical Hygiene Plan, and on the "Group Links" section of the Stucky Group Website (<http://web.chem.ucsb.edu/~stuckygroup/stuckygroup/grouplinks.html>). **It is your responsibility to read, understand and sign SOPs pertaining to the chemicals, processes and physical hazards that you plan on using in the lab BEFORE working with those chemicals.** If your experimental procedure deviates from the approved SOP you are responsible for writing and getting PI approval of an acceptable SOP BEFORE using that chemical. You are also responsible for generating SOPs for the chemicals indicated below if an SOP does not already exist.

There should be complete, approved and signed SOPs for chemicals* listed in Exhibit 1 of the Settlement Agreement & OSHA List of Particularly Hazardous Substances (PHS) which includes Known/suspected human carcinogens (Groups 1, 2A, 2B); Known/suspected carcinogens; Human reproductive toxins: CA Proposition 65 List; Acutely toxic chemicals (LD50 < 50 mg/kg) that are in use in the Stucky lab. They may be labeled with a blue label "SOP on file: please sign before use", but it is your responsibility to find, read, understand and sign any available SOPs for the chemicals that you are using. Chemicals on the list, but not currently in use should be sealed with tape or with a yellow label "storage only: SOP needed for use" or "in storage: requires approved SOP before use". * There are searchable Excel files in the "Chemical Lists Relevant to SOPs" section of the Department Safety Program Page (<http://www.chem.ucsb.edu/about/safety>). It is recommended that you check all chemicals that you plan on using with the "CAS Checker.xls" (found on the Stucky Group website) to determine if any of them are on the Chemical Lists Relevant to SOPs.

A Stucky lab specific SOP template ("Stucky_UCSB_SOP Template.doc") has been generated which can be found on the Stucky Group website. Fill in all information and in the "Protocol/Procedure" section describe the experimental situation(s) in which this material is used in the Stucky laboratory. Discuss minimum and maximum quantities of materials and other reaction parameters. Use the following filename structure for your SOP: "Chemical Name_CAS #_SOP.doc" (e.x. "Bromine_7726-95-6_SOP.doc").

Online Material Safety Data Sheets (SDS or MSDS) can be accessed at <http://ehs.ucsb.edu/units/labsfty/labrsc/chemistry/lchemmsds.htm> or from the chemical supplier. Paper MSDS's are located the "MSDS Binder" in PSBN 3622. If you order a chemical for which there is no MSDS in the "MSDS Binder" it is your responsibility to print out and file a paper MSDS.

Stucky Group Laboratory Safety Check-In

Incoming group members must go over the following items with the Laboratory Check-In Coordinator BEFORE beginning work in ANY of the Stucky Group Labs.

1. Completed the online EH&S safety course (print out quiz results and attach).
 - a. <http://ehs.ucsb.edu/4DAction/WebCourseSessionList>
2. Complete the in-person EH&S safety course as soon as it is offered.
 - a. Signup at website listed above.
3. Read and understand the Laboratory Safety Program including:
 - a. EH&S Chemical Hygiene Plan.
 - b. Standard Operating Procedures for the Stucky Group.
4. Sign/date the master copy of the Laboratory Safety Program in the entryway to room PSBN 3622.
5. Is familiar with the proper procedure for starting new experiments in the lab.
 - a. Review the MSDS for all chemicals that you will be using. This should be done every time that you begin work with a new chemical, and all MSDS should be kept in a personal file (paper version) and/or individually bookmarked on your computer.
 - b. Identify Particularly Hazardous Substances that are to be worked with. This should be done every time that you begin work with a new chemical. Information on PHS can be found at
<http://www.ehs.ucsb.edu/units/labsfty/labrsc/chemistry/lischphazsubstance.htm>.
 - c. Incorporate the proper safety precautions for dealing with the hazards identified into your experimental protocol (i.e. necessary personal protective equipment (PPE), hazardous waste disposal, proper labeling).
 - d. DO NOT START WORK until you are certain that you have taken all of the hazards into account. You can contact the Stucky safety committee or EH&S at any time if you are uncertain about how to control the hazards associated with any substance.
6. Can locate emergency response items (safety showers, spill control kit, fire extinguisher, etc.)
7. Is familiar with emergency building evacuation procedure:

- a. Location of nearest stairwells (the west end of PSBN and the one nearest to the old Chem building).
 - b. All personnel must assemble on the South Lawn and *remain present* so they can be accounted for. (Bring your keys and laptop if possible)
- 8. Can locate hazardous waste storage area and is familiar with how to properly fill out a waste label.
- 9. Is familiar with the areas where work with Particularly Hazardous Substances (PHS) is conducted.
 - a. Areas where PHS work is conducted must be labeled clearly to inform others of potential hazards they may encounter.
- 10. Is equipped with the proper personal protective equipment (lab coat, safety glasses, gloves).
- 11. Is familiar with EH&S contact phone numbers and website.
 - a. Emergency contact info is posted in each lab room, usually close to the doorway.
- 12. Is familiar with the members of the group safety committee.
 - a. 5 members, one from each lab room.
- 13. Is familiar with the procedure for reporting unsafe activity;
 - a. First step: Approach unsafe individual directly and discuss your concerns with him or her.
 - b. Second step: If you still have safety concerns after 1st step, contact group safety committee.
 - c. Third step: If safety concern still exists, contact Galen directly.
 - d. Fourth step: If safety concern still exists, contact EH&S directly.

Once the checklist is complete, it must be signed by the individual, the principal investigator, and the check-in coordinator and kept on file in Room 3633.

New Group Member (print name) Date

Check-In Coordinator Date

Principal Investigator Date

Signature

Signature

Signature

Stucky Group Move-Out Procedure

You should let the Laboratory Check-out Coordinator know your move-out date at least one week before you leave. You have to schedule two meetings with them: a first meeting in order to identify everything that you are responsible for cleaning before you leave, and a second meeting to show that you have cleaned all of your spaces. After you have demonstrated that you have followed the move-out procedure, Galen will sign you off.

Note that it usually takes at least 3 full days for people to complete this procedure. Please plan accordingly.

Thank you in advance for your cooperation!

1. All samples must be properly dealt with
 - a) **All old samples must be disposed of**
 - b) Any samples that absolutely must be kept in the lab must have a **label** indicating exactly what they are (date, composition, reference to your lab notebook page). **These samples must be given to a current group member for storage.**
 - c) If there is no lab member who will take responsibility for your samples, you are **not allowed** to leave samples in the lab.
2. All personal chemicals must be entered into the group chemical inventory and placed in the appropriate location
 - a) Please dispose of any chemicals that are dangerous to store (ie peroxides) or entrust their safe storage to a current group member.
 - b) If you need help putting your chemicals away, you can ask lab members for help and offer them lunch/dinner to thank them.
 - c) You should create a spreadsheet that follows the format of our chemical inventory and record all the chemicals that you are placing in the group shelves. An electronic version of this list must be handed to the person in charge of the chemical inventory.
3. **All supplies must be returned to the appropriate place**
 - a) Thoroughly **clean all glassware** in your possession and **return to the group glassware collections.**
 - b) **Return any tools** in your area to the group toolbox.
 - c) Remove all contents from your personal drawers and **return items to group storage** or other group members.
4. **Your areas must be cleaned**
 - a) Wipe down drawers, countertops and shelving with IPA/paper towels.

- b) Thoroughly **clean any hood space, fridges, vacuum lines and glove boxes** that you have used during your time here.
- c) Clean all common lab space (counters, scales, glove-boxes, spin-coater, TEG, Nanosizer, etc) for any messes you may have made.
- d) Clean your desk/office space. **Recycle all old papers or journals** or specifically give them to another lab member.

5. You must deal with all your data

- a) Remove all data from common lab computers. Back-up important data to CDs and deposit with your lab notebook.
- b) Clearly label and deposit your lab notebooks and backed-up data in the designated lab location.

6. Gas cylinders must be returned and the stockroom notified.

7. Transfer group duties and instrument responsibilities to other group members. Train others as necessary.

8. Check with current Internal Lab Safety Coordinator that all safety requirements have been met. Report any issues that you do not know how to deal with.

9. Meet with Galen after you have completed cleaning everything.

Biohazard Area User Guide

Before work can begin the user must take Biosafety, Bloodborne Pathogens and Medical Waste course LS02 offered through EH&S. Information can be found on the EH&S website under training.

- Gloves and safety goggles must be worn in biohazard area; lab coats are highly recommended
- Everything marked with a biohazard label must stay in biohazard area
- Do not bring non-biohazard equipment into biohazard area
- Gloves used in biohazard area must be disposed of in biohazard labeled receptacle (small red biohazard trash can)
- Any material used in biohazard area must be disposed of in biohazard labeled receptacle (TEG cups, plasma, human blood, etc.)
- Use Biohazard bags to line biohazard waste receptacles
- If gloves are exposed to biohazard material, remove and dispose of properly and then replace with new gloves (ex. Liquid plasma)
- Replace gloves often to limit contamination and exposure
- To clean biohazard area: make a 10% bleach solution and spray area generously. Allow to sit for at least 15 min. before wiping up
- Biohazard waste will be picked up 2x/month by Medical Waste Environmental Engineers Inc., a receipt will be left and if more frequent pickups are needed then call them to schedule it

TEG USER GUIDE

Turn on TEG and let warm up for 30 minutes

Turn on computer, open TEG software (Username: Administrator Password: TEG)

TEG Program → Maintenance: E-Test

Level 1 and Level 2 (In refrigerator)

Green vial = Water, Blue = Powder

Dump water into powder, DO NOT SHAKE, mix gently

TEG Button on software

Channel 1 → level 1 & 2 control

→ lot number

→ 20 uL 0.2 CaCl₂

The channel that is highlighted will go first

Loading TEG cups:

Put cup in slot

Push cup up into machine

Push button underneath tray 3X while holding the machine down

Pull tray back down

Push cup all the way into slot

Loading TEG Sample:

20 uL CaCl₂ into cup first

340 uL Plasma sample into cup second

Push tray up into machine

Turn the switch to TEST

Push F10 on computer to start

Cleaning Channels:

Clean channels based on how often machine is used

Qtip broken in half

Hydrogen peroxide on tip and clean channel

Alcohol pad on broken Qtip stick and clean channel

Preparing Plasma:

Remove sample from -80°C Freezer

Put into beaker of water at 37°C for 20 minutes

Run sample

Lyophilizer Operation Procedure

1. Make sure the top cover is on the lyophilizer and all output valves are off (the bevel on each knob should be positioned away from the sample port).
2. Turn ON the main power switch on the right-hand side of the lyophilizer.
3. Press the **Refrigeration Man** switch on the control panel. This will start the refrigeration system. Wait until the temperature reaches -40°C (takes about 20 min).
4. Turn on the vacuum pump by pressing the **Vacuum** switch on the control panel. Wait until the vacuum indicator light is green (takes a few hours).
5. Add the pre-frozen sample to a freeze drying flask and attach it to the sample port. * Pre-freezing temperature must be at least 10° to 20°C below the eutectic temperature of the sample. Typically, let the sample sit in the -80°C freezer overnight.
6. Turn the plastic valve knob to the vacuum position to open the valve. The bevel on the knob should be positioned toward the sample port to apply vacuum to the sample.
7. Wait until the sample is completely dry. Typically, the sample is nearly dry when all the frost disappears from the outer surface of the sample container. To be certain of low final moisture content, dry the sample for several hours past this point.
8. To remove the container after drying is complete, turn the plastic knob on the valve to the vent position, which closes the valve and vents the container. The sample container can now be removed.

Shut Down Procedure

1. Release the system vacuum by turning the plastic knob on a valve to the open position.
2. Press the **Vacuum** switch on the control panel to turn the vacuum pump off.
3. Press the **Refrigeration Man** switch to turn off the refrigeration system.
4. Remove the top cover off the lyophilizer.
5. Turn off the main power switch.

UV-Vis User Guide

You must be trained by the responsible lab member before you can have full access to the machine

1. Turn on computer
2. Enter username and password
3. Turn on UV-Vis
4. Open UV-Vis software (Instrument 1 online)
5. Turn on both lamps: Instrument → Lamps
6. Let the machine warm up for a minimum of 30 min
7. Run blank (Click blank button on screen)
8. Run samples
9. Save spectra to your individual folder
10. Turn off lamps: Instrument → Lamps
11. Shut down software
12. Turn off computer
13. Turn off UV-Vis

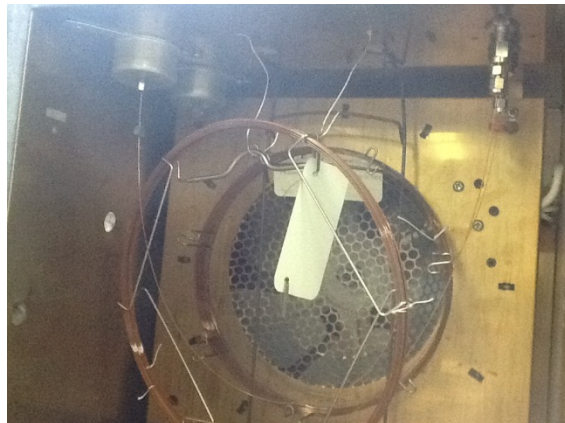
GC User Guide

By: Young-Si Jun (ysjun@chem.ucsb.edu)

Front : Injector A and Detector A (TCD)

Back : Injector B and Detector B(FID)

1. Check the columns first before you start your operation. Normally, we use TCD column (molecular sieve 5A) for permanent gas analysis, for example, hydrogen, oxygen, nitrogen, and carbon monoxide and FID columns for hydrocarbons, for example, methane, methanol, and higher hydrocarbons. We also have PEG-based column (ZB-WAXSplus), with which we are able to analyze organics in aqueous solutions.



2. If you need to use the column which is not installed, you have to install your column. We use graphite (single use only, HP 1/16"×0.4mm) fittings for injector connection. Once you load rubber septum, SS connection fitting, graphite septum, you need cut the end part of column by using ceramic ruler because the column might be blocked. Try to scratch (just once) the column about 0.5 cm with the ruler, and cut it by hands softly. Then, connect inlet part first. To be sure, check whether there is leak by immersing the other end into acetone. Open the He regulator and increase the total flow valve (the left side of the oven) of detector A for TCD and Detector B for FID. Once you see the bubbles, then you can be sure that the injector part is well connected without leak or block.

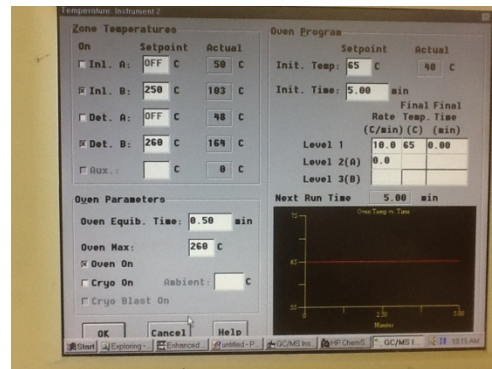


3. Connect the detector part in the same way. Graphite septum will fit into the SS fittings because graphite is kind of soft material compared to SS. So, please do not tight the fittings too much.
4. Turn on GC and open the gas regulators. He only for TCD and He, air, and H2 for FID. Air and H2 are flame gases of FID. Open the FID gas valves in the left panel of GC.



5. Once you see the sign in the control panel indicating that GC passed self test, then execute the software in PC. GC2(2) is for HP5890. Chemstation (ver. B.01.00) does not support HP5890A. But, ignore the warning sentences. We are using multiple GCs. The other icons are for other GCs. Once you see that GC is connected with the software, load method for your analysis.

6. To program your own method, go to method menu, click edit entire method. Click OKs until you see the temperature setup menu. Set temperature for Injector, oven, and detector according to the instructions for your column. You can change the ramping rates or other setting values later once you see the peaks that you want. Go to detector menu. select Detector A for TCD or Detector B for FID. In case you are interested in H₂ analysis, you can set negative polarity. Since we are using He as a carrier gas, the peak of H₂ will be shown as as negative peak. Less than 6 wt % of H₂ in He will be shown as a positive peak. So, there is converting concentration. Go to signal and select signal 1 and det. A for TCD and Det. B. and signal 2. for FID.

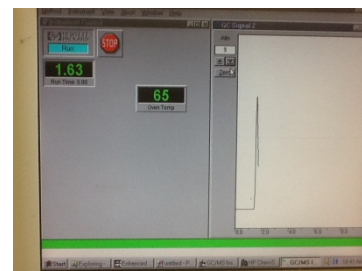


7. FID only. Once the detector and injector temperature reach the setting values, ignite the flame by pressing FID ignitor. You might hear the sound. Press twice the button "SIG 2" on the right panel of GC. You will see the signal of FID detector. If you see the signal jumped up to some values like 30 or 20, it means that FID is ignited. Stabilize your signal for more than an hour. Increase the total flow up to 10 psi (column head pressure). We normally increase the total flow after we ignite the FID because it might interrupt the ignition. We are not able to use both FID and TCD detectors simultaneously. please do not flow He into the other detector which you don't use by closing all of the valves of the other detector.



8. Inject your samples by using GC syringes. We have syringes for liquid samples. We also have gas tight syringes (100 and 250 micro liter) for gas samples. Press green arrow button and then provide the sample information. Press start and then you will see another button appearing. After you inject your samples, press the start button as soon as possible. Retention time of your sample might be slightly different depending on your injection speed and volume.

9. You can see that there is red rubber septa inside the injector. If you inject several times, then the rubber will be destroyed. You might see the column head pressure



going down to zero during your operation. Then, you need to change the septum. You first need to decrease all of the temperatures to room temperature. You should not touch the injector with your bare hands because it is very hot (200-300 degree C).

10. You can see the real time signal. You can also bring your analysis result into the screen by using data analysis software.
11. To integrate the signal in the data analysis software, use the right click of mouse to set the starting and ending point of the peak. Software will tell you the peak retention time, area, and height.
12. When you are done with the samples, load stand by method in order to cool down the injector, oven, and detector. You have to wait until all of the temperatures reach to room temperature. Turn off GC first, close software, then finally close all of the gas regulators.

Solar Simulator User Guide

By: Young-Si Jun (ysjun@chem.ucsb.edu)

Wear protection glass first. Light from solar simulator includes very strong UV which will burn your eyes.

Turn on the power supply.

Click display/select button until WATTS led turns on.

Click set/enter to set the power value you want. It depends on what kind of lamp you are using. We normally use 300 W Xelamp. It provides the intensity more than that of 2 sun. Please do not set the values as you want, which will severely damage the lamp. You can control power by placing neutral density filters in front of light house and controlling sample position

Place filters. We have neutral density filters and band pass filters (300, 400, and 420 nm) and also IR filters.

Press lamp start or lamp off button. Stabilize the lamp for more than an hour before you illuminate your samples. Please do not turn on or turn off several times in a day. Instead you just leave your lamp as turned on.

When you are done with the lamp, turn off the lamp and then wait till the bulbs are cooled down. You have to wait until the fan on the side of the light house stops.



Glove Box User Guide

To Take a sample into the Glove Box:

Close all open valves, only one valve may be open at a time (doors count as valves)
Open Refill valve of small/big chamber, fill all the way
Close refill valve
Open chamber door
Put in sample
Close chamber door
Evacuate chamber
Refill Chamber to 50 (1 purge cycle)
3 Total purge cycles
Refill chamber to 0
Adjust Glove Box pressure → Function → Lab pressure control → low pressure limit (0.5) →
Escape → High pressure limit (3.0) → Escape
Adjust pressure as you put hands into the box
Open inside Chamber and remove sample
Close chamber

To Take a sample out of the Glove Box:

All valves closed
Open refill valve, fill to 50
Close refill valve
Evacuate chamber (1 purge cycle)
3 Purge cycles total
Open refill valve and fill to 0
Adjust glove box pressure
Adjust pressure as you put hands into the box
Remove chamber door inside box
Load sample into chamber
Close chamber door
Remove hands from box
Open outside chamber door
Remove sample
Close chamber door
Evacuate chamber
Purge chamber 1 time
Adjust Glove Box pressure (High = 4.5, Low = 1.5)

Done → Open both evacuation valves and record what you did on the chart

Change pump oil once a month: Close all valves, turn pump off, drain all pump oil into hazardous waste plastic container, fill pump half way with oil and let run for couple minutes, drain pump oil out (3X), Fill pump full and turn back on, open evacuation valves.

We have three glove boxes. And they each have their own rules of what chemicals may be introduced. On the outside of each of these boxes, there is a list of general rules. I do not have an electronic copy of these rules and won't be able to copy it all down until Monday.

Yichi is in charge of the "electrochemistry" glovebox. You will want to ask him for more specific details about that box.

Young-Si is in charge of the "wet" glovebox. This means certain "dry" solvents may be introduced.

I am in charge of the "dry" glovebox. Absolutely no solvents may be introduced. There are air and water sensitive chemicals in this box.

Schlenk Line Operation and Maintenance

Schlenk lines are an apparatus useful for performing a variety of common laboratory techniques under air-free conditions. A well-maintained Schlenk line will allow you to do the following: flame dry glassware, vacuum distillation, freeze-pump-thaw degassing, removal of residual solvent, vacuum transfer purification, and run a reaction under an inert or reagent gas. The apparatus consists of two long glass cylinders (lines) with valves on the bottom, a vapor trap to the right attached to the vacuum pump, and a gas bubbler to the left. The incoming gas line, (Ar or N₂) passes through a drying tube filled with Drierite, and the indicator should be blue.

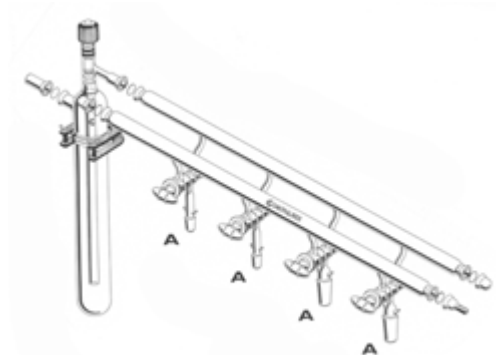


Figure 1: The Schlenk line

Operating Vacuum lines:

Close all valves, and then turn on the vacuum pump. Starting with the valve closest to the pump, slowly open to fill the line with vacuum. Before opening your reaction to vacuum, place Dewar's filled with liquid N₂ on the traps.

Operating Inert lines:

Close all valves. Turn on gas (Ar or N₂) tank and use the bubbler attached to the line to adjust the gas flow, typically only need one bubble ever one or two seconds.

Check for leaks:

It is important to check for leaks at the joints of the line to prevent contamination of your reaction, collecting explosive liquid O₂ in your traps, or damaging the vacuum pump. This can be done by lightly applying a dilute soap solution to the joints to check for bubble formation. Leaky joints should be greased and tightened.

Clean the trap.

You should make sure the trap is cleaned *on a daily basis* when in use. After you've performed your experiment, you should remove the N₂ Dewar, close the valve to the pump, immediately after isolating the trap, quench the vacuum by opening a stopcock, allow the trap to warm to RT, clean it out with acetone or another solvent appropriate to your reaction conditions, blow

forced air through it to dry, reassemble the trap, and put the trap and line under vacuum by opening the valve to the left of the trap

The Line: Under normal circumstances, this should require minimal maintenance. Periodic cleaning with degreasing solvent, acetone, water, and then IPA (once a year) is recommended. You should clean it as needed – if you inadvertently pull a compound into the line (including HCl, a common mistake), or oil from your bubbler (this will happen if you're not paying attention during evacuation/backfill cycles).

Safety Considerations: There are multiple ways to “blow up your world”. Memorize them and avoid them at all costs.

1. Explosion: can happen as a result of several easily avoidable pathways.
 - i. Overpressure in the Line: One must make sure that the system is not closed when the gas supply is open – always make sure your bubbler is open, and when using the lines in 565, make sure the vacuum line is on.
 - ii. Condensed Gases: Some commonly used gases are easily trapped at liquid nitrogen temperatures. These include carbon monoxide/dioxide, ethylene, and argon. For example, ~10 mL of liquid carbon monoxide at -195 °C corresponds to 6500 mL as it is warmed to its boiling point just 4° warmer – a huge change in volume! One might mistakenly condense argon if the flask on Schlenk line is open to the argon line rather than vacuum during freeze-pump-thaw.
 - iii. Runaway Reactions: Some reactions are highly exothermic and could cause a low-boiling solvent (like ether or DCM) to boil. Other reactions evolve gases as by-products (like the Swern Oxidation). Be sure there is an outlet for the pressure!
 - iv. Heating a Closed System: This can be dangerous. Consider using a blast shield if your reaction requires heating a closed system over the boiling point of your solvent. Never heat a closed reaction vessel containing a reaction that evolves gas! (example: thermal Boc deprotection)
2. Implosion: If you are putting a flask on your vacuum line, check for star cracks or defects that can lead to implosion.
3. Liquid O₂: A condensed gas so dangerous it gets its own section. This is formed when air is continuously pulled into a liquid nitrogen trap. *Liquid O₂ reacts violently with almost anything including vacuum grease and Teflon tape.* Avoiding liquid O₂ condensation is why I've repeatedly stressed that you must check for leaks before cooling the trap. If you remove the trap and see a pale blue liquid, put the trap back on, tell your lab mates to evacuate the lab and alert EH&S.

Common Techniques on the Schlenk Line:

1) Flame Drying Glassware: The glassware/apparatus to be dried is fitted with either a rubber septum and a needle line going to the Schlenk line, a ground glass hose adaptor and a tube to the Schlenk line, or directly attached to the line by the ground glass. The Schlenk line vacuum should be active and inert gas should be on. Put the flask under vacuum. Apply a flame to the exterior of the flask, taking care not to scorch the septum or any other plastic parts. Shut off the vacuum. Open the inert gas line. As you open the inert gas, Watch your bubbler and either make sure the oil is not sucked into the line. Repeatedly evacuate and backfill three times. Allow the flask to cool to RT and continue with the experiment.

2) Freeze-Pump-Thaw (FPT): Turn on the vacuum and inert gas lines as described above. Cool the trap just in case you mistakenly pull vacuum on the liquid. Attach your material to be degassed to the line either directly, or via a hose and ground glass hose connector – *absolutely no septa allowed*. Grease your joints to obtain maximum vacuum. Cool the flask in a bath sufficient to freeze the solvent well below the melting point. Dry ice/IPA will suffice for some, but many will require liquid nitrogen. Once the material is frozen solid, open the flask to the vacuum line, and open the Teflon screw on the top of the flask. Evacuate for 5-10min. Close the Teflon screw on the top of the flask and remove the flask from the cold bath. Allow the solid inside to melt. Repeat the above steps at least 3 times (4 cycles). On the final cycle, you may backfill with Argon if desired. Seal the Schlenk flask and proceed with your experiment.

3) Removing Residual Solvent: Turn on the vacuum and inert gas lines as described above. Cool the trap. Attach a flask containing your material to the Schlenk line. If it is a solid, used a hose connector containing a frit. If you desire maximum vacuum, grease your joint - this is especially important if you want to use other taps on your line to do FPT or vacuum distillation. Allow the material to stand until you are satisfied residual solvent has been removed. Backfill, remove your flask, and remember to CLEAN THE TRAP WHEN DONE. This is no point in having a trap if you don't clean it after you've pulled something in there.

ZetaSizer User Guide – 1/13/2010

1. Instrument Reservations and Sign-In Log
 - a. YOU MUST BE PROPERLY TRAINED by the Stucky group member responsible for the ZetaSizer prior to using the instrument.
 - i. Attending the Malvern training session does not qualify you to be an independent user or train other members of your research group.
 - ii. Each member of your research group must be trained by the Stucky group member responsible for maintaining the instrument.
 - iii. You will be given the computer log-in password for the ZetaSizer at the time of your training. **DO NOT GIVE OUT THIS PASSWORD TO OTHER USERS. If you are caught distributing this password without permission, then your instrument privileges may be revoked.**
 - b. You must reserve time on the instrument by signing your name on the reservation sheet taped to the desk. If the dates on the sheet are not current, please erase them by wiping the sheet down with ethanol and fill-in the appropriate dates.
 - c. You must fill out the “ZetaSizer User Log” every time you use the instrument. This will help us keep track of which groups are using the instrument most frequently and identify the source of any problems that may occur.
2. Instrument Manuals and Application Guides
 - a. Manuals for the ZetaSizer and the Auto-Titrator are located both on the shelf above the ZetaSizer and in a large white binder sitting on the desk behind the computer monitor.
 - b. There are pdf copies of the manuals located in the “Malvern ZetaSizer Files” folder located on the desktop of the computer.
 - c. The “Malvern ZetaSizer Files” also includes many helpful documents from Malvern such as training presentation, application notes, sample preparation guidelines, measurement techniques, and zeta potential and dynamic light scattering theory.
 - i. Please refer to these files if you have questions regarding specific instrument applications.
 - ii. You may also call the **Malvern Help Desk at (508) 768-6450** and talk to an Applications Specialist to get information specific to your sample/measurement type.
 - iii. Feel free to take copies of the electronic files with you, but do not erase them from the ZetaSizer computer.
3. Instrument Startup
 - a. The “on/off” switch for the ZetaSizer is located on the back of the instrument. Turn the switch on and let the instrument warm-up for **AT LEAST 30 minutes** before running a measurement.
 - b. You should hear one loud beep when you turn the switch on. Then, you will hear three additional beeps as the instrument goes through the self-check process.
 - i. If you do not hear all three self-check beeps (4 total) then the instrument is not working properly and you should not run any measurements. Please

notify the person responsible for maintaining the ZetaSizer
IMMEDIATELY!

- c. When the self-check process is completed, the light around the lid button should turn green.
 - i. If the light does not turn green it could be for one of two reasons:
 - The software used to control the ZetaSizer is not open and the instrument is not recognizing the computer.
 - The instrument failed the self-check process
 - ii. Always check to make sure that the software is open and the instrument is properly connected to the computer if you do not get a green light.
 - iii. If this does not fix the problem, then do not run any measurements and notify the person responsible for maintaining the ZetaSizer IMMEDIATELY!
 - iv. **Amber light = Not ready to run, Red light = Instrument bad – Call Malvern, Green light = Ready to measure.**
- d. The “on/off” switch for the Auto-Titrator is located on the back of the instrument. The small, round light on the front of the instrument should light up when the instrument is on.

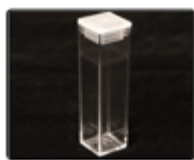
4. Software and Data Storage

- a. There is a shortcut to the software that runs the ZetaSizer on the computer desktop. It is a bright pink square with two white triangles on the front. The software icon is label “ZetaSizer”.
- b. Double-click on this logo to open the software. If the instrument has already been turned on, then it should automatically recognize the connection to the computer.
- c. Alternatively, you can open the software on the computer before turning the instrument on and the connection should be recognized automatically.
- d. The software will automatically open the last measurement file used. If this is the not the file you would like your data to be stored in, then close the measurement file and open or create a new one.
- e. You can open an existing measurement file by going to “File → Open → Measurement File.” This will take you to the “Measurement Data” folder where all measurement files are stored.
- f. If you do not have your own folder in the “Measurement Data” folder, please make one and store all of your measurement files there. DO NOT store measurement files on the computer desktop.
- g. The “Measurement Data” folder can is stored in “C:\ProgramFiles\MalvernInstruments\DTS\MeasurementData”. There is also a shortcut to the “Measurement Data” folder in the “Malvern ZetaSizer Files” folder on the computer desktop.
- h. To create a new measurement file go to “File → New → Measurement File”. This will automatically open the “Measurement Data” folder. Select, or create, a folder with your name on it and then save the new measurement file in that location.
- i. You will not be able to set up a manual measurement of an SOP until you have opened a measurement file.

- j. Make sure that the opened measurement file is the file that you would like your data to be stored in!!!!

5. Sample Cells and Measurement Types

- a. All sample cells are designed for AQUEOUS samples only. DO NOT use organic solvents.
- b. There are a limited number of disposable cells available in the Stucky group. If you are going to use the disposable cells regularly, your group should purchase their own supply from Malvern (www.malvern.com). There is a parts price list available in the white binder that contains the ZetaSizer manual. The disposable 12 mm cuvettes available from Malvern are a higher quality polystyrene than those available from Fisher Scientific and, therefore, are more expensive.
- c. Size and Molecular Weight Measurements



12 mm polystyrene cuvette with lid.

The cell needs to be filled between a height of 10 and 15 mm. There is a sample height indicator inside the lid covering the ZetaSizer sample cell for reference. Each cell should be rinsed once with EtOH or MeOH followed by 2 or 3 rinses with DI water. The final rinse should be your dispersant solution. **MAKE SURE THAT THE OUTSIDE OF THE CUVETTE IS COMPLETELY CLEAN AND DRY** before placing it inside the ZetaSizer and always keep the cover in place to prevent dust from settling inside your samples. These are disposable cuvettes and should not be re-used.



Small volume polystyrene cuvette with lid.

The cell needs to be filled between a height of 10 and 15 mm. There is a sample height indicator inside the lid covering the ZetaSizer sample cell for reference. Each cell should be rinsed once with EtOH or MeOH followed by 2 or 3 rinses with DI water. The final rinse should be your dispersant solution. **MAKE SURE THAT THE OUTSIDE OF THE CUVETTE IS COMPLETELY CLEAN AND DRY** before placing it inside the ZetaSizer and always keep the cover in place to prevent dust from settling inside your samples. These are disposable cuvettes and should not be re-used.



Low volume, 3 mm, batch quartz cuvette with screw cap.

This cell is designed to use the minimum volume of sample possible for a size or molecular weight measurement. The sample must be pipette carefully into the

bottom of the cuvette, so it is filled from the bottom up. The minimum volume that can be used is 12 μL . This will only partly fill the visible cell volume. After filling, carefully inspect the cell for trapped bubbles. **BE VERY CAREFUL** when removing air bubbles. Any chips or cracks on the outside of the cuvette could interfere with measurement quality.

MAKE SURE THE OUTSIDE OF THE CUVETTE IS CLEAN AND DRY before placing the cell in the ZetaSizer. Wipe the outside of the cuvette with lens paper **ONLY** to prevent scratches on the outer surface. This is **NOT** a disposable cuvette, so it must be cleaned before and after use, as well as between each measurement.

Cleaning Procedure: Rinse the cuvette once or twice with DI water. Fill the cuvette with EtOH or MeOH and submerge in an ultrasonic bath for 5 minutes. Rinse the cuvette several times with EtOH or MeOH, and then several times with DI water. You can dry the inside of the cuvette by flushing with an aeroduster (canned air). Dry the outside of the cuvette by wiping with **LENS PAPER ONLY**. Rinse once or twice with your sample dispersant just prior to loading your sample.

Make sure that the sample cell is completely clean and dry when you are finished with your measurements. Always store the sample cell in the case provided when not in use.



Low volume, 3mm, flowing quartz cuvette with tubing.

This cell is designed to use the minimum volume of sample possible for a size or molecular weight measurement. This cuvette can also be connected to the auto-titrator for flow-through measurements. If you do not need to do a series of measurements using the auto-titrator, then you should use the batch cell described above.

This cell can be filled by connecting the cell to the auto-titrator and following the procedure for “cleaning” the sample cell. Make sure that there are no air bubbles in the sample cell or the connected tubing. **BE VERY CAREFUL** when removing air bubbles. Any chips or cracks on the outside of the cuvette could interfere with measurement quality.

MAKE SURE THE OUTSIDE OF THE CUVETTE IS CLEAN AND DRY before placing the cell in the ZetaSizer. Wipe the outside of the cuvette with lens paper **ONLY** to prevent scratches on the outer surface. This is **NOT** a disposable cuvette, so it must be cleaned before and after use, as well as between each measurement.

Cleaning Procedure: Rinse the cuvette once or twice with DI water. Fill the cuvette with EtOH or MeOH and submerge in an ultrasonic bath for 5 minutes. Rinse the cuvette several times with EtOH or MeOH, and then several times with DI water. You can dry the inside of the cuvette by flushing with an aeroduster (canned air). Dry the outside of the cuvette by wiping with LENS PAPER ONLY. Rinse once or twice with your sample dispersant just prior to loading your sample.

Make sure that the tubing and the sample cell are completely clean and dry when you are finished with your measurements. Always store the sample cell in the case provided when not in use.

d. Zeta Potential Measurements



Disposable folded capillary cell.

This cell can be used either for batch zeta potential measurements or flow-through size and zeta potential measurements using the auto-titrator. There are detailed instructions for the cleaning and filling procedures taped to the bench top next to the ZetaSizer. Always rinse your cell with EtOH or MeOH, then DI water, and then your dispersant before adding your sample to the cell.

You need at least 1 mL of sample to use this cell. Make sure that the cell is completely filled with your sample and carefully remove any air bubbles present in the cell or on the surface of the electrodes. Always use the cell with the caps in place, unless you are connecting the cell to the auto-titrator. **MAKE SURE THAT THE OUTER SURFACE OF THE CELL IS CLEAN AND DRY** before placing the cell in the ZetaSizer sample holder. When placing the cell into the sample holder, press the cell down gently. Pushing the cell into the sample holder roughly could cause the electrodes on the instrument to break!

These cells can be reused for a limited number of uses. Always check the surface of the gold electrodes inside the cell to make sure they haven't turned black. If they turn black, then either your sample or your solvent has reacted with the electrode surface and the cell is no longer good. If the electrodes are still a shiny gold color, then the cell is good and you can continue using it.

Clean the cell between uses by flushing with DI water or your sample dispersant. You can also submerge the cell in an ultrasonic bath to facilitate cleaning. The cell can be thrown away when it is no longer usable.



Dip cell with glass cuvette.

The dip cell is designed to do batch zeta potential measurements on small samples, or in solutions that will readily react with the gold electrodes on the folded capillary cells. The electrodes in the dip cell are palladium and can easily be cleaned between measurements.

The dip cell is designed to be used with the glass cuvette that comes with the dip cell kit. This cuvette IS NOT disposable and should be cleaned before and after each use. The dip cell is usually stored inside a disposable polystyrene cuvette to protect the electrodes. The polystyrene cuvette should not be used for measurements.

To clean the glass cuvette, rinse once or twice with EtOH or MeOH and then once or twice with DI water. You can also fill the cuvette with EtOH or DI water and submerge in an ultrasonic bath for 5 minutes for a more thorough cleaning. You should rinse the cuvette with DI water and then your sample dispersant before adding your sample. **MAKE SURE THE OUTSIDE OF THE CUVETTE IS CLEAN AND DRY** before inserting the cell in the ZetaSizer. Wipe the outside of the cuvette with **LENS PAPER ONLY**.

The cuvette should be filled to a minimum sample height of 7 mm and a maximum sample height of 10 mm. Overfilling the cuvette will cause the cuvette to overflow when the dip cell is inserted. Overfilling can also cause thermal gradients in the sample which will interfere with the accuracy of the instrument temperature control. The minimum sample height corresponds to approximately a **0.7 mL volume**. Tap the cell gently after adding your sample to remove any air bubbles.

Cleaning the dip cell: NEVER COMPLETELY IMMERSE THE CELL IN LIQUID. If the electrodes at the top (outside) of the dip cell are submerged in water (or any other liquid) the cell will not function properly!!!

Immerse the bottom electrodes in your sample dispersant and sonicate for 5 to 15 minutes. Rinse the electrodes with DI water, and then sonicate in DI water for an additional 5 to 15 minutes. Rinse the electrodes with DI water again and gently pat the electrodes dry with a kimwipe. Make sure the dip cell is completely clean and dry before returning it to the storage case.

6. Filtering Samples and Titrants

- a. Malvern recommends that all titrants and samples be filtered with a 0.22 μm filter before taking measurements. This ensures that any dust or debris that may be in your solutions does not interfere with measurement data.

- b. The easiest way to do this is to filter your solutions with a Corning vacuum filter container or a Stericup.
 - c. If you would like to filter your solutions or samples on a smaller scale, you can use a syringe and the 0.22 μm syringe filters that are located on the shelf above the ZetaSizer.
 - d. Anyone using the Auto-Titrator must provide their own titrants. Typical titrant solutions include 0.25 M, 0.025 M, 0.1 M, and 0.01 M NaOH or HCl, depending on the pH increments you choose and whether you are doing a basic or an acidic titration. Making your own titrants will prevent cross-contamination of solutions.
7. Using the Auto-Titrator
- a. If you would like to obtain a titration curve that includes the full pH range, then it is recommended that you perform two separate titrations for the basic and acidic ranges.
 - i. Example: Your solution starting pH is 7.5, so you would do one titration using sodium hydroxide that covers pH 7.5 – pH 14. Then, you would start with a new sample and do a titration with hydrochloric acid to cover pH 7.5 – pH 2.
 - ii. This will prevent over-filling of the sample cup during the titration.
 - b. As stated above, make sure all of your titrants have been filtered with a 0.22 μm filter before using them.
 - c. You may degas your titrants by bubbling them with N_2 for 10 – 15 minutes prior to use. This may yield more accurate results when doing size and zeta potential measurements. It is recommended that you degas your solutions just before use.
 - d. Setting up the Auto-Titrator:
 - i. There are detailed instructions on the auto-titrator functions in the MPT2 manual located in the “Malvern ZetaSizer Files” folder on the computer desktop and in the white binder located behind the computer monitor.
 - ii. The “on/off” switch for the auto-titrator is located on the back of the instrument. When you turn the auto-titrator on, the status indicator on the front of the instrument should be amber.
 - Amber = Standby
 - Red = Error
 - Green = In Operation
 - iii. Please inform the Stucky group member responsible for the ZetaSizer if the status light is red!
 - iv. The titrants should be empty and the fluid lines should be clean and dry when you start the set-up.
 - v. Add your titrants to the titrant containers and loading them into the Auto-Titrator.
 - Titrant containers are located in the large cardboard box beneath the ZetaSizer.
 - Fill the titrant container to the line marked on the top of the tube.
 - Make sure the fluid lines are completely submerged in your titrants so air bubbles are not introduced

- vi. The next step is to prime the titrants, which fills the auto-titrator with your solutions and purges any air bubbles that are in the tubing or the manifold assembly.
 - Go to the manual control panel for the MPT2 (icon located in the toolbar of the Malvern software) and select the “prime” tab.
 - You can prime all of the titrant lines, or select individual lines to prime. For example, if you only used 2 out of the 3 titrant lines then you only need to prime those lines. If you want to prime all of the titrant lines, then select “all”.
 - Set the number of cycles for 6 cycles.
 - Place a waste beaker under the sample holder and press the “prime” button.
 - The instrument will purge the fluid lines between the titrant containers and the sample cup.
 - Carefully watch the lines to make sure:
 - a. The air bubbles are being removed from the system
 - b. No new air bubbles are being introduced
 - c. There are no leaks in the fluid lines or fittings
 - Perform the prime 2-3 times to make sure the lines are thoroughly flushed with your titrants.
- vii. Attach the folded capillary cell to the sample lines for zeta potential and size measurements, or the quartz flow-through cell for size measurements.
 - Rinse the cell once with EtOH and once with DI water before attaching to the sample lines.
 - Select the “clean” tab under the manual control panel for the MPT2.
 - NEVER ADJUST THE FLOW RATE ABOVE 40% WHEN cleaning or filling your cell.
 - Place a beaker of DI water under the sample holder and press the “clean” button.
 - This will circulate water through the fluid lines that go from the sample holder, to the sample cell, and back to the sample holder again.
 - Carefully tap the sample cell to make sure that all of the air bubbles are being removed
 - Remove the beaker of DI water from the sample holder and press the “clean” button so air can circulate through the sample lines and sample cell
 - Repeat this process 2-3 times to ensure the sample lines and sample cell are completely clean.
 - The final “clean” cycle should be with air to make sure the sample lines and sample cell are empty.
- viii. Calibrating the pH meter:
 - Add the desired buffer to a sample container and load the in the sample holder.
 - Uncover the fill hole on top of the pH electrode. This should be covered with parafilm when not in use.

- Open the pH calibration control window by clicking on the “pH” icon in the software toolbar.
 - If the buffer that you’re using doesn’t show up in the dialogue box, then click “add” and add the desired buffer.
 - To calibrate a buffer place the electrode in that buffer and wait for the pH probe output (mV) to stabilize. Then, press the “measure” button. When the calibration is complete, then that pH value will have a green check mark beside it. Repeat this process for the other buffers of interest.
 - Make sure to rinse the pH probe between buffers.
- ix. Loading your sample:
- Fill the polystyrene sample tube with **10 mL** of your sample and then load the sample tube onto the auto-titrator. The maximum sample volume that can be used with the auto-titrator is 15 mL. Exceeding this volume will cause the sample tube to over flow!!
 - Bring up the “clean” tab on the auto-titrator control panel and flush the sample lines with your sample by pressing the “clean” button.
 - Gently tap the folded capillary cell while the sample is cleaning to remove any air bubbles that are present.
 - Check the fluid lines while the sample is pumping to make sure there are no air bubbles trapped inside.
 - **DO NOT SET THE CLEAN RATE ABOVE 40%.**
 - Once you are sure that all of the air bubbles are removed from the zeta cell and the fluid lines, and then you can load the zeta cell into the instrument sample cell holder.
 - GENTLY press the folded capillary cell into the sample holder. Be careful not to bend the electrodes on the sample cell holder.
 - Correctly seat the sample fluid lines in the sample cell pinch valve and check to make sure that fluid still flows freely to the cell.
 - If there are no leaks or blockages in the fluid lines then you can close the lid to the sample holder and set up your measurement.
- x. Set up your titration using the manual measurement dialogue box or a titration SOP.
- xi. Cleaning the auto-titrator:
- Once your titration is finished, you should remove your sample from the sample holder and place a waste beaker under the sample port.
 - Press the “clean” button to remove all of your sample from the fluid lines and the folded capillary cell.
 - Place a beaker of DI water under the sample holder and “clean” the sample lines and capillary cell with DI water.
 - Repeat this process three times, with the last “clean” cycle being air so that the fluid lines are completely empty.
 - Detach your folded capillary cell and discard or save for another use.
 - Remove your titrants from the titrant holder and press the “prime” button to remove your titrants from the fluid lines. Make sure you have

a beaker placed underneath to the sample port to catch any acid or base that is pumped out.

- Place DI water in the titrant holder and press the “prime” button to flush the lines with DI water.
- You should repeat this process 5 times, alternately priming the lines with air and DI water. The number of cycles for each prime should be set from 7-10.
- Make sure that your last prime cycle is with air to completely evacuate all of the fluid lines.
- NEVER LEAVE YOUR TITRANT OR DI WATER in the fluid lines when you are finished with the auto-titrator. This will cause corrosion inside the auto-titrator and lead to costly repairs.

8. Running Standard Size and Zeta Potential Measurements

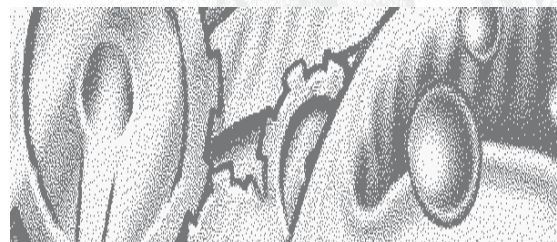
- a. There is a NIST certified standard size sample available to run as a quality control measurement if you chose. The sample is made of polystyrene spheres of 59 nm +/- 2 nm diameter.
- b. To run a size standard, add one drop of the polystyrene spheres to a cuvette filled to the appropriate level with DI water. You may need to filter your DI water with a 0.2 μm filter to get accurate results.
- c. If the instrument does not measure the particles as 59 nm (within 2 nm), then make sure your cuvette is completely clean and there are no air bubbles present. Run the measurement again.
- d. If the instrument fails to measure the standard accurately again, please let the person in charge of the ZetaSizer know. This could indicate a larger problem with the instrument.
- e. There are also a batch of -60 mV (+/- 5 mV) standards available if you would like to run a quality control sample for zeta potential measurements.
- f. Simply fill either a folded capillary cell or the glass cuvette with the appropriate volume of the standard and run a zeta potential measurement. The zeta potential should be -60 mV, within the 5 mV tolerance.
- g. If the instrument fails the quality control check, please let the person in charge of the ZetaSizer know. This could indicate a larger problem with the instrument.

9. Clean up and Instrument shutdown

- a. Make sure that all fluids have been completely flushed from the auto-titrator as described above.
- b. Remove all samples from the ZetaSizer work space. Clean and dry any non-disposable cuvettes used and place them back in the desk drawer.
- c. Dispose of any disposable cuvettes, syringes, or filters that are in the ZetaSizer workspace.
- d. Make sure that the lid to the ZetaSizer sample cell is closed.
- e. Close the software to the ZetaSizer and turn off both the ZetaSizer and the Auto-Titrator.
- f. Make sure to log off the computer when you are finished with the instrument. This will prevent unauthorized users from using the instrument during off-hours.

Zetasizer Nano Series User Manual

MAN0317 Issue 1.0 June 2003



© **Malvern Instruments Ltd. 2003**

Malvern Instruments makes every effort to ensure that this document is correct. However, due to Malvern Instruments policy of continual product development we are unable to guarantee the accuracy of this, or any other document after the date of publication. We therefore disclaim all liability for any changes, errors or omissions after the date of publication. No reproduction or transmission of any part of this publication is allowed without the express written permission of Malvern Instruments Ltd.

Head office:

Malvern Instruments Ltd.
Enigma Business Park,
Groveswood Road,
Malvern,
Worcestershire. WR14 1XZ
United Kingdom.

Tel + [44] (0)1684-892456

Fax + [44] (0)1684-892789

Windows 2000 and XP are registered trademarks of the Microsoft Corporation.

Zetasizer, NIBS and M3-PALS are registered trademarks of Malvern instruments.

Printed in England

Table of Contents

Part 1 - Operators guide

CHAPTER 1 - Introduction to this manual

Introduction to this manual	1-1
How to use this manual	1-2
Access to the Instrument	1-3
Assumed information	1-4
Where to get help	1-4

CHAPTER 2 - What is the Zetasizer Nano?

Introduction	2-1
What does the Zetasizer Nano do?	2-1
The Zetasizer Nano range	2-1
What is Particle Size, Zeta potential and Molecular weight?	2-3

CHAPTER 3 - How does the Zetasizer Nano work?

Introduction	3-1
How is a Zetasizer measurement performed?	3-1
What does the Zetasizer consist of ?	
- Identifying the Hardware	3-4
- Navigating the Software	3-13

CHAPTER 4 - Making measurements - A tutorial

Introduction	4-1
Quick guide to making a measurement	4-2
Powering up the system	4-2
Sample preparation	4-3
Choosing the correct Cell	4-3
Filling the Cell	4-8
Inserting the Cell	4-11
Making an SOP measurement	4-14
Making a manual measurement	4-16
The Measurement display	4-17
Editing the result	4-23

CHAPTER 5 - Records and Reports - Viewing the results

Introduction	5-1
Displaying the results	5-1

CHAPTER 6 - Sample Preparation

Introduction	6-1
Preparing the sample	
- Size	6-1
- Molecular weight	6-5
- Zeta potential	6-6

CHAPTER 7 - Maintenance

Introduction	7-1
Cleaning the instrument	7-1
Cleaning the Cells	7-2
Replacing the system fuse	7-4

Part 2 - Supervisors guide**CHAPTER 8 - Security**

Introduction	8-1
Initial start-up - set up the administrator	8-2
Enabling security	8-3
User groups	8-3
Users	8-5

CHAPTER 9 - Using SOPs

Introduction	9-1
Creating an SOP	9-2
All SOPs	9-4
Size SOPs	9-10
Molecular weight SOPs	9-18
Zeta potential SOPs	9-22
Trend & Protein melting point SOPs	9-27
Extracting an SOP	9-29
Modifying an SOP	9-29
Distributing an SOP	9-30



CHAPTER 10 - Measurement file window - Workspace management

Introduction	10-1
Measurement file window	10-2

CHAPTER 11 - Exporting results

Introduction	11-1
Exporting results	11-1
Creating an export template	11-3

CHAPTER 12 - Creating custom reports

Introduction	12-1
Overview	12-1
Opening a report	12-2
Creating a report	12-2
Laying out a report	12-2
Customising and editing the report	12-4
A finished report	12-11
Viewing the new report	12-12

CHAPTER 13 - Size theory

Introduction	13-1
What is Dynamic Light Scattering?	13-1
Operation of the Zetasizer Nano - Size measurements	13-6

CHAPTER 14 - Molecular Weight theory

Introduction	14-1
What is Static light scattering?	14-1
The Debye plot	14-4

CHAPTER 15 - Zeta Potential theory

Introduction	15-1
What is Zeta Potential?	15-1
Laser Doppler Velocimetry	15-5
The M3-PALS technique	15-7
Operation of the Zetasizer Nano - Zeta potential measurements	15-11

Part 3 - Appendices

APPENDIX A - Health and Safety

APPENDIX B - Specification

APPENDIX C - Site requirements

Introduction	C-1
Environmental conditions	C-1
Space required	C-2
Power requirements	C-3
Additional services	C-3
Computer specification	C-4
Laser Safety	C-4

APPENDIX D - Unpacking instructions

APPENDIX E - Installation

Introduction	E-1
Installing the Zetasizer Nano	E-1
Changing the computer	E-2
Installing the Titrator	E-2

APPENDIX F - Regulatory Statements

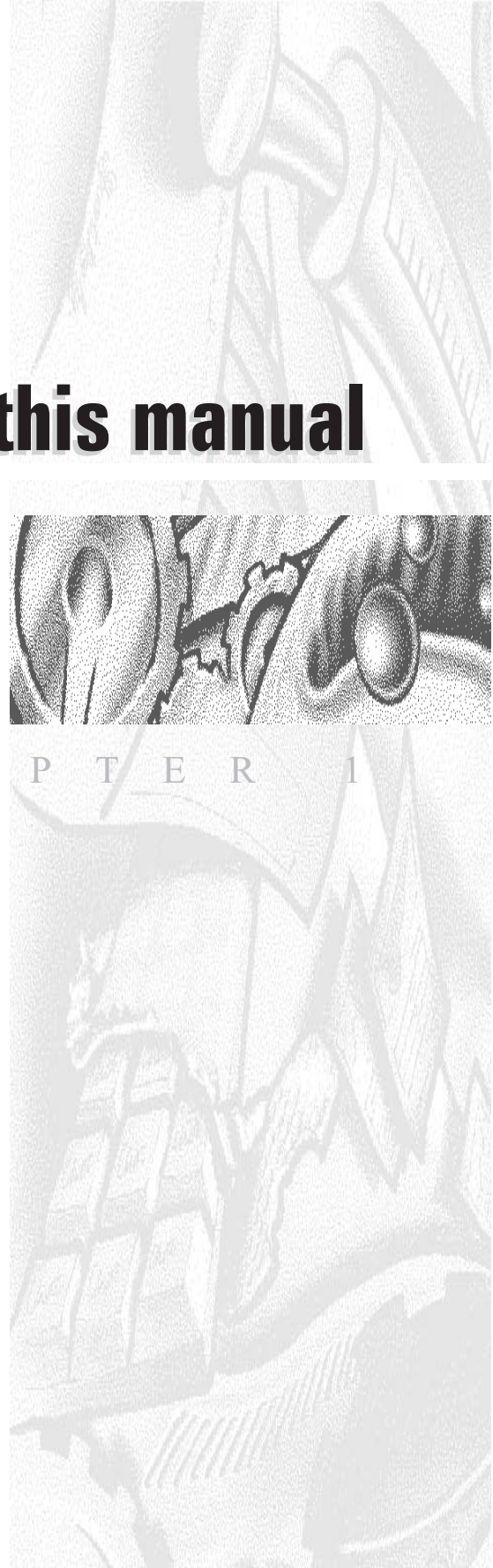
CE Declaration of Conformity	F-1
Federal Communications Commission (FCC) Notice	F-2
Canadian Regulatory Information	F-3

Part 1 - Operators guide



Introduction to this manual

C H A P T E R 1




Introduction to this manual

This manual covers the operation and maintenance of the Zetasizer Nano particle analyser series.

Zetasizer Nano instrument	Model number	Description
<i>Nano S (Red badge)</i>	<i>ZEN1600</i>	<i>Size measurement particle sizer</i>
<i>Nano S (Green badge)</i>	<i>ZEN1500</i>	<i>Size measurement particle sizer</i>
<i>Nano Z (Red badge)</i>	<i>ZEN2600</i>	<i>Zeta potential particle sizer</i>
<i>Nano Z (Green badge)</i>	<i>ZEN2500</i>	<i>Zeta potential particle sizer</i>
<i>Nano ZS (Red badge)</i>	<i>ZEN3600</i>	<i>Size and Zeta potential particle sizer</i>
<i>Nano ZS (Green badge)</i>	<i>ZEN3500</i>	<i>Size and Zeta potential particle sizer</i>
<i>Nano S90 (Red badge)</i>	<i>ZEN1690</i>	<i>Size measurement particle sizer - 90° optics</i>
<i>Nano S90 (Green badge)</i>	<i>ZEN1590</i>	<i>Size measurement particle sizer - 90° optics</i>
<i>Nano ZS90 (Red badge)</i>	<i>ZEN3690</i>	<i>Size and Zeta potential particle sizer - 90° optics</i>
<i>Nano ZS90 (Green badge)</i>	<i>ZEN3590</i>	<i>Size and Zeta potential particle sizer - 90° optics</i>

Instruments that have a red oval badge fitted to the instrument cover are fitted with a 633nm 'red' laser; instruments that have a green badge are fitted with a 532nm 'green' laser.

Build options exist for each of the above instruments, these are described in chapter 2.

 **Note** ■ The Zetasizer model, Serial number, software and firmware version can be found by left-clicking the Nano icon in the right corner of the status bar.

The aim of this manual is to:

- Identify what the instrument is.
- Explain in simple terms how it works.

- Explain how the instrument should be used to make a measurement.
- Identify the user maintenance procedures.

How to use this manual

It is important to read the Health and Safety information in appendix A before operating the instrument.

It is recommended this manual is read fully before you start your first measurement, though if more familiar with particle size analysers, jump straight to chapter 4 - “Making measurements - A tutorial”. This chapter gives practical details on making measurements.

The manual is divided into 3 sections.

Part 1 – Operators guide

Contains all the information required for the operator to use the Zetasizer Nano instruments.

Topics covered are: What are the Zetasizer Nano instruments, what are the components of the Zetasizer Nano and what do they do, instructions on using the instrument and the software, basic measurement and maintenance procedures.

Part 2 – Supervisors guide

The Supervisors guide concentrates on the administration and creation of the Zetasizer functionality. It provides a greater insight into the measurement procedures and results expanding the analysis theories.

Topics covered are: security aspects, use of **Standard Operating Procedures (SOPs)**, and organising the measurement files, and discussion on each of the analysis theories used – Zeta potential, Size and Molecular weight.

It is recommended that the supervisor should also read Part 1 – Operators guide

Part 3 - Appendices

Contains supplementary information not necessary for the general operation of the system.

More detail on the Zetasizer software can be found by using the online **Help** within the software.

The Zetasizer Nano measures three different particle characteristics; the text within each chapter has therefore been structured to, detail the instrument functions as applicable to all measurements types, or individually if the function only applies to one, i.e. if concerned only with zeta potential measurements ignore all references to size and molecular weight unless otherwise directed.

Access to the Instrument

Within this manual, reference is made to the various people who will have access to the instrument.

Malvern personnel

Malvern personnel (service engineers, representatives etc.) have full access to the instrument and are the only people authorised to perform all service procedures that may require the removal of the covers.



Warning!

Removal of the covers by unauthorised personnel will invalidate the warranty of the instrument.

Supervisor

The supervisor is the person responsible for the management and safety of the instrument and its operation. The supervisor is responsible for the training of the operators. They can perform all user maintenance routines identified in chapter 7.

Under no circumstances, should the supervisor remove the main cover of the instrument.

Operator

An operator is a person trained in the use of the system. The operator can perform all user maintenance routines identified in chapter 7, except changing the fuse.

Under no circumstances, should the operator remove the main cover of the instrument.

**Warning!**

Failure to follow these guidelines could result in exposure to hazardous voltages and laser radiation.

Assumed information

Naming convention

The Zetasizer Nano will either be referred to in full, as the Zetasizer, or as the ‘instrument’.

The combination of the Zetasizer Nano instrument, the computer and Zetasizer software will be referred to as the "System".

Cells and Cuvettes

Any device for holding and measuring the sample in the instrument will generally be referred to as a “cell”. This includes dip cells and all kinds of cuvettes used (i.e. glass, small volume, disposable) unless a proper description is more appropriate.

Menu commands

Menu commands from the Zetasizer software are referred to in the form **main menu-menu item**. As an example, the command **Configure-New SOP** refers to selecting the **New SOP** item in the **Configure menu**. Menu commands are always shown in bold text.

Where to get help

Manual and Help files

The primary source of help for the Zetasizer system is from this manual and the online help system within the software. This manual is designed to give an overview of the system as a whole, while the online help system is designed to give detailed information on the Zetasizer software.

Each dialogue within the Zetasizer software has a **Help** button that gives information specific to that dialogue. A secondary type of help exists within the dialogue; this is the **Advice** button and contains more sample related content.

Help desk

All queries regarding the system should initially be directed to the local Malvern representative. Please quote the following information:

- Model and serial number of the instrument (located on the rear panel and the front of the cuvette holder).
- The build option fitted: a smaller label attached alongside the model and serial number labels identifies any options fitted.
- The Zetasizer software version (select **Help-About** within the software).

Contact the United Kingdom help desk if the local Malvern representative is not available. The direct line to the United Kingdom Helpdesk is +44 (0) 1684 891800. It should be noted that this help line is primarily English speaking.

Remote support

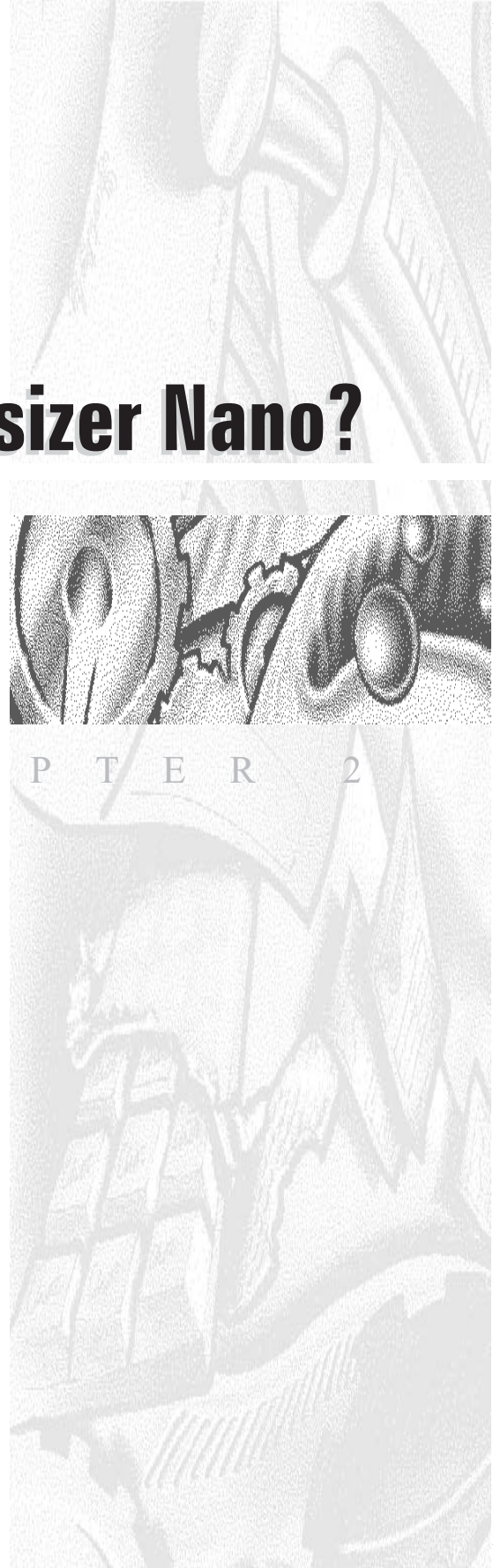
Malvern Instruments offers a remote support service, delivered by an Internet connection. Benefits include fast and efficient fault diagnosis, reducing downtime and costs.

On-line user training is also available, plus software updates. A direct Internet connection LAN must be available to make use of this facility.



What is the Zetasizer Nano?

C H A P T E R 2



Introduction

What is the Zetasizer Nano system and what is it supposed to do?

This chapter gives a brief overview of the Zetasizer Nano instrument range: what the Zetasizer Nano does and simple explanations about the measurement technique.

What does the Zetasizer Nano do?

The Zetasizer Nano range of instruments provides the ability to measure three characteristics of particles or molecules in a liquid medium.

These three fundamental parameters are **Particle size**, **Zeta potential** and **Molecular weight**. By using the unique technology within the Zetasizer system these parameters can be measured over a wide range of concentrations. The Zetasizer system also enables determination of the **Protein melting point** plus the ability to perform **Trend** measurements.

The Zetasizer range features pre-aligned optics and programmable measurement position plus the precise temperature control necessary for reproducible, repeatable and accurate measurements. In addition facility is included for measurements of other key parameters such as pH and concentration.

The Zetasizer range has been designed with simplicity in mind, so that a minimal amount of user interaction is necessary to achieve excellent results. The use of **Standard Operating Procedures (SOPs)** and features such as the **Folded capillary cell** alleviate the need for constant attention.

The Zetasizer Nano range

There are ten instruments in the Zetasizer Nano particle analyser range: five different models fitted with either a 633nm 'red' or 532nm 'green' laser. The models and their measurement specifications are described in the table below, with instrument options following.

Zetasizer	Size range	Size range for Zeta potential	Size range for Molecular weight	Instrument option (see below)
Nano S	–	3nm to 10 μ m	–	ⒶⒷⒸ
Nano Z	0.6nm to 6 μ m	–	1000 to 2x10 ⁷ Daltons	Ⓐ
Nano ZS	0.6nm to 6 μ m	3nm to 10 μ m	1000 to 2x10 ⁷ Daltons	ⒶⒷⒸ
Nano S90	2nm to 3 μ m	–	–	Ⓐ
Nano ZS90	2nm to 3 μ m	3nm to 10 μ m	–	ⒶⒷⒸ

Laser fitted

The Zetasizer Nano series is available with either a 633nm ‘red’ laser or a 532nm ‘green’ laser fitted. The laser fitted is identified by the colour of the oval badge on the cover.

- The 633nm laser is **least** suitable for **blue** samples.
- The 532nm laser is **least** suitable for **red** samples.

90° optics

The instruments above with the suffix 90 indicate the optics have a 90° scattering angle. These models have been included in the Zetasizer Nano instrument range to provide continuity with other instruments with ‘classical’ 90° optics.

Instrument options

A range of accessories and options are also available for more advanced measurement strategies.

Ⓐ Narrow band filter

This filter improves the signal for samples that fluoresce at the wavelength of the laser fitted. If fitted an option label will be attached to the front of the cuvette holder.

Option label**Option fitted**

ZEN1003

*Narrow band filter for all instrument options***Ⓑ Universal ‘Dip’ cell**

Used to provide repeatable measurements of non-aqueous samples such as solvents. Also suitable for measurements of valuable aqueous samples where minimal sample quantity is important.

Ⓒ MPT2 Autotitrator

Used to perform sample, pH, conductivity and additive titrations.

What is Particle Size, Zeta potential and Molecular weight?

This section will describe, “basically”, what Particle Size, Zeta potential and Molecular weight are and why they are important. Greater detail on the measurement techniques is given in the theory chapters (13, 14 and 15).

What is Size?

Particle size is the diameter of the sphere that diffuses at the same speed as the particle being measured.

The Zetasizer system determines the **size** by first measuring the **Brownian motion** of the particles in a sample using **Dynamic Light Scattering (DLS)** and then interpreting a size from this using established theories - see chapter 13.

Brownian motion is defined as:

“The random movement of particles in a liquid due to the bombardment by the molecules that surround them”.

The particles in a liquid move about randomly and their speed of movement is used to determine the size of the particle.

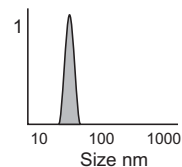
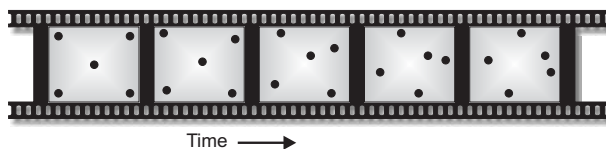
It is known that small particles move quickly in a liquid and large particles move slowly. This movement is carrying on all the time, so if we take two ‘pictures’ of the sample separated by a short interval of time, say $100\mu\text{S}$, we can see how much the particle has moved and therefore work out how big it is.

If there has been a minimal movement and the particle positions are very similar, then the particles in the sample will be large; similarly if there has been a large

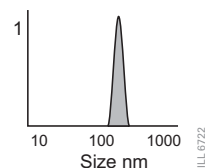
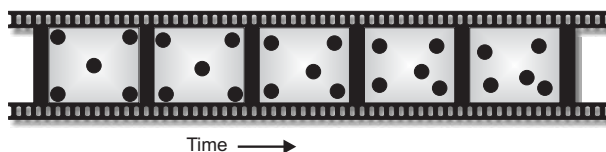
amount of movement and the particle positions are quite different, then the particles in the sample are small.

Using this knowledge and the relationship between diffusion speed and size the size can be determined.

Small particles moving quickly



Large particles moving slowly



ILL 6722

The above is a **very** simplistic explanation. A more detailed explanation can be found in chapter 13 - Size theory.

Why do we use it?

Toners and Liquid inks

Image quality, viscosity and the tendency to aggregate and clog ink delivery nozzles are all influenced by particle size. Controlling the size of ink and toner products has a direct effect on image properties, ink permanence and adhesion.

Pigments

Knowledge of Particle size is important in developing stable formulations of pigments. Pigment colour and hue are highly related to particle size, this has applications in determining a pigments properties.

What is Molecular weight?

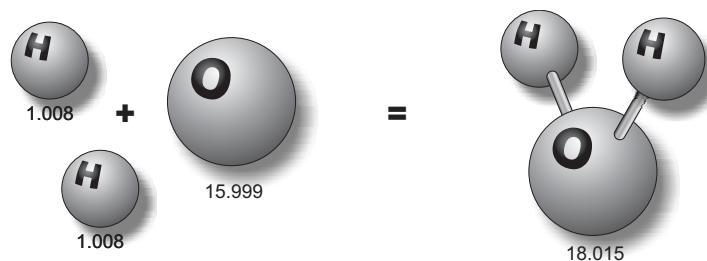
The molecular weight of a substance is the weight in **atomic mass units (amu)** of all the atoms in one molecule of that substance. Mathematically the **molecular weight** can be calculated from the **molecular formula** of the substance; it being the **sum** of the **atomic weights** of all the atoms making up the molecule.

If we take as an example the molecular formula H_2O (water) we can work out the molecular weight.

In each molecule of water there are two atoms of Hydrogen (H₂) and one atom of Oxygen (O).

Now the atomic weight of hydrogen is 1.008 amu and that of oxygen is 15.999.

Therefore the molecular weight of water is 18.015 i.e. $(1.008 \times 2) + 15.999$.



ILL 6721

This is a calculation using a known molecular formula and applying the values from the periodic table.

With the Zetasizer Nano series of instruments the molecular weight can now be determined by use of **Static Light Scattering (SLS)** measurement techniques.

This technique will be more explained in chapter 14 - Molecular weight theory.

Note ■ Malvern uses **Daltons** to identify the molecular weight.

Why do we want to know it?

We need to know the molecular weight, so we can determine how many grams there are in 1 mole of a substance. The mole being the chemistry standard term for '1 molecular weight', e.g. one mole of water is 18.015g).

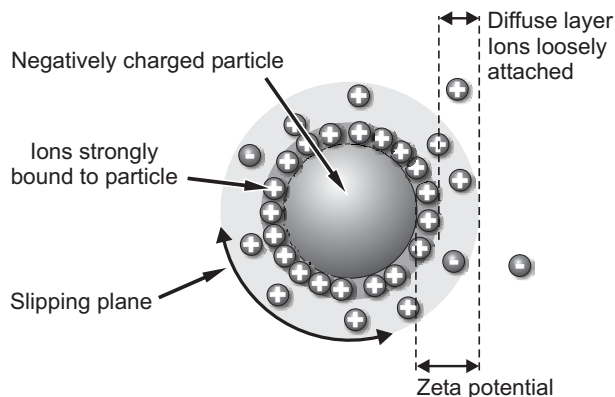
In an application, knowing the molecular weight of polymer compounds will aid in determining many of their physical characteristics such as density, flexibility and strength.

What is Zeta potential and Electrophoresis?

Most liquids contain **Ions**; these can be negatively and positively charged atoms called **Cations** and **Anions** respectively. When a charged particle is suspended in a liquid ions of an opposite charge will be attracted to the surface of the suspended particle.

i.e. - a negatively charged sample attracts positive ions from the liquid and conversely a positive charged sample attracts negative ions from the liquid.

Ions close to the surface of the particle, will be strongly bound while ions that are further away will be loosely bound forming what is called a **Diffuse layer**. Within the diffuse layer there is a notional boundary and any ions **within** this boundary will **move** with the particle when it moves in the liquid; but any ions **outside** the boundary will **stay** where they are – this boundary is called the **Slipping plane**.



ILL 6747

A potential exists between the particle surface and the dispersing liquid which varies according to the distance from the particle surface – this potential at the slipping plane is called the **Zeta potential**.

Zeta potential is measured using a combination of the measurement techniques: **Electrophoresis** and **Laser Doppler Velocimetry**, sometimes called **Laser Doppler Electrophoresis**. This method measures how fast a particle moves in a liquid when an electrical field is applied – i.e. its velocity.

Once we know the velocity of the particle and the electrical field applied we can, by using two other known constants of the sample – viscosity and dielectric constant, work out the zeta potential.

This technique will be further explained in chapter 15 - Zeta theory.

Why do we use it?

The zeta potential of the sample will determine whether the particles within a liquid will tend to flocculate (stick together) or not.

Knowledge of zeta potential is therefore useful in many industries such as:

Ceramics:

A high zeta potential is required to ensure the ceramic particles are densely packed. This gives added strength to the end product.

Waste water treatment.

The flocculation state of waste water is altered by changes in pH, the addition of chemical flocculants, such as charged polymers, and the presence of aluminium chloride or other highly charged salts. Measurement of zeta potential in combination with these parameters is fundamental in the development and maintenance of optimized water treatment protocols.

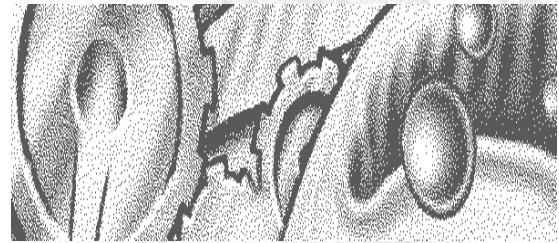
Emulsions.

Zeta potential is used to study the chemistry involved in determining whether or not an emulsion will remain stable in the environment where it will be used.



How does the Zetasizer Nano work?

C H A P T E R 3



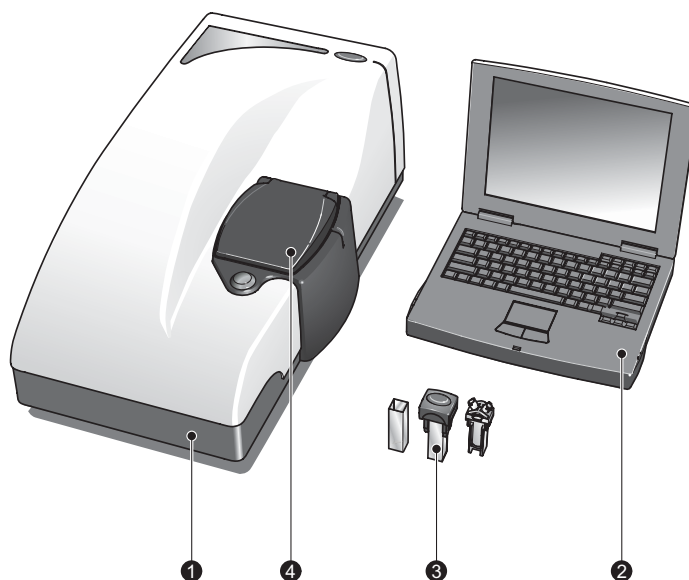
Introduction

Previously we identified the instrument and described the various measurement processes that can be performed. This chapter introduces the hardware and software features, that the instrument incorporates.

The initial section, “How is a Zetasizer measurement performed?”, will briefly describe what is involved in making a measurement; what the major components of the system are and how the software performs the task. This is followed by two sections identifying the major hardware components and the software used.

The complete measurement process for size, zeta potential and molecular weight measurements will be described in later chapters.

How is a Zetasizer measurement performed?



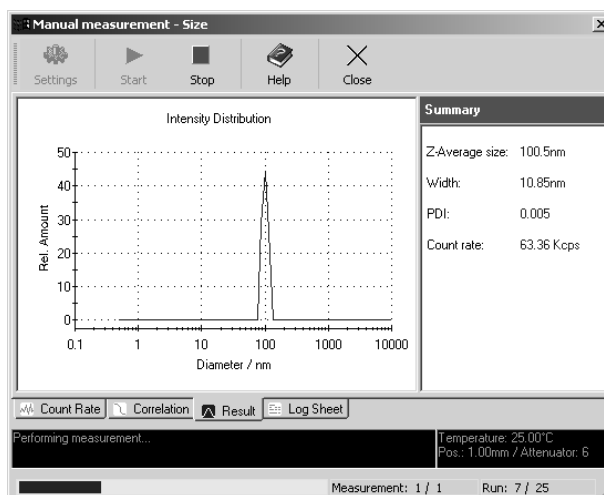
A typical system, shown above, comprises the Zetasizer instrument ① and a computer with the Zetasizer software installed ②. A cell ③ is filled with the sample and loaded into the cell area on the top of the instrument ④.

The software is used to control the measurement of the sample, there are two basic ways to make a measurement:

- **SOP measurements.** A **Standard Operating Procedure (SOP)** is like a template that pre-defines all the measurement settings. This ensures that measurements made on the same type of sample are made in a consistent way. SOPs are ideal if the same type of sample is regularly measured, inputting the same parameters each time a measurement is made is tedious and runs the risk of making errors.

SOPs can be created or modified as required.

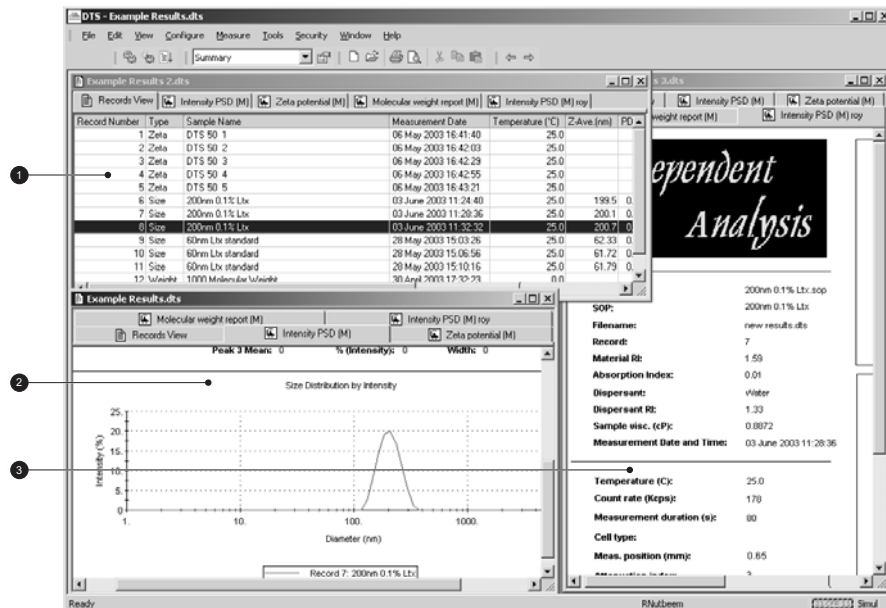
To perform an SOP measurement, select **Measure-Start SOP** from the menu bar and select an SOP to use. With an SOP chosen the **Measurement display** will appear (below). The measurement will begin by pressing the **Start** (▶) button.



- **Manual Measurement.** A manual measurement is where all the measurement parameters are set immediately before the measurement is performed. This is ideal if measuring many different types of sample, or experimenting with the measurement parameters.

To perform a manual measurement, select **Measure-Manual** from the menu bar. A manual measurement dialogue window will appear where the measurement settings can be chosen, and if required saved as an SOP. Once chosen the measurement can begin by simply pressing the **Start** (▶) button on the **Measurement display**.

Once the measurement is complete the results can be viewed; either in a **Record view** ①, by selecting one of the Malvern pre-set reports ②, or a user defined report ③.



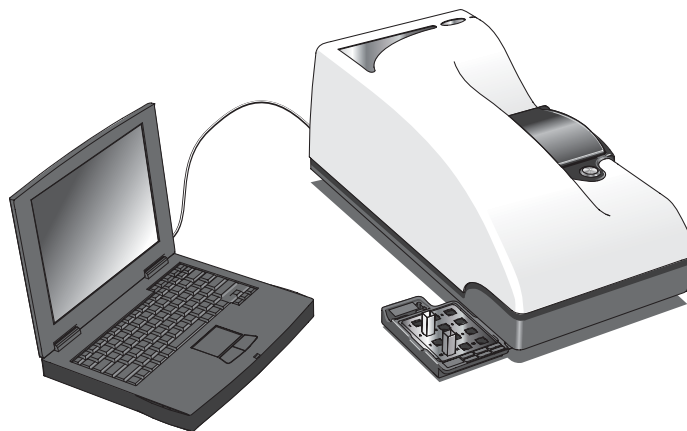
The measurement results will be automatically saved to a measurement file.

Note ■ The measurement file must be selected **before** the measurement is started as the measurements will be saved in the file currently open.

What does the Zetasizer consist of ?

- Identifying the Hardware

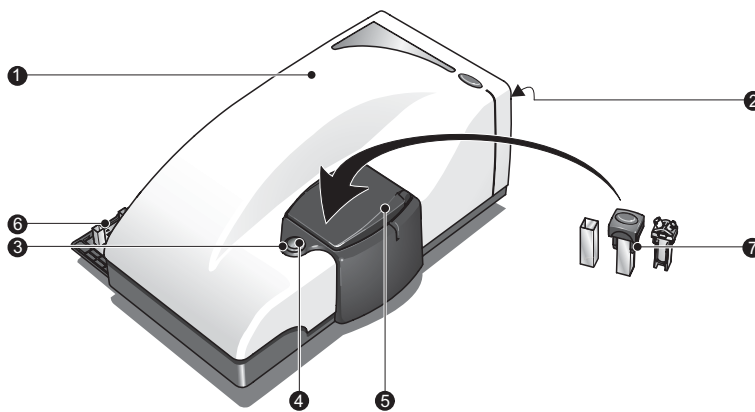
The diagram below shows a typical system with its key modules of the Zetasizer instrument, and a computer system with the Zetasizer software installed. It is preferable the computer is dedicated to just running the Zetasizer software.



ILL 6739

The software controls the Zetasizer and any accessories used and analyses the data from the instrument to give either the size, molecular weight or zeta potential for the sample measured.

The Zetasizer Nano instrument



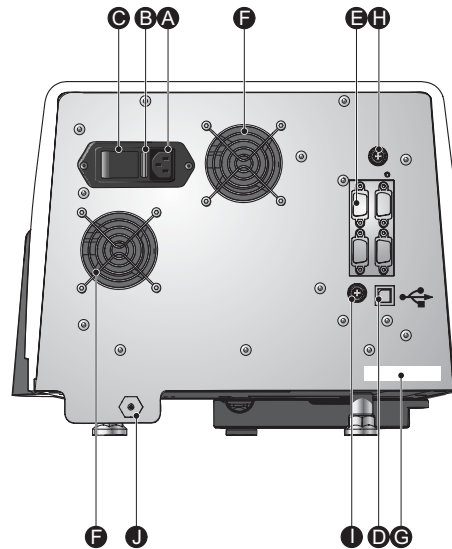
ILL 4022

① Optical unit

Positioned on the cover are two labels - one to identify the instrument and another to identify the instrument model; refer to the identification table in chapter 2.

② Rear Panel

The rear panel provides all the connections. These are identified below.



ILL 6737

Ⓐ Power input socket

Mains power input socket for the instrument.

Ⓑ Fuse holder

Fuse for the instrument. Details on changing the fuses can be found in chapter 7 - Maintenance.

Ⓒ Power switch

The on/off power switch for the instrument. On switch on the “**status indicator**” ③ around the **Cell access button** ④ will illuminate.

Ⓓ Computer connection

The **USB** cable from the computer is connected here.

Ⓔ Accessory connections

Two types of connection are available.

CAN Ports ① and ②

Use these ports to connect any Malvern supplied accessory that requires a CAN connection (Controller Area Network). Consult the respective accessory manual for details.

RS232 (I0I0I) Ports ① and ②

Use these ports to connect any Malvern supplied accessory that requires an RS232 connection. Again consult the respective accessory manual for details.

Control of any accessory is done via Zetasizer software.

Ⓕ Cooling fans

In conjunction with ventilation slots underneath the instrument, the fans provide cooling to the internal components of the Zetasizer.



Do not obstruct the ventilation slots underneath the instrument, nor the fans on the rear panel.

Ⓖ Serial number and Model number label

Identifies the actual Zetasizer Nano model and its serial number. Please quote all numbers in any correspondence with Malvern Instruments.

The option label indicates any instrument options fitted into the Zetasizer.

For an explanation refer to the identification table in chapter 2.

Ⓗ Accessory output

A 12v output supply is provided on the rear panel to connect to any Malvern supplied accessory that requires an external voltage source. Consult the respective accessory manual for details.



Only connect Malvern approved accessories.

① Green laser PSU input

If the instrument uses a 532nm 'green' laser, connect the laser PSU to this connection.

② Purge connection

If a Nitrogen supply is used the instrument must be located in a well ventilated environment. The purge supply expels 50ml of Nitrogen a minute. Ensure the supply is turned **off** when not in use.

If measuring samples at low temperatures there is a risk of condensation occurring on the cell; this occurs when the measurement temperature is less than the 'dew point' of the ambient air surrounding the cell. This is particularly prevalent in humid climates. If it is suspected that this may be a problem then the

purge inlet port can be used to connect an air or nitrogen supply. This will remove any moisture in the air immediately surrounding the cell and prevent condensation.

If using the purge inlet connector then the air or nitrogen supply must conform to the following specification:

- Compressed air to DIN 8573-1
- Oil = Class 1
- Water = Class 3
- Particulate = Class 3
- Pressure = 200 ± 20 kPa g

On entry into the Zetasizer the flow rate of the purge air is controlled by a built in regulator.



Caution

It is important that the purge air line supply conforms to the above specification. Failure to meet this specification may result in permanent damage and invalidation of the warranty.

③ Status indicator

The status indicator is an illuminated ring (or bezel) positioned around the **Cell access button** ④, and shows the operational state of the instrument.

<i>Indicator colour and state</i>	<i>Function</i>
<i>Amber - Flashing</i>	<i>Shows the start-up initialisation routine is running.</i>
<i>Amber</i>	<i>Shows that the instrument is at "Standby". The instrument is functioning correctly but is either not connected to the computer or the software has not been started.</i>
<i>Green</i>	<i>Indicates the instrument is functioning correctly and is ready to start a measurement.</i>
<i>Green - Flashing</i>	<i>Occurs when the instrument is performing a measurement.</i>

Red

Indicates if the instrument has detected an error. The measurement will be stopped.

Amber is a combination of red and green lights.

④ Cell access button

Positioned in the middle of the **Status indicator** ③, pressing the button will open the cell area lid.


⑤ Cell area

The system is capable of heating the cell to high temperatures. Care should be taken when removing the cells if a measurement has been performed at high temperatures. It is recommended that the cell area is allowed to cool before removing the cell.

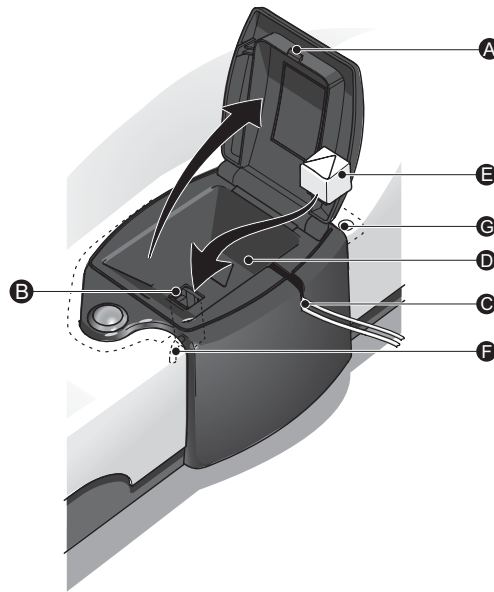
A **warning triangle** is provided in the cell basin.

The cell area is where all cells are inserted to undertake a measurement. The cell area is completely self enclosed and controls the sample temperature over the range 2°C to 90°C.

If the lid is opened with the cell area temperature **above** 50°C the instrument will ‘beep’ twice every few seconds to warn of high temperatures.

-
-  **Note** ■ When the Zetasizer is initially switched on the cell area will be driven to a “default” temperature of 25°C. This will also happen if the software is closed, but the instrument is not switched off.
-

Other important features of the cell area are described below.



ILL 4022

Ⓐ Cell area lid

On pressing the **cell access button** the lid will slowly raise allowing access to the cell holder. On opening two safety interlocks are activated.


- **Laser safety interlock.** This interlock prevents any laser light from entering into the cell area.
- **Electrode voltage interlock.** This interlock immediately turns off any voltage to the cell electrodes

To close the lid push it down until it locks. No measurements can be performed unless the lid is fully closed.

Ⓑ Electrodes

The electrodes perform two functions.

- Provide voltage for zeta potential measurements. Voltage is immediately turned off when the cell lid is opened.


 **Note** ■ The maximum working voltage on the electrodes is $\pm 160\text{v}$ (Measurement category I).

- Identifies the zeta cell fitted.

Depending upon the measurement to be performed, either a size or zeta potential cell will be required.

When a zeta potential cell is inserted, the electrodes positioned on either side of the cell holder will enable the type of cell to be detected automatically. During the measurement the electrodes supply the voltage necessary to perform electrophoresis.

If a size cell is inserted no electrode contact is made and the Zetasizer assumes a size cell is fitted.

-
-  **Note** ■ The software will indicate if the wrong cell has been inserted for the measurement.
-

© Access for flow cell tubes

A channel incorporated into the cell area allows sample tubes to be connected to the measurement cell. This facility is used with the MPT2 Autotitrator accessory.

The channel includes a pinch valve to hold the tubes in place and stop sample movement during the measurement. To fit the tubes slide them into the channel until clamped by the pinch valve.

Ⓓ Cell basin

The cell basin is made of an insulating material which provides protection from the heated cell holder and in conjunction with the **Thermal cap** Ⓔ gives temperature stability when heating and cooling the sample.

A warning label indicates that high temperatures may exist in the cell area.

Ⓔ Thermal cap



Warning

With the thermal cap removed both the metal lining of the the cap and the top of the cell holder will be exposed. Care should be taken when removing the cells after a measurement has been performed at high temperatures. It is recommended that the cell area is allowed to cool before removing the cell.

A **warning triangle** is provided on the top of the cap.

The thermal cap gives increased temperature stability for size measurements when heating and cooling the sample during size measurements. This is important when measuring at either end of the temperature specification. The thermal cap is placed over the cell to ensure the temperature requested is reached.

A parking position is provided in the cuvette holder to store the thermal cap.

Note ■ The thermal cap is not compatible with the folded capillary cell.

Ⓕ Drain port

In case of spillage within the cell area, there is a drain incorporated into the base of the cell holder.

Any spillage will exit onto the bench area underneath the Zetasizer.

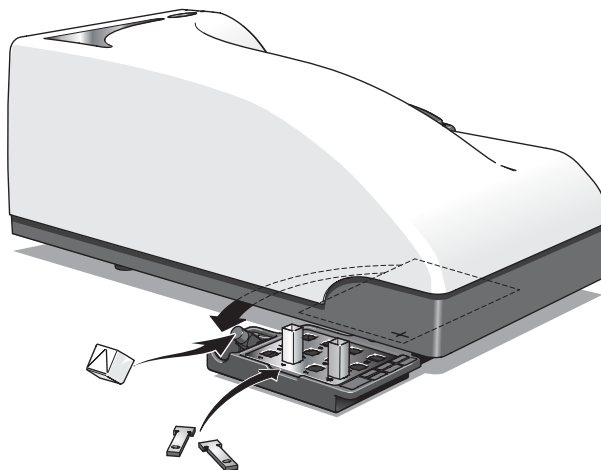
Ⓖ Drain channel

Likewise in case of spillage on the cover a drainage channel is provided around the outside of the cell area, this is hidden from view under the main cover. Any spillage will flow along the drain into a hole positioned at the back of the cell area.

Any spillage will exit onto the bench area underneath the Zetasizer.

Ⓒ Cuvette holder

The cuvette holder is for storing the cells before and after use. The cuvette holder swings out from under the instrument and up to 12 cuvettes can be stored.



ILL 6735



Caution


Ensure the thermal cap is lowered and all cuvettes have been removed before swinging the holder back under the instrument base.

The two trays that hold the cuvettes can be removed for cleaning.

The cuvette holder provides a place to store the **thermal cap** during changeover of cells. The cap is released by raising the cap and lifting off the cap post.

Similarly storage is provided for the two cell “**thermal contact plates**” used with the folded capillary cell. These work in the same manner as the **thermal cap** ⑤ above and provide thermal accuracy and stability. Place these in the holder to the left of the tray.

The cuvette holder includes a **serial** number, **model** number and **option** labels. These identify the instrument and should be quoted in any correspondence with Malvern Instruments. Refer to the identification table in chapter 2.

-  **Note** ■ The Zetasizer Nano model, serial number, software and firmware version can be found by left-clicking the Nano icon in the right corner of the status bar.

⑦ Cells and Cuvettes

A range of cells and cuvettes are available to use with the Zetasizer instrument. Full details are given in the making measurement chapters, but briefly the following cells can be used.

Cell	Application
<i>Disposable “polystyrene” cuvettes – Standard and Small volume</i>	<i>For Size and Zeta potential (with dip-cell)</i>
<i>Quartz glass cuvettes – Square – Standard, Low and ultra-low volume, flow</i>	<i>For Size, Molecular weight and Zeta potential (with dip-cell)</i>
<i>Folded capillary cell</i>	<i>For Zeta potential, Size and Molecular weight</i>
<i>Universal ‘Dip’ cell</i>	<i>For Zeta potential</i>

Navigating the Software

The Malvern Zetasizer software controls the system during a measurement and then processes the measurement data to produce either a size, zeta potential or molecular weight result. It displays the results and allows reports to be printed.

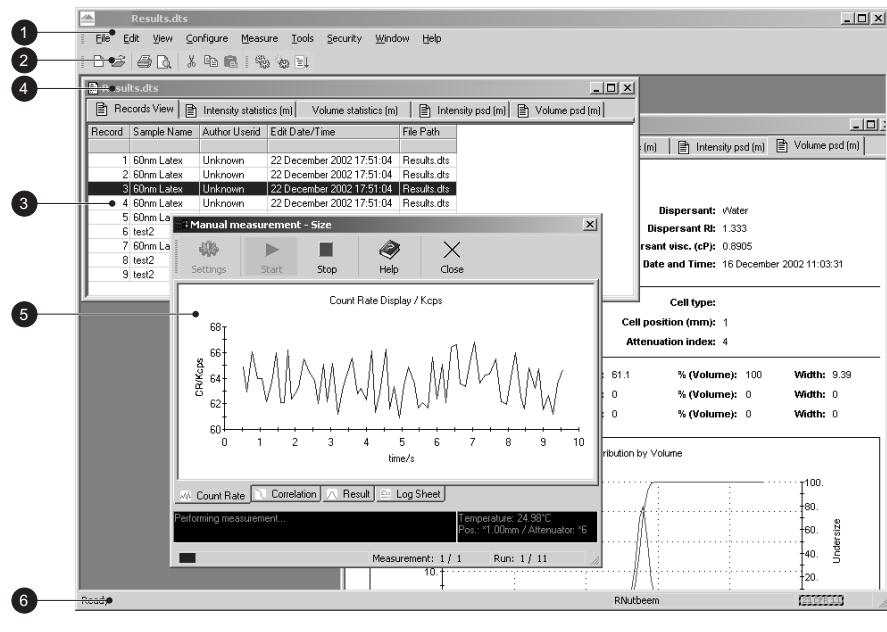
There are two modules that are incorporated into the standard Zetasizer software.

- The main Zetasizer application which is described below
- A secondary module known as Report Designer which enables custom reports to be created to display the results. The features of Report Designer are detailed in chapter 12.

The next section describes the key features of the main application.

Quick guide to the Zetasizer Nano software application

A typical screen is shown below. The features and their function are described in the following sections.



① Menu bar.

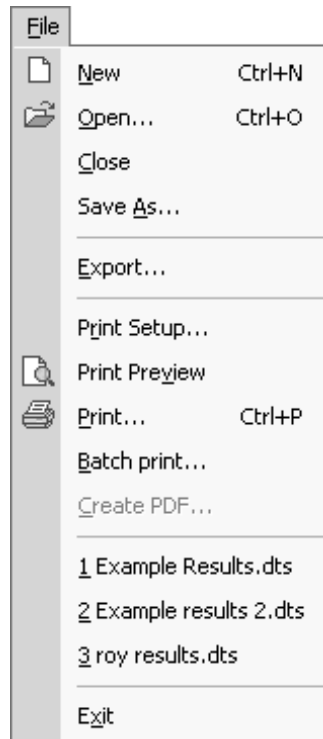
The menu bar contains the main menu headings for all software functions.

Items which end with a row of dots (...) will cause dialogue boxes to appear. Similarly any items which end with an arrow (▶) will cause a second menu to appear.

Items that are shown in grey indicate that they are not available. Grey items indicate that some security setting has been activated, or that the item is not relevant to the system connected.

The Menus available are shown below, together with a brief overview.

File Menu



The **File** menu is used to **Open...** or create a **New** measurement file. The measurement file is where all the measurement records (results) will be stored. Select **Save as...** to store a measurement file under a different file name.

Once a measurement file has been created, use **Export ...** to export the measurement details to other software packages such as Excel or Wordpad.

Select **Batch print...** to simultaneously print a number of measurement records.

Create PDF... is only enabled if the 21 CFR part 11 feature key has been installed.

As a shortcut, a list of the most recently used measurement files are shown at the bottom of the menu for immediate opening.

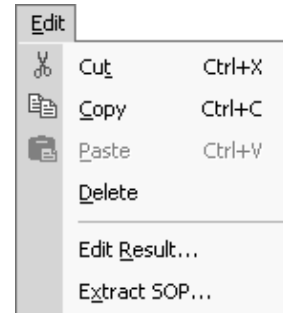
Exit will close down the software.

Edit Menu

Allows movement and manipulation of records in the Measurement file window(s). Records can be **cut**, **copied**, **pasted** and **deleted** into their own or other measurement files.

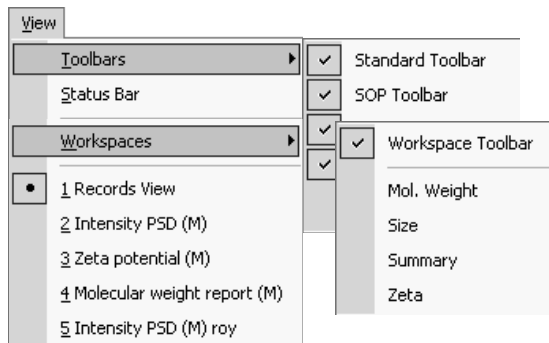
Edit result... allows an existing measurement record to be reanalysed using different dispersant and particle properties. Comments on the editing reason added. The edited measurement will then be added to the bottom of the Records view listing.

To view the settings for any particular measurement record, select the record and then **Extract SOP....** The SOP dialogues will appear showing the original measurement settings. These can then be saved as an SOP so measurements can be made again with the same settings. This is useful if the parameters of the measurement record are not already saved in the SOP directory.



Note ■ The Edit menu will appear when the right mouse button is pressed anywhere in the measurement file window.

View Menu



This selects which reports will be shown in the measurement file window and which **Toolbars** are to be displayed.

Workspaces will select the workspace toolbar, and which measurement workspace is to be displayed.

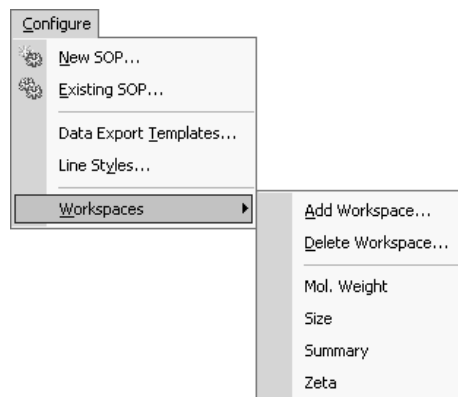
The reports available are those selected when creating the workspace with the **Configure-Workspace** dialogues. The reports available will change to match the workspace chosen.

Select **Status bar** to toggle the status bar from being displayed or not.

Configure Menu

Use this menu to create or edit the measurement settings in an SOP prior to performing the measurement.

New SOP... opens the SOP creation wizard, while **Existing SOP...** allows a previously created SOP to be altered.



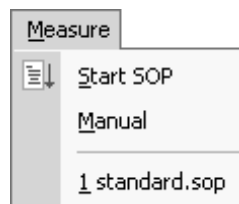
Data Export Templates...

opens a dialogue that defines the parameters and the format in which the record data will be exported. Once a template is created the measurement data can be exported to other software packages such as Excel or Wordpad, by using **File-Export data**.

Line styles... allows the colour and style of lines to be changed in the report graphs.

The reports available, and the parameters shown in the **Records view** tab, are those selected when creating the measurement file workspace with the **Workspace...** dialogues. The dialogues enable workspaces to be **deleted** and **Added**, or previously created ones to be displayed.

Measure Menu



Select this menu when ready to perform a measurement.

There is a choice of using an existing measurement SOP or manually setting up the measurement and sample details.

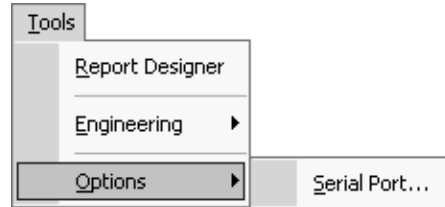
Once the measurement details have been entered or an SOP has been chosen, the **Measurement display** will appear.

Tools Menu

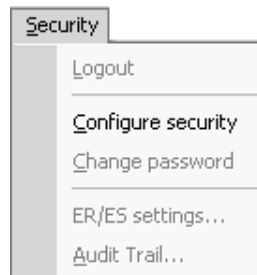
The operation of **Report Designer** is explained in chapter 12.

Serial port... allows the USB communications port to which the instrument is connected to be chosen. All instruments connected will be shown in the related dialogue.

Engineering allows Malvern authorised service engineers to perform maintenance tasks. The engineering screens are password protected.



Security Menu

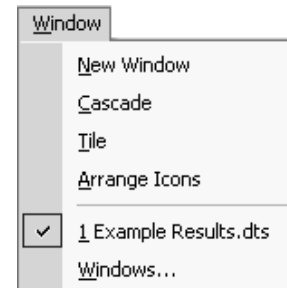


To prevent unauthorised changes, the Malvern software can be configured to limit each users access to various functions - e.g. modifying an SOP. Users are assigned operating **permissions** that allow, or restrict, access; this will be fully explained in chapter 8.

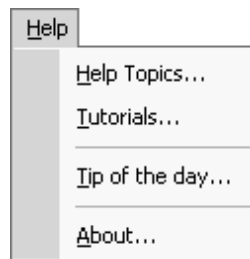
Window Menu

Use this menu to alter the view characteristics of any measurement file windows that are open - i.e. minimise, tile, and cascade the measurement file windows as required.

Select **Window-Windows...** to open the view dialogue.



Help Menu



Help Topics... give access to the help files.

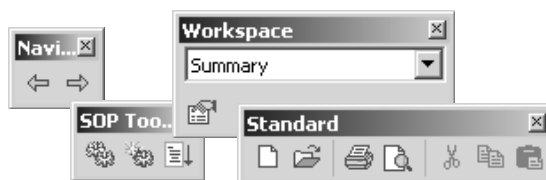
Tutorials... provides a list of easy to follow tutorials describing various aspects of the system.

Tip of the day... will give hints on how to use the Zetasizer software; a different tip will appear each time this is selected. An option is given for turning off the Tip of the day dialogue that appears when the software starts.

About... gives details on the software version installed. It is helpful to quote this if contacting Malvern Instruments.

② Toolbars

The toolbars contain a selection of tools that can be used to perform the most popular operations. Each tool will have its equivalent commands within the menu bar. For example, using the **Open** tool is equivalent to using the **File-Open** menu item.



To identify each tool's function, move the cursor over a tool - a tool tip will be displayed under each tool with a short description of its action displayed in the status bar.

As with the menu bar, if a tool is not available it will be shown "greyed out".

The content and appearance of the toolbar can be changed using the **View-Toolbars-Customise** option.

③ Measurement file window.

The measurement file window displays all the information for 'one' measurement file. More than one measurement file window can be displayed at a time. The contents of the window will change when a **Record** or **Report tab** is selected.

Manipulation of the measurement file windows is described in chapter 10.

Measurement file workspace

When performing zeta potential measurements, it may be unnecessary to see parameters associated with size measurements in the measurement file window. A **measurement file workspace** called **zeta potential** is available that displays only parameters associated with zeta potential measurements.

Workspaces allow configurable selection of record view parameters and reports that are only relevant to a particular measurement type. Similarly a user can create a personalised workspace so that only parameters and reports relevant to themselves will be shown.

Record and Report tabs.

Measurement records are viewed with the **Records view** tab; this gives a listing of all the measurement records in any measurement file. The **Records view** tab is always shown as the prominent report tab when a new measurement file is opened.

The parameters shown are selected by the **Record View parameters** tab in the **Workspace** dialogues.

Selecting a **report tab** will display a predefined report as selected by the **Report pages** tab in the **workspace** dialogues. Malvern supplies several reports that give different views of the measurement settings and results, whilst custom reports can be generated using the **Report Designer**.

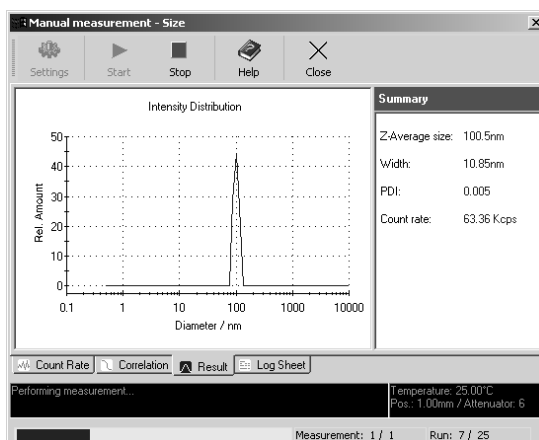
Details on interpreting the reports can be found in chapter 6.

④ Title bar.

The title bar displays the software name and the file name of the currently selected measurement file.

⑤ Measurement display

When a measurement is being performed a measurement display (below) will appear showing the progress of the measurement. The screen display shown depends on the type of measurement being performed and the view tab selected.



ILL 6601


⑥ Status bar and 21 CFR 11 icon

The status bar gives an indication of the instrument's current operating state and gives an extended description of the tool icons. If required, use the **View** menu to disable.

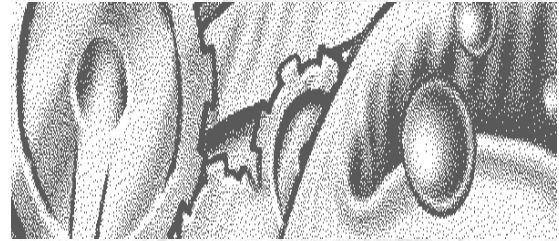
Double-clicking the Nano icon will display the Zetasizer Nano model, serial number, software and firmware version of the instrument (only if the instrument is connected and switched on).

If the 21 CFR part 11 feature key is installed, the “21 CFR 11” icon will appear in yellow on the right of the status bar. Double-clicking the icon will display the feature key number. Please note that the 21 CFR part 11 option is not detailed in this manual.

Malvern defined features

Within the software, various parameters, settings or reports will have either a small Malvern logo () or an (M) alongside. This identifies it as Malvern defined and cannot be overwritten. The Malvern defined parameters can be used as a template that can be altered and saved under a different name.

Making measurements - A tutorial



C H A P T E R 4

Introduction

After reading this chapter a user should be able to make simple measurements. The chapter goes through the basics from turning on the system to displaying the results of a measurement.

The first section, “**Quick guide to making a measurement**” will run through the basic steps, giving an overview of the measurement process. The rest of the chapter will go through the same steps but in more detail.

Once a measurement has been completed, the result can be edited to check the effects if one of the measurement parameters was altered - refer to **Editing the result** at the end of the chapter.

Manual and SOP measurements

It was mentioned in Chapter 3 that there are two basic measurement methods: **Manual** measurements and **Standard Operating Procedure (SOP)** measurements. It is important to understand and consider these methods before proceeding.

- A **Manual** measurement is basically a one-off measurement where all the measurement parameters are set up immediately prior to the measurement. This is ideal if measuring many different types of sample, or experimenting with the measurement parameters.
- An **SOP** measurement uses pre-set parameters (that have previously been defined) to ensure that measurements made on the same type of sample are made in a consistent way; this is useful in quality control environments. SOPs are also ideal if measuring the same sample in slightly different ways; having to type a majority of identical parameters each time a measurement is made is tedious and runs the risk of making errors in the settings. Instead, alter an existing SOP and just change the required parameters.

Note that most of the settings and dialogues used for a manual measurement are the same as those used in an SOP measurement.

The sections that follow, “Quick guide to making a measurement” etc, will focus on SOP measurements. Chapter 9 will give details on creating and managing your own SOPs.

Quick guide to making a measurement

This section will give a brief overview of the measurement process using an SOP. More information on each stage can be found later in this chapter.

- Close the lid and **Turn on the instrument** and wait 30 minutes for the laser to stabilise.
- **Start the Zetasizer software.**
- **Prepare the sample** following the sample preparation guidelines.
- **Choose the cell(s)** appropriate for the sample and measurement type.
- **Fill the cell(s)** with the prepared sample.
- **Make an SOP measurement.**

If necessary **Open or create a new measurement file.**

Select **Measure-Start SOP** from the Zetasizer software.
Select the **SOP** required and select **Open.**

Follow any onscreen instructions that appear.

The **Measurement display** will now be shown.

- When requested, **insert the cell** into the instrument and wait for the temperature to stabilise.
- Click **Start (▶)**. The measurement will be made and the results displayed and saved to the open measurement file.

Powering up the system


To power up the system, **Turn on the instrument** and then **Start the software.**

Turning on the instrument

On switch on an **initialisation routine** is performed that checks the instrument is functioning correctly.

Close the lid and **turn on** the optical unit, switch on the power at the power socket and turn the power switch at the rear of the unit on.

A “beep” will occur to indicate the instrument has been turned on and the initialisation routine will begin, followed by a second “beep” once the instrument has finished the routine. Two further beeps will be heard to indicate the instrument has reached the “default” temperature of 25°C.

 **Note** ■ **Important!** All laser based measuring instruments should be left powered up for approximately 30 minutes before measurements are made. This is to prevent any thermal equilibration problems affecting the measurement results.

Starting the Zetasizer Nano software

Double click on the icon to start the software.

If the desktop icon is not available, select **Start-Programs-Malvern Instruments-DTS-DTS** to start the program.



Sample preparation

The process of making a measurement is very simple - insert the sample into the instrument and then use the software to run either an SOP or manual measurement. However, the preparation of the sample before it is inserted into the instrument is paramount.

Please refer to Chapter 6 for sample preparation guidelines for the different measurement types.

Choosing the correct Cell

Malvern offers a range of cells for performing measurements with the Zetasizer system. Choice of cell is dependent upon the type of measurement being performed and the sample that will be measured.

The choices for each measurement type are outlined below with some discussion on their use.

General advice

Generally, for “easy to perform” measurements, such as with samples that scatter a reasonable amount of light (latex with 0.01% mass or higher, high scattering intensity etc.) the disposable polystyrene cuvettes can be used.

Disposable cuvettes are easily scratched though and should never be used more than once.

Disposable polystyrene cuvettes are not resistant to organic solvents, thus non-water based samples should generally be measured in glass or quartz type cuvettes.

The optical quality of the cells is vitally important when performing molecular weight and protein measurements, therefore glass or quartz type cuvettes should be used to ensure the optimum signal is achieved.

All the cells mentioned below are available from Malvern and should be used with the supplied cell caps. Using the caps will ensure greater thermal stability of the sample, as well as preventing dust introduction and possible spillage.



Caution!

Due to the risk of melting, polystyrene cuvettes must not be used for measurements above 50°C.

Size measurements

	<i>“Size & Zeta” Folded Capillary cell (DTS1060)</i>	<i>Disposable polystyrene (DTS0012)</i>
<i>Typical solvent</i>	<i>Water, Water/alcohol</i>	<i>Water, Water/ethanol</i>
<i>Optical quality</i>	<i>Good to very good</i>	<i>Good to very good</i>
<i>Minimum Sample volume</i>	<i>0.75ml</i>	<i>1ml</i>
<i>Advantages</i>	<i>Low cost. Single use disposable (no cleaning) Use with MPT2 Autotitrator No sample cross-contamination Fast sample change over</i>	<i>Low cost Single use disposable (no cleaning)</i>
<i>Disadvantages</i>	<i>Not resistant to organic solvents Unsuitable for use at high temperatures (above 70°C)</i>	<i>Not resistant to organic solvents Unsuitable for use at high temperatures (above 50°C)</i>

	Disposable low volume polystyrene (DTS0112)	Glass - round aperture (PCS8501)
Typical solvent	Water, Water/alcohol	Water, most organic and inorganic solvents
Optical quality	Good to very good	Excellent
Minimum Sample volume	375µl (100µl when using insert)	1ml
Advantages	Low cost Low volume Single use disposable (no cleaning)	Highest optical quality Can use nearly any dispersant
Disadvantages	Requires careful filling to avoid bubbles Not resistant to organic solvents Unsuitable for use at high temperatures. (above 50°C)	Requires cleaning after measurement
	Glass - square aperture (PCS1115)	Low volume quartz (DTS2145)
Typical solvent	Water, most organic and inorganic solvents	Water, most organic and inorganic solvents
Optical quality	Excellent	Excellent
Minimum Sample volume	1ml	20µl
Advantages	Highest optical quality Can use nearly any dispersant Reusable	Highest optical quality Can use nearly any dispersant Low sample volume
Disadvantages	Requires cleaning after measurement	Requires cleaning after measurement Requires careful filling to avoid bubbles

Low volume Glass flow cuvette (ZEN0023)

Typical solvent	Water, most organic and inorganic solvents
Optical quality	Excellent
Minimum Sample volume	75 μ l plus tubing
Advantages	Highest optical quality Can use nearly any solvent (tubing dependent) Use with Autotitrator
Disadvantages	Requires cleaning after measurement With manual use requires careful filling to avoid bubbles

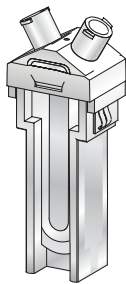
Molecular weight measurements

	Glass - round aperture (PCS8501)	Glass - square aperture (PCS1115)
Typical solvent	Water, most organic and inorganic solvents	Water, most organic and inorganic solvents
Optical quality	Excellent	Excellent
Minimum Sample volume	1ml	1ml
Advantages	Highest optical quality Can use nearly any dispersant Reusable	Highest optical quality Can use nearly any dispersant Reusable
Disadvantages	Requires cleaning after measurement	Requires cleaning after measurement

Zeta potential measurements

“Size & Zeta potential” Folded Capillary cell (DTS1060)

Description



ILL 6733

This is a maintenance-free capillary cell primarily designed for zeta potential measurements.

It has been designed to be used for a single measurement or series of measurements, then discarded rather than cleaned. This removes the chances of cross-contamination. The cell can be inserted either way round.

The cell provides a low-cost alternative to previous reusable quartz capillary cells.

The stoppers can be replaced with ‘Luer’ connectors to provide leak-free connection to the optional MPT2 Autotitrator.

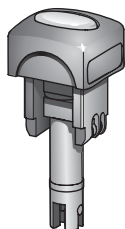
Size measurements can also be performed without having to remove and reposition the cell.

Sample details can be written on the textured area on the side of the cell with a permanent pen.

Application	<i>The cell is used for measurements of aqueous based samples</i>
Typical solvent	<i>Water, Water/alcohol</i>
Optical quality	<i>Good to very good</i>
Minimum Sample volume	<i>0.75ml</i>
Advantages	<p><i>Low cost</i></p> <p><i>Single use disposable (no cleaning)</i></p> <p><i>Use with Autotitrator</i></p> <p><i>No sample cross-contamination</i></p> <p><i>Fast sample change over</i></p>
Disadvantages	<p><i>Not resistant to organic solvents</i></p> <p><i>Unsuitable for use at high temperatures (above 70°C)</i></p>

Universal 'Dip' cell (ZEN1002)

Description



ILL 6764

The Universal 'Dip' cell is used to provide a method to measure the zeta potential of both aqueous and non-aqueous samples - A number of samples can be prepared and the Dip cell inserted to measure each one in turn.

For aqueous samples the dip cell can be used in conjunction with the disposable polystyrene (DTS0012), and for non-aqueous samples use the reusable Glass - square aperture (PCS1115). These cells are described above.

Application

The 'Dip' cell can be used for measurements of aqueous and non-aqueous based samples.

To avoid cross-contamination between aqueous and non-aqueous sample, the dip cells are fitted with colour-coded caps and the ability to be identified as such by the software. It is suggested that the blue capped cell is used for aqueous samples, and the green capped cell used for non-aqueous.

Filling the Cell

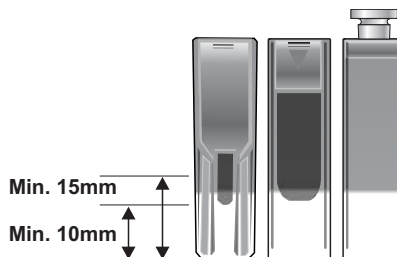
When filling the cell there are several actions to consider; some that applies to all cells and other actions that are only applicable to the measurement type and the cell chosen.

General advice

- Only clean cells should be used.
All size and zeta potential cells should be rinsed/cleaned with filtered dispersant before use.
All molecular weight cells should be rinsed/cleaned with the filtered standard (i.e. Toluene) or solvent before use.
- The cell should be filled slowly to avoid air bubbles from being created. Ultrasonication can be used to remove air bubbles - but only if the sample is suitable for use with ultrasonics.
- If using syringe filters for the dispersant, never use the first few drops from the syringe, in case there are any residual dust particles in the filter that may contaminate the dispersant.

Size and Molecular weight measurements

A minimum sample volume must be provided. However, this minimum volume depends on the actual cell type and it is easier to ensure a certain **depth** of the sample in the cell. This **minimum** is **10mm** from the bottom of the cell (the measurement is made 8mm from the bottom of the cell).



ILL 6731

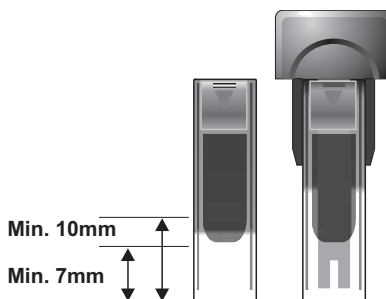
Do not overfill the cell, about **15mm maximum**, as this can produce thermal gradients within the sample that will reduce the accuracy of the temperature control.

Zeta potential measurements

The two cells used for zeta measurements are the folded capillary cell and the dip cell; the dip cell will use square cuvettes to hold the sample. Though filling either cell is a simple task, there are a number of precautions to be aware of.

Universal 'Dip' cell

With the insertion of the dip cell the sample will be displaced upwards within the cuvette. If too much sample is placed into the cuvette prior to insertion of the dip cell there is a risk that the cuvette will overflow.



ILL 6730

To ensure a minimum sample volume is provided for the sample to be measured, but protect against overfilling we recommend the cuvette is filled to a depth of

between **7mm** and **10mm** (**before** the dip cell is inserted). The minimum level relates to approximately **0.7ml** of sample.

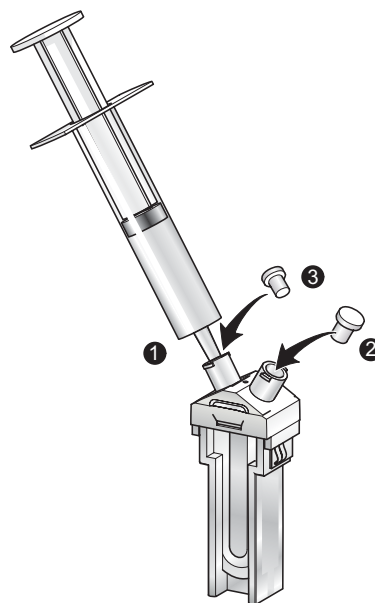
Do not overfill the cell; as well as overflowing the cuvette once the dip cell is inserted, this can also produce thermal gradients within the sample that will reduce the accuracy of the temperature control.

If necessary tap the cell lightly to dislodge any bubbles that may be caught between the electrodes.

Folded capillary cell

Fill the cell as described below

- Prepare the sample in a syringe of at least 1ml capacity.
- Place the sample syringe into one of the sample ports.
- **Slowly** inject the sample through the cell ①, checking that all **air bubbles are removed**.
If a bubbles forms under the sample port, pull the syringe plunger back to draw the bubble into the syringe body and then reinject.
- Once sample starts to emerge from the second sample port, insert a **stopper** ②.
- Remove the syringe and replace with a second **stopper** ③.
- **No** bubbles should be seen within the clear capillary area of the cell. If necessary tap the cell lightly to dislodge them. Check that the cell electrodes are still completely covered.
- Remove any liquid that may have spilt onto the electrodes.



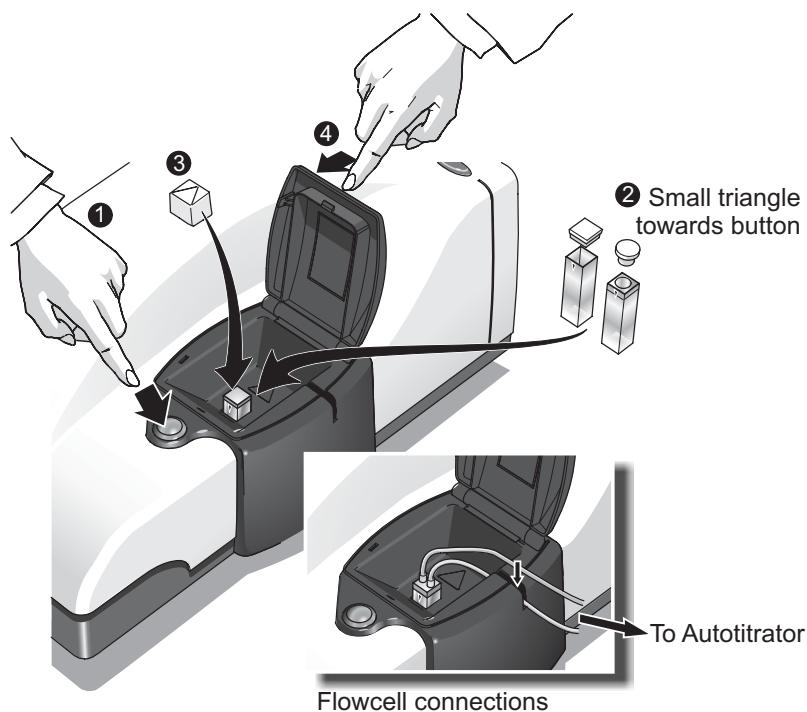
ILL 6734

Note ■ The stoppers **must** be fitted before a measurement is performed.

Inserting the Cell

In the status bar, the software will prompt when the cell needs to be inserted. This will always be after the SOP has been started - see next section. When and how the cell is inserted will depend on the application, and the measurement choices selected.

Size and Molecular weight measurements



- ① Open the cell area lid by pushing the button in front of the lid.
- ② Push the cell into the cell holder until it stops. Some cells have opaque surfaces as well as polished optical surfaces. A polished optical surface must be facing the front of the instrument (towards the button). Most cells have a small triangle at the top to indicate the side that faces the front. This is especially critical for molecular weight measurements.

If a **flowcell** is used, insert the sample tubes into the threaded inserts and screw into the top of the flowcell, and then push **both** tubes down into the pinch valve on the side of the cell area.

- ③ Place the thermal cap over the cell; Do not fit if using the flowcell.
- ④ Close the cell area lid.

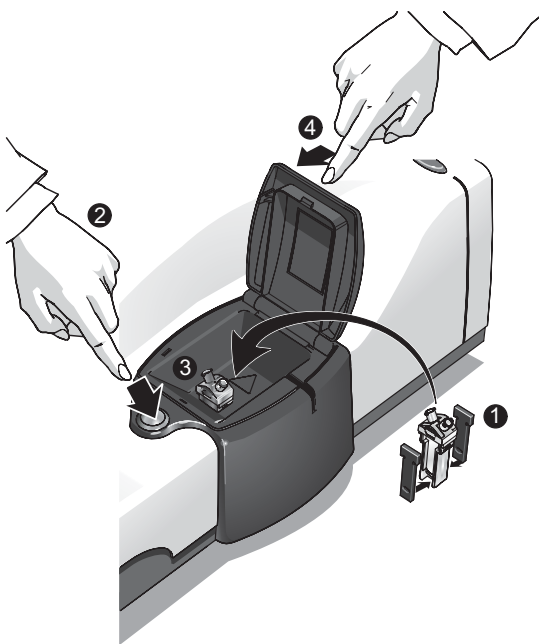
Zeta potential measurements

The two cells used for zeta potential measurements are the folded capillary cell and the dip cell; the dip cell needs to be used with a cuvette with a square aperture. Both cell types involve slightly different insertion routines.

- Note** ■ The electrode contacts on each cell, as well as applying the measurement voltage, provide identification to the software of which zeta potential cell is fitted.

Inserting the Folded capillary cell

- ① Place a **thermal contact plate** into the recess on either side of the folded capillary cell. The plates provide increased temperature stability.



- ② Open the cell area lid by pushing the button in front of the lid.

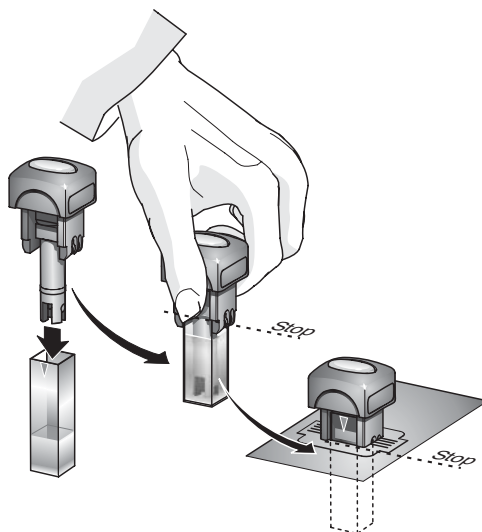
ILL. 4022

- ③ Hold the cell near the top, away from the lower measurement area, and push into the cell holder until it stops. The cell can be inserted either way round.
- ④ Close the cell area lid.

Inserting the Universal 'Dip' cell

Insertion of the dip cell, with cuvette, into the cell holder is the same procedure as above, but first the dip cell must be placed into the sample cuvette.

Place the dip cell fully into the cuvette. Some cells have opaque surfaces as well as polished optical surfaces. Ensure the small **triangle** at the top of the cell faces the front of the instrument. Check that the sample does not overflow the cuvette when the dip cell is fully inserted.



ILL 6736

Holding the base of the dip cell cap and the top of the cuvette simultaneously, push the cell into the cell holder until it stops - a '**stop**' on the dip cell **must** rest on the top of the cell holder.

**Caution.**

When removing the dip cell, and cuvette, ensure that both the dip cell cap **and** cuvette are held when the cell is withdrawn. If only the dip cell is removed there is a risk of drops of sample falling from the bottom of the dip cell onto the instrument and surrounding area. This is especially important with solvent based samples.

Making an SOP measurement

If a measurement is being made using an SOP, then all the hard work has already been done. The instrument has been turned on and the software started; the sample has been prepared and added to the cuvette. Now all that remains is to open or create a measurement file, open the required SOP, place the filled cuvette into the instrument and finally to press the **Start** (▶) button.

This process is outlined below. Chapter 9 gives all the detail required to create new SOPs.

Opening or creating a Measurement File.

Each time a measurement is made, the measurement data will be saved to a measurement file. How the measurement files are managed is down to preference. As an example:

- One measurement file may be used for all the measurement records (not recommended).
- Separate files are used for each type of sample i.e. one for titanium dioxide and one for carbon black.
- A separate measurement file is used for each week or month.
- A separate measurement file is used for each user.



Note ■ If more than one measurement file window is open, the measurement record will be saved to the measurement file currently active. When the software starts it will automatically open the last measurement file used.

► **To open an existing measurement file:**

Select **File-Open**.

A dialogue will appear allowing selection of a measurement file.


Select **Open**.

► **To create a new measurement file:**

Select **File-New**.

A dialogue will appear allowing the new measurement file to be named and specify where it will be saved.

Select **Save**.

 **Note** ■ All measurement files have the extension **.DTS**. This is added automatically to all new files.

Starting an SOP measurement


Everything should now be ready to make the actual measurement.

To start an SOP measurement, select **Measure-Start SOP**. The **Open SOP** dialogue will appear. Select the SOP that will be used and select **Open**. If an SOP has not been specified for the sample, read chapter 9 for details on how to create one.

Pre-measurement instructions may appear to advise of any actions that need to be performed before the measurement can proceed.

This may be followed by a **Labels** dialogue, allowing the measurement to be named (displayed as the **Sample name** in the records view). This dialogue also allows any other information about the measurement to be entered in the **General notes** box, such as a batch number etc. Once the measurement record has been named and any comments added, select the **OK** button.

The **Measurement display**, discussed below, will now appear.

 **Note** ■ It may be that the SOP was not configured to automatically show the **Labels** dialogue. If the dialogue does not appear, but is required, select the **Settings** button in the measurement display.

Follow the instructions on the status line of the measurement display - i.e. **Insert the Cell** (described above) and press the **Start (▶)** button to start the measurement.

The progress of the measurement can be viewed in the measurement display. The measurement may take anything from 2 minutes to over an hour per measurement, depending on the settings within the SOP.

Once the **measurement sequence** (below) is complete the measurement display can be closed and the new record will be shown in the measurement file window. The results can now be viewed - see **Displaying the results** in chapter 5.


Making a manual measurement

Making a manual measurement is essentially the same as making an SOP measurement, except, where in an SOP measurement all the measurement options are pre-specified, it will be necessary to set them immediately. All the dialogues are available at once in a tabbed format.

Follow the measurement procedures described above. Instead of starting an SOP, select **Measure-Manual**. This will open the **Manual measurement settings** dialogue allowing any measurement types to be chosen and the settings to be configured.

The dialogues are virtually identical to those used to define a new SOP. To save repeating the same information here, please refer to Chapter 9 - Managing SOPs, for more details.

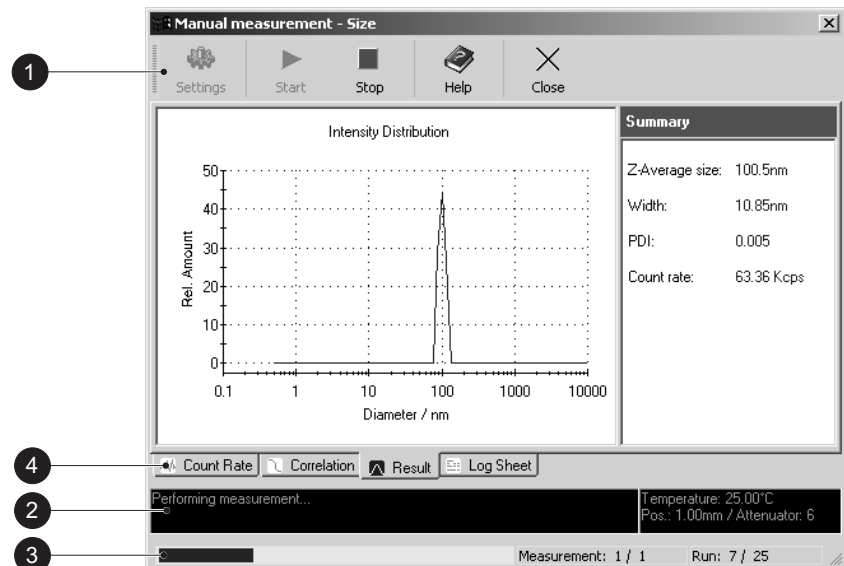
Once all settings have been made select the **Save as SOP...** button, if required, to store the settings. Click the **OK** button to close the manual measurement settings dialogue and return to the measurement display.

 **Note** ■ The manual measurement settings can be viewed and subsequently saved by selecting **Edit-Extract SOP**.

The Measurement display

When an SOP or manual measurement is started the measurement display will appear showing the progress of the measurement.

The measurement display for all measurement types is generally the same and shows a number of dialogues representing the progress of the **measurement sequence**. The dialogues displayed is dependent upon the measurement type selected. The diagram below shows the display for a size measurement.



The features of the measurement display are:

① Button bar

The button bar provides the control for the measurement operation.

Settings

Opens the measurement settings dialogue. Extra comments and changes to the measurement parameters can be added prior to the measurement being started.

Start (▶) and Stop (■)

Starts and stops the measurement. If **Stop** is pressed while performing a measurement then the measurement must be started again from the beginning. **Stop** does not act like a pause.

Help

Opens the Help file.

Close

Closes the measurement display and returns to the record view. If close is pressed while a measurement is in progress the screen will close and all measured data will be lost; a warning box will appear asking **“Are you sure you wish to abort the measurement”**.

② Status bar

The status bar shows instructions and the current operation in the measurement sequence, plus the temperature, measurement position and attenuator settings

③ Progress meter

The progress meter shows how far the measurement has progressed plus the number of measurements performed and the measurement runs completed.

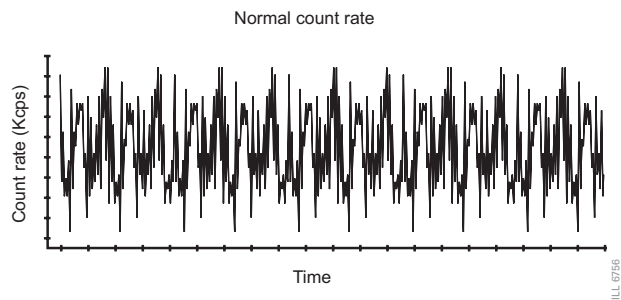
④ Tab dialogues

The **Tab** dialogues alter depending upon the measurement type selected. The dialogues available are:

Size measurements**Count rate**

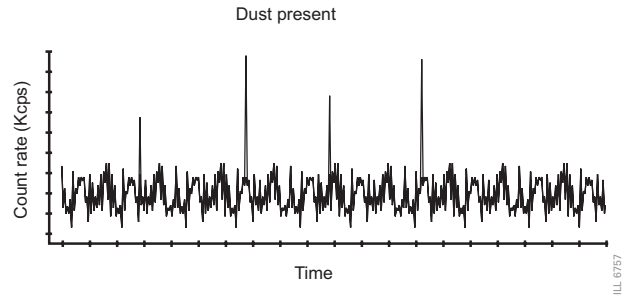
Displays the number of photons detected per second. The count rate is useful for monitoring the sample quality.

*Normal count rate
display*

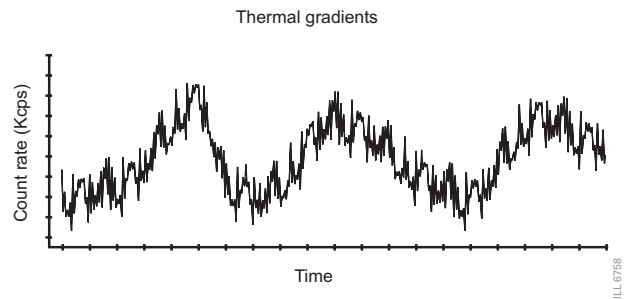


If dust is present then sharp spikes will be observed.

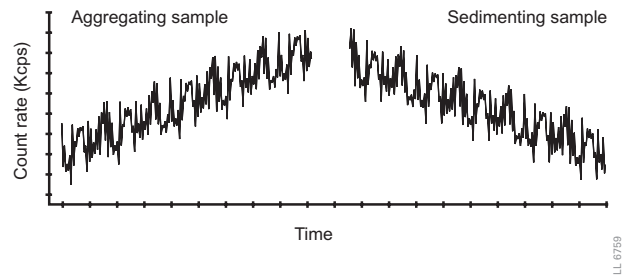
Measurement runs with dust present will be removed from the final measurement calculation by a dust filtration algorithm.



A wildly fluctuating count rate may indicate that thermal gradients are present in the sample, and further time is required for temperature equilibration.



A steadily increasing count rate will indicate an aggregating sample, while a decreasing count rate will indicate a sedimenting sample.

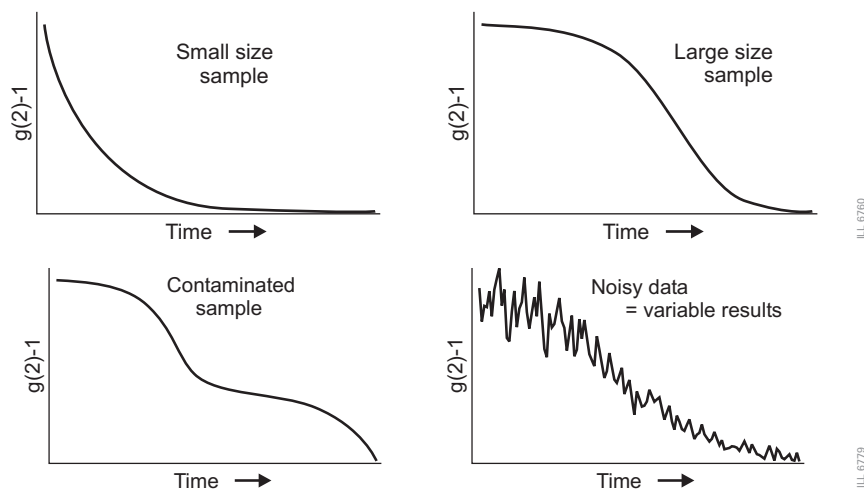


Log sheet

Displays the progress of the measurement.

Correlation function

The correlation function helps the experienced user to interpret any problems with the sample.

**Result**

The result view will be updated after every run of the measurement. The result shown will be the sum of the acceptable data collected. When a result is available to view the result tab will turn blue.

Molecular weight measurements**Count rate**

Please refer to size description above.

Correlation function

Please refer to size description above.

Result

The result view will be updated after each of the individual concentration measurements. The result shown will be an evolving value from the data collected so far.

Debye

Displays the current result as a Debye plot. The Debye plot displayed will show an evolving plot generated from the data collected so far.

Log sheet

Displays the progress of the measurement.

Zeta measurements**Count rate**

Displays the number of photons detected in kilo-counts per second.

Zeta potential

Displays the zeta potential result. The view will be updated after every individual measurement run, with the result being the sum of the completed measurement runs .


Log sheet

Displays the progress of the measurement.

Trend and protein melting point measurements

The trend and melting point dialogues are generally the same as those used with either a size or zeta measurement SOP is chosen. The difference is the inclusion of a **Trend** tab - this will show an evolving plot as the measurement progresses.

Measurement sequence

 **Note** ■ The status bar will prompt for certain actions during the course of the measurement.

Before the measurement sequence begins the cell temperature will change to the starting temperature requested in the SOP.

The measurement will then continue with an optimisation or initialisation stage, where the cell positions, compensation and attenuator settings for the cell, sample and measurement type will be determined.

Monitoring the **status bar** or clicking on the **Log sheet** tab will give more detail about what is happening during this procedure. The **progress meter** indicates how far the system is through the optimisation stages.

Once these stages have been completed, the measurement proper will start; again the actual measurement sequence will depend upon the measurement being performed.

Size measurements

The cell is inserted, **Start** is pressed and data collection begins. The progress meter indicates the measurement progress, while **Measurement** and **Run** show the number of runs completed and measurements performed.

The measurement is divided into a number of 'runs', This is done to allow data filtering. At the end of data collection the data quality of each 'run' is assessed; the runs that contain the poorest data are rejected while the remaining runs are analysed and used in the final measurement calculation.

As soon as a run is completed the result tab changes to blue to indicate a preliminary size result is available to view (by clicking on the tab). As more runs are made and assessed the quality of the result will improve.

Molecular weight measurements

The molecular weight measurement sequence requires a series of intensity measurements to be made, first of a standard to establish the reference scattering intensity, and then of each of a number of prepared sample concentrations. At each part of the sequence the user will be prompted for the insertion of the next concentration. As this requires more interaction than for size and zeta potential measurements, the sequence has been described below

- Press **start** to begin the dark count measurement.
The laser is turned off and a measurement is taken of the background light level.
- Insert the scattering standard cell (i.e. Toluene) and press **start** when ready.
The measurement will measure the scattering intensity of the scattering standard used.
- Once the standard has been measured a dialogue box will appear to prompt insertion of the first sample concentration (i.e. the pure solvent). Insert the first sample concentration and press **start**.
- The software displays another dialogue where the sample concentration can be entered. Type in the concentration and press **enter**.
The measurement continues.
- On completion of the first sample measurement, a dialogue is displayed - answer **Yes** to “Repeat measurement of concentration 1?” or **No** to continue with the second concentration.
- Continue as above until all sample concentrations have been measured.
- On completion of the last concentration the final result will be calculated.


The progress meter indicates the measurement progress during each stage.

Zeta potential measurements

The cell is inserted and **Start** is pressed. The cell is first checked to identify the cell type fitted, and that it agrees with that selected in the SOP. Once identified the measurement sequence continues automatically. The status bar will indicate the instrument is now “**Performing the measurement**”.

Each **complete measurement** is divided into a number of **measurement runs**. All the individual measurement runs are accumulated together and then summed to give a final **Zeta result**.

As soon as **one** measurement run is completed the zeta result tab changes to blue to indicate a preliminary zeta result is available to view (by clicking on the tab). As more measurements runs are made, the zeta result will change as more runs are accumulated and averaged until the final result is achieved.

-
-  **Note** ■ To shorten the measurement sequence, select the '**Auto**' measurement duration in the SOP; the change in zeta potential will now be monitored as the measurement progresses. The default number of runs in 'auto' measurement duration is 30, but for a stable sample as few as 10 runs may be required. The measurement will then complete even though the displayed run total has not been achieved.
-

During a measurement sequence it is possible to select and view the information on any of the displayed tabs.


The final result is displayed and saved to the current open file. This report pages can be viewed when the measurement display has been closed.

Editing the result

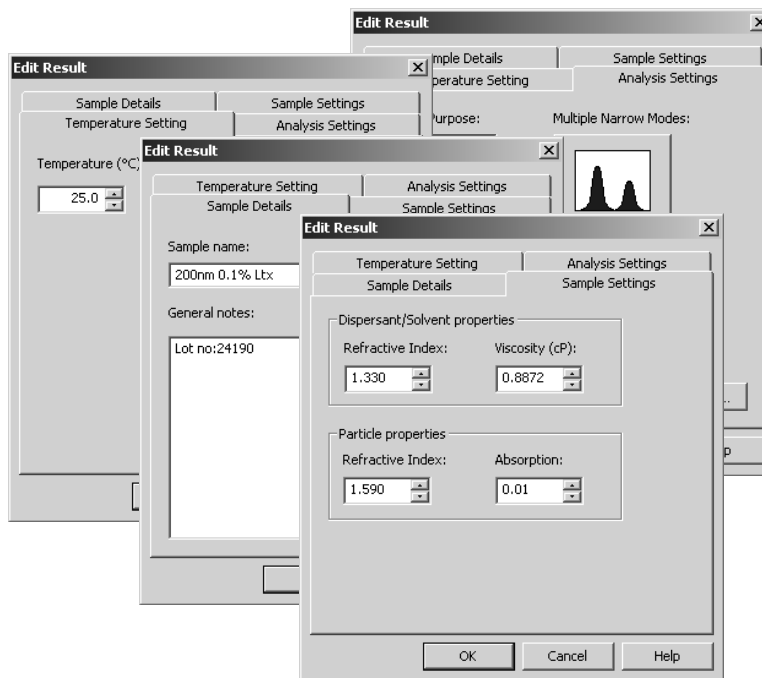
It is possible to re-analyse a measurement record using different measurement parameters. The re-analysed record will be added to end of the current file. Comments on the reasons for editing can be added and viewed in the report views.

This option allows measurements to be reanalysed without the need for the instrument to be connected.

Right-click on a measurement record and select **Edit Result**; the dialogue below will appear. The **Edit result** tabs will show choices similar to those within the SOP editor.

-
-  **Note** ■ Each edit result dialogue will be slightly different depending upon the measurement type originally performed. The picture below shows the size view.
-

Alter the appropriate parameter and press **OK**. It is advisable to add the modified parameter to the records list so the altered records can be instantly identified.



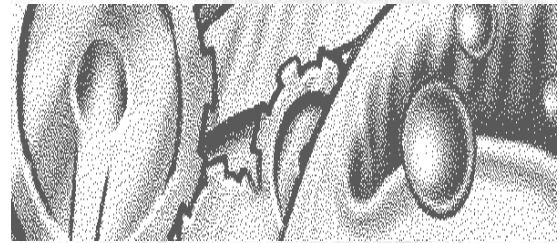
If the 21 CFR part 11 feature is installed, a **Reason for change** dialogue will appear so comments can be entered detailing what changes have been made.

The reasons for change, can be displayed in the **Records view** tab by selecting the **Measurement-Audit information-Reason** in the Workspace settings dialogue (Select **Configure-Workspaces-“.. workspace choice..”** and then the **Record view** parameters tab).

The result will instantly be reanalysed and the result added to the **Record view**.

Records and Reports - Viewing the results

C H A P T E R 5




Introduction

Once a measurement has finished the results will need to be reviewed. This chapter details the displaying of the final measurement result.

The final result is displayed in the measurement file window as either a measurement record or report.

Displaying the results

The results are displayed in two ways. The records view that shows a list of the measurement records in a measurement file, and the reports tabs which show all measurement details of a selected measurement record.

 **Note** ■ The **Records view** parameters and **Report** tabs that are displayed are dependent upon the **Workspace settings** selected- refer to chapter 10.

Records view

Once the measurement is complete, a new measurement record will be shown in the **Records view** of the measurement file window. The records will be sequentially numbered. Records are automatically assigned a record number on completion of the measurement.


Record Number	Type	Sample Name	Measurement Date	Temperature (°C)	Z.Ave.(nm)	PDI	Zeta Potential (mV)	Mobility (µmcm ² /Vs)	Molecular Weight (kDa)	Result origin
1	Zeta	DTS 50 1	06 May 2003 16:41:40	25.0			47.37	-3.735		Nano series
2	Zeta	DTS 50 2	06 May 2003 16:42:03	25.0			-51.29	-4.044		Nano series
3	Zeta	DTS 50 3	06 May 2003 16:42:29	25.0			-48.99	-3.862		Nano series
4	Zeta	DTS 50 4	06 May 2003 16:42:55	25.0			-50.9	-4.013		Nano series
5	Zeta	DTS 50 5	06 May 2003 16:43:21	25.0			-46.06	-3.633		Nano series
6	Size	200nm 0.1% Lix	03 June 2003 11:24:40	25.0	199.5	0.001				Nano series
7	Size	200nm 0.1% Lix	03 June 2003 11:28:36	25.0	200.1	0.006				Nano series
8	Size	200nm 0.1% Lix	03 June 2003 11:32:32	25.0	200.7	0.022				Nano series
9	Size	60nm Lix standard	28 May 2003 15:03:26	25.0	62.33	0.024				Nano series
10	Size	60nm Lix standard	28 May 2003 15:06:56	25.0	61.72	0.038				Nano series
11	Size	60nm Lix standard	28 May 2003 15:10:16	25.0	61.79	0.038				Nano series
12	Weight	1000 Molecular Weight	30 April 2003 17:32:23	0.0						1.08 Edited
13	Weight	Polystyrene in toluene 96	29 April 2003 15:57:12	0.0						88.93 Edited

The parameters shown will be those selected using the **Configure-Workspaces** dialogues, the record view shown above depicts the default **summary** workspace (see chapter 10)

Report tabs - a typical view


Selecting a measurement record and then selecting any of the **report tabs** will display the measurement information for that record.

This includes the pre-measurement settings such as sample name, the SOP and the SOP parameters used; the system settings for the measurement to be performed and the measurement results. A graph or table is also included at the bottom of the report.

-
-  **Note** ■ For each report two views are created. One view shows the **printed** version, the other view shows the **computer screen** version. This is done to accommodate for the different aspect ratios of the printed page and the computer screen.
-

In addition to the information described in the body of the report, the footer of the printed report will show the software version and serial number of the Zetasizer, the filename and record number of the measurement and the date printed, plus the Malvern contact phone number.

Each measurement type has a 'standard' report associated to it. The same result information is present in both the computer and printed versions, except for the diagnostic reports which contain extended information on the printed version.

-
-  **Note** ■ To show multiple results on one report, hold down **Shift** or **Ctrl** and select the required records and then click the required report tab.
-

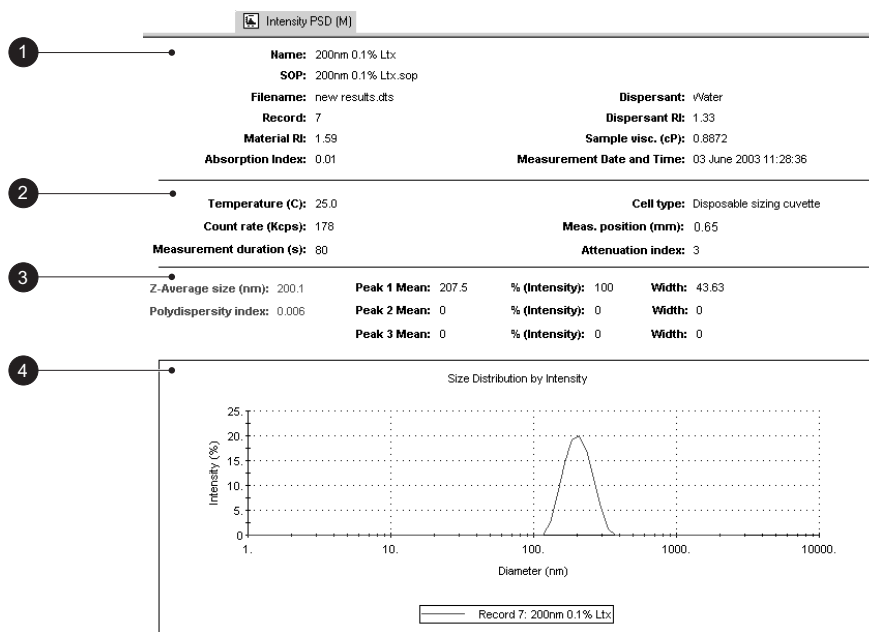
Size measurements - standard report

The standard report for Size measurements is **Intensity PSD (M)**. (**PSD** stands for **P**article **S**ize **D**istribution).

The report is divided into four areas; these are described below.

① Sample details

This section gives details of parameters relating to the sample. This includes the measurement name, record number, time of measurement, sample/dispersant refractive indices, viscosity, etc. The information shown is generally that which was entered by the user into the SOP measurement dialogues.



② System details

This section gives details on instrument settings for this particular measurement. Specifically, these are:

Temperature

Measured temperature at the start of the measurement.

Count rate

Average count rate for the measurement.

Measurement duration

This is the measurement time in seconds.

Cell type

This displays the cell type selected.

Attenuation index

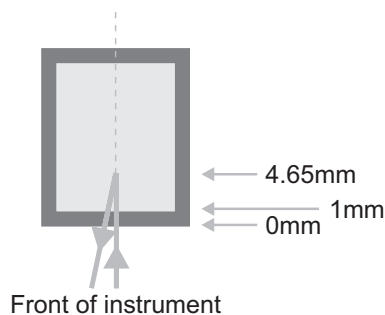
The laser power is automatically attenuated so that the count rate from the sample, especially high scattering samples, is within acceptable limits. An attenuation index of 11 denotes no attenuation (full laser power), while 0 denotes full attenuation (total laser block).

The attenuation range is shown in the following table; the transmission value is the percentage of laser light that enters the sample cuvette.


Attenuator Index	Transmission (% Nominal)
0	0
1	0.0003
2	0.003
3	0.01
4	0.03
5	0.1
6	0.3
7	1
8	3
9	10
10	30
11	100

Measurement position

The measurement position within the system is automatically moved to allow a large range of sample concentrations to be measured. The default measurement position is 4.65mm from the cell wall when a 12mm square cuvette is used. Numbers lower than this indicate that the measurement position is closer to the cuvette wall.



ILL 6774

-  **Note** ■ The attenuator index and measurement position are automatically adjusted by the software, though for more experienced users there is an advanced button that allows both to be set manually.

③ Results

This section gives the results of the measurement. The values given here will be based either on intensity, volume or number, depending on which view tab is selected.

The results section gives three pieces of information:

Z-average size (also known as the “cumulants mean”)

In dynamic light scattering this is the most important and stable number produced by the technique. This is the size to use if a number is required for quality control purposes.

It will only be comparable with other techniques if the sample is monomodal (i.e. only one peak), spherical and monodisperse (i.e. no width to the distribution), and the sample is prepared in the correct dispersant.

In any other case, the Z-average size can only be used to compare results with samples measured in the same dispersant, by the same technique, i.e. by Dynamic Light Scattering (DLS).

The cumulants analysis only gives two values, a mean value for the size, and a width parameter known as the **Polydispersity**, or the **Polydispersity Index (PDI)**. It is important to note that this mean size (often given the symbol Z or z-average) is an intensity mean. It is not a mass or number mean because it is calculated from the signal intensity.

The cumulants analysis is actually the fit of a polynomial to the log of the G1 correlation function.

$$\ln[G1] = a + bt + ct^2 + dt^3 + et^4 + \dots$$

The value of b is known as the second order cumulant, or the z-average diffusion coefficient. This is converted to a size using the dispersant viscosity and some instrumental constants.

Only the first three terms a,b,c are used in the standard analysis to avoid over-resolving the data; however this does mean that the Z-average size is likely to be interpreted incorrectly if the distribution is very broad (i.e. has a high polydispersity).

Polydispersity index

The coefficient of the squared term, c , when scaled as $2c/b^2$ is known as the polydispersity, or polydispersity index (PDI).

The calculations for these parameters are defined in the ISO standard document 13321:1996 E.

Peak means

Displays the size and percentage by either intensity, volume or number for up to three peaks within the result.

In summary, the cumulants analysis gives a good description of the size that is comparable with other methods of analysis for spherical, reasonably narrow monomodal samples, i.e. with polydispersity below a value of 0.1. For samples with a slightly increased width, the Z-average size and polydispersity will give values that can be used for comparative purposes. For broader distributions, where the polydispersity is over 0.5, it is unwise to rely on the Z-average mean, and a distribution analysis should be used to determine the peak positions.

④ Graph

The results are also shown in graphical form.

The format of the graph can be altered by moving the cursor over the graph and right clicking the mouse. The **Graph control properties** dialogue will appear. This dialogue allows the following attributes to be altered:

Display

The **Display** tab allows a choice of graph type and how it is to be displayed. i.e. Either as a histogram or curve, or particular statistics shown.

The **Options** tab allows the graph key position to be chosen. A graph tips option can also be set, this allows the setting of pop up tips (flags showing data points on the graph) on the report itself.

Axis settings

Allows both the X and Y axis settings to be defined. Whether **logarithmic** or **linear** axis are required and the axis scales - user defined or auto-scaling. **Graticule**, or grid lines can also be shown on the graphs..

Font

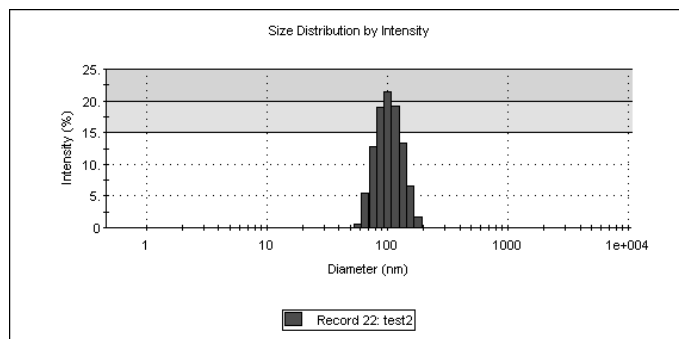
Allows the font style to be altered. The setting will apply to all annotations used on the graph.

Upper/Lower limits

Further tabs allow the setting of **Upper** and **Lower** warning or action **limits** so the value shown can be checked to be within desirable limits.

- Note** ■ Though it is possible to change the appearance of the graph on the screen, when the report is printed out it will print the original default view. To print out a different graph a new report will have to be created using report designer.

The graph below has been configured using the dialogue to display a size distribution by Intensity graph as a histogram, with logarithmic X-axis and linear Y-axis settings. Graticule, or grid, lines have been included and upper action and warning limits have been set.



ILL 6006

It is possible to zoom into a graph report. Simply hold down the left mouse button, and move the mouse to draw a “Marquee” (from top left to bottom right) around the area to be enlarged. To zoom back out, simply click the left mouse button on the graph.

Other Size reports

Other Malvern views available for size measurements are:

Intensity Statistics (M)

Volume psd (M)

Volume Statistics (M)

Number psd (M)

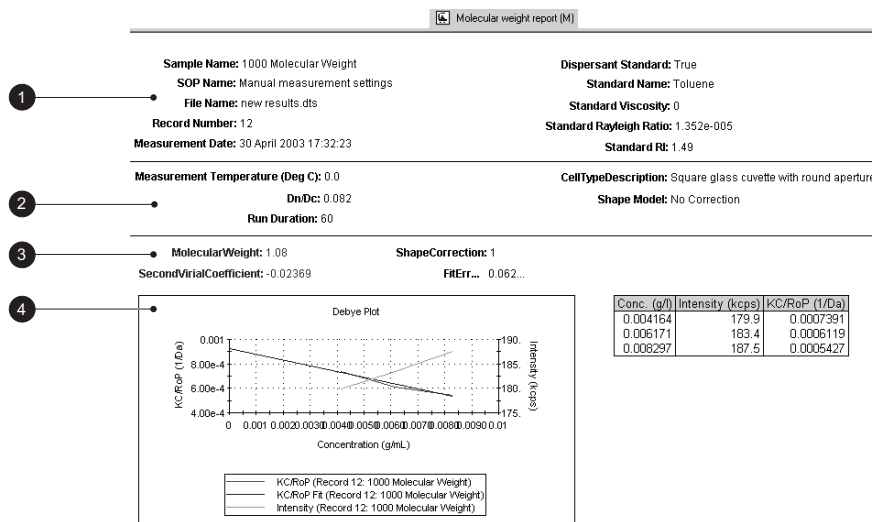
Number Statistics (M)

Size diagnostics report (M)

This report shows six graphs: The size distribution by intensity and volume, size and cumulants residuals by time, and the data and cumulants fit. These graphs are helpful for more experienced users to determine the quality of their measurement data.

Molecular weight measurements - standard report

The standard report for molecular weight measurements is **Molecular weight report (M)**.



ILL 67769

The report is divided into four areas; these are described below.

① Sample

The **Sample** section gives details of parameters relating to the sample.

Please see the **size** description for details.

② System details

The System section gives details on settings configured during the measurement process.

Please see the **size** description for details about **Temperature**, **Cell type** and **Run duration**.

Settings specific for molecular weight are:

Dn/Dc

This is the differential refractive index increment; the change in refractive index as a function of the change in concentration.

Shape model

Shows the shape correction model used for the measurement. With knowledge of the sample configuration it is possible to improve the result of the measurement by adding the value that most closely corresponds to the probable sample shape; i.e. sphere, coil, cylinder or no shape correction.

③ Results

The results section gives four pieces of information:

Molecular weight

Shows the measured weight of a molecule within the measured sample expressed in atomic mass units in Daltons.

Shape correction

Displays the shape correction model used for the calculation as mentioned above.

Second Virial Coefficient

A property describing the interaction strength between the molecule and the solvent.

Fit error

This is an indication of the quality of the measurement. The lower the fit error the better the measurement.

④ Graph

The results are also shown in graphical form.

Please see the **size** description above for details on altering the graph.

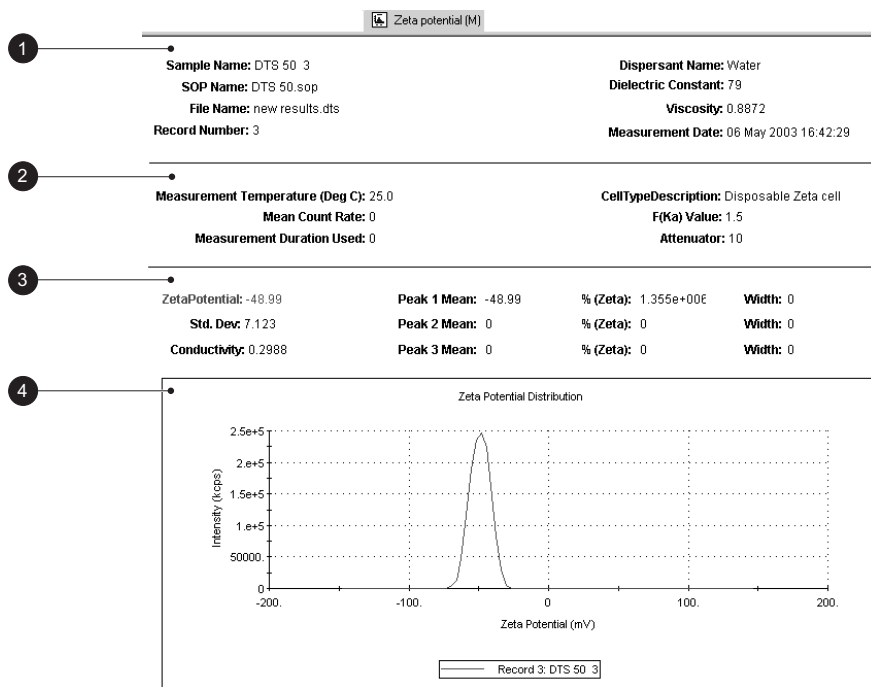
Zeta potential measurements - standard report

The standard report for zeta potential measurements is **Zeta potential (M)**.

The report is divided into four areas; these are described below.

① Sample

The **Sample** section gives details of the sample parameters. This includes the sample name, record number, measurement date and time, dispersant name, The SOP used and the measurement file name. The information shown is generally that which was inputted into the SOP measurement dialogues.



② System

The system section gives details on settings configured during the measurement process.

Please see the **size** description for details about **Temperature**, **Cell type**, **Run duration** and **Attenuator**.

Settings specific for zeta measurements are:

Mean Count rate

Average count rate for the measurement.

F(ka) value

Displays the Henry function or ‘approximation’ used during the measurement. A value of **1.5** means the **Smoluchowski** approximation was used, while a value of **1** means the **Huckel** approximation was used.

③ Results

The results section gives four pieces of information:

Zeta potential

Displays the complete zeta potential result of the measurement in mV.

Std Dev. (Standard deviation).

The standard deviation displays 1 standard deviation of the zeta distribution around the mean result, in millivolts (mV).

Conductivity.

The ability of a sample to conduct electrical current. Higher salt concentrations generally allow higher currents to pass and so have higher conductivities. Conductivity can be temperature dependent.

Peak means.

Displays the mean zeta potential for up to three peaks within the result.

④ Graph

The results are also shown in graphical form.

Please see the **size** description above for details on altering the graph.

Other Zeta potential reports

Other Malvern views available for zeta potential measurements are:

Electrophoretic mobility (M)

This report is effectively the same as the zeta potential report described, except the zeta potential result and graph are replaced by the electrophoretic mobility.

Trend and protein melting point measurements

These measurements can be viewed using the report **Melting point (M)**. The report gives the same information as seen in a standard size or zeta report, plus the following:

Trend Temperature Start (°C)

The temperature defining the beginning of the measurement.

Trend Temperature End (°C)

The temperature that the measurement will end at.

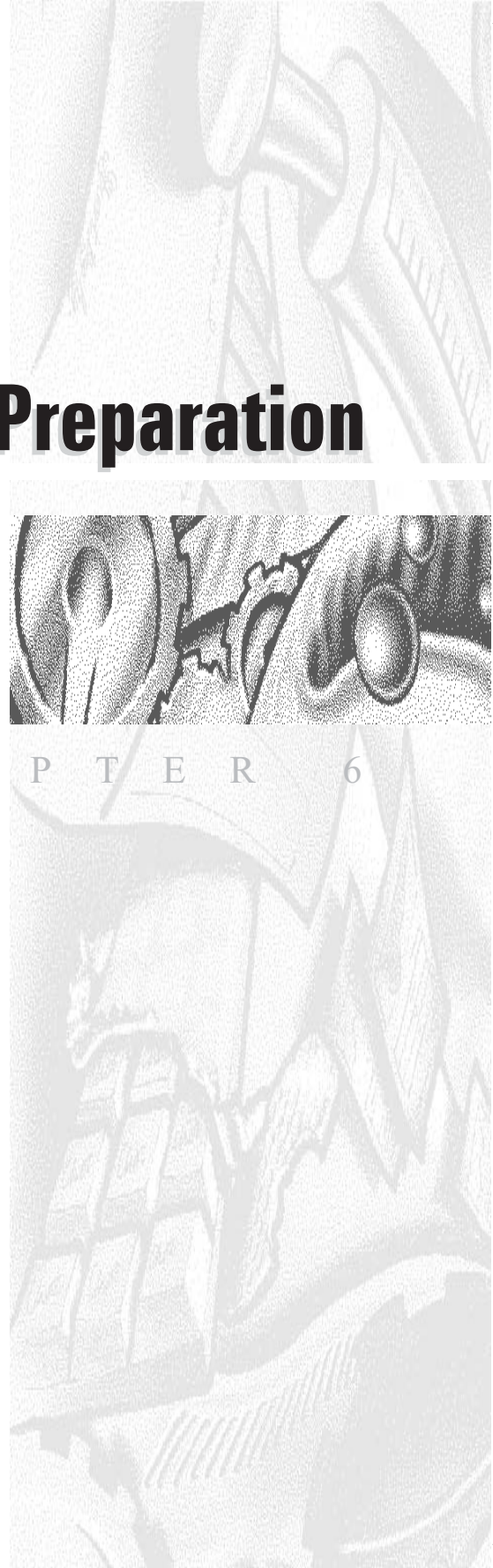
Melting point (°C)

The temperature at which the melting point is achieved.



Sample Preparation

C H A P T E R 6



Introduction

Before filling the cell or cuvette that will be used in the measurement, the sample will need to be prepared. To ensure reliable and accurate measurements proper sample preparation is extremely important.

Preparation of the sample for the different measurement types will involve specific preparation techniques. For each measurement type follow the guidelines described.

Preparing the sample - Size

Consideration must be given to the physical properties of the sample such as its particle size and sample concentration. This section outlines the basic considerations for sample preparation.

Sample concentration

Each type of sample material has its own ideal range of sample concentration for optimal measurements.

- If the sample concentration is too low, there may not be enough light scattered to make a measurement. This is unlikely to occur with the Zetasizer except in extreme circumstances.
- If the sample is too concentrated, then light scattered by one particle will itself be scattered by another (this is known as multiple scattering).
- The upper limit of the concentration is also governed by the point at which the concentration no longer allows the sample to freely diffuse, due to particle interactions.

An important factor in determining the maximum concentration the sample can be measured at, is the size of the particles.

The table below can be used as an approximate guide to determine the maximum and minimum concentrations for different sizes of particles. The figures given are approximate values for samples with a density near to 1g/cm^3 , and where the particles have a reasonable difference in refractive index to that of the dispersant, e.g. a refractive index of 1.38 against water which has a refractive index of 1.33.

Particle size	Min. Concentration (Recommended)	Max. Concentration (Recommended)
< 10nm	0.5g/l	Only limited by the sample material interaction, aggregation, gelation etc.
10nm to 100nm	0.1mg/l	5% mass (assuming a density of 1g/cm ³)
100nm to 1μm	0.01g/l (10 ⁻³ % mass)	1% mass (assuming a density of 1g/cm ³)
> 1μm	0.1g/l (10 ⁻² % mass)	1% mass (assuming a density of 1g/cm ³)

Whenever possible, the sample concentration should be selected such that the sample develops a slightly milky appearance - or in more technical terms, gets slightly turbid.

If such a concentration cannot be selected easily (for example, the particle size of the sample may be so small that even concentrated dispersions show no turbidity), various concentrations of the sample should be measured in order to detect and then avoid concentration dependent effects (i.e. particle interactions etc). A concentration should be chosen such that the result is independent of the concentration chosen. However, these effects do not normally appear at concentrations below 0.1% by volume.

Be aware that particle interactions may occur at sample concentrations larger than 1% by volume - particle interactions will influence the results.

Considerations for small particles

Minimum concentration

For particle sizes smaller than 10nm, the major factor in determining a minimum concentration is the amount of scattered light that the sample generates. In practice, the concentration should generate a minimum count rate of 10,000 counts per second (10kcps) in excess of the scattering from the dispersant. As a guide, the scattering from water should give a count rate in excess of 10kcps, and toluene in excess of 100kcps.

Maximum concentration

For samples with small particle sizes, a maximum concentration does not really exist (in terms of performing Dynamic Light Scattering (DLS) measurements). However, in practice, the properties of the sample itself will set the maximum value. For example, the sample may have the following properties:

- **Gelation.** A gel is unsuitable for measurement using the Zetasizer (this is true for all instruments based on Dynamic Light Scattering).

- **Particle interactions.** If there are interactions between the particles then the diffusion constant of the particles usually changes, leading to incorrect results. A concentration should be chosen to avoid particle interactions.

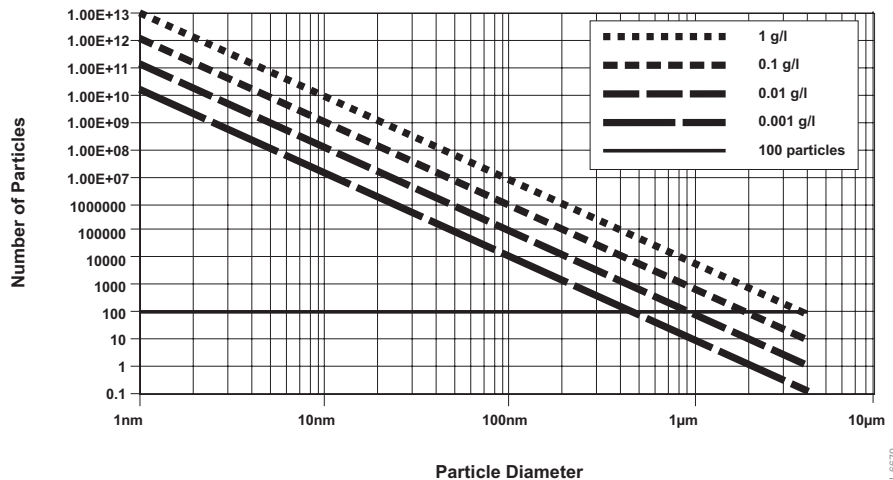
Considerations for large particles

Minimum concentration

Even for larger particles, the minimum concentration is effectively still a function of the amount of scattered light, though the additional effect of “number-fluctuation” must be taken into account.

As an example, if a sample of large particles (say 500nm) were to be measured at low concentration (say 0.001 g/l ($10^{-4}\%$)), the amount of scattered light generated would be more than sufficient to perform a measurement. However, the number of particles in the scattering volume is so small (fewer than 10) that severe fluctuations of the momentary number of particles in the scattering volume will occur. These fluctuations are not the type assumed by the calculation method used, or will generally be misinterpreted as larger particles within the sample.

Such fluctuations must be avoided and this determines the lower limit for the required concentration and for a lower limit in the number of particles. At least 500 particles should be present, however, a minimum of 1000 particles is recommended. See the figure below for an estimate plot of the number of particles per scattering volume for different concentrations assuming a density of 1 g/cm^3 .



Maximum concentration

The upper limit for the sample concentration for larger particles is determined by their tendency to cause multiple-scattering. Although the Zetasizer is not very sensitive to multiple-scattering, with increased concentration, the effect of the multiple-scattering becomes more and more dominant, up to a point where so much multiple-scattering is generated that the results of the measurement will be affected. Of course, such a high concentration should not be used for accurate measurements, and rough estimates are given for maximum concentrations for the different size classes in the table above.

As a general rule, measure at the highest concentration possible before multiple scattering and particle interactions affect the result. Dust contamination in the sample can be presumed equal for high and low concentrations, and thus, the amount of scattered light from the sample increases in relation to the scattering from the dust contamination as the concentration increases.

Filtration

All liquids used to dilute the sample (dispersants and solvents) should be filtered before use to avoid contaminating the sample. The size of the filter will be determined by the estimated size of the sample. If the sample is 10nm, then 50nm dust will be an important contaminant in the dispersant. Aqueous dispersants can be filtered down to 0.2 μ m, while non-polar dispersants can be filtered down to 10 or 20nm.

Samples are not filtered if at all possible. Filters can remove sample by absorption as well as physical filtration. Only filter the sample if aware of larger sized particles, such as agglomerates, that need to be removed as they are not of interest, or cause result variations.

Using ultrasonics

Ultrasonication can be used to remove air bubbles or to breakup agglomerates - however, this must be applied carefully in order to avoid damaging the primary particles in the sample. Limits for the use of ultrasonication in terms of intensity and application time are strongly sample dependent. Minerals such as titanium dioxide are ideal candidates for dispersion by high powered probes, however, the particle size of some materials, such as carbon black, may depend on the power and length of time ultrasonication is applied. Some materials can even be forced to aggregate using ultrasound.

Emulsions and liposomes should not be ultrasonicated.

Preparing the sample

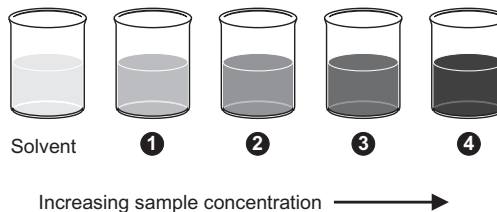
- Molecular weight

Preparation of a molecular weight sample is similar to that followed for a size sample, though there are other aspects to consider.

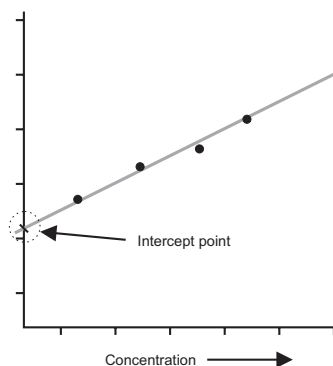
The technique is very sensitive to dirt or dust in the sample and therefore great care is required in sample preparation. All solvents must be filtered to $0.02\mu\text{m}$ or better several times, and the prepared solutions allowed to stand for a period dependent upon the sample, this may be over 24 hours, to several days to ensure adequate solvation. All glassware and apparatus **must** be rigorously clean and free of scratches. Preparation of samples and storage of apparatus in a laminar flow cabinet to ensure minimisation of dust contamination is strongly recommended. Failure to carry out these routine procedures are certain to end in a poor or wrong result.

Very small samples such as proteins in aqueous solutions will often require filtering.

A number of concentrations of the sample must be prepared (typically 0.25 to 1 g l^{-1}). The polymer must be fully soluble and dust must be excluded.



The solvent scattering is measured followed by the various concentrations of sample. From these measurements a Debye plot can be generated.



ILL 6766

ILL 6767

This is a plot of the variation in average intensity versus the concentration. The intercept of the extrapolation to zero concentration is calculated.

Minimum concentration

The minimum concentration of sample that should be used is defined by the excess scattering over the solvent which should be a minimum of 30%, e.g. If the solvent is toluene with a count of 150 Kcps then the lowest sample concentration should be greater than 150×1.3 kcps (195Kcps). Careful sample preparation procedures can make it possible to measure a sample with only 10% excess scattering but this is not ideal.

Maximum concentration

The maximum concentration will be sample dependent and will be determined by the onset of particle interactions. Typically it is best to keep the maximum concentration to below 0.1w/v%

Preparing the sample - Zeta potential

The optical arrangement for zeta potential measurements in the Zetasizer Nano series means that the concentration requirements are not as strict as those used for size and molecular weight measurements.

An auto attenuator fitted inside the instrument ensures that the sample count rate is suitable for the requirements of the detector. However if the sample is too concentrated a low sample count rate error will be displayed. For example, for a 300nm oil in water emulsion this will be the case for concentrations higher than 0.5w/v%.

Too low a concentration will be indicated by the system setting an attenuator index position of 11, and the generated results being variable.

In extreme cases, or where there is a bubble in the cell, a 'low sample concentration' error may be displayed.

Many samples will require dilution and this procedure is absolutely critical in determining the final value measured. For meaningful measurements the dilution medium is crucially important. A measurement result given with no reference to the medium in which the material is dispersed is meaningless. The zeta potential is as dependent on the composition of the disperse phase as it is on the nature of the particle surface.

Dilution Medium

The continuous phase of most samples can arbitrarily be put into one of two categories:

- **Polar** dispersants are defined as those with a dielectric constant greater than 20 e.g. ethanol and water.
- **Non-polar** or low polarity dispersants are defined as those with a dielectric constant less than 20, e.g. hydrocarbons, higher alcohols.

Aqueous/Polar Systems

The aim of sample preparation is to preserve the existing state of the surface during the process of dilution. There is only one way to ensure this is the case. This is by filtering or centrifuging some clear liquid form the original sample and using this to dilute the original concentrated sample. In this way the equilibrium between surface and liquid is perfectly maintained.

If extraction of a supernatant is not possible, then just letting a sample naturally sediment and using the fine particles left in the supernatant is a good method. Zeta potential is not a size-dependent parameter using the approximation of the Smoluchowski theory.

Another method is to imitate the original medium as closely as possible. This should be done with regard to:

- pH.
- Total ionic concentration of the system.
- Concentration of any surfactants or polymers present.

Non-Polar Systems

Measuring samples in insulating media such as hexane, isoparaffin, etc. is far from easy. It requires the use of the universal dip cell. This is required because of its chemical compatibility and the close spacing of the electrodes which allows the generation of high field strengths without using excessively high voltages.

Sample preparation for such systems will follow the same general rules as for polar systems. As there will be generally fewer ions in a non polar dispersant to suppress the zeta potential, the actual values measured can seem very high, as much as 200 or 250mV. In such non-polar systems, equilibration of the sample after dilution is the time dependent step, equilibration can take in excess of 24 hours.





Maintenance



C H A P T E R 7

Introduction

This instrument has been designed so that supervisor/operator maintenance is kept to a minimum. It should be fully understood that no one, except a qualified Malvern representative, should remove the main cover.

This chapter explains the routine user maintenance procedures that can be performed. These procedures are:

- Cleaning the instrument.
- Cleaning the cells.
- Replacing the system fuse.


Cleaning the instrument



Caution!

The surfaces of the instrument may be permanently damaged if samples or dispersants are spilt onto them. If a spillage should occur, then the system should be disconnected from the power supply before scrupulously cleaning up the spillage.

Periodically the covers should be thoroughly cleaned using a mild soap solution.

 **Note** ■ Always ensure that the instrument is disconnected from the power supply and all electrical cables are disconnected.

- **Never** use excessive liquid to clean the instrument and always avoid electrical components (connectors, etc).
- **Always** ensure that the instrument is completely dry before applying power.
- The paint has a solvent resistant finish, however it is good practice never to use a solvent based solution as this may result in damage to the painted surfaces.
- **Never** use an abrasive cleaner to clean the instrument as this may result in damage to the painted surfaces.

- **Never** use compressed air.

Cleaning the Cells

It is very important that the cells are cleaned thoroughly between measurements and especially between different types of sample. Any cross-contamination between samples can seriously affect the results.

Cleaning the Universal 'Dip' cell



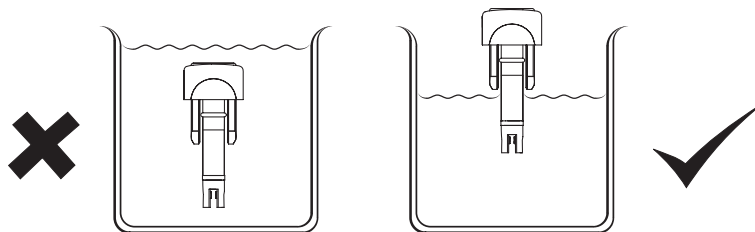
Caution

Do **not** immerse the complete cell. Only the electrodes must dip in to the dispersant as shown below.

The cell electrodes are made of palladium and can be cleaned physically and chemically.

Cleaning procedure:


- Immerse the electrodes in a gentle ultrasound bath (30Watts) for 5 to 10 minutes before use. For the cleaning fluid, use the dispersant used for the previous sample. If the dispersant contains additives such as surfactants, follow this by ultrasonication for two minutes in the pure solvent. Take account of any health and safety issues, as ultrasonication can produce a fine aerosol of the liquid in the bath.



1116783

- On removal from the bath rinse electrodes with pure solvent.
- Before a measurement, rinse the electrodes and the cuvette with the sample you are measuring.

- Each time you change the sample, the electrode needs to be thoroughly rinsed with pure dispersant to ensure cleanliness.
- A cotton bud may be used for **gentle** cleaning of the electrode.

 **Note:** ■ The electrode holder is made from PEEK (polyetheretherketone) which is resistant to a wide range of chemical products, however, it is advisable to seek advice from Malvern and the sample manufacturer before using strong acid or strong base.

Cleaning Cuvettes

There are two main types of cuvette available, disposable polystyrene and reusable glass or quartz. Never attempt to clean and re-use disposable cuvettes. Cleaning disposable cuvettes will cause small surface scratches that will give inaccurate results.

The cleaning procedure for glass or quartz cuvettes is dependent on the sample that was measured, therefore specific instructions can not be given here.

However, the following advice should be followed:

- Rinse the cuvette with the same dispersant that was used for the measurement, i.e. if the sample was dispersed in water - use clean water to rinse it.
- Try submerging the cuvette in an ultrasonic bath of clean solvent.
- Once clean, wipe the cuvette with a lint free tissue (photographers lens cleaning tissues are recommended).
- The smaller and more dilute the sample being measured, the more important the cleanliness of the cuvette.

Cleaning the Folded capillary cell

The folded capillary cell is intended to be used once then discarded, but can easily be used again if required. Using syringes wash the cell with the same dispersant as used for the measurement, or de-ionised water.

Never attempt to clean the outside of the folded capillary cell as this will cause small surface scratches that will give inaccurate results.

Replacing the system fuse



Warning!

Fuses must not be replaced by the operator. Only the supervisor or a Malvern representative should attempt to change the fuse.

If the instrument does not power up, check the system fuses. The system fuses are located on the rear panel of the instrument in the mains power switch.

Before changing the fuses, disconnect the instrument from the mains power supply.

Pull the fuse holders out and replace any faulty fuses with ones of the following specification:

Rating : *T 2A L 250v* (*T = Time delay*)

Size : *5mm x 20mm*

Part 2 - Supervisors guide





Security



C H A P T E R 8



Introduction


The systems may be used by users of varying skill levels. With the Zetasizer it is possible to limit user access so that certain functions such as editing SOPs, deleting records and editing results can be reserved for specific users only.

Within the Malvern **Security** system, one or more persons are set up as the administrator. The administrator then controls access to the instrument by defining 'User groups' and 'Permissions'

- A **User group** is one or more persons that have the same access rights.
- **Permissions** are the access rights that are allowed for each user group, these will range from allowing SOPs to be edited to disabling view selection.

The administrator adds operators to a group and assigns each operator a password. Each operator's identification and password are entered to enable access to the software.

The first time the software is run the security system will be disabled and an administrator user and administrators group will be created by the system. This is so at least one user will have permission to configure the security system.

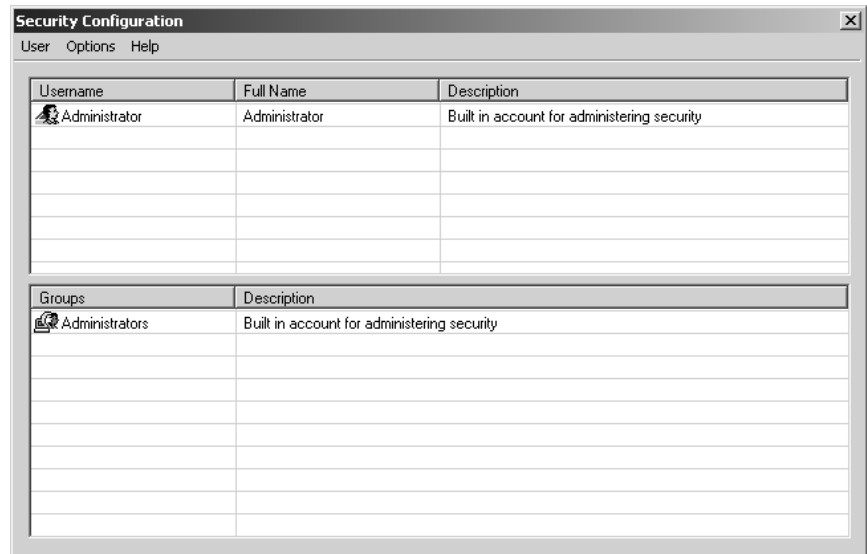
-
-  **Note** ■ The Malvern security system can be upgraded to 21 CFR part 11 compliance by installing a "feature key". Once this is installed 21 CFR part 11 security settings can be applied and 'Audit trails' can be viewed. If the feature key has been installed the grey 21 CFR 11 icon on the status bar will turn to yellow. Please note that this manual will not detail the 21 CFR part 11 option but will concentrate only on the standard security software.
-

The first task is to set up the administrator account.

Initial start-up - set up the administrator:

-  **Note** ■ It is vital that at least one user has access to the configuration of the security system.

Select **Security-Configure Security**.



The first time the system is run, there will be only one user (Administrator) and only one group (Administrators). The administrators group is originally set to only allow configuration of the security system and to deny access to all other features of the system.

On first start up of the software the security system defaults to a member of the administrators group with no password.

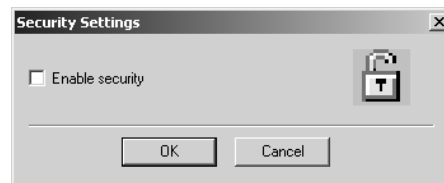
To secure the system, a password must first be specified for the administrator account (detailed later) and then the security enabled using the **Options-Security settings...** menu.

-
- Note** ■ It is preferable to have at least two users assigned to the administrators group. The users user name and password should be stored in a secure location. This is to safeguard against accidental lockout or deletion of permissions that may prevent the security settings being available.
-

Remember that after any changes to the security system - either adding users, changing of permissions, etc, the new changes must be saved (**User-Save**). A dialogue will appear on exiting to give a reminder on saving changes.

Enabling security

Within the **Security configuration** dialogue select **Options-Security settings** and select the **Enable security** check box.



ILL 0556

With security enabled each user must login when the software starts. Once logged in only their relevant permissions will be accessible, all other functions will be greyed out.

-
- Note** ■ With the 21 CFR part 11 feature key installed a different dialogue will be displayed. Once 21 CFR part 11 security is enabled it cannot be disabled.
-

If changing operators when the software is already open select **Security-Logout** and then **Security-Login** and enter the appropriate passwords.

User groups

-
- Note** ■ Only users assigned to the administrator group can add or edit the user group properties.
-

Adding/editing a group

Select **Security-Configure security** to enter the **Security configuration** dialogue. Select **User-New Group...** to display the **Group properties** dialogue, alternatively double-click on an empty row. When editing double-click on an existing group to display the dialogue.

Members:	
Administrator	Administrator

Permissions:

- Configure communications settings
- Configure ER/ES settings
- Create and edit export templates
- Create and edit reports
- Create and edit SOPs
- Delete results
- Edit user settings
- Make a measurement

ILL 6557

Enter a **Group name** and a **Description** of the group's purpose. Example names might be:

Operators - General users of the system.

Supervisors - Skilled operators responsible for configuration and SOP creation.


Administrators - Users authorised to configure the security system.

The **Members'** section of the dialogue shows a list of all the users currently assigned to the group. To add a user press **Add** and a list of all users not currently allocated to that group will be shown. Selecting one or more users (hold down the control key to select multiple users) and press **OK** to add those users to the group.

To remove a member, simply select the member and press the **Remove** button. Note that members are only removed from the group, they are not removed from the system.

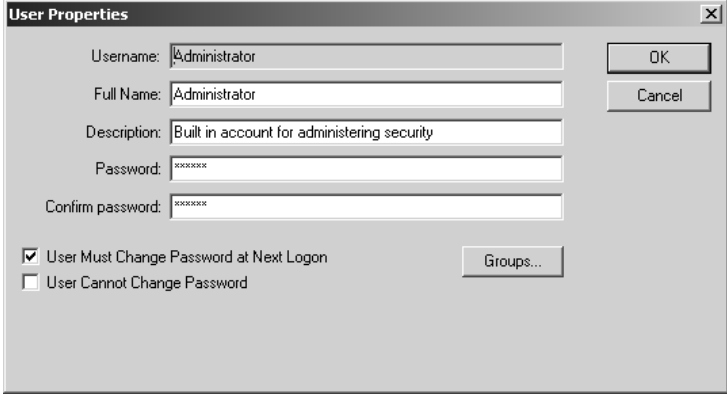
The **Permissions** section of the dialogue allows functions of the software to be enabled/disabled for the group. To set the access permissions for the group, simply scroll down the list of permissions and check the boxes for the permissions required. This can also be done with a mouse or by highlighting a permission and pressing the space bar. The permissions list can be navigated using the up and down keys once selected.

Users

 **Note** ■ Only users assigned to the administrator group can add or edit user properties.

Adding/editing a user

Select **Security-Configure security** to enter the **Security configuration** dialogue. Select **User-New User...** to display the **User properties** dialogue, alternatively double-click on an empty row. When editing double-click on an existing user to display the dialogue.



ILL 0558

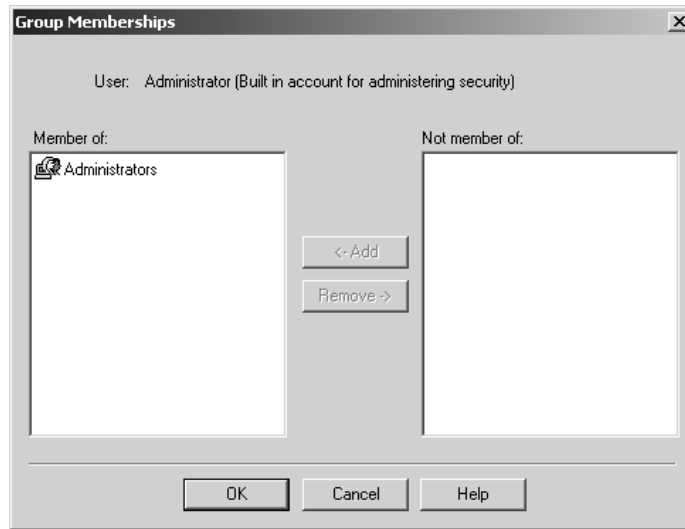
Each user must have a unique **Username**. This, along with the user **Password**, forms the unique key required to identify each individual using the system. The **Username** is commonly an abbreviated form of the individual name or a unique identifier such as an employee code.

The **Full Name** is used to hold the full printed name of the individual and can be used on reports to identify the user if an employee code is used as a **Username**.

The **Description** field is optional and is just used to add some descriptive text to the user details.

Generally it will be the administrator entering these details. The administrator can enter the password but this would require the administrator to know a users password beforehand. A more secure approach is for the administrator to specify a previously arranged password, such as the users name, and force the user to change their password the next time they log on using the password check boxes.

Press the **Groups** button to allocate the user to the required groups. Use the **Add** and **Remove** buttons to allocate the groups or groups appropriate.



ILL 0550

Password options

■ User must change password at next logon

The change password dialogue will be shown when the user next logs in, allowing the user to change their password. This allows the security administrator and a user to set up a user account without the administrator knowing the users final password.

Users can change their own passwords once logged in by using the **Security-Change password** menu.

To change the user password; enter the current password and specify a new password with confirmation. Pressing **OK** makes the change to the security settings.



Set password

Change your password.

User name: Administrator

Current Password:

New Password:

Confirm New Password:

OK Cancel

ILL 6560

- **User can not change password**

This option prevents a user from changing a password once it has been initially set.

For a secure system, it is advisable for users to regularly change their passwords to prevent unauthorised access. The only possible exception is the security administrator's account where forgetting the password to this account could prevent any further configuration of the security system.





Using SOPs

C H A P T E R 9

Introduction

An SOP measurement uses **pre-set** parameters, to ensure that measurements made on the same type of sample are made in a consistent way. SOPs are ideal if repeatedly measuring the same type of sample. Having to set the same parameters each time a measurement is made is tedious and also adds the risk of introducing errors into the settings - using SOPs avoids this.

Chapter 4 gave instructions on how to use an SOP to make measurements - but this assumes that an SOP already exists for the sample to be measured. If no SOP exists for the sample then one needs to be created; there are three ways to do this:

- Create a new SOP by using the SOP creation wizard - see **Creating an SOP** below.
- Create a new SOP by editing an existing one - see **Modifying an SOP** later in this chapter.
- Optimise the measurement settings by making manual measurements, then **extract the SOP** from the record and save.

This chapter focuses on how to create a new SOP and details all the measurement settings that are available. Almost all the options for creating an SOP are the same as those available for making a manual measurement, so this chapter will also be of benefit to those who require an explanation of the options available for a manual measurement.

As well as describing the SOP creation for the three major measurement types (size, molecular weight and zeta potential), it is also possible to create SOPs for **Trend** and **Melting point** measurements. These will be described towards the end of this chapter as they generally use the same SOP dialogues as those described for the major measurement types.

Once a measurement has been completed it will be added to the list in the **Records view** tab of the **Measurement file** window. The SOP used can be viewed by right-clicking over the measurement record and selecting **Extract SOP**.

In each of the SOP dialogues there are **Help** or **Advice** buttons that will advise on how to complete the SOP.

Creating an SOP

To create a new SOP, select **Configure-New SOP**. This will open up the SOP creation wizard. The SOP creation wizard consists of several dialogues that can be stepped through by using the **Next** button.

Most of the SOP dialogues are common for all measurement types; these are identified in the All SOPs section. Other SOP dialogues are **unique** to the **measurement type** selected and are discussed in individual **Size, Molecular weight, Zeta potential, Trend** and **Protein melting point SOP** sections.

The SOP dialogues available are:

Measurement type

Allows selection of the type of measurement that the SOP will perform, i.e. Size, Protein melting point, Zeta potential, Molecular weight or Trend.

Labels

This dialogue allows the measurement record to be named. All measurements made using this SOP are given the sample name entered in the SOP by default, but you can give an option to change this when you make the measurement. Information can be entered in the **General notes** box, such as a batch number.

This is a common dialogue for **all** SOPs.

Instructions

Enables instructions on preparing and measuring the sample to be shown before and after each measurement. This dialogue is not available when performing a manual measurement.

This is a common dialogue for **all** SOPs.

Cell

This dialogue allows selection of the cell that will be used.

This is a common dialogue for **all** SOPs.

Sample

This dialogue allows the physical properties of the sample that will be measured, to be set. The refractive index and viscosity can be entered for both the material and dispersant used.

This is a common dialogue for **all** SOPs with unique features dependent upon the measurement type.

Trend

Enables the selection of the temperature trend parameters, i.e. start and end temperatures, and intervals between measurement steps.

For **Trend** SOPs only. Common dialogue for size and zeta trend measurements.

Measurement

This dialogue allows the duration of the measurement to be set, and to make multiple measurements of the same sample.

This is a common dialogue for **all** SOPs with unique features dependent upon the measurement type.

Result calculation

If characteristics are already known about the sample being measured, this dialogue will allow a more appropriate analysis model to be applied.

This is a common dialogue for **all** SOPs with unique features dependent upon the measurement type.

Reports

This dialogue enables reports to be automatically printed after each measurement and/or after a run of measurements has been completed. An option is also available to make PDF versions of the results (21 CFR part 11 feature key required).

This is a common dialogue for **all** SOPs.

Export

Enables the measurement results to be exported, as a text file, to third party software packages such as Excel or Wordpad.

This is a common dialogue for **all** SOPs.

► To create an SOP

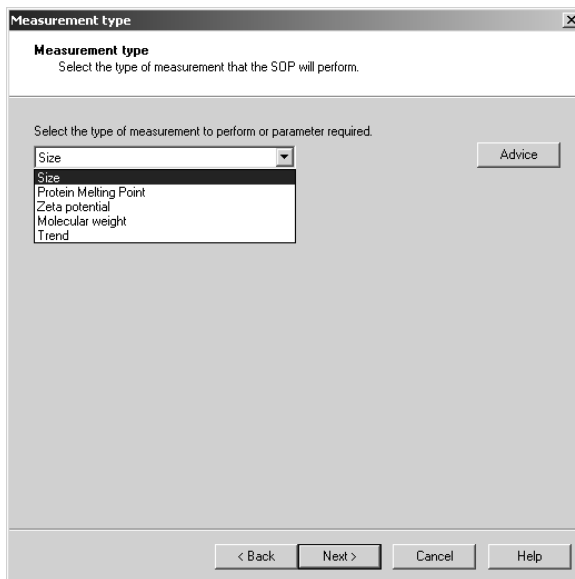
- Select **Configure-New SOP**.
- Change the settings within the SOP dialogues.
- Press **Next** on the final **Export** dialogue to display the SOP Wizard Complete dialogue.
- Press **Finish**, and name and save the new SOP.

All SOPs

The dialogues below are the **same** for **all** SOP types. Note that **Report** and **Export** occur at the end of the SOP creation wizard, after defining the measurement type dialogues.

Measurement type

The **initial** dialogue is always the **Measurement type** selection. From the drop-down list select the type of measurement the SOP will perform: either Size, Protein melting point, Zeta potential, Molecular weight or Trend (and then the type of trend measurement to perform).



Labels

ILL 6608

① Sample name.

The name entered here should be a description of the sample being measured. If no name is entered the sample name entry in the records view will be left blank.

② General notes.

The general notes area allows comments about the measurement to be entered.

Note that any comments written during the SOP creation will appear in all measurement records that use the SOP unless the **Show this page when the SOP is started...** check box is selected and the text modified.

③ Show this page when the SOP is started...

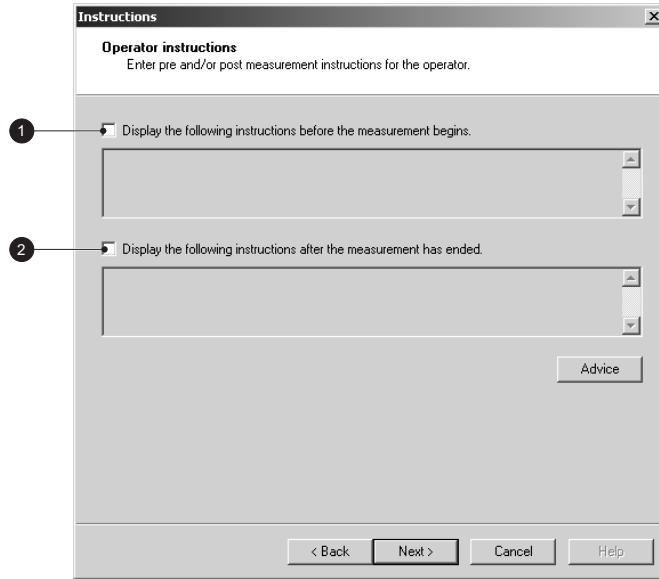
With this check box selected the **Labels** dialogue will appear when the SOP is started. The user can modify the sample name and add or modify any comments about the measurement.

If this check box is not selected the measurement display will appear immediately when the SOP is started. To add comments press the **Settings** button on the display.

Instructions

This dialogue allows instructions to the user to be displayed both before and/or after running an SOP.

This dialogue will not appear when doing a manual measurement.



ILL 0609

① Display the following...before...

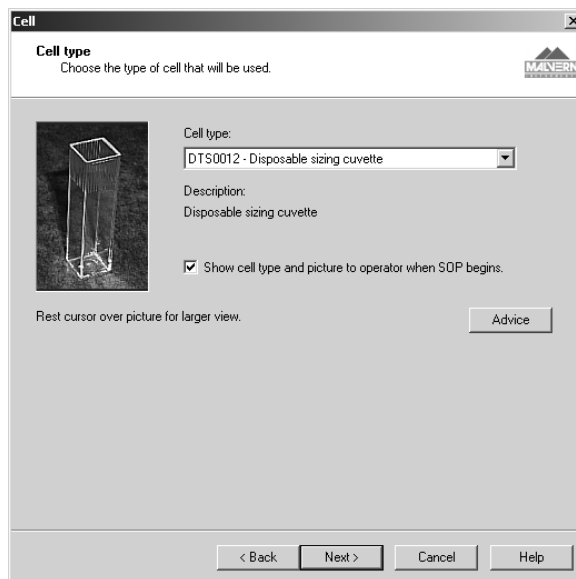
This area is useful to give details that will allow measurements to be made correctly and consistently; e.g:

- The SOP creator may give comments that inform that this SOP should only be used for certain batches of sample.
- Detail can be provided to describe how to prepare the sample.

② Display the following...after...

With this check box selected post-measurement instructions can be displayed on any administration or sample handling details that could not be detailed in the SOP dialogues.

Cell



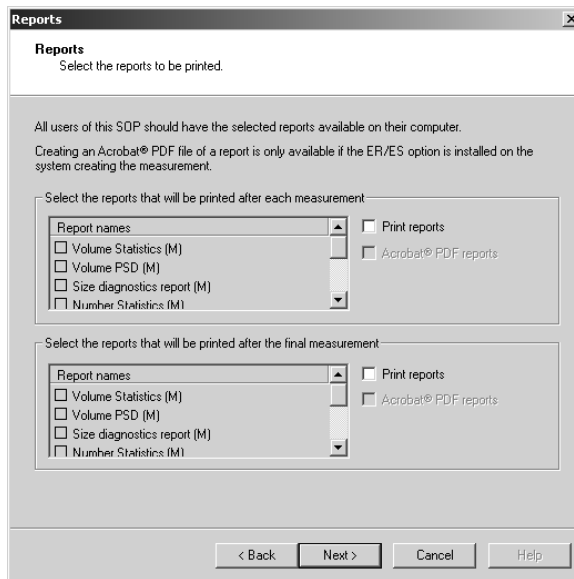
Cell type.

Select the appropriate cell from the list. The choices match the range of cells available from Malvern Instruments. Once selected a description of the cells is given. For measurements using other cell types please contact Malvern for compatibility.

The software will automatically determine the measurement conditions for the cell type selected, i.e. cell position for size cells and applied voltage for the zeta potential cells.

Note that only the cells appropriate for the measurement type selected will be displayed, i.e if a **size** measurement is selected, then only the size cells will be listed.

Reports



ILL 6615

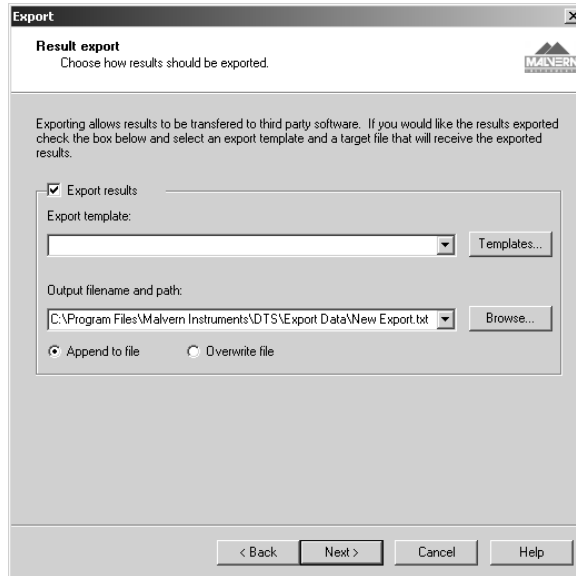
The reports dialogue enables various reports to be selected and then printed automatically once the measurement has finished.

Two selection boxes are available. One to enable a report to be printed after **each** measurement is completed and the other to print out a report after **all** measurements have been completed. For each, select the **Print reports** check box and then the reports required. Any reports created using **Report Designer** will automatically be added to this list.

If the 21 CFR part 11 feature key is installed reports can be converted to Acrobat® PDF format and saved.

Export

Enables the measurement results to be exported to third party software packages such as Excel or Wordpad.



With the **Export results** check box selected measurement parameters can be defined and exported on completion of the measurement. Select the required **Export template** from the list or create or modify a template using the **Templates** button; this will display the export dialogues described in chapter 11. Refer to this chapter for full details on creating the export templates.

The parameters will be exported as a text file to the specified **output filename...** and saved.

Append to file will add the exported results to the existing file while **Overwrite file** will replace any previous measurement results so only the last measurement will be available.

Size SOPs

The measurement dialogues described below are **unique** for **size** measurements.

Sample


① Material and Dispersant properties.

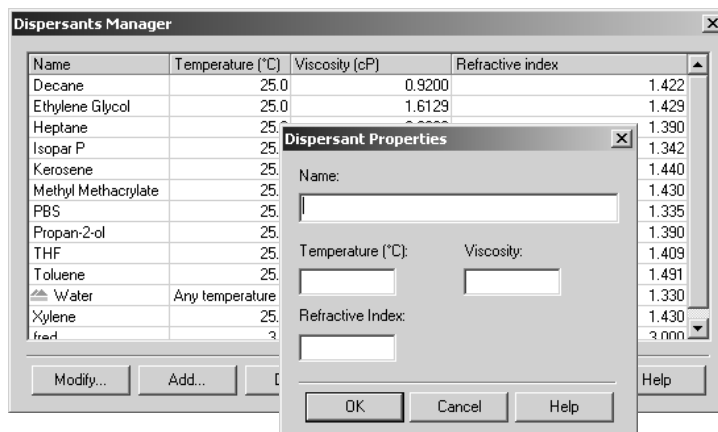
The Zetasizer software requires certain information about the physical properties of the material and dispersant that make up the sample. By selecting the **Material** or **Dispersant** tab and pressing the **Add...** button these properties can be defined.

Dispersant properties

To define a new dispersant, press the **Add...** button.

The **Dispersant properties** dialogue will appear, allowing new dispersants to be defined. The dispersant name, refractive index and viscosity can all be specified.

-
-  **Note** ■ Viscosity is temperature dependent. The Malvern defined dispersants include a built in viscosity calculation that determines the correct viscosity of the dispersant at any sample temperature.
-



The viscosity can only be specified at a certain temperature. If the measurement is to be done at another temperature, then the viscosity value must be changed to match.

It is often more practical to find a value for viscosity at a particular temperature, and then change the temperature of the measurement to match. Alternatively create multiple dispersants with varying viscosity values, the dispersant name containing the temperature for that viscosity, e.g. Propanol 25C.

To **modify** a dispersant, select the dispersant from the list and press the **Modify...** button (or double-click the dispersant in the list). The dispersant properties dialogue will appear, allowing the parameters to be changed.

A dispersant can be **deleted** by selecting the dispersant from the list and pressing the **Delete...** button.

Note that it is not possible to modify or delete the dispersants that were specified by Malvern.

Material properties

The physical properties required for the sample material.

Sample refractive index.

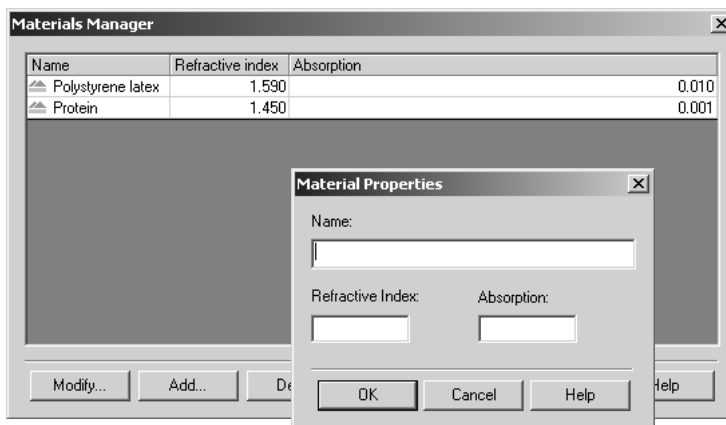
Only required to display or print the result as a volume distribution. It is not required for calculation of the Z-average size.

Absorption.

This is the quantity of light absorbed by the material when suspended in the dispersant. Generally clear samples will have a low or zero absorption while coloured or black samples will have a high value.

Example absorption values:

Emulsions :	0.00
Latex :	0.01
Coloured samples :	0.3
Blue/black samples :	0.9



Material properties can be added, modified and deleted in the same way as the dispersants detailed previously.

② Sample viscosity options

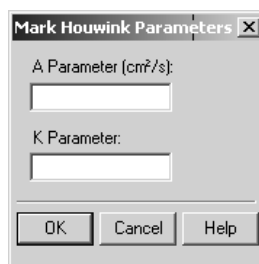
This area is useful for customising the viscosity parameters.

If working at low sample concentrations it may be useful to use the **dispersant viscosity** as **sample viscosity**. At high dilutions, the assumption that the sample viscosity is the same as the dispersant viscosity is usually valid. If this is not the case, or at higher sample concentrations, over 0.1%, then the sample viscosity should be measured and this value used.

If the viscosity of the sample is known only at certain temperatures select the **Prompt operator to enter viscosity** button. This allows the user to enter both the viscosity and the temperature, prior to the measurement being started.

③ Mark-Houwink parameters.

By selecting the **Define** button the **Mark-Houwink parameters** dialogue appears.



This allows the Mark-Houwink parameters to be entered to enable a molecular weight to be calculated based on the dynamic light scattering data.

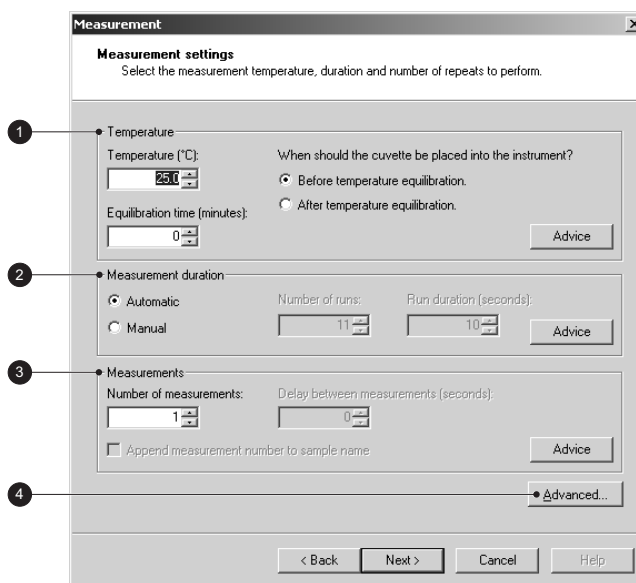
In the Mark-Houwink relationship: $\eta = KM_p^a$

From knowledge the viscosity η and the **a** (A) and **K** parameters (both available in many reference books) the molecular weight can be determined.

Once entered, both a molecular weight calculation and size measurement will be available for display.


Measurement

This dialogue allows the temperature and duration of the measurement to be defined, and to make multiple measurements of the same sample.



ILL 6614

① Temperature

-
-  **Note** ■ The Zetasizer is capable of heating or cooling the sample to allow the measurement to be made at a specific temperature. The measurement will not start until the sample has reached the temperature specified.
-

Input the **Temperature** required, and how long the sample should have to equilibrate before the measurement is started.

Equilibration time adds a delay before the start of each measurement to ensure the sample temperature is equal to the cell area temperature. The delay starts as soon as the instrument has reached the operating temperature requested. Allowing the sample to be fully temperature equilibrated ensures the Brownian motion of the particles is measured and not thermal motion due to temperature gradients. If you find that the first result of a series of repeat measurements is different, try increasing the equilibration time.

Note that it takes time for the sample to reach the required temperature - the further away the required temperature is from current temperature, the longer it will take for the sample to reach a stable temperature.

When should the cuvette be placed into the instrument? A choice is given to either insert the cell **Before...** or **After the start temperature is reached.**

- **Before...** should be chosen if the sample needs to either heat or cool to the requested temperature, before the measurement is started.
- **After...** should be chosen if the sample has been stored at the required temperature (e.g. using a water bath); there is therefore no need to wait for the sample temperature to equilibrate before the measurement is started. This avoids the sample temperature changing during the measurement process.

The status bar in the Measurement display will indicate when to insert the cell.

-
-  **Note** ■ For **Manual** measurements the insertion choice is not available.
-


② Measurement Duration

The measurement duration setting affects the accuracy and repeatability of the results.

With **Automatic** selected, the software automatically determines the most appropriate measurement duration. For most samples this can be left as the

default. Automatic measurements will be divided into a number of runs of at least 10 seconds in length.

Using **Manual** the time may be reduced for the measurement a latex standard, or increased to improve the repeatability of the measurement of particularly polydisperse samples. Manually set run durations can be set between 1 and 600 seconds. The number of runs from 1 to 600. Longer measurement durations will increase the quality of data obtained and will generally give better results, as those runs that contain the poorest data will be rejected, with the remaining good runs used in the final measurement calculation.

 **Note** ■ If repeat measurements with the **Manual** setting still show differences in the distribution with an increased measurement time then the sample preparation method should be reconsidered, as large particles may be disrupting the measurement.

③ Measurements

This option allows a sample to be measured more than once; possibly to investigate the effect on particle size over time, or to prove repeatability. Set the **Number of measurements** to that required. Add a delay between measurements in the **Delay between measurements** text box if required.

The **Append measurement number...** check box will append an incremental number, i.e. 1, 2, 3 etc, to the record name for each measurement made. The incremental number is reset back to 1 each time the SOP is started.

④ Advanced

The **Advanced** dialogue contains functions to alter the cell and attenuator position settings. For normal operations both will be at the **default** setting.

Measurement Position

Typically the measurement position that is automatically determined by the software will be appropriate for most sample measurements. In certain circumstances it may be beneficial to fix the position to reduce the total measurement time, or establish a precise cell position for subsequent measurements of the same sample.

By changing the cuvette position the effect of performing a measurement at different positions within the cell can be investigated.

Attenuator

Fixing the attenuator setting can be used to enable the count rates of two samples

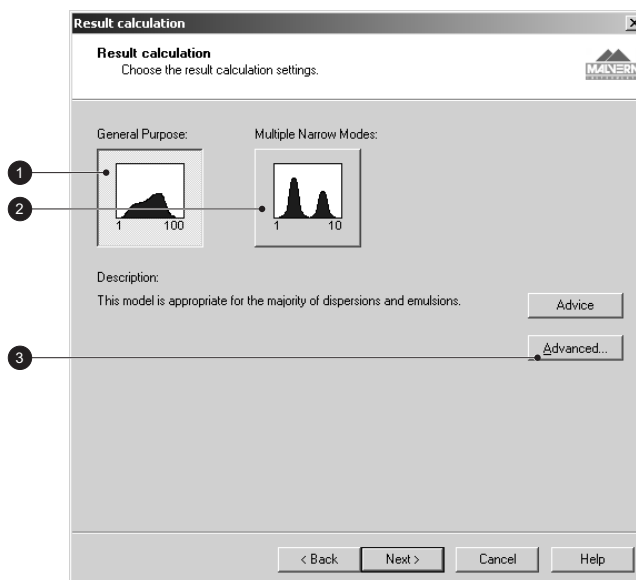
to be compared, or to speed up the optimisation stage prior to a measurement being performed.

Please see the **Advice** button in the **Advanced** dialogue for more information.

Result calculation

If characteristics are already known about the sample being measured, this dialogue will allow an appropriate analysis model to be applied and so optimise the measurement calculation. Size ranges and measurement thresholds can be applied to the analysis to filter spurious peaks out prior to the analysis being performed.

Please see the **Advice** button for more information.



① General Purpose

Select this model if the characteristics of the sample to be measured are unknown.


② Multiple Narrow Modes

If it is known that the sample to be measured will give a distribution of one or more narrow peaks (i.e. a multi-modal distribution of latices) then select this model.

③ **Advanced**

The **Advanced** button dialogue enables both the measured **size range** and the measurement **thresholds** to be set.

If it is known that all particles within the sample will fall within a certain size range, then the **size range** can be set to ignore data or artifacts at each end of the distribution. The transformations to volume and number will then ignore the data outside the set limits. Similarly lower and upper **thresholds** can be applied to eliminate undesired populations that are below a defined percentage of the particle population.

-
-  **Note** ■ If the **Size** or **Thresholds** values are different from the default settings the **Advanced** button will be shown with a **red exclamation mark**. Moving the cursor over the button will display the message “**Advance settings not at default**”.
-

Molecular weight SOPs

Though the majority of the **Sample** and **Measurement** dialogues described below are **unique** to **Molecular weight** measurements; when indicated please refer to the description in the size SOP section.

Sample

The screenshot shows the 'Sample' dialog box with the following elements:


- Sample Settings**: Select solvent, standard, material and other sample properties.
- Molecular weight by static light scattering**: A section containing a text input field for 'Refractive Index Increment - dn/dc (mL/g)' and an 'Advice' button.
- Material**: A section with tabs for 'Material', 'Solvents', and 'Standard'. The 'Material' tab is selected, showing a 'Material Name' field with 'Protein' and an 'Advice' button.
- Navigation**: '< Back', 'Next >', 'Cancel', and 'Help' buttons at the bottom.

① Molecular weight by static light scattering

Input here the differential refractive index increment (dn/dc) of the solvent. This is the change in refractive index as a function of the change in concentration. For many sample/solvent combinations this may be available in literature; while for novel combinations the dn/dc can be measured by use of a sensitive differential refractometer.

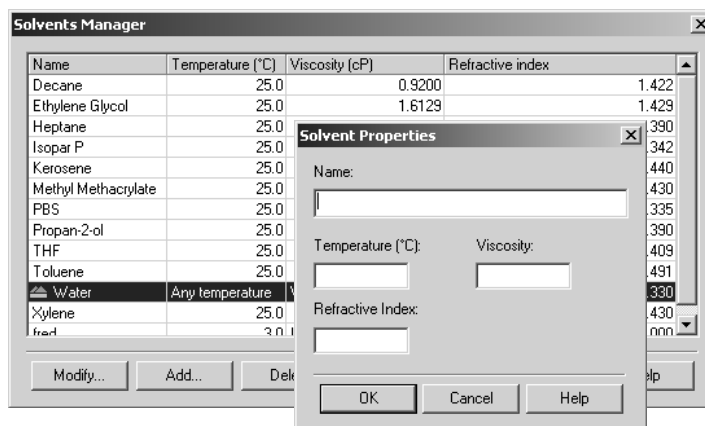
 **Note** ■ This will require a refractometer capable of measuring to an accuracy of 6 decimal places.

② Material, solvent and standard properties

The Zetasizer software requires certain information about the physical properties of the material and solvent that make up the sample that is to be measured, plus information about the “Standard” used for laser intensity calibration. By selecting either the Material, Solvent or Standard tab and selecting the  button these properties can be defined.

Solvent properties tab

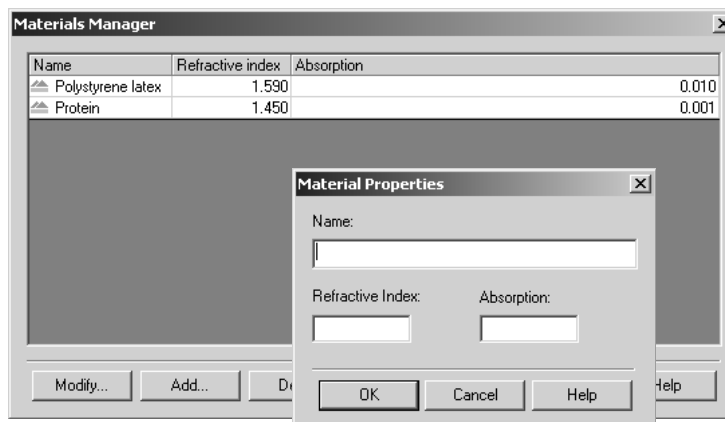
Selecting this tab enables new solvents to be added and defined.



Please refer to the **Material and dispersant properties** description in the **size SOP** section for details.

Material properties tab

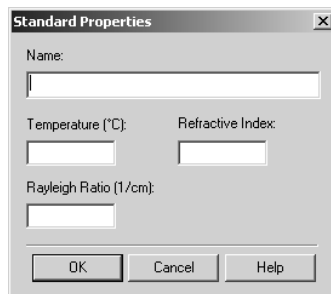
Selecting this tab allows new material to be added and selected.



Define a new material by pressing the **Add** button and entering the material name.

Standard properties tab

Input and define the scattering “standard” properties of the liquid to be used in the measurement: **Temperature**, **Refractive index** and **Rayleigh ratio**. This will nearly always be Toluene.



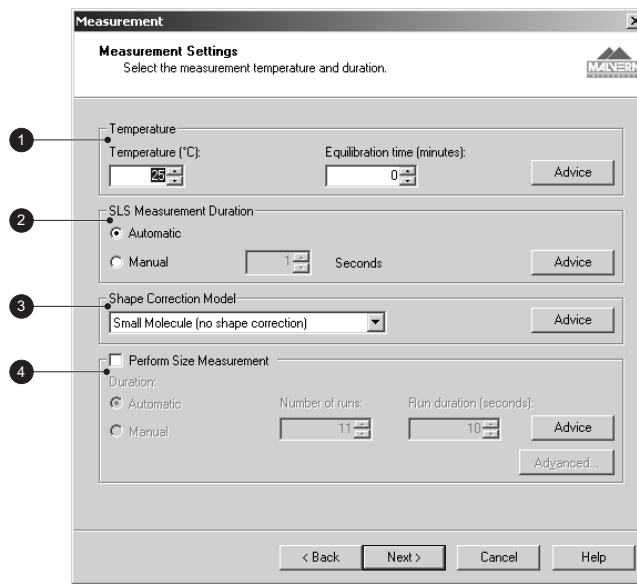
The 'Standard Properties' dialog box contains the following fields and buttons:

- Name: [Text input field]
- Temperature (°C): [Text input field]
- Refractive Index: [Text input field]
- Rayleigh Ratio (1/cm): [Text input field]
- Buttons: OK, Cancel, Help

The standard properties can be added, modified and deleted in the same way as the solvents detailed previously.

Measurement

This dialogue allows the duration of the measurement to be set, and to make multiple measurements of the same sample.



The 'Measurement Settings' dialog box includes the following sections and controls:

- Temperature:** Temperature (°C) [25] and Equilibration time (minutes) [0]. Includes an Advice button.
- SLS Measurement Duration:** Radio buttons for Automatic (selected) and Manual [1] Seconds. Includes an Advice button.
- Shape Correction Model:** Dropdown menu set to 'Small Molecule (no shape correction)'. Includes an Advice button.
- Perform Size Measurement:** Unchecked checkbox. Includes sub-sections for Duration (Automatic/Manual), Number of runs [11], and Run duration (seconds) [10]. Includes Advice and Advanced... buttons.

Navigation buttons at the bottom: < Back, Next >, Cancel, Help.

① **Temperature**

Please refer to the **Temperature** description in the **Size SOP** section.

② **SLS measurement duration**

The measurement duration setting affects the accuracy and repeatability of the results.

With **Automatic** selected, the software will automatically determine the measurement duration.

With **Manual** selected the measurement will use the user defined settings.

③ **Shape correction model**

This option allows selection of the shape correction model used for the measurement. With knowledge of the sample structure it is possible to improve the result of the measurement by adding the value that most closely corresponds to the probable sample shape; i.e. sphere, coil, cylinder or no shape correction.

④ **Perform Size measurement**

With the check box selected a size measurement will be performed simultaneously with molecular weight.

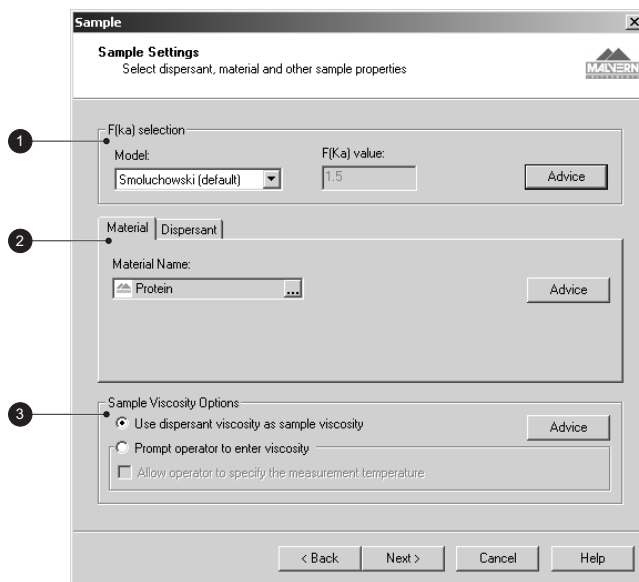
The **Duration** setting affects the accuracy and repeatability of the size results.

Please refer to the **Measurement duration** description in the size SOP section.

Zeta potential SOPs

Though the majority of the **Sample** and **Measurement** dialogues described below are **unique** for **zeta** measurements; when indicated please refer to the description in the size SOP section.

Sample




① F(ka) selection

Input the F(ka) model that will be used in the Henry's function.

- For small particles in **aqueous** media select **Smoluchowski : 1.5**.
- For **non-aqueous** measurements the **Huckel** approximation should be used : **1.0**.

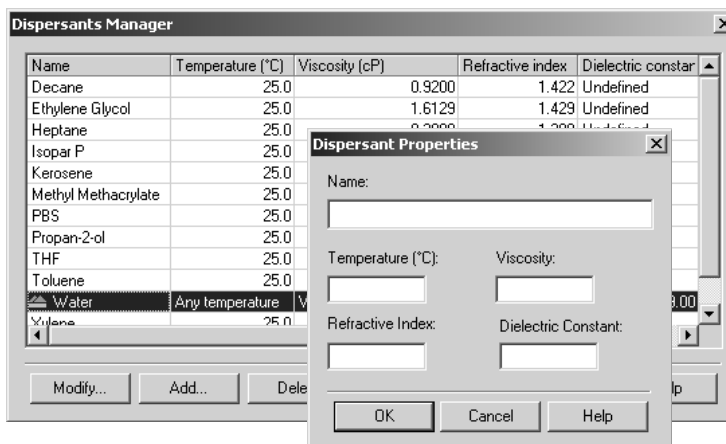
Please refer to chapter 15 for more information on the F(ka) model.

② Material and Dispersant properties

The Zetasizer software requires certain information about the physical properties of the material and dispersant that make up the sample that is to be measured. By selecting either the material or dispersant tab and pressing the  button these properties can be defined.

Dispersant properties tab

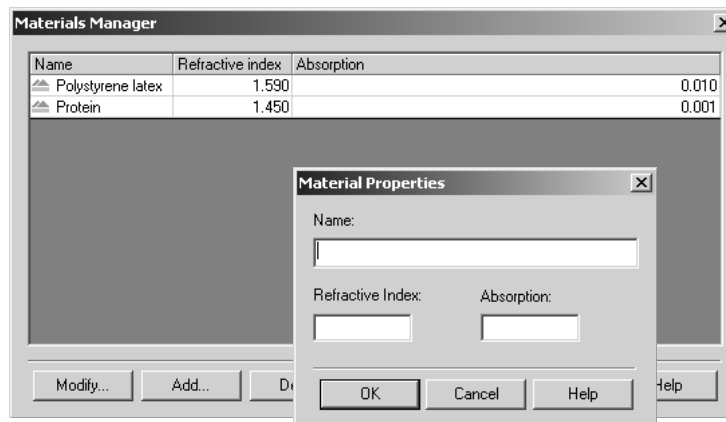
Selecting this tab enables new dispersants to be added and defined.



Please refer to the **Material and Dispersant properties** description in the size SOP section for details.

Material properties tab

Selecting this tab allows new material to be added and selected.



Define a new material by selecting the **Add** button and entering the material name. This is only required to enable the material to be displayed on the report.

③ Sample viscosity options

This area is useful for customising the viscosity parameters.

Please refer to the **Sample viscosity options** description in the **Size SOP** section.

Measurement

This dialogue allows the duration of the measurement to be set, and to make multiple measurements of the same sample.

Measurement

Measurement settings
Select the measurement temperature, duration and number of repeats to perform.

1 Temperature

Temperature (°C): 25.00

Equilibration time (minutes): 0

When should the cuvette be placed into the instrument?

Before temperature equilibration.

Alter temperature equilibration.

Advice

2 Measurement duration

Automatic

Manual

Number of runs: 20

Advice

3 Measurements

Number of measurements: 1

Delay between measurements (seconds): 0

Append measurement number to sample name.

Advice

< Back Next > Cancel Help

① Temperature

Please refer to the **Temperature** description in the size SOP section.

② Measurement Duration

The measurement duration setting affects the accuracy and repeatability of the results.

With **Automatic** selected, the software will automatically determine the measurement duration. This will be suitable for the majority of samples and can simply be left as the **default**.

The default number of runs in 'auto' measurement duration is 30, but for a stable sample as few as 10 runs may be required. The measurement will then complete even though the displayed run total has not been achieved.

With **Manual** selected the measurement will use the user defined settings. The time may be reduced for the measurement of a latex standard, or increased to improve the repeatability of the measurement of particularly polydisperse samples. Manual measurements can be up to 10,800 runs. For a zeta potential measurement all the individual runs are accumulated and then averaged to give a final zeta potential result. Therefore the more runs performed the better the repeatability. Naturally the more runs selected the longer the duration of the complete measurement. However if the sample has a high conductivity, measuring for too long can affect the sample and the electrodes.

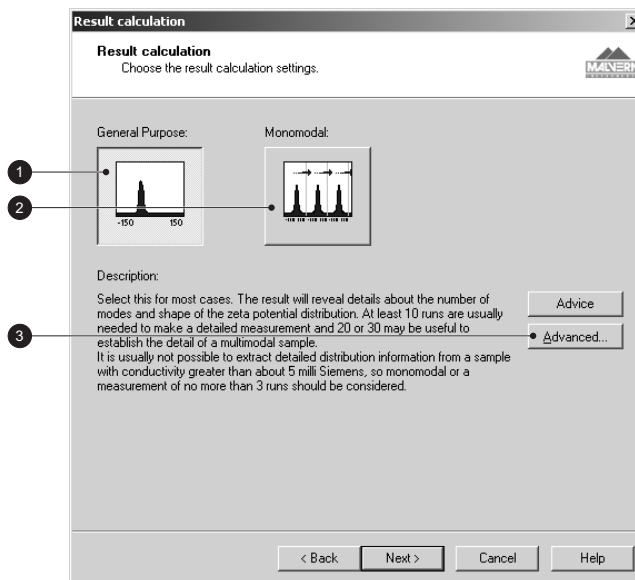
③ Measurements

This option allows a sample to be measured more than once.

Please refer to the **Measurement duration** description in the size SOP section.

Result calculation

If characteristics are already known about the sample being measured, this dialogue will allow an appropriate analysis model to be applied and so optimise the measurement calculation.



Size ranges and measurement thresholds can be applied to the analysis to filter spurious peaks prior to the analysis being performed.

Please see the **Advice** button for more information.

① General Purpose

Select this model for most cases, especially if the sample to be measured is unknown. The result will reveal details about the number of modes and the shape of the zeta potential distribution.

To obtain a detailed measurement a minimum of 10 runs should be performed. If the sample is thought to generate a multi-modal distribution then 20 to 30 measurement runs should be performed.

② Monomodal


The monomodal model should be used if only the zeta potential mean value is required. In this case only 5 to 10 runs are required to obtain a detailed measurement.

This mode is recommended for time, pH and temperature trend measurements as well for samples known to have a conductivity greater than 5milliSiemens.

③ Advanced

The **Advanced** button dialogue enables both the measured **Zeta potential range** and the measurement **thresholds** to be set.

If it is known that all particles within the sample will fall within a certain Zeta potential range, then the **Zeta range** can be set to improve repeatability of the measurement result; similarly a lower **threshold** can be applied to eliminate undesired particles that are below a defined percentage of the particle population.

 **Note** ■ If the **Zeta range** or **Threshold** values are different from the default settings the **Advanced** button will be shown with a **red exclamation mark**. Moving the cursor over the button will display the message “**Advance settings not at default**”.

Using the **Smoothing** function, it is possible to control the sharpness and shape of the plotted zeta potential distribution, i.e. with an increased smoothing figure, two distinct peaks will begin to blend together to show a gentler distribution.

Please see the **Advice** button for more information.

Trend & Protein melting point SOPs

The **Trend** SOP allows size and zeta measurements to be performed and plotted as a function of temperature, while the **Protein melting point** SOP enables the protein **denaturation** point to be determined; again this is done against temperature.

Trend and melting point SOPs are the same as the standard size and zeta SOPs, the only differences being the inclusion of a trend and altered measurement dialogues.

Trend

Enables the selection of the temperature trend parameters, i.e. start and end temperatures, and intervals between measurement steps.

Trend

Trend - Temperature range
Choose the temperature range for the trend measurement.

Temperature Trend

Start Temperature (°C): End Temperature (°C):

Temperature Interval (°C): Number of Steps:

Stop when melting point found
 Return to Start Temperature

When should the cuvette be placed into the instrument?
 Before the start temperature is reached.
 After the start temperature is reached.

Estimated Experiment Length: 3 hour(s), 8 minute(s), 5 seconds

< Back Next > Cancel Help

Start Temperature

Input the temperature that the **first** measurement is to be made at. The instrument will initially cool, or heat, to this temperature before starting the measurement.

End Temperature

Input the final required temperature. Once this temperature is reached the measurement will stop.

Temperature Interval / Number of steps

The measurement can be configured to change either in specific measurement intervals (e.g. every 5°C), or in a specific number of steps (e.g. 10 steps). Select the button to identify which is required.

When should the cuvette be placed into the instrument? A choice is given to either insert the cuvette **Before...** or **After the start temperature is reached.**

- **Before...** should be chosen if the sample can be allowed to either heat or cool to the requested temperature, before the measurement is started.
- **After...** should be chosen if the sample has been stored at the required temperature (e.g. using a water bath); there is therefore no need to wait for the sample temperature to equilibrate before the measurement is started.

The status bar in the measurement display will indicate when to insert the cuvette.

Melting point measurements only

Stop when melting point found

If this check box is selected the measurement will stop once the melting point has been determined.

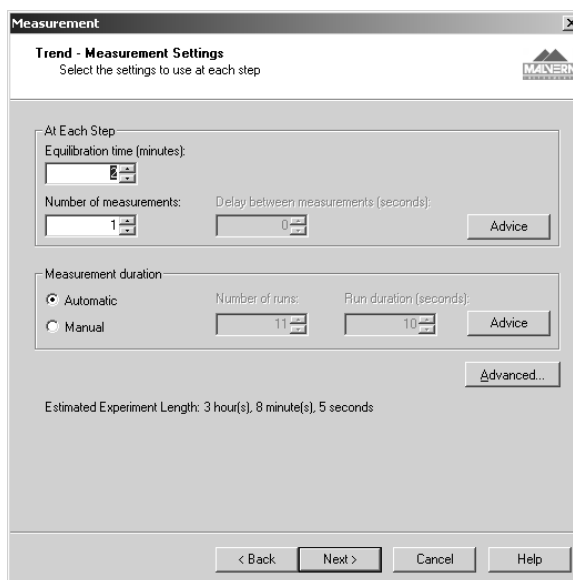
Return to Start temperature

If this check box is selected, once the melting point has been determined the measurement will return to the original starting temperature defined above.

Measurement

The trend and melting point measurement dialogues are generally the same as those used with either a size or zeta measurement SOP. The differences are:

- Removal of the “When should the cuvette be placed into the instrument?” question - now part of the trend dialogue above.
- **Each step** of the trend measurement gives an option to control the equilibration time and measurements performed.
- **Optimise Measurement position** enables the cell position to be checked and verified before each individual measurement is performed. This option is **not** available with the melting point dialogue.



ILL 4022

Please refer to the size and zeta SOPs section for details of the measurement dialogue functions.

Extracting an SOP

The SOP (or manual settings) used when performing a measurement can be viewed by right-clicking on the measurement record and selecting **Extract SOP**. This will display the SOP dialogues.

If required the SOP can be modified and saved under a new name.

Modifying an SOP

To modify an SOP, select **Configure-Existing SOP**. The same dialogues as explained above will be displayed, allowing any settings to be changed. In this case they will be displayed in tabbed dialogue format instead of the SOP wizard views.

Alter the settings within these tabs and select OK. A dialogue will appear allowing the SOP to be named and saved.

If measuring similar samples, it is often easier to modify an existing SOP and then save it under a different name.

Distributing an SOP

SOPs are designed to allow the measurement of the same type of sample in a consistent way - if monitoring batches of sample in a quality control environment, the results will be meaningless if different measurement parameters have been chosen for each batch.

Consider an application where a manufacturer is producing the same type of sample in different factories. It will be important that the measurement protocol is consistent between factories.

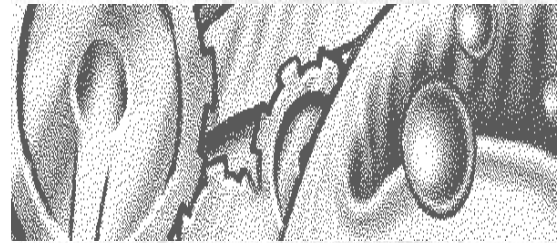
An SOP can be created and copied into the SOP directory on another Zetasizer Nano instrument so that measurements are done consistently. Simply copy the **.SOP** file to all computers running a Zetasizer Nano system.

If the SOP has a non-standard dispersant defined then this will not exist on the target computer. When the SOP is run on the target computer, the software will detect this and give the following choices:

- Add the dispersant to the dispersant database.
- If the dispersant already exists, the software will ask if the existing dispersant parameters should be over-written or saved under a new name. It should be noted that if the dispersant parameters are over-written, the new parameters will then be used for all SOPs that use that dispersant.

Measurement file window - Workspace management

C H A P T E R 1 0



Introduction

The **Measurement File window** is where all results (or records), and reports for each measurement file can be viewed, moved and analysed. Depending upon the **Window** options set, multiple measurement files can be viewed at the same time.

The screenshot displays the DTS software interface with multiple windows open. The main window shows a list of records with columns for Record, Sample Name, Date, Type, PDI, Z-Average, and Result Origin. A detailed report window is open for record 27, showing parameters such as Dispersant (Acetone), Dispersant RI (1.356), Sample wsc. (cP) (0.1000), Date and Time (29 May 2003 17:26:18), Cell type (Disposable sizing cuvette), Meas. position (mm) (4.65), and Attenuation index (5). A size distribution graph is also visible, showing a peak at approximately 1000 nm diameter.

Record	Sample Name	Date	Type	PDI	Z-Average	Result Origin
MAL060016	Size	2.00.008 alpha 3	0		12 February 2003 10:27:27	
MAL060016	Size	2.00.008 alpha 3	0		12 February 2003 10:30:33	
MAL060016	Size	2.00.008 alpha 3	0		12 February 2003 10:41:38	
SIM-NANO	Size	1.00.007 alpha 1	0		07 April 2003 12:46:20	
SIM-NANO	Size	1.00.007 alpha 1	0		07 April 2003 12:49:39	
SIM-NANO	Zeta	1.00.002 alpha 2	0		29 April 2003 16:21:59	
SIM-NANO	Melting Point	1.00.002 alpha 2	46		29 April 2003 16:25:29	
SIM-NANO	Zeta	1.00.004 alpha 3	0		01 May 2003 17:21:07	
SIM-NANO	Melting Point	1.00.004 alpha 3	46		13 May 2003 16:57:39	
SIM-NANO	Size	1.00.004 alpha 3	46		13 May 2003 17:00:11	
SIM-NANO	Size	1.00.004 alpha 3	46		13 May 2003 17:01:40	
SIM-NANO	Size	1.00.004 alpha 3	46		13 May 2003 17:02:13	
SIM-NANO	Size	1.00.004 alpha 3	46		13 May 2003 17:02:49	
SIM-NANO	Size	1.00.004 alpha 3	46		13 May 2003 17:03:26	
SIM-NANO	Size	1.00.004 alpha 3	46		13 May 2003 17:04:02	
SIM-NANO	Size	1.00.004 alpha 3	46		13 May 2003 17:04:37	
SIM-NANO	Size	1.00.004 alpha 3	46		13 May 2003 17:05:14	
SIM-NANO	Size	1.00.004 alpha 3	46		13 May 2003 17:05:50	
SIM-NANO	Size	1.00.004 alpha 3	46		13 May 2003 17:06:27	

Once a result has been calculated, the data can be displayed as either a record or report - by selecting the appropriate tab.

Malvern provides several default reports (identified with (M) in the name) that will be sufficient for most users. The user also has the ability to create their own custom reports to satisfy their individual needs. Details on creating custom reports (using **Report Designer**) are given in chapter 12.

Generally a computer screen displays information in a landscape format while a printer uses a portrait format. To accommodate this, each report is generated in two formats - one for displaying the report on the screen, and another configured for a printer. It is possible for the screen and print formats to be configured to report different information. A report file has the extension **.PAG.** and will hold both the screen and print variations. The report page files can be found in the **\Pages** folder.

Measurement file window

Initially a measurement file must be opened by selecting **File-Open**. Once a file has been selected, a measurement window will appear.

Record Number	Type	Sample Name	Measurement Date	Temperature (°C)	Z-Ave (nm)	PDI	Zeta Potential (mV)
1	Zeta	DTS 50 1	06 May 2003 16:41:40	25.0			-47
2	Zeta	DTS 50 2	06 May 2003 16:42:03	25.0			-51
3	Zeta	DTS 50 3	06 May 2003 16:42:29	25.0			-48
4	Zeta	DTS 50 4	06 May 2003 16:42:55	25.0			-5
5	Zeta	DTS 50 5	06 May 2003 16:43:21	25.0			-46
6	Size	200nm 0.1% Ltx	03 June 2003 11:24:40	25.0	199.5	0.001	
7	Size	200nm 0.1% Ltx	03 June 2003 11:28:36	25.0	200.1	0.006	
8	Size	200nm 0.1% Ltx	03 June 2003 11:32:32	25.0	200.7	0.022	
9	Size	60nm Ltx standard	28 May 2003 15:03:26	25.0	62.33	0.024	
10	Size	60nm Ltx standard	28 May 2003 15:06:56	25.0	61.72	0.038	
11	Size	60nm Ltx standard	28 May 2003 15:10:16	25.0	61.79	0.038	
12	Weight	1000 Molecular Weight	30 April 2003 17:32:23	0.0			
13	Weight	Polystyrene in toluene (96kDa) Manual 30seconds	29 April 2003 15:57:12	0.0			

The way the measurement information is displayed is dependent upon which tab along the top of the measurement file window has been selected.

- The first tab is always **Records view**; this tab lists all individual measurements contained in the opened measurement file.
- Subsequent tabs are the **Report tabs** - the tab giving the name of the report viewed. To view a measurement as a report, first select the measurement, or group of measurements, in the records view tab and then select the appropriate report tab. The report tabs will be greyed out if no measurement is selected.

What measurement information is presented in the **Report** and **Record** tabs is controlled by the **Workspace** selection dialogues (below). The tabs can be configured to display either all the measurement file records and associated reports (i.e. Summary), or only those that are relevant to the measurement type performed (i.e. Zeta).

The following sections deal with the various aspects of the measurement file window. The sections will deal primarily with the **Workspace** selection chosen as **Summary**, as this will show a 'complete' measurement file.

 **Note** ■ The Workspace view will apply to all open measurement files.

Workspaces

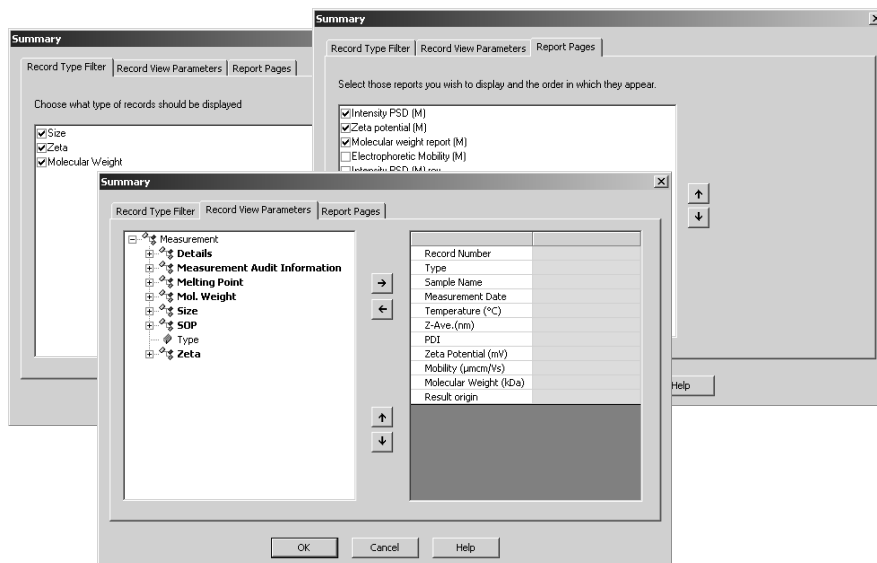
Instead of the measurement file windows showing every single measurement, it is possible to customise the window views so that, for example, only zeta potential measurements from that file are shown. Similarly a user can create a personalised workspace so that only parameters and reports relevant to themselves will be shown.

Initially only **Size**, **Molecular weight**, **Zeta potential** and **Summary** workspaces are available, with **Record view** parameters and **Report pages** already configured.



- To **display** an **existing** workspace window, select **View-Workspaces-“..workspace choice..”** or use the **Workspace** toolbar.



- To **create** a **new** workspace select **Configure-Workspaces-Add Workspace...** Type in a **Name** for the new workspace and press **OK**. This will display the **Workspace settings** dialogue.



- To **modify** an existing workspace select the workspace name from the list.

-  **Note** ■ Pressing the workspace configure icon  will display the **Workspace Settings** dialogue **only** for the workspace currently viewed.

Use the **Workspace settings** dialogue to display what is required.

- Use the **Record type filter** to display only specific measurement types in the **Record View**.

If only the size records are required, select **only** the **size** check box, and leave the zeta and molecular weight ones blank.

If all three measurement types are selected, then all size, zeta and molecular weight measurement records will be displayed.
- Use the **Record view parameters** to specify what parameters will be viewed as the column headings.
- **Report pages** allows selection of both Malvern created and customised reports.

Once completed the new workspace can be selected using the **View** menu or **Workspace** toolbar.

The workspace toolbar can be turned on and off by selecting **View-Workspace-Workspace Toolbar**.

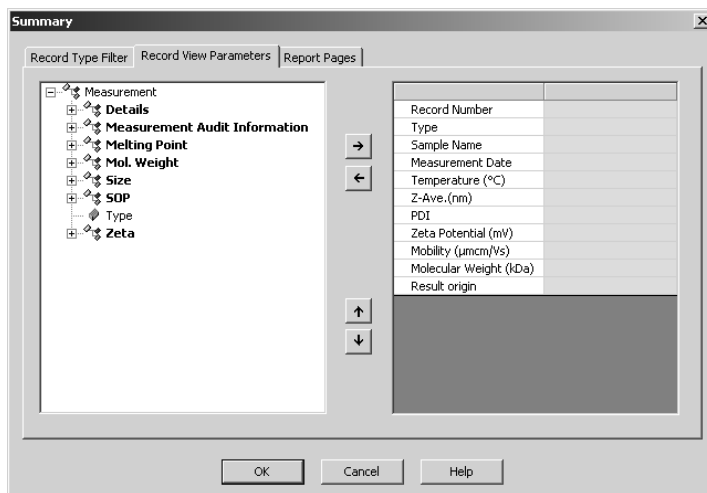
Record view

With the **Summary** workspace selected, the default parameters displayed when the software is first installed are : **Record, Type, Sample Name, Measurement Date, Temperature (°C), Z-Ave(nm), PDI, Zeta potential (mV), Mobility (μmcm/Vs) and Molecular weight (Daltons)**.



For each workspace the record view can be customised by adding or removing any of the parameters that are stored with the measurement record. The order in which the parameters are displayed can also be rearranged.

Adding and deleting a parameter in the record view

The **Record view** parameters dialogue is accessed by selecting **Configure-Workspaces-“.workspace choice..”** and then the **Record view** parameters tab.



The list on the left displays all parameters available for a measurement record. The list on the right displays all parameters that are currently displayed in the **Records view** and the order in which they appear.

Parameters can be added or removed from this list by using the **Add** and **Remove** keys  .

Change the order in which the parameters are displayed by selecting a parameter, and pressing the **Move up** or **Move Down**   buttons.

Press **OK** to exit and return to the **Records view**.

Parameters with arguments

Certain parameters allow specific values (or arguments) to be added.

A d(99) percentile can be viewed by adding **Measurement -Size-Percentile** and then typing 0.99 as the argument value.

Column titles can be altered by double-clicking on them e.g. Double-click on Percentile and alter to Percentile d(99) to reflect the particular percentile size viewed.

Column Title	Argument Value
Record	
Sample Name	
Author Userid	
Edit Date/Time	
File Path	
Percentile d(99)	
Percentile	0.99

ILL 6568

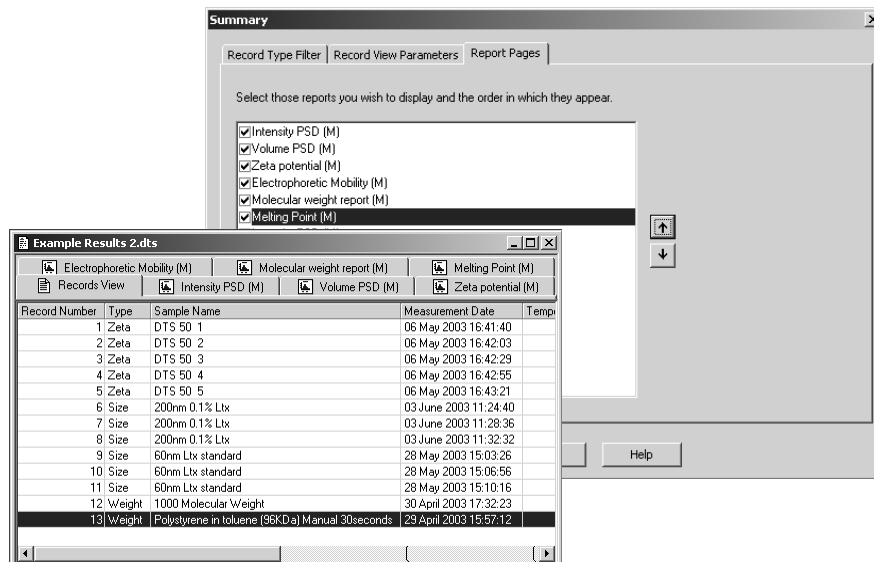
Note ■ If the software is subsequently updated with a new version, the changed parameter names will be preserved.

Report tabs


The report tabs show every report that has been selected for a workspace. With the **Summary** workspace selected the default reports are **Intensity PSD (M)**, **Zeta potential (M)** and **Molecular weight (M)**. These and other reports can be added or removed using the workspace dialogues.

Selecting a report to view

Select **Configure-Workspaces- “..workspace choice..”** and then the **Report Pages** tab.



Select the check box of the required reports and press **OK**.

Use the **Move up / Move down**  buttons to alter the screen order of the viewed reports.

Any reports created with **Report Designer** will automatically be added to the list.

Handling the measurement files

In the **Records view**, depending upon the workspace settings chosen, all measurement records for that file will be shown. It is possible within the **Records view** to move, delete and copy individual records in the measurement file window or between different files, plus the ability to edit the result.

Moving, Copying and deleting records

There are several ways to handle the information.

- One or more records can be copied from a **Records view** tab and “dragged and dropped” directly into another open measurement file.

Select a record then move the cursor slightly towards the edge of the selection until a small rectangle and plus sign attaches. Press the mouse button down and ‘drag’ the selected record(s) to the other file, then ‘drop’ - release the mouse button.

- A record can also be copied by selecting the item then selecting **Edit-Copy**. The item can then be pasted into another file (**Edit-Paste**).
- Selecting **Edit-Delete** will instantly remove any selected records from the measurement file. They will not be deleted from the software, but be stored in a **.DEL** file in the **DTS\Measurement data** directory. This file has the same name as the original measurement file but with a **.DEL** extension. If required the deleted records can be copied back to the original file. Use the Windows file manager to delete the complete **.del** file.

Records can be selected by either clicking the cursor on the required record, or by using the **Navigation** toolbar - press the arrows to move up and down the record list.



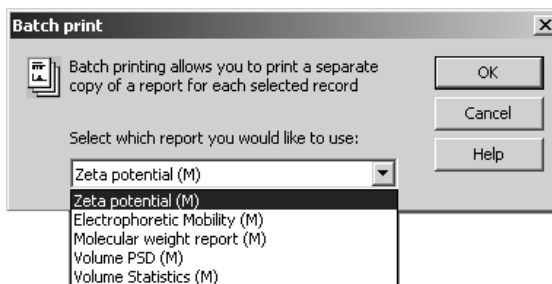
Sorting


The records within a measurement file window can be sorted into alpha-numeric order.

For example, In record view click on the **Measurement Date** header to list all records into numeric “measured date” order. Click on the column header again to toggle between ascending (Δ) and descending order (∇).

Batch printing

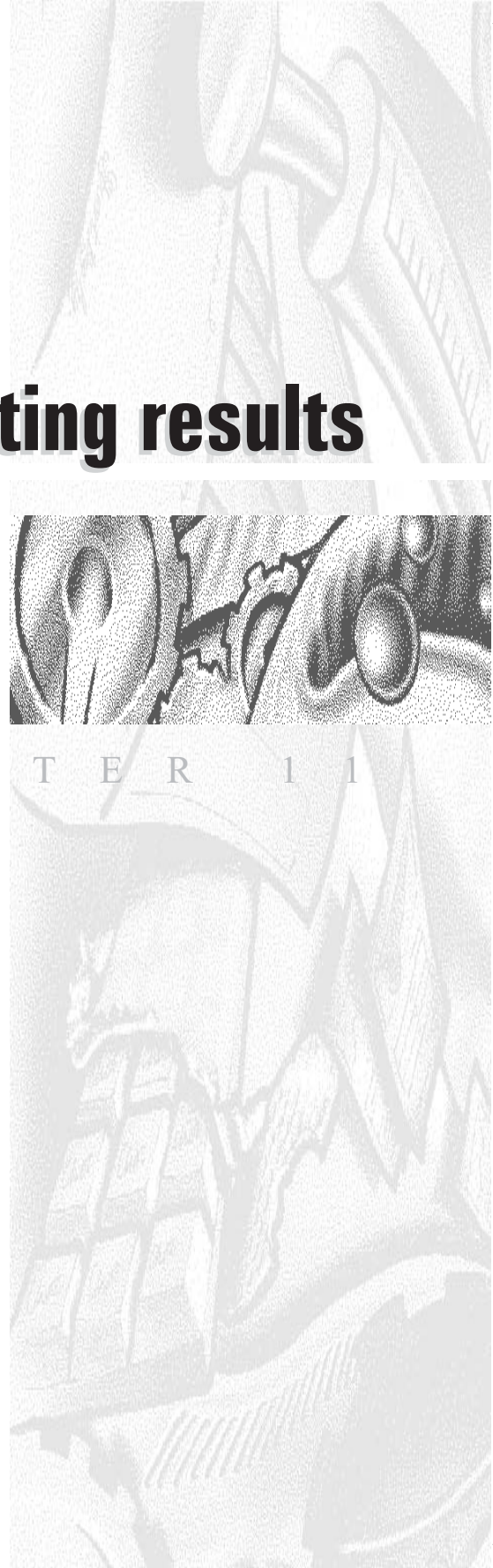
To immediately print reports from more than one record choose the required measurement records, and select **File-batch print...** The **Batch print** dialogue will appear requesting which report template to print out.



-  **Note** ■ The measurement file records can be chosen using standard Windows™ procedure. Either click a single record, or hold down **Shift** or **Ctrl** and choose either sequential or non-sequential records.

Exporting results

C H A P T E R 1 1



Introduction

The Zetasizer software allows any of the parameters from one or more records, as well as graph and table data to be exported to another application such as Excel, Word or Wordpad.

There are several ways to export the required information:

- One or more records can be selected in the **Records view** tab and moved via “drag and drop” directly into another ‘visible’ application (provided that the application supports tab or comma separated formats e.g. Excel).
- A table or graph can be dragged and dropped into another application by holding the **Ctrl** key, selecting the item and dragging into the application.
- A table or graph can be copied by selecting the item then selecting **Edit-Copy**. The item can then be pasted into another application.
- The Zetasizer software allows any of the parameters from one or more records to be exported as a text file. This file can then be loaded into a word document or a spreadsheet. A template is used to specify which record parameters are exported. The **Configure-Data export templates** dialogue allows you to create and modify your own templates.
- A complete measurement file, or selection of records, can be exported using **File-Export....** The following section will guide you through this.

Exporting results

To export a complete measurement file, or selection of records, first open the required file and select **File-Export...**

Step through the tabs - **File**, **Parameter** and **Settings** - and select the exporting options.

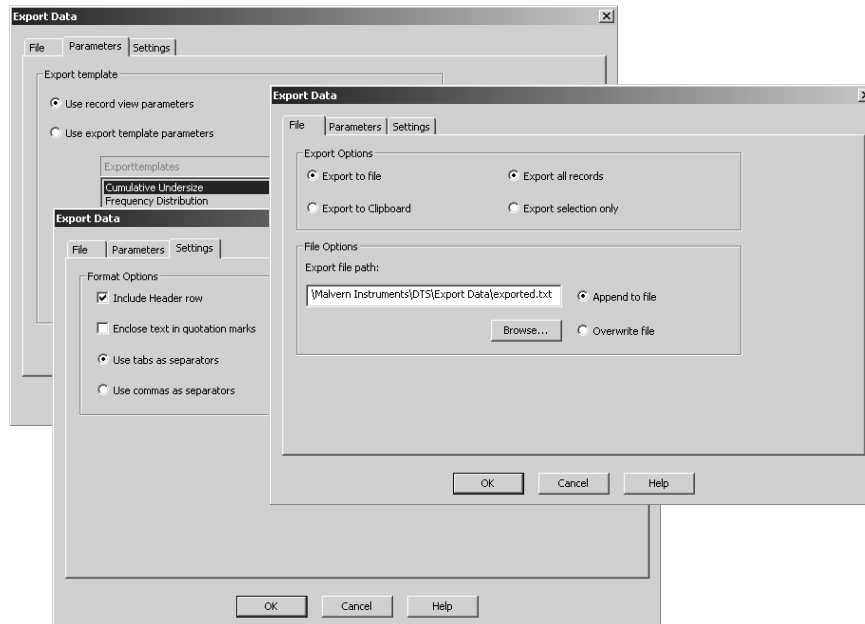
- **File tab**

The data can be either **Exported to file** or **Exported to Clipboard** by selecting the appropriate radio button.



To **Export to file**, type the destination filename into the **File options** selection box and decide whether to **Append** (add) to the existing file or **Overwrite** it. Overwrite will delete any previous measurement results so only the last measurement will be available.

Export to Clipboard will **copy** the data into the ‘clipboard’, where it can then be **pasted** into another application. The **File options** selection box will be greyed out when this option is chosen.

Export all records will export the complete measurement file, whereas **Export selection only** will export only the records that have been highlighted.



■ Parameters tab

Enables exporting of selected parameters. Selecting **Use records view parameters** export parameters currently displayed in the Record view. User defined parameters can be exporting by selecting **Use export configuration parameters** and choosing the required template. If necessary, a new template can be created  or an existing one edited . This will be discussed later in this chapter - **Creating an export template**.

■ Settings tab

This tab determines how the data is exported. The **Format options** specifies the separator character that is used between each parameter.

Different spreadsheet packages require different field separation characters to correctly space the data output. Commas are the most usual but these should not be used if they are numerical separators in the localised Windows version (e.g. French Windows).

Drag and Drop

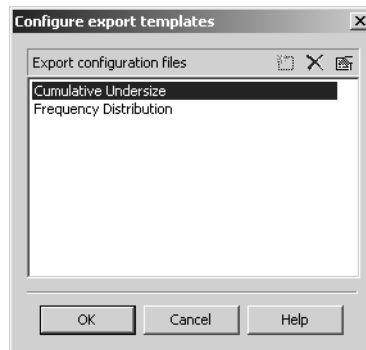
An alternative method to export data is to “drag and drop”. As the name suggests this is simply a matter of dragging the records to another application and then dropping them.

Select a record then move the cursor slightly until a small rectangle and cross attaches. Press the mouse button down and drag the selected record(s) to the other application and release - i.e. drop.

The parameters exported will be those shown in the **Records view**.


Creating an export template.



New export templates can be created (or existing ones modified) by selecting **Configure-Data exports templates**.



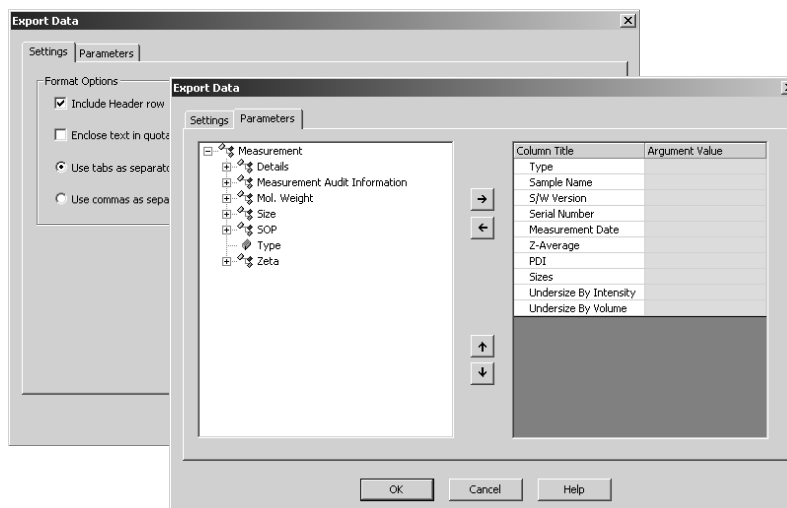
ILL 6672

Two export templates are supplied as standard: **Cumulative Undersize** and **Frequency Distribution**.

To create a new export template select **New (insert)**  and type in a name. The name of a template can be changed by double-clicking the cursor over the name and typing a new one.

Once created, a template can be highlighted and either altered by pressing the **Edit**  button, or deleted by pressing the **Delete**  button.

When the Edit  button is pressed the dialogue below will appear.



The list on the left displays all available parameters. The list on the right displays all parameters that are to be included in the export template.

Parameters can be added or removed from this list by using the **Add** and **Remove** keys.

Change the order in which the parameters are displayed by selecting a parameter, and using the **Move up** or **Move Down** buttons.

Press **OK** to exit and return to the **Data exports templates** dialogue. Press **OK** again to save the template.

Parameters with arguments

Certain parameters allow specific values (or arguments) to be added.

Column Title	Argument Value
Type	
Sample Name	
S/W Version #	
Serial #	
Date	
Z-Average Mean	
Polydispersity	
Sizes	
Undersize By Intensity	
Undersize By Volume	
Percentile d(99)	
Percentile	0.99

ILL 6589

A d(99) percentile can be viewed by adding **Measurement -Size-Percentile** and then typing 0.99 as the argument value.

Column titles can be altered by double-clicking on them e.g. Double-click on Percentile and alter to Percentile d(99) to reflect the particular percentile size viewed.

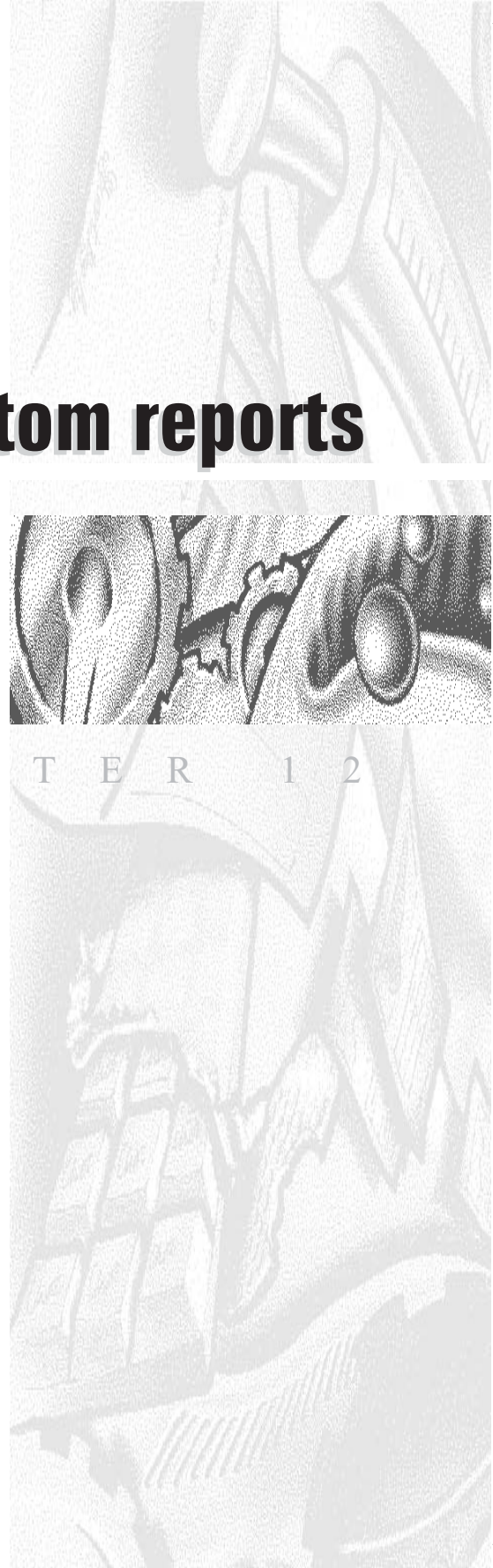
The **Settings** tab works the in the same way as previously described.

 **Note** ■ The Export templates can also be selected and edited using the SOP creation procedures - **Export** dialogue.



Creating custom reports



C H A P T E R 1 2



Introduction

Malvern provides a range of default reports (identified with (M) appended to the name - e.g. Intensity PSD (M)) that are sufficient for most users. For some users though the default reports will not display all the required parameters. With **Report Designer** the user has the ability to create their own custom reports to satisfy their individual needs.

Overview

Two views must be created due to the different aspect ratios of the printed page and computer screen. The **Page Layout**  shows the **printed** version and the **Screen Layout**  shows the computer screen version. Both views are saved under the same **.pag** file name.

The two views do not have to be identical; for example company logos can be added to the printed reports while not showing them on the display.

Report Designer enables the user to add and alter the following items to a report. This is done with the aid of a **Control Palette** (or **Tools**) dialogue.

Data

The data in a selected record can be added to the report in the form of a table or a graph.

Text

User defined text can be added.

Picture Objects

Bitmaps, GIF, JPEG, Metafiles and Icons can all be added to customise the report.

Calculations

Custom calculations using system parameters can be added. These could be used to provide pass/fail decisions.

Parameters

Any parameter stored in a Zetasizer record can be added to the report.

 **Note** ■ During the creation of a report, dummy data is used.

Opening a report

Report designer is opened from within the main Zetasizer software. Select **Tools-Report Designer** to start the application and open a report with the menu option **File-Open...**

To save a report select **File-Save...** All reports are saved with default file extension **.pag**.

A report **must** be saved in the **pages** directory if the report is to be listed in the reports tabs of a measurement file.

Creating a report

A new report can be created in two ways.

- From a blank page by selecting **File-New** and adding the content.
- Alternatively it is often easier to create a new report by opening an existing one, customising it to suit and then saving it under a different name.

For both ways, formatting the layout of a report and customising the content is the same.

Laying out a report

The Report Designer **Control palette** (or **toolbox**) is a pop up window from which the items such as graphs, data and expressions can be added to the report.



ILL 0574

When a button is selected an area can be dragged on the report which will then contain the selected item.

The control palette can be moved anywhere on the screen. It can be switched on and off using the **View-Control palette** option on the menu.

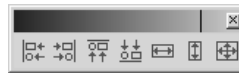
The items that can be added using the toolbox are:

- Text
- Picture
- Parameter
- Frame
- Calculation

- Graph
- Table

Once objects are placed on the report screen they can be manoeuvred by selecting and dragging. Once selected an object can also be reduced or expanded using the black squares.

Report Designer has many size and alignment tools that are available from the toolbar or using the **Layout** option on the menu. Right clicking the mouse button on the selected objects will also bring up the layout options.




ILL 6575

The following functions operate on two or more selected objects; the **last** object selected, highlighted with **six dark squares**, provides the reference position for alignment.

- Align left
- Align right
- Align top
- Align bottom
- Make same width
- Make same height
- Make same size

The **Layout** menu also contains extra functions to **Space evenly** and **Centre** the object. When two or more objects are overlaid, the selected object can be moved to the front or back of the overlay.

Individual objects are selected by a left click on the mouse button, while multiple object selection is made by holding down the **Shift** or **Ctrl** key while clicking the mouse button on the objects, or by “marquee selection”  - dragging a box over a group of objects. If an object has been overlaid by another item and cannot be highlighted; select any object and use the **tab** key to step through all the objects one-by-one until it appears.

Customising and editing the report

Once an object has been inserted into the report its properties can be customised using the **Properties** dialogue box. This dialogue can be displayed in a number of ways. Select the required object and then:

- Double-click on it.
- Right-click and select **Properties** from the menu.
- Select **Alt-enter** on the keyboard.
- Select **Edit-Properties** from the drop-down menu.

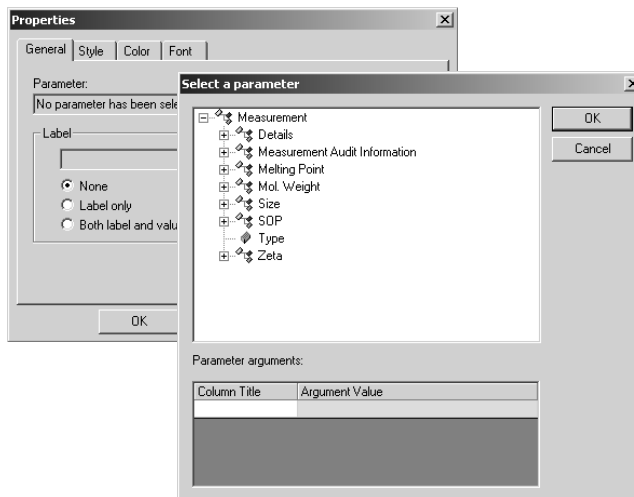
The sections below show the properties each object can display.

Text

Within the text properties dialogue the text can be edited, and its font, alignment, colour and size altered.

Parameter

The parameter properties dialogue is the same as that for the **text** dialogue, plus a **General** parameters tab that enables selection of the parameter.



Any available parameter can be selected and inserted into the report.

Within the **General** tab of the properties dialogue, **Select** the appropriate parameter and press **OK**.

Parameters with arguments

Certain parameters allow specific values (or arguments) to be added.

A d(99) percentile can be viewed by adding **Measurement -Size-Percentile** and then typing 0.99 as the argument value.

Parameter titles can be altered by double-clicking on them e.g. Double-click on Percentile and alter to Percentile d(99) to reflect the particular percentile size viewed.

Column Title	Argument Value
Record	
Sample Name	
Author Userid	
Edit Date/Time	
File Path	
■ Percentile d(99)	
..... Percentile	0.99

ILL 6568

The **Label** selection area enables the parameter name to be added alongside the parameter value.

Picture

The properties dialogue enables pictures to be inserted and deleted.

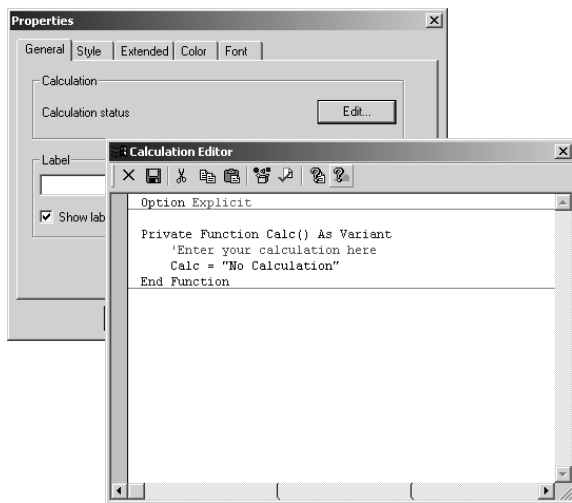
Frame

The frame tool is useful to split the report into relevant sections. By using the properties dialogue the frame style can be chosen - etched, raised, sunken, etc and its colour changed. Horizontal and vertical lines can be inserted into the report to divide sections.

Calculation


As well as entering any of the defined parameters described in the parameters list, it is possible to enter custom calculations. To enter a calculation, select the calculator button from the tool box and draw a marquee where you require the calculation. Right click on the marquee and select **Properties**. The properties dialogue is as for a text object, plus a **General** calculation tab that enables editing of the calculation.


Press the **Edit** button to open the **Calculation editor**.



Calculation editor

Within the Calculation editor, existing calculations can be loaded (and modified if required) or a new calculation created. The language used is Sax Basic.

Selecting the browse icon  to choose functions from the library lists, which can be used to build up calculations. All details about a measurement and how it was performed can be accessed in the available data type lists, i.e. size results, measurement and result details.

Help on writing sax basic calculations is given within the editor by selecting the  icon.

The calculation will only display a value for records that have been created since the calculation was set up. To obtain a result in a calculation on a record created prior to the set up of the calculation, the record will need to be reanalysed (using **Edit-Result...**).

Graphs

A number of data graphs can be added to a report using the report designer control palette.

Select the graph icon and then choose one of the following graphs from the drop-down menus. The menus are split into **Trend**, **Size**, **Molecular weight** and **Zeta potential** with accompanying graph choices.

Trend Graph.

Trend graphs allow the measurement data from multiple records to be compared to investigate any trends in the information. Any parameter can be chosen for the X-axis, and any two other numerical parameters for the Y1 and Y2 axes.

Size graphs

The following size graphs can be inserted.

Result

This shows the size result of the measurement. It can be displayed in different ways using its properties box.

Correlogram

Displays the correlation function, which is the output of the contents of each of the correlation channels.

Residuals

This is the plot of the difference between the actual correlation function measured and the correlation function that is back calculated from the size distribution displayed.

Statistics

The statistics graph will show the mean, standard deviation, maximum and minimum of a group of selected measurement records. The statistics shown on the table can be altered using the properties dialogue.

Fit

This is an overplot of the actual correlation function measured and the correlation function that is back calculated from the size distribution displayed. The closeness of the fit is an indicator of the success of the algorithm used and the quality of the measurement.

Cumulants fit

Plots the difference between the correlation data and the polynomial that is the best fit to that data.

Cumulants residuals

Shows a plot of the difference between the cumulants fit and the measurement data.

Z Average trend

Shows a plot of the Z-average against selected records.

Molecular weight graphs

The following molecular weight graph can be inserted.

Debye

Displays the calculated molecular weight as a Debye plot - the variation in intensity versus the concentration.

Zeta potential graphs

The following zeta potential graphs can be inserted.

Zeta Potential

This shows the Zeta potential distribution generated by the measurement. It can be displayed in different ways using its properties box.

Statistics

The statistics graph will show the mean, standard deviation, maximum and minimum of a group of selected measurement records. The statistics shown on the table can be altered using the properties dialogue.

Graph properties dialogue

This dialogue allows the attributes of the graph to be altered.

- **Display or Option.** Depending on the graph type inserted, **Display** or **Option** will appear in the properties dialogue.


The **Display** tab allows a choice of graph type and how it is to be displayed, i.e. as a histogram or curve.

The **Options** tab (or selection box in the above **Display** tab) allows the graph key position to be chosen. A graph tips option enables pop up tips (flags showing data points on the graph to appear on the report (not on the screen shown in the Report Designer).

- **Axis settings.** Allows both the X and Y axis settings to be defined. Whether **logarithmic** or **linear** axis are required and the axis scales - defined or auto-scaling. **Graticule** (or grid) lines can also be shown on the graphs..
- **Font face.** Allows the font style to be altered. The setting will apply to all annotations used on the graph.
- **Upper/Lower limits.** Further tabs allow the setting of **Upper** and **Lower** warning or action **limits**. The value shown is checked to be within the limits.

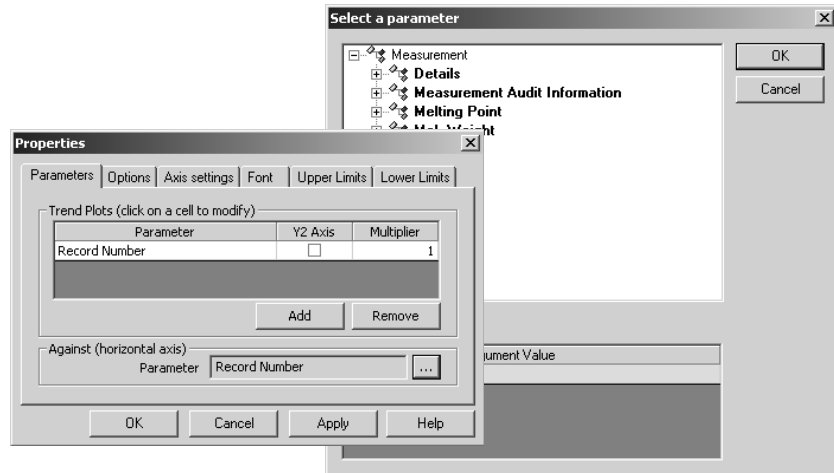
Trend graph properties

The **Trend** graph properties dialogue is the same as above but also contains an extra **Parameters** tab from which all required parameters can be selected for viewing.

The **Trend plots** selection area is used for selecting the Y axes parameters. The Y1 parameter column is used to select the axis on the left of the graph and Y2, the axis on the right. To choose a parameter press the **Add** button, select the appropriate parameter and press **OK**. Either highlighting the chosen parameter and pressing the  icon, or double-clicking the parameter will again display the **Select a parameter** dialogue.

The **Against** selection area is used for selecting the X axis parameter. Selection is the same as for the Y axes.

As before with the **Parameters** properties dialogue certain parameters can have specific values (or arguments) to be added, and their titles altered.



Line styles

Select **Configure-Line styles** to display a line styles dialogue. This enables each measurement result line in a graph to be either coloured, increased in thickness or have its line style changed. Select the **Line style** from the list and alter as required.

Tables

A number of data tables can be added to the report using the control palette. The data is taken from the current selected measurement record, or group of selected records for the trend and statistics tables.

Select the table icon and select one of the following tables from the drop-down menu. The menus are split into **Trend**, **Size**, **Molecular weight** and **Zeta potential** with accompanying table choices.

Trend tables

Trend tables allow the measurement data from multiple records to be compared to investigate any trends in the information. Any two numerical parameters can be compared.

Size tables

The following size tables can be inserted.

Result

This shows the size result of the measurement. It can be displayed as Size/Intensity, Size/Volume and Size/Number using its properties box.

Statistics

The statistics graph will show the mean, standard deviation, maximum and minimum of a group of selected measurement records. The statistics shown on the table can be altered using the properties dialogue.

Molecular weight tables

The following molecular weight table can inserted.

Debye

Shows the data used to generate a Debye plot in a tabular format.

Zeta potential tables

The following zeta potential tables can be inserted.

Zeta Potential

This shows the result of the measurement. It can be displayed as either zeta potential or mobility using its properties box.

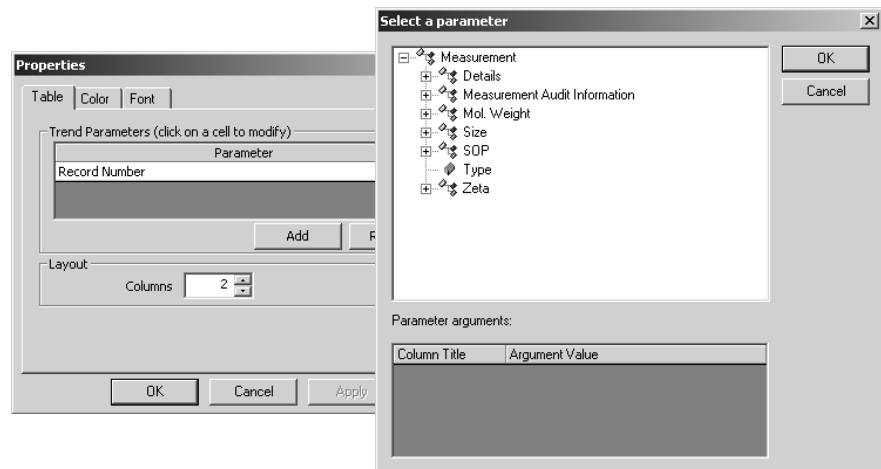
Statistics

The statistics graph will show the mean, standard deviation, maximum and

minimum of a group of selected measurement records. The statistics shown on the table can be altered using the properties dialogue.



Tables properties dialogue

This dialogue is the same as that for the **text** dialogue, plus a **Table** tab that defines the type of table displayed and how many columns to use. The **Trend** table properties dialogue also contains a **Trend** Parameters selection area from which the required parameter can be selected - press the **Add** button, select the appropriate parameter and press **OK**. Refer to the **Parameters properties dialogue** for more details.




Any available parameter can be selected and inserted into the report. The parameter value displayed is taken from the selected measurement record.

A finished report

Using the tools described above a custom report can be created. For example a printed **Page layout**  is shown below with the corresponding **Screen layout**  alongside. The dotted lines indicate the edge of the printed page.

Remember if the custom report is to be printed then a **Page** version must also be created. Both screen and page versions of each report will be saved in the **..\DTS\pages** directory.



SYSTEM


Temperature (C): -0.255e+001
 Count rate (Kcps): not implemented
 Run duration: not implemented
 Cell type: 7 3435873036
 Cell position (mm): not implemented
 Attenuation index: 3435873036

RESULTS

Z-Average size (nm): -1.074e+008
 Polydispersity index: -1.074e+008
 Peak 1 mean: 0
 Peak 2 mean: 0
 Peak 3 mean: 0

SAMPLE DETAILS

Name: Dummy Data 5
 SOP: Size
 Record: 5
 Sample ID: -0.255e+001
 Dispersant ID: -0.255e+001
 Date: 06/11/2002 14:44:08



SYSTEM

Temperature (C): -0.255e+001
 Count rate (Kcps): not implemented
 Run duration: not implemented
 Cell type: 7 3435873036
 Cell position (mm): not implemented
 Attenuation index: 3435873036

RESULTS

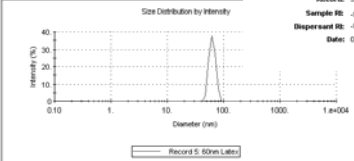
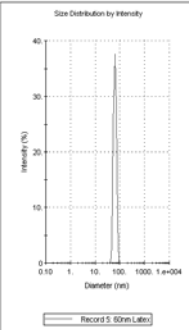
Z-Average size (nm): -1.074e+008
 Polydispersity index: -1.074e+008
 Peak 1 mean: 0
 Peak 2 mean: 0
 Peak 3 mean: 0

SAMPLE DETAILS

Name: Dummy Data 5
 SOP: Size
 Record: 5
 Sample ID: -0.255e+001
 Dispersant ID: -0.255e+001
 Date: 06/11/2002 14:44:08

Size	% Intensity
0.4	0
0.4552	0
0.5101	0
0.5599	0
0.671	0
0.7036	0
0.809	0
0.909	0
1.125	0
1.281	0
1.450	0
1.659	0
1.888	0
2.149	0
2.445	0
2.783	0
3.167	0
3.604	0
4.102	0
4.680	0
5.312	0
6.045	0
6.88	0
7.83	0
8.911	0
10.14	0
11.54	0
13.13	0
14.95	0
17.01	0
19.26	0
22.03	0
25.07	0
28.53	0
32.47	0

Size	% Intensity
36.95	0
42.06	0
47.88	2.966
54.47	24.85
61.99	37.69
70.68	27.54
80.28	7.153
91.37	0
104	0
118.3	0
134.7	0
153.3	0
174.4	0
198.5	0
225.9	0
256.6	0
291.1	0
330	0
373.9	0
423.2	0
480.0	0
550.5	0
636.6	0
733.4	0
853.2	0
996.9	0
1166	0
1363	0
1591	0
1852	0
1788	0
2005	0
2316	0
2636	0
3000	0

ILL 6588

An easy way to create a screen report from a page report (or vice versa) is to use **Edit>Select all** from the menu, then copy and paste the complete page from the printed layout and directly paste into the screen version; all that is then required is some rearranging and formatting to line up all the objects.

Viewing the new report

Once a new report has been created and saved it can be viewed in the main Zetasizer software by selecting **Configure-Workspaces**, and then selecting the workspace for the new report to be associated with. In the **Report Pages** tab, select the check box of the newly created report, and press **OK**.

When the appropriate workspace is selected, the report will be shown as a tab on the measurement file window screen.

Note ■ If a report is edited that is currently displayed by the main application, then saving the edited report will immediately refresh the view in the main application; any changes therefore can instantly be seen.



Size theory

C H A P T E R 1 3

Introduction

The aim of this chapter is to describe the basic **Size** principles behind the Zetasizer Nano series. This will help in understanding the meaning of the results achieved.

The chapter is divided into two major sections. **What is Dynamic light scattering?** and **Operation of the Zetasizer Nano - Size measurements.** The first section describes the theory, while the second describes the physical operation of how a size measurement is performed.

What is Dynamic Light Scattering?

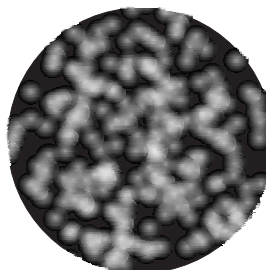
The Zetasizer Nano series performs size measurements using a process called **Dynamic Light Scattering (DLS)**

Dynamic Light Scattering (also known as **PCS - Photon Correlation Spectroscopy**) measures **Brownian motion** and relates this to the size of the particles. It does this by illuminating the particles with a laser and analysing the intensity fluctuations in the scattered light.

Scattering intensity fluctuations

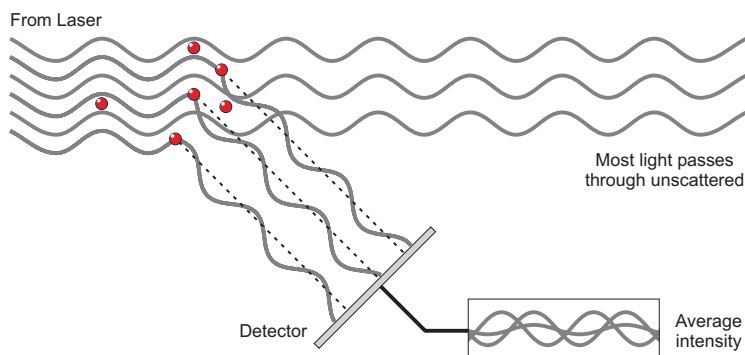
If a small particle is illuminated by a light source such as a laser, the particle will scatter the light in all directions.

If a screen is held close to the particle, the screen will be illuminated by the scattered light. Now consider replacing the single particle with thousands of stationary particles. The screen would now show a speckle pattern as shown below.



The speckle pattern will consist of areas of bright light and dark areas where no light is detected.

What causes these bright and dark areas? The diagram below shows the propagated waves from the light scattered by the particles. The bright areas of light are where the light scattered by the particles arrive at the screen with the same phase and interferes constructively to form a bright patch. The dark areas are where the phase additions are mutually destructive and cancel each other out.



The scattered light falling on the detector.

ILL 4022

In the above example we said that the particles are not moving. In this situation the speckle pattern will also be stationary - in terms of both speckle position and speckle size.

In practice, particles suspended in a liquid are never stationary. The particles are constantly moving due to Brownian motion. Brownian motion is the movement of particles due to the random collision with the molecules of the liquid that surrounds the particle. An important feature of Brownian motion for DLS is that small particles move quickly and large particles move more slowly. The relationship between the size of a particle and its speed due to Brownian motion is defined in the **Stokes-Einstein** equation.

As the particles are constantly in motion the speckle pattern will also appear to move. As the particles move around, the constructive and destructive phase addition of the scattered light will cause the bright and dark areas to grow and diminish in intensity - or to put it another way, the intensity appears to fluctuate. The Zetasizer Nano system measures the rate of the intensity fluctuation and then uses this to calculate the size of the particles.

Interpreting scattering intensity fluctuation data

We know that the Zetasizer measures the fluctuation in scattering intensity and uses this to calculate the size of particles within the sample - but how does it do this?

Within the instrument is a component called a digital correlator. A correlator basically measures the degree of similarity between two signals over a period of time.

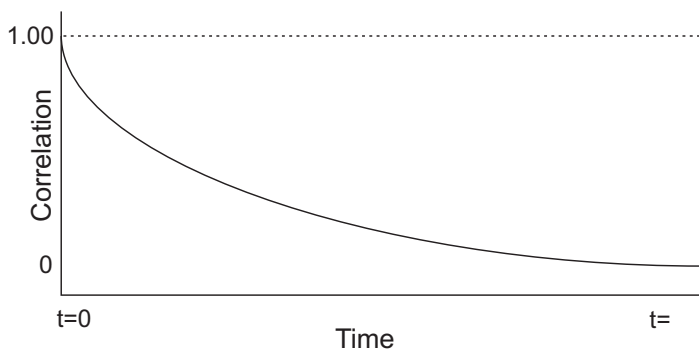
If we compared the intensity signal of a particular part of the speckle pattern at one point in time (say time = t) to the intensity signal a very short time later ($t + \Delta t$) we would see that the two signals are very similar - or strongly correlated. If we then compared the original signal a little further ahead in time ($t + 2 \Delta t$), there would still be a relatively good comparison between the two signals, but it will not be as good as at $t + \Delta t$. The correlation is therefore reducing with time.

Now consider the intensity of the signal at ' t ', with the intensity at a much later time - the two signals will have no relation to each other as the particles are moving in random directions (due to Brownian motion). In this situation it is said that there is no correlation between the two signals.

With DLS we are dealing with very small time scales. In a typical speckle pattern the length of time it takes for the correlation to reduce to zero is in the order of 1 to 10's of milliseconds. The "short time later" (Δt) will be in the order of nanoseconds or microseconds!

If we compare the signal intensity at (t) with itself then we would have perfect correlation as the signals are identical. Perfect correlation is reported as 1 and no correlation is reported as 0.

If we continue to measure the correlation at ($t + 3 \Delta t$), ($t + 4 \Delta t$), ($t + 5 \Delta t$), ($t + 6 \Delta t$), etc, the correlation will eventually reach zero. A typical correlation function of correlation against time is shown below.



ILL-4022

Using the correlation function to get size information

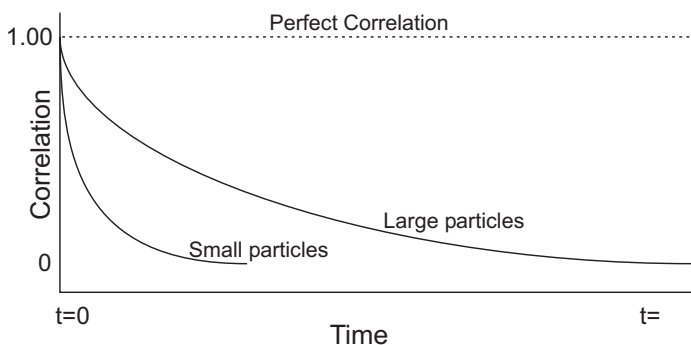
How does the correlation function relate to the particle size? We mentioned earlier that the speed of particles that are being moved by Brownian motion is

related to size of the particles (Stokes-Einstein equation). Large particles move slowly, while smaller particles move quickly. What effect will this have on the speckle pattern?

If large particles are being measured, then, as they are moving slowly, the intensity of the speckle pattern will also fluctuate slowly.

And similarly if small particles are being measured then, as they are moving quickly, the intensity of the speckle pattern will also fluctuate quickly.

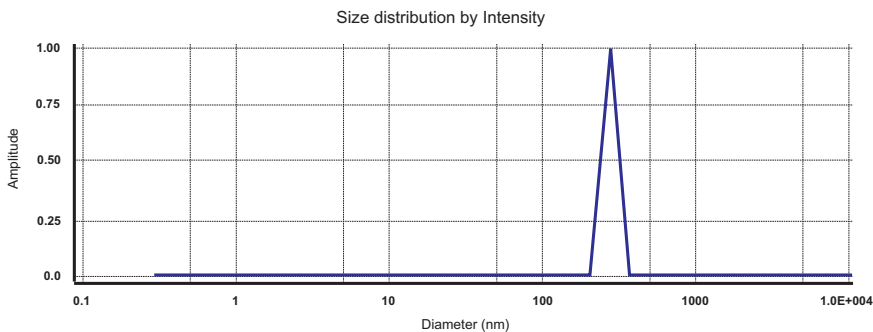
The graph below shows the correlation function for large and small particles. As can be seen, the rate of decay for the correlation function is related to particle size as the rate of decay is much faster for small particles than it is for large.



ILL 4022

After the correlation function has been measured this information can then be used to calculate the size distribution. The Zetasizer software uses algorithms to extract the decay rates for a number of size classes to produce a size distribution.

A typical size distribution graph is shown below. The X axis shows a distribution of size classes, while the Y axis shows the relative intensity of the scattered light. This is therefore known as an intensity distribution.



ILL 4022

Although the fundamental size distribution generated by DLS is an intensity distribution, this can be converted, using Mie theory, to a volume distribution. This volume distribution can also be further converted to a number distribution. However, number distributions are of limited use as small errors in gathering data for the correlation function will lead to huge errors in distribution by number.

Intensity, volume and number distributions

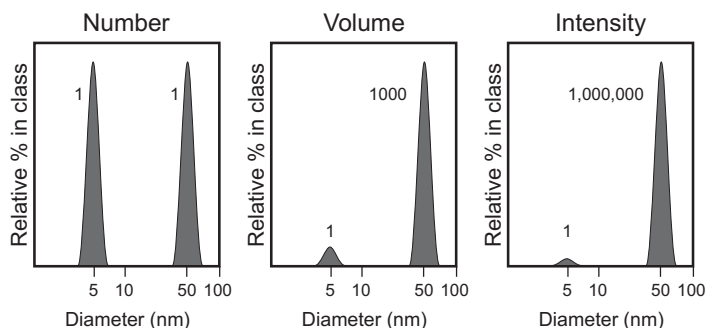
What is the difference between intensity, volume and number distributions?

A very simple way of describing the difference between the intensity, volume and number distributions is to consider a sample that contains only two sizes of particles (5nm and 50nm) but with equal numbers of each size particle.

The first graph below shows the result as a number distribution. As expected the two peaks are of the same size (1:1) as there are equal number of particles.

The second graph shown the result of the volume distribution. The area of the peak for the 50nm particles is 1000 times larger the peak for the 5nm (1:1000 ratio). This is because the volume of a 50nm particle is 1000 times larger that the 5nm particle (volume of a sphere is equal to $\frac{4}{3} (r)^3$).

The third graph shows the result of an intensity distribution. The area of the peak for the 50nm particles is now 1,000,000 times larger the peak for the 5nm (1:1000000 ratio). This is because large particles scatter much more light than small particles (the intensity of scattering of a particle is proportional to the sixth power of its diameter - (from Rayleigh's approximation).



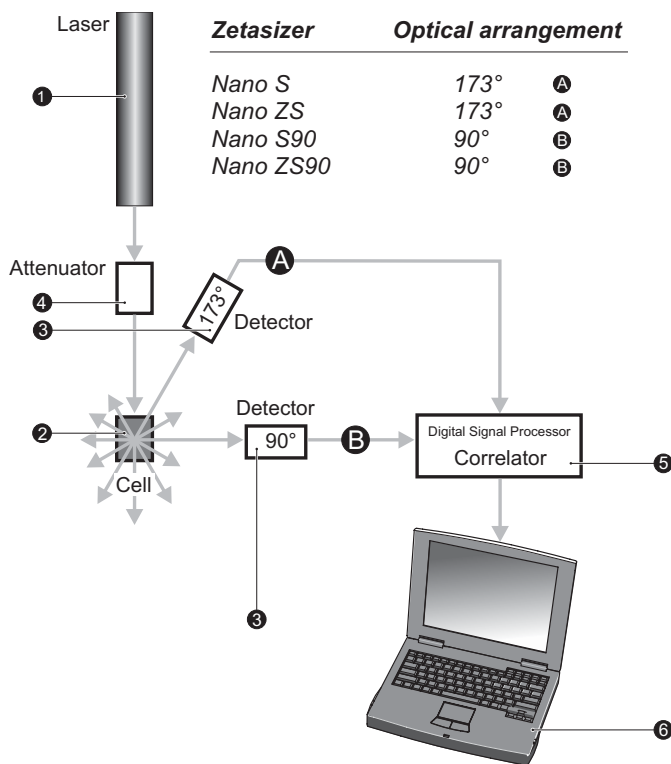
ILL-4022

It is worth repeating that the basic distribution obtained from a DLS measurement is intensity - all other distributions are generated from this.

Operation of the Zetasizer Nano - Size measurements.

A typical DLS system comprises of six main components. First of all a **laser** ① is used to provide a light source to illuminate the sample particles within a **cell** ②. Most of the laser beam passes straight through the sample, but some is scattered by the particles within the sample. A **detector** ③ is used to measure the intensity of the scattered light. As a particle scatters light in all directions, it is (in theory), possible to place the detector in any position and it will still detect the scattering.

With the Zetasizer Nano series, depending upon the particular model, the detector position will be at either 173° or 90°.



The intensity of the scattered light must be within a specific range for the detector to successfully measure it. If too much light is detected then the detector will become overloaded. To overcome this an “**attenuator**” ④ is used to reduce the intensity of the laser and hence reduce the intensity of the scattering.

- For samples that do not scatter much light, such as very small particles or samples of low concentration, the amount of scattered light must be increased. In this situation, the attenuator will allow more laser light through to the sample.
- For samples that scatter more light, such as large particles or samples of higher concentration, the amount of scattered light must be decreased. This is achieved by using the attenuator to reduce the amount of laser light that passes through to the sample.

The appropriate attenuator position is automatically determined by the Zetasizer during the measurement sequence.

The scattering intensity signal for the detector is passed to a digital signal processing board called a **correlator** ⑤. The correlator compares the scattering intensity at successive time intervals to derive the rate at which the intensity is varying.

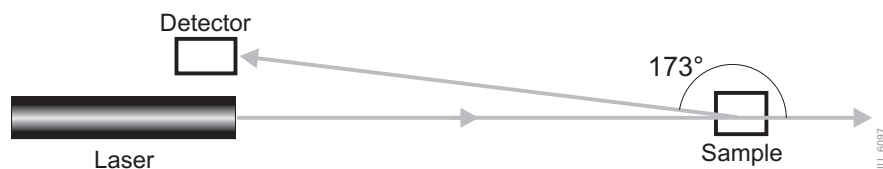
This correlator information is then passed to a **computer** ⑥, where the specialist Zetasizer software will analyse the data and derive size information.

As mentioned earlier, depending upon the Zetasizer Nano model the detection optics will either be arranged at positions of either 173° or 90°.

173° detection optics - Backscatter detection

The Zetasizer Nano Z, S and ZS measure the scattering information close to 180°. This is known as backscatter detection.

The application of the Backscatter detection is by a patented technology called **NIBS (Non-Invasive Back-Scatter)**.



Why measure backscatter? There are several advantages to doing this:

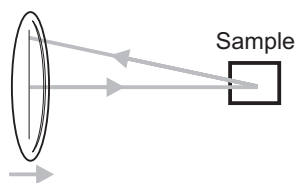
- Because the backscatter is being measured, the incident beam does not have to travel through the entire sample. This reduces an effect known as multiple scattering, where the scattered light from one particle is itself scattered by other particles. As the light passes through a shorter path length of the sample, then higher concentrations of sample can be measured.
- Contaminants such as dust particles within the dispersant are typically large compared to the sample size. Large particles mainly scatter in the forward

direction. Therefore, by measuring the backscatter, the effect of dust is greatly reduced.

- The effect of multiple scattering is at a minimum at 180° - again, this allows higher concentrations to be measured.

Moveable lens

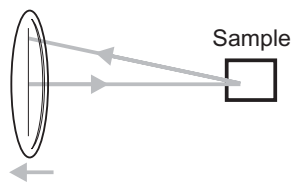
Within the Zetasizer Nano system, a movable lens allows the focus position within the cell to be changed. This allows a much larger range of sample concentrations to be measured.



ILL 6098

For small particles, or samples of low concentration, it will be beneficial to maximise the amount of scattering from the sample.

As the laser passes through the wall of the cell and into the dispersant, the cell wall will cause "flare". This flare may swamp the scattering signal. Moving the measurement point away from the cell wall, towards the centre of the cell will remove this effect.



ILL 6099

Large particles or samples of high concentration, scatter much more light. In this situation, measuring closer to the cell wall will reduce the effect of multiple scattering.

In this instance the flare from the cell wall will have less impact. Any flare will be proportionally reduced compared to the scattering signal.

The measurement position is automatically determined by the Zetasizer software.

90° detection optics - Classical arrangement

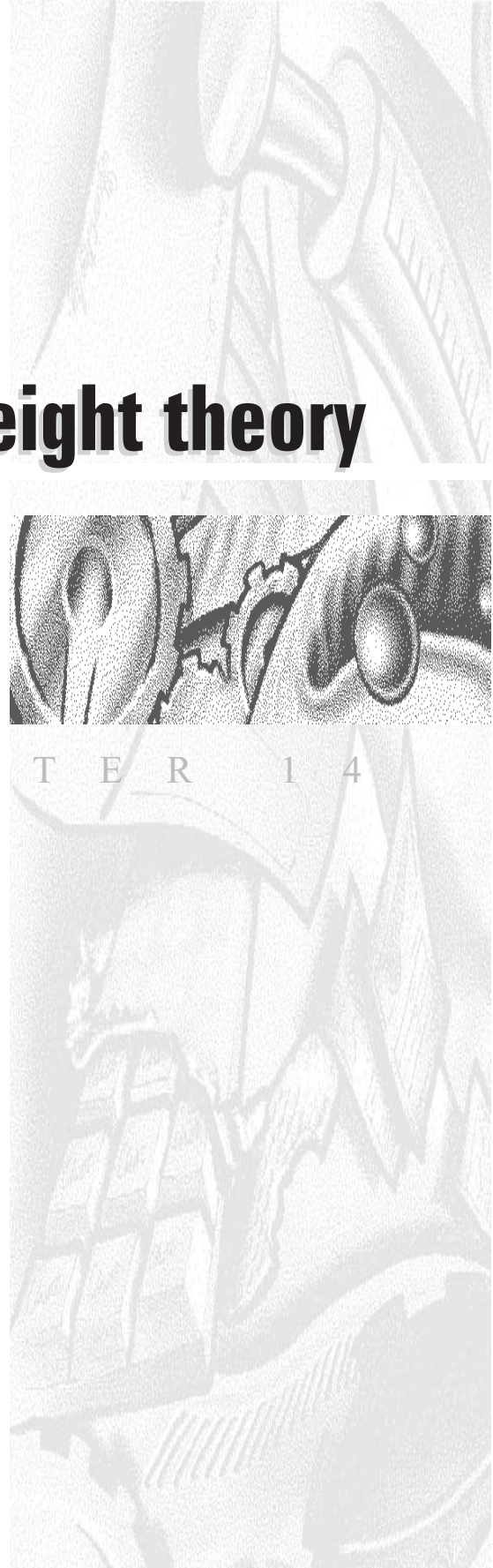
The 90° models, Zetasizer Nano S90 and ZS90, have been included in the Zetasizer Nano instrument range to provide continuity with other systems that have 90° detection optics.

These models do not utilise a movable measurement arrangement but use the 'classical' fixed detection arrangement of 90° to the laser and the centre of the cell area.

This arrangement reduces the detectable size range on these models. Please see the specification table in appendix B for the measurement ranges.

Molecular Weight theory

C H A P T E R 1 4



Introduction

The aim of this chapter is to describe the basic **Molecular weight** principles behind the Zetasizer Nano. This will help in understanding the meaning of the results achieved.

The chapter is divided into two major sections. **What is Static light scattering?** and **The Debye plot**. The first section describes the molecular weight theory, while the second shows how a molecular weight measurement is displayed.

What is Static light scattering?

The Zetasizer Nano series performs Molecular weight measurements using a process called **Static Light Scattering (SLS)**.

Static Light Scattering (SLS) is a non-invasive technique used to characterise the molecules in solution.

In a similar way to Dynamic light scattering - the Size theory in chapter 13 - the particles in a sample are illuminated by a light source such as a laser, with the particles scattering the light in all directions. But, instead of measuring the time dependent fluctuations in the scattering intensity, Static light scattering makes use of the time-averaged intensity of scattered light instead.

The intensity of light scattered over a period of time, say 10 to 30 seconds is accumulated for a number of concentrations of the sample. This time averaging removes the inherent fluctuations in the signal, hence the term 'Static Light Scattering'.

From this we can determine the **Molecular weight (MWt)** and the **2nd Virial Coefficient (A_2)**.

The **2nd Virial Coefficient (A_2)** is a property describing the interaction strength between the particles and the solvent or appropriate dispersant medium.

- For samples where $A_2 > 0$, the particles 'like' the solvent more than itself, and will tend to stay as a stable solution.
- When $A_2 < 0$, the particle 'likes' itself more than the solvent, and therefore may aggregate.
- When $A_2 = 0$, the particle-solvent interaction strength is equivalent to the molecule-molecule interaction strength – the solvent can then be described as being a **theta** solvent.

Static light scattering - theory

The molecular weight is determined by measuring the sample at different concentrations and applying the **Rayleigh equation**. The Rayleigh equation describes the intensity of light scattered from a particle in solution.

The **Rayleigh equation** is :

$$\frac{KC}{R_{\theta}} = \left(\frac{1}{M} + 2A_2C \right) P(\theta)$$

- **R_θ** : The Rayleigh ratio - the ratio of scattered light to incident light of the sample.
- **M** : Sample molecular weight.
- **A₂** : 2nd Virial Coefficient.
- **C** : Concentration.
- **P_θ** : Scattering angle.
- **K** : Optical constant as defined below.

$$K = \frac{2\pi^2}{\lambda_o^4 N_A} \left(n_o \frac{dn}{dc} \right)^2$$

N_A : Avogadro's constant.

R_g : Radius of gyration.

λ_o : Laser wavelength.

n : Solvent refractive index..

dn/dc : The differential refractive index increment. This is the change in refractive index as a function of the change in concentration. For many sample/solvent combinations this may be available in literature; while for novel combinations the dn/dc can be measured by use of a differential refractometer.

The standard approach for molecular weight measurements is to first measure the scattering intensity of the analyte used relative to that of a well described 'standard' pure liquid with a known Rayleigh ratio.

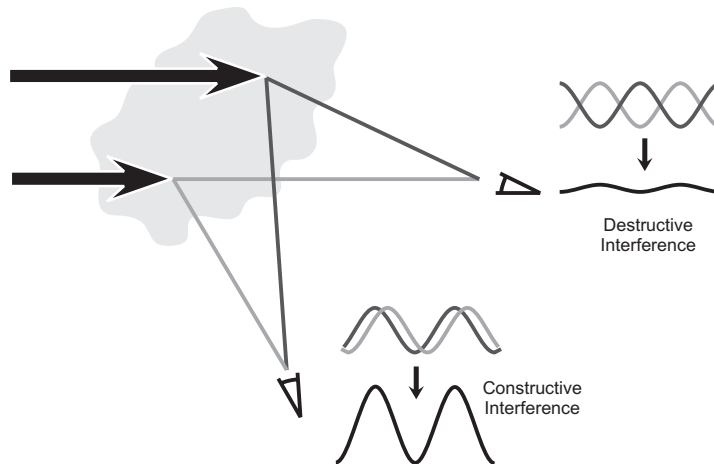
A **common standard** used in Static light scattering is **Toluene**, for the simple reason that the Rayleigh ratios of toluene are suitably high for precise measurements, are known over a range of wavelengths and temperatures and, maybe more importantly, Toluene is relatively easy to obtain. The Rayleigh ratio of Toluene can be found in many reference books, but for reference purposes the expression used to calculate the sample Rayleigh ratio from a toluene standard is given below.

$$R_{\theta} = \frac{I_A n_o^2}{I_T n_T^2} R_T$$

- I_A : Residual scattering intensity of the analyte (i.e. the sample intensity – solvent intensity).
- I_T : Toluene scattering intensity.
- n_o : Solvent refractive index.
- n_T : Toluene refractive index.
- R_T : Rayleigh ratio of toluene.

Rayleigh scattering

The scattering angle P in the Rayleigh equation embodies the angular dependence of the sample scattering intensity. The angular dependence arises from constructive and destructive interference of light scattered from different positions on the same particle, as shown below. This phenomenon is known as **Mie scattering**, and it occurs when the particle is large enough to accommodate multiple photon scattering.



However when the particles in solution are much smaller than the wavelength of the incident light, multiple photon scattering will be avoided. Under these conditions, P_{θ} will reduce to 1 and the angular dependence of the scattering intensity is lost. This type of scattering is known as **Rayleigh scattering**.

The Rayleigh equation will now be:

$$\frac{KC}{R_{\theta}} = \left(\frac{1}{M} + 2A_2C \right)$$

We can therefore stipulate that if the particle is small, Rayleigh scattering can be assumed and the Rayleigh approximation used.

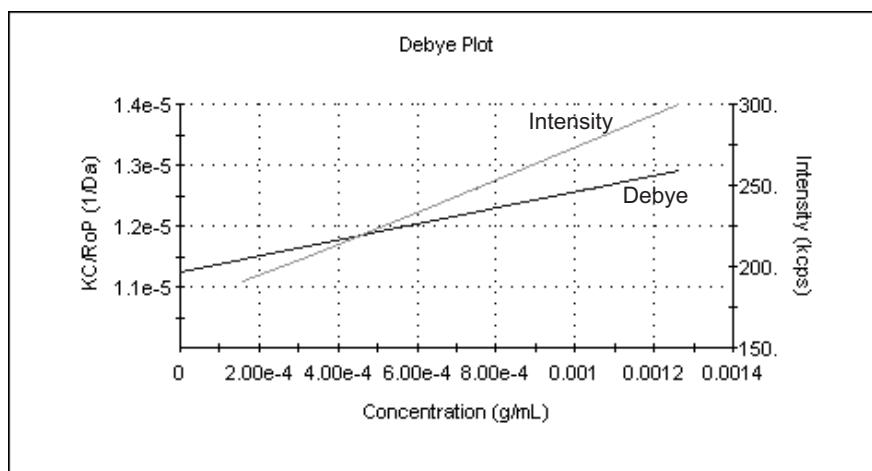
With the Zetasizer Nano series the applicable molecular measurement weight range is from a few hundred g/mol to 500,000 for linear polymers, and over 20,000,000 for near spherical polymers and proteins.

The Debye plot

The intensity of scattered light that a particle produces is proportional to the product of the weight-average molecular weight and the concentration of the particle.

The Zetasizer Nano S and ZS measure the **Intensity of scattered light** (K/CR_θ) of various **concentrations** (C) of sample at **one** angle; this is compared with the scattering produced from a standard (i.e. Toluene).

The graphical representation of this is called a Debye plot and allows for the determination of both the Absolute Molecular Weight and 2nd Virial Coefficient.

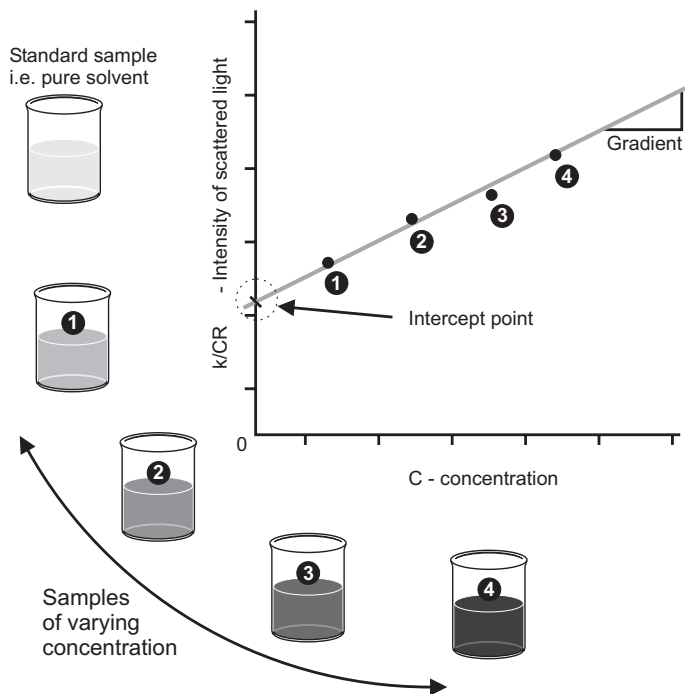


The **Molecular weight (Mwt)** is determined from the intercept point on the X axis. i.e. $K/CR_\theta = 1/MWt$ in **Daltons**.

The **2nd Virial Coefficient (A₂)** is determined from the gradient of the Debye plot.

Each plot and molecular weight measurement is performed by doing several individual measurements; from just the solvent used (a zero concentration measurement), through sample preparations of various concentrations.

The diagram below shows how the Molecular weight and 2nd Virial Coefficient are derived from the Debye plot.

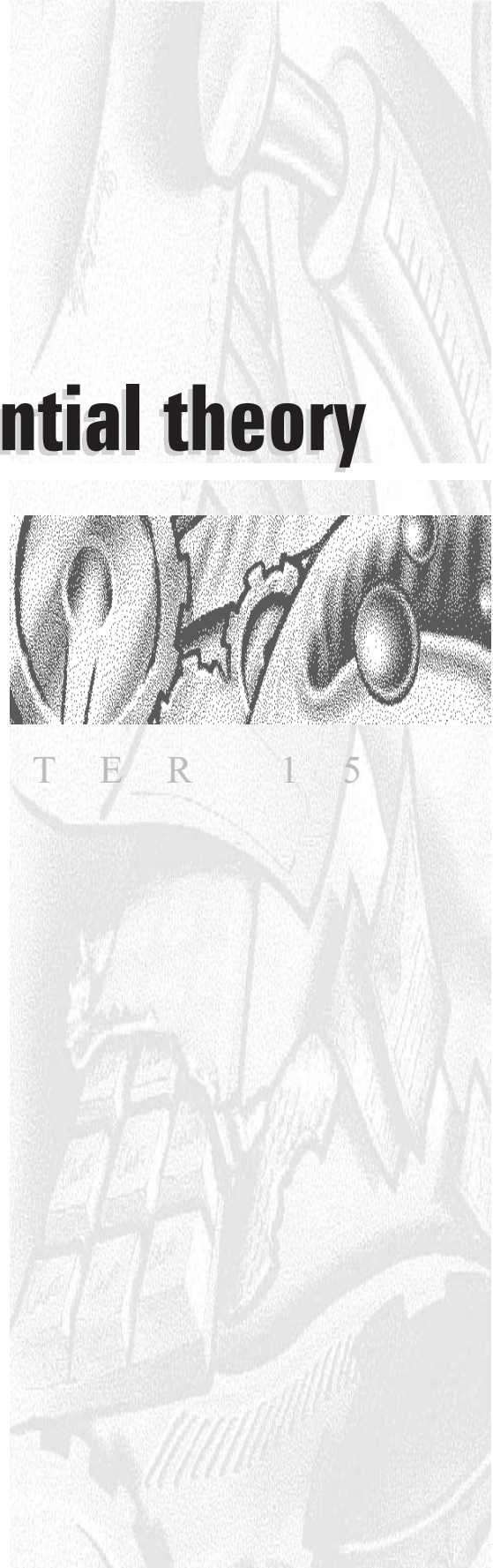


As only one measurement angle is used in this case, a plot of K/CR_{θ} versus C should give a straight line whose intercept at zero concentration will be $1/M$ and whose **gradient** will be A_2 .



Zeta Potential theory

C H A P T E R 1 5



Introduction

The aim of this chapter is to describe the basic **Zeta potential** measurement principles behind the Zetasizer Nano. This will help in understanding the meaning of the results achieved.

The chapter is divided into two major sections. **What is Zeta Potential?** and **Operation of the Zetasizer Nano - Zeta potential measurements**. The first section describes the zeta potential theory, while the second describes the physical operation of how a zeta potential measurement is performed.

What is Zeta Potential?

The Zetasizer Nano series calculates the zeta potential by determining the **Electrophoretic Mobility** and then applying the **Henry equation**. The electrophoretic mobility is obtained by performing an electrophoresis experiment on the sample and measuring the velocity of the particles using **Laser Doppler Velocimetry (LDV)**.

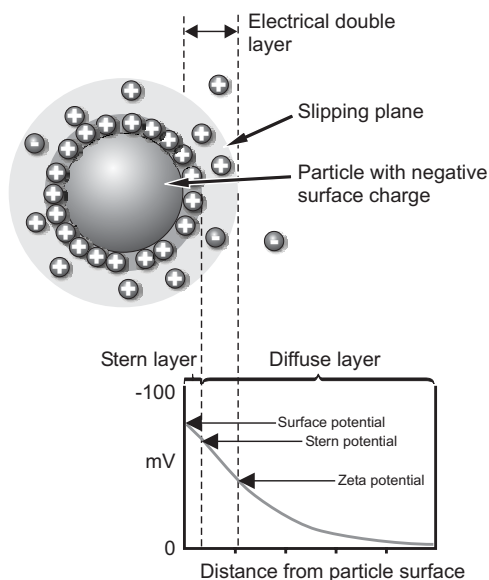
These techniques are described in the following sections.

Zeta potential and the Electrical double layer

The development of a net charge at the particle surface affects the distribution of ions in the surrounding interfacial region, resulting in an increased concentration of counter ions (ions of opposite charge to that of the particle) close to the surface. Thus an **electrical double layer** exists around each particle.

The liquid layer surrounding the particle exists as two parts; an **inner** region, called the **Stern layer**, where the ions are strongly bound and an **outer**, diffuse, region where they are less firmly attached. Within the diffuse layer there is a notional boundary inside which the ions and particles form a stable entity. When a particle moves (e.g. due to gravity), ions **within** the boundary move with it, but any ions beyond the boundary **do not** travel with the particle. This boundary is called the surface of hydrodynamic shear or **slipping plane**.

The potential that exists at this boundary is known as the **Zeta potential**.



ILL 6748

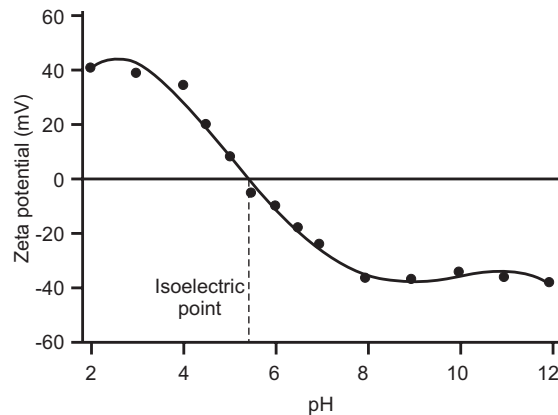
The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. A colloidal system is when one of the three states of matter: gas, liquid and solid, are finely dispersed in one of the others. For this technique we are interested in the two states of: a solid dispersed in a liquid, and a liquid dispersed in a liquid, i.e. an emulsion.

If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there is no tendency to flocculate. However, if the particles have low zeta potential values then there is no force to prevent the particles coming together and flocculating. The general dividing line between stable and unstable suspensions is generally taken at either +30mV or -30mV. Particles with zeta potentials more positive than +30mV or more negative than -30mV are normally considered stable.

The most important factor that affects zeta potential is **pH**. A zeta potential value on its own without a quoted pH is a virtually meaningless number.

Imagine a particle in suspension with a negative zeta potential. If more alkali is added to this suspension then the particles will tend to acquire a more negative charge. If acid is then added to this suspension a point will be reached where the negative charge is neutralised. Any further addition of acid can cause a build up of positive charge. Therefore a zeta potential versus pH curve will be positive at low pH and lower or negative at high pH.

The point where the plot passes through zero zeta potential. This point is called the **Isoelectric point** and is very important from a practical consideration. It is normally the point where the colloidal system is least stable. A typical plot of zeta potential versus pH is shown below.



ILL 6749

Electrokinetic effects

An important consequence of the existence of electrical charges on the surface of particles is that they will exhibit certain effects under the influence of an applied electric field. These effects are collectively defined as **electrokinetic effects**. There are four distinct effects depending on the way in which the motion is induced. These are:

- **Electrophoresis :**
The movement of a charged particle relative to the liquid it is suspended in under the influence of an applied electric field.
- **Electroosmosis :**
The movement of a liquid relative to a stationary charged surface under the influence of an electric field.
- **Streaming potential :**
The electric field generated when a liquid is forced to flow past a stationary charged surface.
- **Sedimentation potential :**
The electric field generated when charged particles move relative to a stationary liquid.

Electrophoresis

When an electric field is applied across an electrolyte, charged particles suspended in the electrolyte are attracted towards the electrode of opposite charge. Viscous forces acting on the particles tend to oppose this movement. When equilibrium is reached between these two opposing forces, the particles move with constant **velocity**.

The **velocity** of the particle is dependent on the following factors :

- Strength of electric field or voltage gradient.
- The Dielectric constant of the medium.
- The Viscosity of the medium.
- The Zeta potential.

The **velocity** of a particle in an electric field is commonly referred to as its **Electrophoretic mobility**.

With this knowledge we can obtain the zeta potential of the particle by application of the **Henry equation**.

The **Henry equation** is :

$$U_E = \frac{2 \varepsilon z f(Ka)}{3 \eta}$$

- **z** : Zeta potential.
- **U_E** : Electrophoretic mobility.
- **ε** : Dielectric constant.
- **η** : Viscosity.
- **f(Ka)** : **Henry's** function.

Two values are generally used as approximations for the **f(Ka)** determination - either **1.5** or **1.0**.

Electrophoretic determinations of zeta potential are most commonly made in aqueous media and moderate electrolyte concentration. **f(Ka)** in this case is **1.5**, and is referred to as the **Smoluchowski** approximation.

Therefore calculation of zeta potential from the mobility is straightforward for systems that fit the Smoluchowski model, i.e. particles larger than about 0.2 microns dispersed in electrolytes containing more than 10^{-3} molar salt.

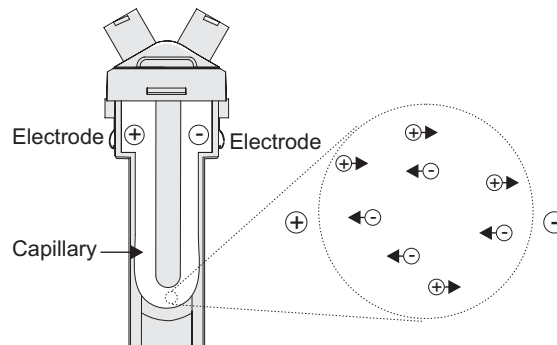
The **Smoluchowski** approximation is used for the folded capillary cell and the universal dip cell when used with aqueous samples.

For small particles in low dielectric constant media $f(Ka)$ becomes **1.0** and allows an equally simple calculation. This is referred to as the **Huckel** approximation. **Non-aqueous** measurements generally use the **Huckel** approximation.

Measuring Electrophoretic Mobility

It is the electrophoretic mobility that we measure directly with the conversion to zeta potential being inferred from theoretical considerations. How is electrophoretic mobility measured?

The essence of a classical micro-electrophoresis system is a cell with electrodes at either end to which a potential is applied. Particles move towards the electrode of opposite charge, their velocity is measured and expressed in unit field strength as their mobility.



ILL 6750

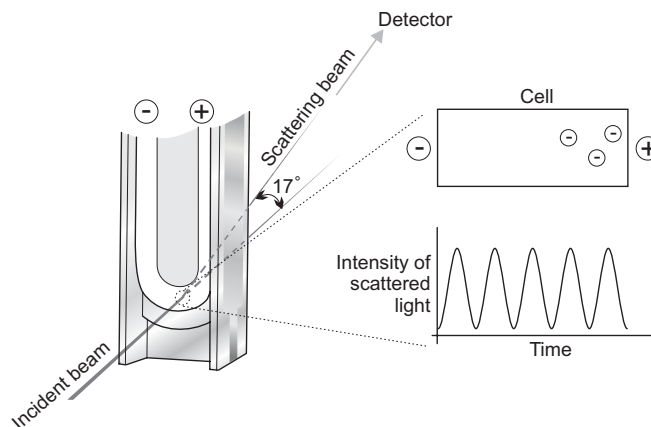
The technique used to measure this velocity in Malvern's Zetasizer Nano series of instruments is **Laser Doppler Velocimetry**.

Laser Doppler Velocimetry

Laser Doppler Velocimetry (LDV) is a well established technique in engineering for the study of fluid flow in a wide variety of situations, from the supersonic flows around turbine blades in jet engines to the velocity of sap rising in a plant stem.

In both these examples, it is actually the velocity of tiny particles within the fluid streams moving at the velocity of the fluid that we are measuring. Therefore, LDV is well placed to measure the velocity of particles moving through a fluid in an electrophoresis experiment.

The receiving optics is focussed so as to relay the scattering of particles in the cell.



The light scattered at an angle of 17° is combined with the reference beam. This produces a fluctuating intensity signal where the rate of fluctuation is proportional to the speed of the particles. A digital signal processor is used to extract the characteristic frequencies in the scattered light.

Optical Modulator

A refinement of the system involves modulating one of the laser beams with an oscillating mirror. This gives an unequivocal measure of the **sign** of the **Zeta potential**.

A second benefit of the modulator is that low or zero mobility particles give an equally good signal, so measurement is as accurate as for particles with a high mobility.

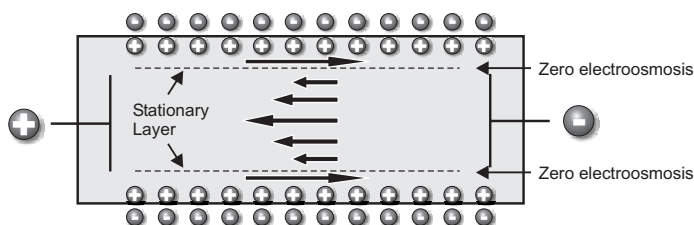
This technique ensures an accurate result in a matter of seconds, with possibly millions of particles observed.

The Electroosmosis effect

The walls of the capillary cell carry a surface charge so the application of the electric field needed to observe electrophoresis causes the liquid adjacent to the walls to undergo electroosmotic flow. Colloidal particles will be subject to this flow superimposed on their electrophoretic mobility. However, in a closed system the flow along the walls must be compensated for by a reverse flow down the centre of the capillary.

There is a point in the cell at which the electroosmotic flow is zero - where the two fluid flows cancel. If the measurement is then performed at this point, the particle velocity measured will be the **true electrophoretic velocity**. This point

is called the **stationary layer** and is where the two laser beams cross; the zeta potential measured is therefore free of electroosmotic errors.



Avoiding Electroosmosis

The stationary layer technique described above has been in use for many years. Because of the effect of electroosmosis the measurement can only be performed at specific point within the cell. If it was possible to remove electroosmosis altogether then it would be possible to performed the measurement on the particles at **any point in the cell** and obtain the true mobility.

This is now possible with the Zetasizer Nano series of instruments. With a combination of Laser Doppler Velocimetry and **Phase Analysis Light Scattering (PALS)** this can now be achieved in Malvern's patented **M3-PALS** technique.

Implementation of M3-PALS enables even samples of very low mobility to be analysed and their mobility distributions calculated.

The M3-PALS technique

To perform measurements at any point within a cell and obtain the electrophoretic mobility Malvern has developed its patented **M3-PALS** technique. This is a combination of Malvern's improved laser doppler velocimetry method - the **M3** measurement technique, and the application of **PALS** (**Phase Analysis Light Scattering**).

The M3 technique

As discussed traditional electrophoretic measurements are performed by measurement of particles at the stationary layer, a precise position near the cell walls. With M3 the measurement can be performed anywhere in the cell, though with the Zetasizer Nano series it is performed in the centre of the cell.

M3 consists of both **Slow Field Reversal (SFR)** and **Fast Field Reversal (FFR)** measurements, hence the name '**Mixed Mode Measurement**'.

Measurement position in the cell

The M3 method performs the measurement in the middle of the cell, rather than at the stationary layer. In principle the M3 measurement could be done at any position in the cell, however there are a number of reasons for choosing to work at the **centre**.

- The measurement zone is further from the cell wall, so reduces the chance of flare from the nearby surface.
- The alignment of the cell is less critical.
- The charge on the cell wall can be calculated.

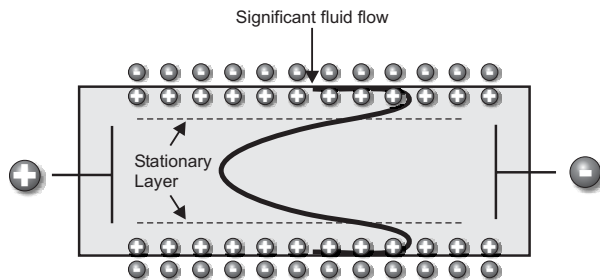
Reversal of the applied field

All systems that measure mobilities using LDV (Laser Doppler Velocimetry) reverse the applied field periodically during the measurement. This is normally just the slow field reversal mentioned below.

However, M3 consists of two measurements for each Zeta potential measurement, one with the applied field being reversed slowly - the SFR measurement; and a second with a rapidly reversing applied field - the FFR measurement stage.

Slow Field Reversal (SFR)

This reversal is applied to reduce the polarisation of the electrodes that is inevitable in a conductive solution. The field is usually reversed about every 1 second to allow the fluid flow to stabilise.



ILL 0753

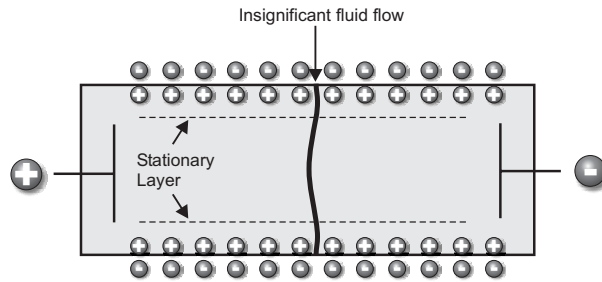
Fast Field Reversal (FFR)

If the field is reversed much more rapidly, it is possible to show that the particles reach terminal velocity, while the fluid flow due to electroosmosis is insignificant. (The residual flow in this diagram below is exaggerated).

This means that the mobility measured during this period is due to the electrophoresis of the particles only, and is not affected by electroosmosis.

The mean zeta potential that is calculated by this technique is therefore very robust, as the measurement position in the cell is not critical.

However, as the velocity of the particles is sampled for such a short period of time, information about the distribution is degraded. This is what is addressed by M3, the PALS technique is used to determine the particle mobility in this part of the sequence.



ILL 6754

M3 measurement sequence

An M3 measurement is performed in the following manner:

- A **Fast field** reversal measurement is performed at the cell centre. This gives an accurate determination of the mean.
- A **Slow field** reversal measurement is made. This gives better resolution, but mobility values are shifted by the effect of electroosmosis.
- The mean zeta potentials calculated from the FFR and SFR measurements are subtracted to determine the electroosmotic flow. This value is used to normalise the slow field reversal distribution.
- The value for electroosmosis is used to calculate the zeta potential of the cell wall.

Benefits of M3

Using M3 the entire Zeta potential measurement is simplified. It is no longer necessary for the operator to select any system parameters for the measurement, as the appropriate settings are calculated as part of the M3 sequence. With a reduction in the number of measurement variables, both measurement repeatability and accuracy is improved. Additionally, alignment is no longer an issue as there are no concerns about the location of the stationary layer.

M3 is now combined with the PALS measurement.

Adding PALS and what is it?

PALS (Phase Analysis Light Scattering) is a further improvement on traditional Laser doppler velocimetry and the M3 implementation described above.

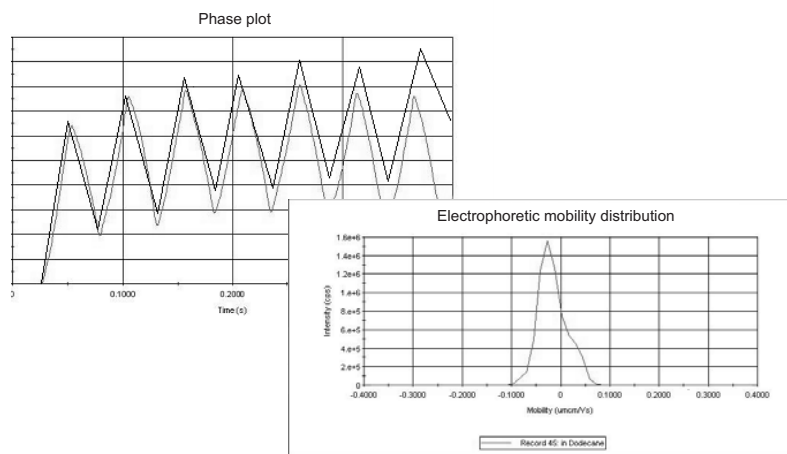
Overall the application of PALS enables accuracy of the measurement of low particle mobilities. This can give an increase in performance of greater than 100 times than that associated with standard measurement techniques.

This allows the measurement of high conductivity samples, plus the ability to accurately measure samples that have low particle mobilities. Low applied voltages can now be used to avoid any risk of sample effects due to joule heating.

How PALS works

By doing a phase comparison of the generated signal with that of a reference frequency - the reference frequency being that of the **optical modulator** - it is possible to see very small shifts (or displacements) in a particles movement that would otherwise be concealed due to natural Brownian motion.

As electroosmosis is insignificant due to the implementation of M3 then the phase between the two frequencies will be constant, so if there is any particle movement then this phase relationship will alter. Detection of a phase change is more sensitive to changes in mobility, than the traditional detection of a frequency shift.



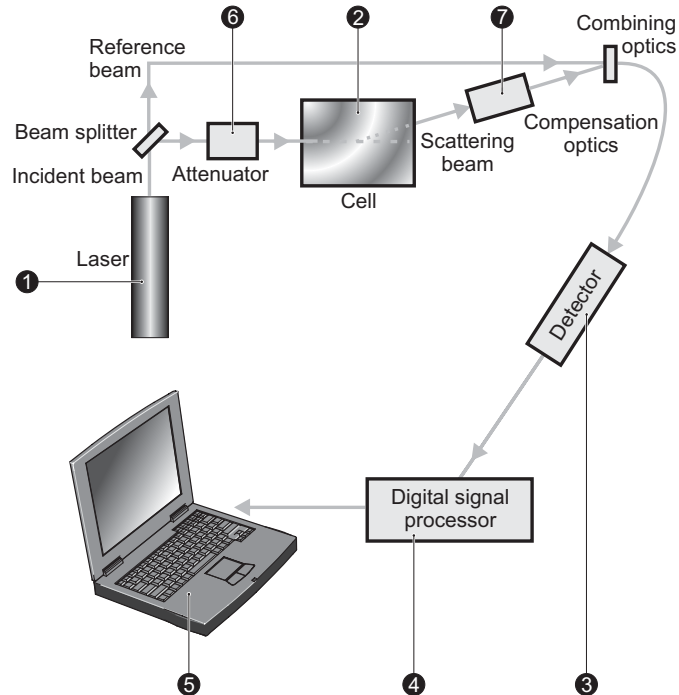
ILL 6776

Electrophoretic mobility and consequently the **Zeta potential** is then determined by summing the phase shifts measured during the FFR part of the measurement.

Operation of the Zetasizer Nano - Zeta potential measurements.

In a similar way to the typical DLS system described in the size theory chapter, a zeta potential measurement system comprises of six main components. First of all a **laser** ① is used to provide a light source to illuminate the particles within the sample; for zeta potential measurements this light source is split to provide an incident and reference beam. The reference beam is also ‘modulated’ to provide the doppler effect necessary.

The laser beam passes through the centre of the sample **cell** ②, and the scattering at an angle of 17° is detected. On insertion of the cell into the cell holder, the cell terminals allow the system to recognise the type of zeta potential cell fitted, and configures the software to use the correct measurement sequence.



ILL 6778

When an electric field is applied to the cell, any particles moving through the measurement volume will cause the intensity of light detected to fluctuate with a frequency proportional to the particle speed.

A **detector** ③ sends this information to a **digital signal processor** ④. This information is then passed to a **computer** ⑤, where the Zetasizer Nano software produces a frequency spectrum from which the electrophoretic mobility and hence the zeta potential information is calculated.

The intensity of the scattered light within the cell must be within a specific range for the detector to successfully measure it. If too much light is detected then the detector will become overloaded. To overcome this an “**attenuator**” ⑥ is used to reduce the intensity of the laser and hence reduce the intensity of the scattering.

For samples that do not scatter much light, such as very small particles or samples of low concentration, the amount of scattered light must be increased. The attenuator will automatically allow more light through to the sample.

For samples that scatter more light, such as large particles or samples of higher concentration, the amount of scattered light must be decreased. The attenuator will automatically reduce the amount of light that passes through to the sample.

To correct for any differences in the cell wall thickness and dispersant refraction **compensation optics** ⑦ are installed within the scattering beam path to maintain alignment of the scattering beams.

Universal Dip cell

When the dip cell is inserted into the cell holder the terminals on the cell allow the system to recognise the type of cell fitted and adjusts the applied voltage and compensation optics appropriately.

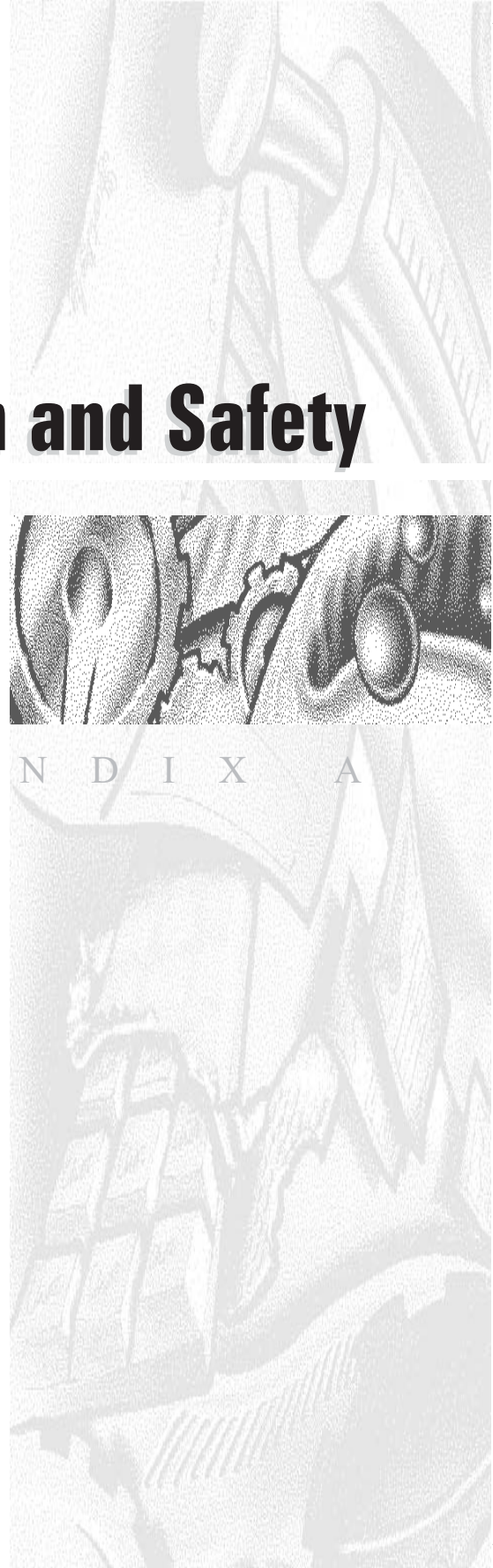
The measurement routine is the same as that for the folded capillary cell except that the FFR measurement is not used. The measurement electrodes on the dip cell are only 2mm apart and positioned right next to the measurement zone. This removes the effect of electroosmosis and therefore the need for the FFR part of the measurement routine.

Part 3 - Appendices



Health and Safety

A P P E N D I X A



Health and safety

General Warnings and Safety Regulations



The instrument or the samples to be measured may be hazardous if misused. Read and fully understand this section before operating the system.



Use of the system in a manner not specified by Malvern Instruments Ltd may impair the protection provided by the system.

The instrument must only be stored or operated in environmental conditions conforming to the specification within appendix C - Site requirements.

Electrical Warnings and Safety Regulations



The Zetasizer Nano series contains high voltage components. Only Malvern trained personnel are permitted to remove the main cover of the instrument.

The Zetasizer Nano series instruments are mains powered device and all power cables and electrical sockets should be treated accordingly. Do not place cables in positions where they are likely to become wet.

Should the instrument become wet (i.e. sample or dispersant has been accidentally spilt), switch off the instrument and disconnect it from the mains power supply immediately. Scrupulously clean and dry the instrument before re-applying power.



This product **must** be connected to a protective earth.
The metal parts of the optical unit and the accessories are earthed via a protective earth connection.

PAT Testing.

If PAT testing is required, connect the earth lead to the earth stud underneath the rear right-hand corner of the instrument.

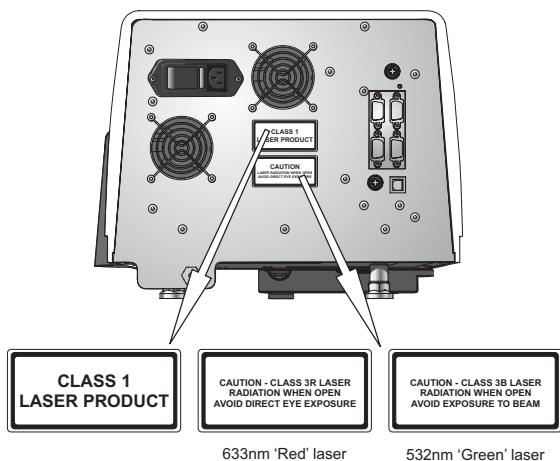
Laser Safety Regulations

The Zetasizer Nano series are Class 1 laser products, and as such, there is no exposure to laser radiation in the normal operation of the instruments.



Use of controls and procedures other than those specified herein may result in hazardous radiation exposure.

The diagram below shows the location of the laser warning labels.



ILL 6729

The standard laser has a maximum cw-power of 4mW at 632.8nm, while the option laser has a maximum cw-power of 50.0mW at 532nm.

Temperature warnings



The warning triangle on the cuvette lid and thermal cap are there to warn the operator of potentially hazardous temperatures within the cell area. The temperature range of the cell area in the Zetasizer Nano instruments is 2°C to 90°C.

Purge warnings



If a Nitrogen supply is used the instrument must be located in a well ventilated environment. The purge supply expels 50ml of Nitrogen a minute. Ensure the supply is turned **off** when not in use.

Sample handling warnings

Always handle all substances in accordance with the COSHH (Control Of Substances Hazardous to Health) regulations (UK) or any local regulations concerning sample handling safety.

Before using any substance, check the Material Safety Data Sheets for safe handling information.

Use the instrument in a well ventilated room, or preferably within a fume cupboard, if the fumes from the sample or dispersant are toxic or noxious.

Wear personal protective equipment as recommended by the Material Safety Data Sheets if toxic or hazardous samples are being handled, particularly during sample preparation and measurement.

Wear protective gloves when handling hazardous materials, or those that cause skin infections or irritations.

Do not smoke during measurement procedures, particularly where inflammable samples are used or stored.

Do not eat or drink during measurement procedures, particularly where hazardous samples are used or stored.

Take care when handling glass (e.g. beakers). Hazardous materials may enter a wound caused by broken glass.

Always test a new sample or dispersant for chemical compatibility before use.

After measuring hazardous samples, scrupulously clean the system to remove any contaminants before making another measurement.

Always label samples for analysis using industry standard labelling, particularly if they are handled by a number of staff or stored for long periods. Clearly mark any operator hazard and associated safety precautions that are required for the handling of dangerous materials.

It is important to keep a record of all hazardous substances used in the system for protection of service and maintenance personnel.

Always adopt responsible procedures for the disposal of waste samples. Most local laws forbid the disposal of many chemicals in such a manner as to allow their entry into the water system. The user is advised to seek local advice as to the means available for disposal of chemical wastes in the area of use.

Recommendations can be found within the Materials Safety Data Sheets.

The surfaces of the system may be permanently damaged if samples are spilt onto them. If spillages should occur, then the system should be disconnected from the power supply before scrupulously cleaning up the spillage.

Moving the system

If it is necessary to move the system, the following guidelines should be followed:

- Always disconnect the computer and power supply before attempting to move the system.
- Always adopt proper lifting techniques to avoid back injury.
- Always lift the instrument by holding it under its base. Never lift an instrument by its covers.
- If the system is to be moved large distances then it is recommended that it is repacked in its original packaging.

Disposing of the instrument and accessories

When the need eventually arises to dispose of the system, it should be done so in a responsible manner. The following are guidelines that should be followed.

- Before disposal of the optical unit, the laser should be disabled in such a manner as to make it impossible for it to be powered up. Seek advice from the local Malvern representative for details.
- The instrument should be decontaminated if hazardous materials have been used in the system.
- Refer to any local regulations on disposal of equipment.



Specification



A P P E N D I X B



Specification

Parameter	Specification
<i>Size range.</i> Nano S and Nano ZS - Concentration range Nano S90 and Nano ZS90 - Concentration range	0.6nm to 6 μ m hydrodynamic diameter 0.1mg/ml lysozyme to 40wt/vol% 2nm to 3 μ m hydrodynamic diameter N.A.
<i>Size range for Zeta potential</i> Nano Z, ZS and Nano ZS90 Minimum sample volume	3nm to 10 μ m 0.75ml
<i>Molecular weight range</i> Nano S and Nano ZS Minimum sample volume - automated with Autotitrator	1000 to 2x10 ⁷ Daltons 20 μ l 3ml
<i>Measurement technique</i> Size - Nano S and ZS - Nano S90 and ZS90 Zeta potential Molecular weight	Dynamic light scattering (NIBS [®]) Dynamic light scattering (90 degrees) M3 - PALS [®] Static light scattering
<i>Laser</i> (Standard - Red) (Option - Green)	He-Ne, 4.0mW, 633nm DPSS, 50.0mW, 532nm
<i>Product laser class</i>	Class 1 compliant, IEC 60825-1(1993)+A1(1997)+A2(2001)
<i>Laser attenuation</i>	Automatic, transmission 100% to 0.0003%
<i>Detector</i>	Avalanche photodiode, Q.E. >50% at 633nm
<i>Condensation control</i>	Purge facility using air/Nitrogen
<i>Temperature range</i>	2°C to 90°C
<i>Dimensions</i>	320mm(W) x 600mm(D) x 260mm(H)
<i>Weight</i>	19.4kg

Parameter	Specification
<i>Power requirements</i>	<i>AC 100-240V, 50-60Hz</i>
<i>Power consumption</i>	<i>Max. 80W</i>
<i>Ambient operating conditions - Humidity</i>	<i>+10 to +35 °C (+50 to 95 °F) 10 to 90% (non-condensing)</i>
<i>Operating systems</i>	<i>Windows XP Professional (Service pack 1a or higher) Windows 2000 (Service pack 3 or higher)</i>
<i>Minimum computer specification</i>	<i>Pentium PC 2GHz, 128MByte RAM, 80MByte free hard disk space, 1024 x 768 screen resolution, CD-ROM drive, One free USB port</i>

** sample dependent*

Site requirements

from MAN0317 Issue 1.0

A P P E N D I X C

© **Malvern Instruments Ltd. 2003**

Malvern Instruments makes every effort to ensure that this document is correct. However, due to Malvern Instruments policy of continual product development we are unable to guarantee the accuracy of this, or any other document after the date of publication. We therefore disclaim all liability for any changes, errors or omissions after the date of publication. No reproduction or transmission of any part of this publication is allowed without the express written permission of Malvern Instruments Ltd.

Head office:

Malvern Instruments Ltd.
Enigma Business Park,
Groveswood Road,
Malvern,
Worcestershire. WR14 1XZ
United Kingdom.

Tel + [44] (0)1684-892456

Fax + [44] (0)1684-892789

Windows 2000 and XP are registered trademarks of the Microsoft Corporation.

Zetasizer, NIBS and M3-PALS are registered trademarks of Malvern instruments.

Printed in England

Introduction

This document outlines all site requirements needed to install a Zetasizer Nano system. Ensure that all site requirements are fulfilled **before** the Malvern Instruments engineer arrives to install and commission your system.


Environmental conditions

When choosing a site for the system, ensure that the following environmental conditions are satisfied.

- The Zetasizer Nano series instruments and accompanying accessories are intended for indoor use only.
- Always position away from strong light sources (e.g. windows).
- Position the optical unit away from strong heat sources (e.g. radiators).
- The bench that carries the system must be horizontal and vibration free. It must also be capable of supporting the weight of the system. The table below lists the weights of the components of the system.

Zetasizer Nano	19.4kg
MPT2 Autotitrator	5.3kg
Computer and Printer	See manufacturers documentation

- Avoid passing electrical cables through areas where liquids can be spilt.
- Rooms must be well ventilated if noxious samples or dispersants are used.
- It is recommended that the computer is positioned to the right of the instrument.

 **Note** ■ Do **not** position the system so power sockets are obstructed, they may need to be disconnected during an emergency.

The system has been designed to be stored and operated in the following conditions.

Operating temperature	+10 to +35 °C (+50 to 95 °F)
Storage temperature	-20 to +50 °C (-104 to +122 °F)

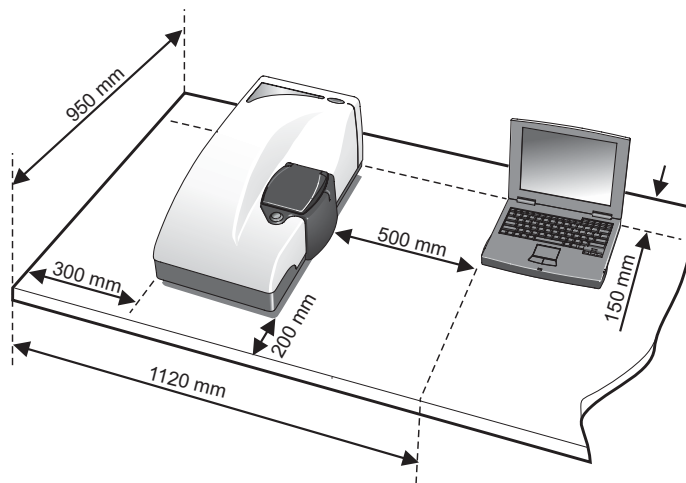
Humidity	10 to 90% (non-condensing)
Altitude	Up to 2000m
Pollution degree	2
Mains supply voltage fluctuations	±10% of nominal voltage

Space required

Enough space should be given to allow easy access to all components of the system. Allow at least 800mm above the bench surface for access to the cell area.

Dimensions of all components of the system are given below.

Component	Width	Depth	Height
Zetasizer Nano	320mm	600mm	260mm
MPT2 Autotitrator	170mm	390mm	260mm
Computer and printer	See manufacturers documentation		



ILL6719

The diagram above shows the **minimum** recommended space required for a typical system. Generally the computer will be placed to the right of the instrument.

Power requirements

The mains power supply must be clean and filtered. If necessary, fit an un-interruptible power supply (UPS) to remove any spikes or noise. The power requirement for each component of the Zetasizer system is given below.

Component	Power requirement	Power sockets required
Zetasizer Nano	~ 100-240V, 50-60Hz	1
MPT2 Autotitrator	~ 100-240V, 50-60Hz	1
Computer	See manufacturers documentation	
Computer monitor	See manufacturers documentation	
Printer	See manufacturers documentation	

Additional services

Purge specification



Warning

A Nitrogen supply must be used in a well ventilated environment.

If measuring samples at low temperatures there is a risk of condensation occurring on the cell; this occurs when the measurement temperature is less than the 'dew point' of the ambient air surrounding the cell being measured. This is particularly likely in humid climates. If it is suspected that this may be a problem then the purge inlet port can be used to connect an air or nitrogen supply. This will remove any moisture in the air immediately surrounding the cell and prevent condensation. If using the purge inlet connector then the air or nitrogen supply must conform to the following specification:

- Compressed air to DIN 8573-1
- Oil = Class 1

- Water = Class 3
- Particulate = Class 3
- Pressure = 200 ± 20 kPa g

**Caution**

It is important that the purge air line supply conforms to the above specification. Failure to meet this specification may result in permanent damage to the instrument and invalidation of the warranty.

Telephone socket specification

The telephone socket for the remote support should have a direct internet connection.

Computer specification

The Zetasizer software can run on a network but it is not recommended as, with any software, running other software at the same time may be detrimental to the speed of the Zetasizer software.

**Minimum
specification of PC**

*Pentium PC 2GHz, 128MByte RAM, 80MByte free hard disk space,
1024 x 768 screen resolution, CD-ROM drive, One free USB port*

Operating platforms

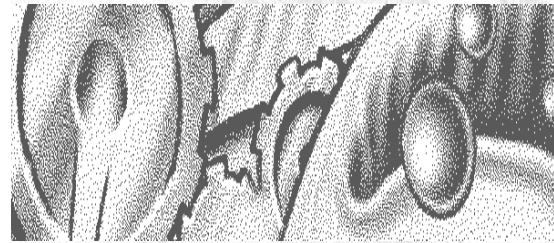
*Windows XP Professional (Service pack 1a or later)
Windows 2000 (Service pack 3 or later)*

Laser Safety

The Zetasizer Nano series instruments are Class 1 laser products and as such, require no special laser safety considerations during normal operation. However, during installation and servicing (which must be performed by a qualified Malvern representative), it is possible that the servicing engineer may be exposed to class 3b or above laser radiation. It is therefore recommended that the administrative controls recommendations of the laser safety regulations (IEC 60825-1(1993) +A1(1997)+A2(2001) are implemented.

Unpacking instructions

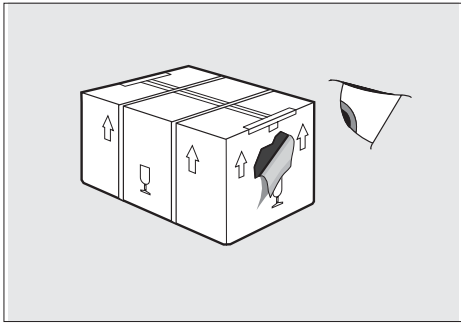
from MAN0317 Issue 1.0



A P P E N D I X D

Déballez l'instrument
Auspacken des Gerates
Desempaquetado del aparato
Desempacotamento do instrumento

1



If there is any sign of damage contact the freight carrier immediately.

Si vous constatez des signes de dommages quelconques, contactez immédiatement le transporteur.

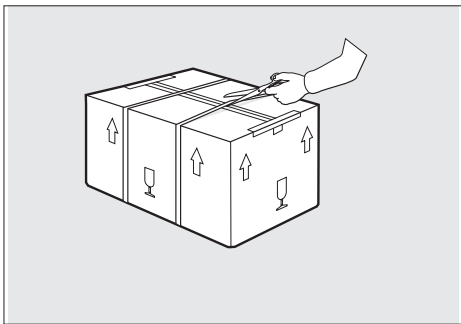
Bei irgendwelchen Zeichen von Transportschäden den Spediteur sofort benachrichtigen.

Si se advierte algún signo de deterioro, contactar inmediatamente con el transportista.

Contacte imediatamente a empresa transportadora se houver qualquer sinal de embalagem danificada.

ILL 6780

2



Cut through the plastic bonding straps.

Découpez les courroies d'attache en plastique.

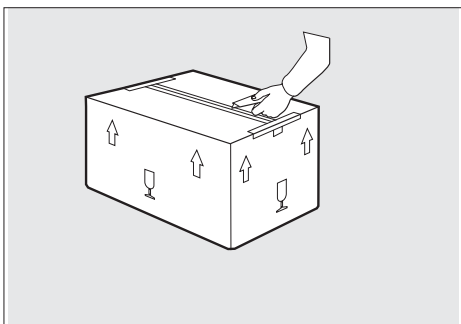
Die Kunststoffbänder durchschneiden.

Cortar las tiras de sujeción de plástico del embalaje.

Corte as tiras plásticas que prendem o conjunto.

ILL 6781

3



Carefully cut the plastic packing tape along the fold lines.

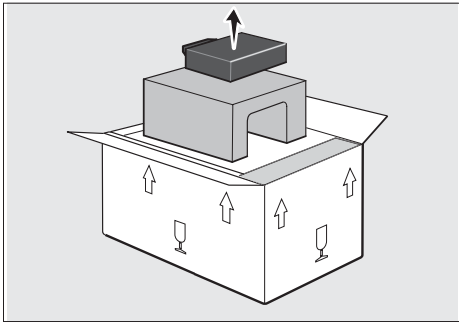
Découpez avec précaution le ruban d'emballage en plastique, le long des pliures.

Die Kunststoffverpackungsbänder vorsichtig entlang den Falzlinien durchschneiden.

Cortar con cuidado la cinta de embalaje de plástico por la líneas de doblado.

Com cuidado, corte a fita de embalagem plástica ao longo das dobras.

ILL 6782

4

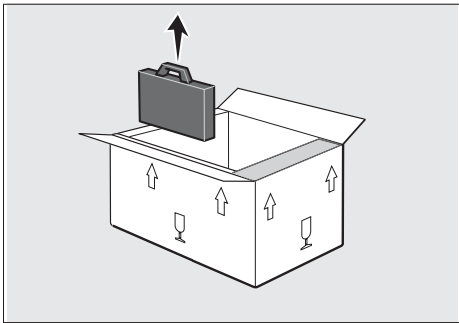
Open the wings of the carton and remove the top foam insert.

Ouvrez les rabats du carton et retirez les blocs de mousse du haut.

Die Klappen des Kartons öffnen und die oberen Schaumeinlagen entfernen.

Abrir los costados de la caja y extraer las piezas intercaladas de espuma de la parte superior.

Abra as abas da caixa e retire o enchimento de espuma que está em cima.

5

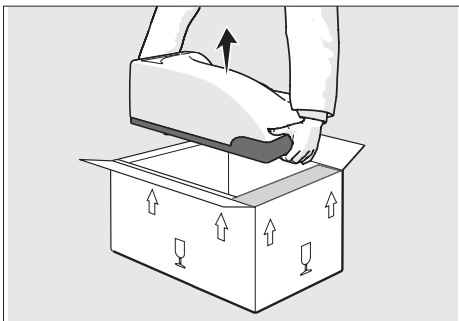
Remove all other packages from the carton and unwrap.

Sortez tous les autres paquets du carton et déballez-les.

Sämtliche andere Pakete aus dem Karton nehmen und auspacken.

Retirar todos los demás paquetes de la caja y desenvolverlos.

Retire todas as outras embalagens da caixa e abra-as.

6

Lift the instrument out of the carton and place on the workbench.

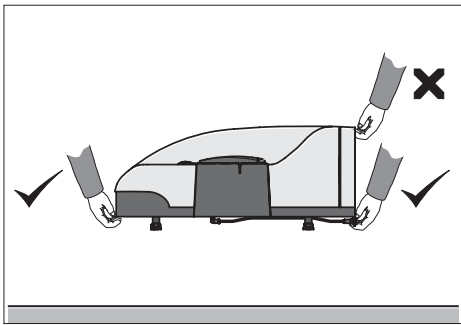
Soulevez l'instrument pour le sortir du carton, et posez-le sur le banc.

Das Gerät aus dem Karton heben und auf der Werkbank aufstellen.

Levantarse el aparato, sacarlo de la caja, y colocarlo sobre el banco de trabajo.

Tire o instrumento da caixa e coloque-o na bancada.

7



Never lift the instrument by its covers.

Pour soulever l'instrument, ne le tenez jamais par ses couvercles.

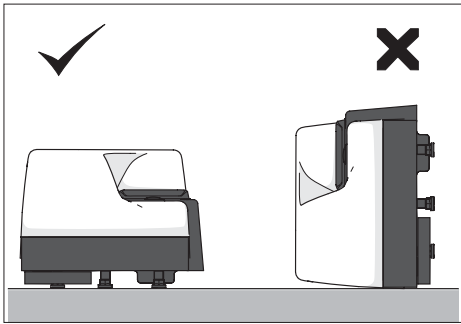
Niemals das Gerät an den Abdeckungen anheben.

No levantar nunca el aparato por sus cubiertas.

Nunca levante o instrumento pegando pelas suas tampas.

ILL 6727

8



Always place the instrument on its feet. Placing it on its side will damage the covers.

Posez toujours l'instrument sur ses pieds. Si vous le posez sur le côté, vous endommagerez les couvercles.

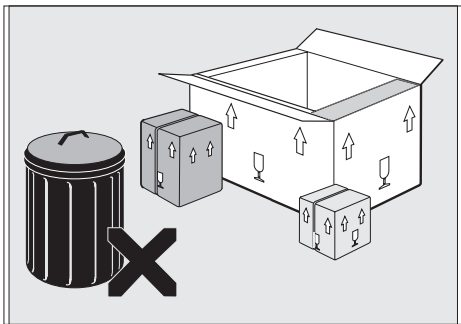
Das Gerät stets auf seinen Füßen aufstellen. Ein seitliches Aufliegen beschädigt die Abdeckungen.

Se debe colocar siempre el aparato sobre sus patas. Depositarlo de costado dañaría las cubiertas.

Coloque sempre o instrumento na vertical, sobre os seus pés. Se o colocar de lado irá danificar as tampas.

ILL 6728

9



Retain all packaging in case the instrument requires transporting in the future.

Conservez tous les emballages, au cas où l'instrument devrait être transporté ultérieurement.

Das gesamte Verpackungsmaterial aufbewahren, falls in Zukunft ein weiterer Transport des Geräts erforderlich wird.

Conservar todo el material de embalaje por si el aparato debiera ser transportado en el futuro.

Guarde todo o material de embalagem, caso seja necessário no futuro transportar o instrumento.

ILL 6783



Installation



A P P E N D I X E

Introduction

It should be noted that the Zetasizer Nano system should initially be commissioned by Malvern trained personnel. This appendix will show how to re-install the system, typically required under the following circumstances:

- **Moving the instrument.**

Should it become necessary to move the system from one laboratory to another, read the information in this appendix to correctly reconnect the system.

- **Changing the computer.**

It may be company policy to periodically upgrade computers. Detail is given on actions required.

Only install the system in a suitable environment as detailed in appendix C - Environmental conditions.



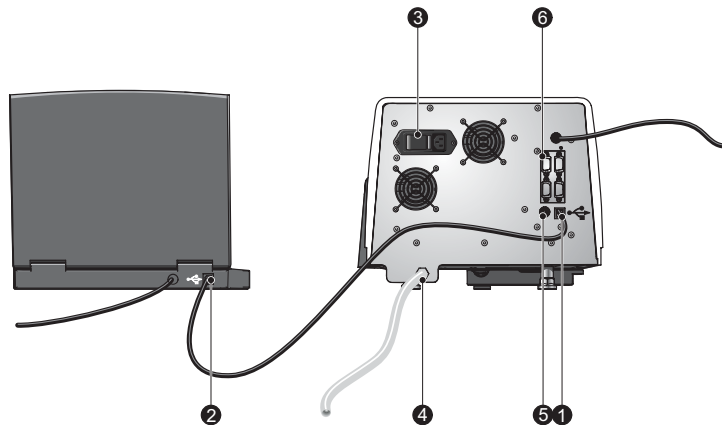
Do not obstruct the ventilation slots underneath the instrument, nor the fans on the rear panel.

Installing the Zetasizer Nano

Installing the Zetasizer Nano is very simple.

► **To install the system:**

- Connect the USB cable to the connector marked **USB** on the rear panel of the Zetasizer Nano instrument ①. Connect the other end of the cable to the USB connection on the computer ②.



ILL 6723

- Connect the power cable to the power socket on the rear panel of the instrument ③.



This product must be connected to a protective earth.

- Make all computer connections (mouse, keyboard, power, etc) by following the instructions provided with your computer.

Installation options

- If purge air is required connect to the purge air port at the base of the rear panel ④. Refer to specification in appendix C.
- If the instrument is fitted with the 532nm 'green' laser option connect the laser PSU to the PSU input on the rear panel ⑤.
- If accessories are used connect to the appropriate ports as described in their respective user manuals ⑥.

Changing the computer

If, at some point in the future, the computer used with the instrument is changed, the following actions will be required.

► To install the software:

- Insert the **Dispersion Technology Software** CD into the CD drive.
- If **Autorun** is enabled on the computer, the software will start to install automatically. Follow all on-screen instructions to complete the installation.
- If Autorun is not enabled, select **Start-Run-Setup.exe** and follow the on-screen instructions.

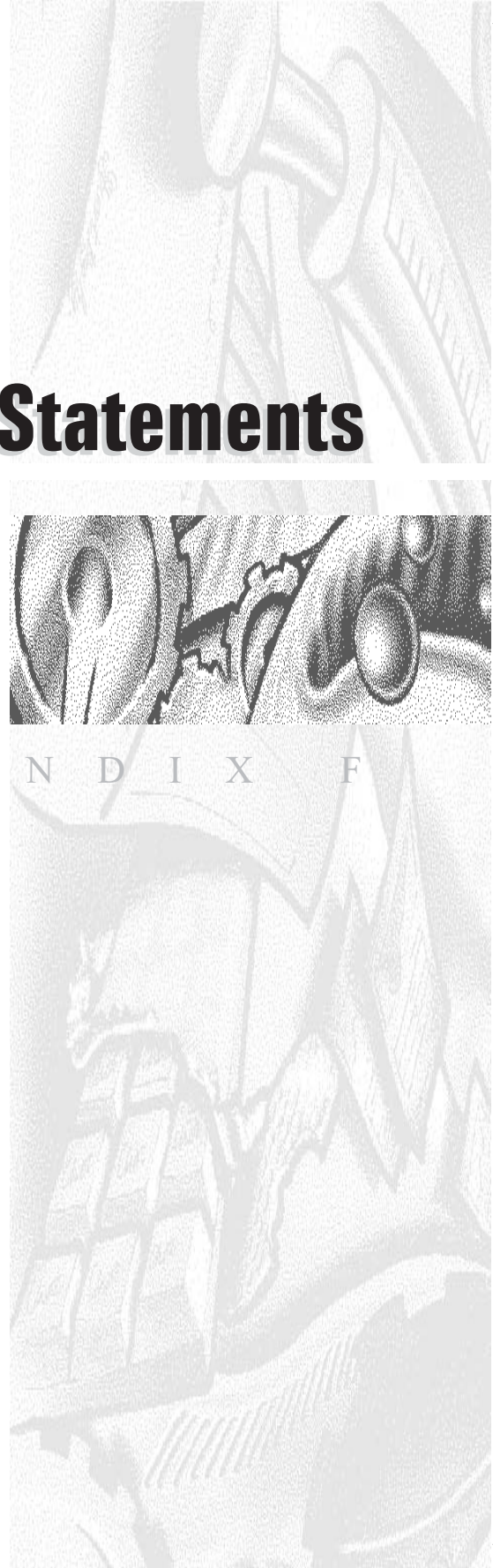
 **Note** ■ If the software is subsequently updated with a new version, any custom reports, parameter settings, SOPs, etc will be preserved.

Installing the Titrator

If the Zetasizer Nano is used in conjunction with a MPT2 Autotitrator follow the instructions in the Autotitrator manual on connecting the accessory to the optical unit and computer.

Regulatory Statements


A P P E N D I X F





CE Declaration of Conformity

The CE badge on this product signifies conformance to European Commission Directives.



CE Declaration of Conformity

Form: CE declaration of conformity for Nano size

Supplier details

Name (NAME OF MANUFACTURER) Malvern Instruments Limited	English company number (REGISTERED) 1020602
Address (ADDRESS OF MANUFACTURER) Enigma Business Park, Groewood Road, Malvern, Worcestershire, WR14 1XZ, UK	

Product details

Product description – name and model

Name : Nano

Model : ZEN1600; ZEN1690; ZEN1500; ZEN1590; ZEN2600; ZEN2500; ZEN3600; ZEN3690;
ZEN3500; ZEN3590

Description : Particle Characterization System

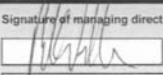
Directive(s) and Standard(s) to which conformity is declared

EMC directive 89/336/EEC as amended by 92/31/EEC and 93/68/EEC
Low Voltage Directive 73/23/EEC as amended by 93/68/EEC

BS EN 61326: 1998 Incorporating Amendments Nos. 1 and 2
BS EN 61010: 2001 Incorporating Corrigendum No. 1

Declaration

I hereby declare that the product specified conforms with the provisions of Council Directive(s) and standard(s) listed above.

Signature of managing director	Date
	25.06.03
Paul Walker	
PRINT NAME	

ILL 6784


Federal Communications Commission (FCC) Notice (US only)


The FCC mark on this product signifies conformance to FCC regulations relating to Radio Frequency Devices. These have been satisfied by testing the product against, and being found to be compliant with:

FCC CFR 47 Part 15: July 2001. Class A digital device.

The device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions:

- 1) This device may not cause harmful interference, and
- 2) this device must accept any interference received, including interference that may cause undesired operation.

 **Note** ■ This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

 **Note** ■ Changes or modifications not expressly approved by Malvern Instruments Limited could void the user's authority to operate the equipment.

Canadian Regulatory Information (Canada Only)

This digital apparatus does not exceed the Class A limits for radio noise emissions from digital apparatus set out in the Radio Interference Regulations of the Canadian Department of Communications.

Note that Canadian Department of Communications (DOC) regulations provide, that changes or modifications not expressly approved by Malvern Instruments Limited could void your authority to operate this equipment.

This Class A digital apparatus complies with Canadian ICES-003.

Cet appareil numérique de la classe A est conforme à la norme NMB-003 du Canada.





Index



Index

17° optics	15-11	Cleaning	7-2
173° optics	13-7	Dip cell	4-8, 15-12
21 CFR part 11	3-21, 4-24, 8-1	Filling	4-8
icon	3-21	Flare	13-8
90° optics	2-2, 13-7, 13-8	Flowcell	3-10
A		Identification	3-10
Absorption	9-11	Inserting	4-11
Access to the Instrument	1-3, 8-1	Molecular weight	4-6
Accessory output	3-6	Size	4-4
Administrator	8-2	Zeta potential	4-7
Advice	1-5	Cell (SOP)	9-2, 9-7
Air purge	3-6	Cell access button	3-8
Aqueous cell	4-8	Cell area	3-8
Aqueous samples	6-7	Cell area lid	3-9
Atomic mass units (amu)	2-4	Cell basin	3-10
Attenuation index	5-3, 5-5	Cell type	9-7
Attenuator	9-15, 15-12	Changing the computer	E-2
Autotitrator	2-3, 3-10, 4-7	Choosing the correct cell	4-3
	C-1	Cleaning cuvettes	7-2
Axis settings	5-6	Cleaning the instrument	7-1
B		Close button	4-18
Backscatter detection	13-7	Compensation optics	15-12
Badge	1-1	Computer	3-5, B-2
Batch printing	3-14, 10-7	Conductivity	5-11
Beeping	4-2	Configure	
Brownian motion	2-3, 13-2	Data exports templates	11-3
Build option	1-5	Security	8-2
Button bar	4-17	View/report selection	3-16, 10-6
C			12-12
CAN Ports	3-6	Configure menu	3-16
Canadian Regulatory Information	F-3	Connections	3-5
CE Declaration of Conformity	F-1	Control palette	12-2
Cell	1-4, 3-12, 4-3	Correlation function	4-19, 13-3
Choosing	4-3	Correlator	13-3, 13-7
		Count rate	4-18, 5-3, 5-10
		Create PDF	3-14
		Creating an export template	11-3
		Creating an SOP	9-2
		Cumulants mean	5-5
		Custom reports	12-1

Cuvette	1-4, 3-12, 4-3	E	
Cleaning	7-2, 7-3	Earth connection	A-2, E-2
Glass	4-4, 4-5, 4-6	Earthing	A-2
	4-8	Edit menu	3-15
Molecular weight	4-6	Edit result	3-15, 4-23
Polystyrene	4-4, 4-8	Electrical warnings	A-1
Position	5-4, 9-15	Electrodes	3-9
Solvents	4-4, 4-5, 4-6	Electroosmosis	15-3, 15-6
	4-7	Avoiding	15-7
Use with solvents	4-4	Electrophoresis	2-5, 15-3, 15-4
Cuvette holder	3-11	Electrophoretic Mobility	15-1, 15-5
Cuvette type	9-7	Engineering	3-18
D		Equilibration	9-14
Daltons	2-5, 14-4	Existing SOP	3-16
Data Export Templates	3-17	Exit	3-14
Debye plot	6-5, 14-4	Export	
Detector	B-1	File	3-14
Diffuse layer	2-6	Parameters	11-2
Digital signal processor	13-7, 15-12	Results	11-1
Dimensions	B-1, C-2	Settings	11-2
Dip cell	2-3, 4-8, 4-9	Template	11-3
	4-13, 15-12	Export (SOP)	9-3, 9-9
Cleaning	7-2	Extract SOP	3-15, 9-1, 9-29
Dispersant properties		F	
Size	9-10	F(ka)	5-10, 9-22
Zeta potential	9-22	Fans	3-6
Dispersion Technology Software	E-2	Ventilation slots	3-6, E-1
Displaying the results	5-1	Fast Field Reversal	15-8
Disposing of the instrument	A-4	FCC	F-2
DLS	2-3, 13-1, 14-1	Federal Communications Commission	
	15-1		F-2
dn/dc	5-8, 14-2	FFR	15-8
Drainage	3-11	File	
DTS	E-2	Open	3-14, 3-19
Dust	4-19	File menu	3-14
Dynamic Light Scattering	2-3, 13-1	Filling the cell	4-8
		Filtration	6-4
		Flare	13-8
		Flowcell	3-10



Folded capillary cell	3-12, 4-4, 4-7	Isoelectric point	15-3
Cleaning	7-3		
Thermal contact plates	3-12	L	
Fuse holder	3-5	Labels (SOP)	4-15, 9-2, 9-5
Fuses	7-4	Laser	2-2
		532nm 'green' PSU connection	3-6
G		Class	B-1
General Purpose model		Safety	A-2, C-4
Size	9-16	Safety interlock	3-9
Zeta potential	9-26	Type	1-1
Graph		Warning labels	A-2
Properties	5-6, 5-9, 5-11	Laser Doppler Velocimetry	15-1, 15-5
Tool	12-6	LDV	15-1, 15-5
Graticule	5-7	Lid	3-9
Green badge	1-1	Lifting	D-3
		Lifting techniques	A-4
H		Limits	5-6
Handling the measurement files	10-7	Line styles	3-17, 12-9
Health and safety	A-1	Log sheet	4-19
Help		Login and logout	8-3
About	1-5	Luer connection	4-7
Desk	1-5		
Menu	3-19	M	
Henry equation	15-1, 15-4	M3	15-7
How to use this manual	1-2	Benefits	15-9
Huckel approximation	15-5	M3-PALS	15-7
Humidity	B-2, C-2	Maintenance	7-1
		Malvern logo / (M)	3-22, 10-1
I		Malvern personnel	1-3
Icon	4-3	Manual measurement	3-2, 4-16
Initialisation routine	4-2	SOP dialogues	4-16
Inserting the Cell	4-11	Mark-Houwink	9-12
Installation	E-1	Material properties	
Installing the Zetasizer Nano	E-1	Molecular weight	9-19
Instructions (SOP)	9-2, 9-6	Size	9-10
Intensity distributions	13-5	Zeta potential	9-22
Interlocks	3-9	Measure menu	3-17
Interpreting scattering intensity	13-2		
Ions	2-5		

Measurement		Molecular weight	
Aborting	4-18	Basic explanation	2-4
Delay	9-15	Cuvette	4-6
Display	3-21, 4-17	Debye plot	14-1, 14-4
Duration	9-14, 9-24	Filling the cell	4-9
File window	3-20, 10-1	Inserting the cell	4-11
Manual	3-2, 4-1, 4-16	Measurements	4-22
Number of	9-15	Range	2-2
Opening a file	4-14	Sample concentrations	6-5
Position	5-4, 9-15	Sample preparation	6-5
Quick guide	4-2	Size range	B-1
Runs	4-21	SOP	9-18
Sequence	4-21	Standard	14-2
SOP	3-2, 4-1, 4-14	Theory	14-1
	4-15	What is...	2-4
Measurement (SOP)	9-3, 9-13, 9-20	Monomodal (Zeta potential)	9-26
	9-24	Moveable lens	13-8
Measurement file window	3-20, 10-1	Moving the system	A-4
Measurement type (SOP)	9-2, 9-4	Multiple Narrow Modes	9-16
Melting point measurements	4-21		
Menu bar	3-14	N	
Configure menu	3-16	Naming convention	1-4
Edit menu	3-15	Nano icon	1-1
File menu	3-14	Narrow band filter	2-2
Help menu	3-19	Navigation toolbar	10-7
Measure menu	3-17	New SOP	3-16
Security menu	3-18	NIBS	13-7, B-1
Tools menu	3-18	Nitrogen purge	3-6
View menu	3-16	Non-aqueous cell	4-8
Window menu	3-19	Non-aqueous samples	6-7
Menu commands	1-4	Non-Invasive Back-Scatter	13-7
Mie theory	13-5	Non-Polar samples	6-7
Model		Number distributions	13-5
Number	1-5, 3-6		
Range	1-1, 2-1	O	
Type	1-1, 5-4	Open file	3-14, 4-14
Modifying an SOP	9-29	Operating conditions	B-2
Modulator	15-6, 15-10	Operator	1-2, 1-3
		Optical quality	4-4, 4-5, 4-6
			4-7

Optical unit	3-5	Warnings	A-3
Optics		Q	
17 deg	15-11	Quick guide to making a measurement	4-2
173 deg	13-7		
90 deg	2-2, 13-7	R	
Option label	2-3	Rayleigh equation	14-2
Oval badge	1-1	Rayleigh scattering	14-3
P		Rear Panel	3-5
PALS	15-7, 15-10	Record view	3-20, 5-1, 10-4
Parameters		Records	
Adding	10-4	Copying	10-7
Deleting	10-4	Deleting	10-7
Export	11-2	Moving	10-7
Record view	10-4	Sorting	10-7
With arguments	10-5, 11-4	Red badge	1-1
Passwords	8-6	Refractive index	9-11
PAT Testing	A-2	Regulatory statements	F-0
PDF	3-14, 9-8	Remote support	1-5
Peak modes	5-6, 5-11	Report	
Permissions (Security)	8-1	Melting point	5-11
pH	15-2	Molecular weight	5-8
Phase Analysis Light Scattering	15-7	Size	5-2
Phase change	15-10	Trend	5-11
Pinch valve	3-10	Zeta potential	5-9
Polar samples	6-7	Report Designer	
Pollution degree	C-2	Calculation	12-5
Polydispersity	5-5, 5-6	Control palette	12-1, 12-2
Positioning the system	C-1	Frame tool	12-5
Power		Graph tool	12-6
Input socket	3-5	Page layout	12-1, 12-11
Requirements	B-2, C-3	Parameter tool	12-4
Switch	3-5	Picture tool	12-5
Print results	9-8	Printed version	12-1
Progress meter	4-18	Screen layout	12-1, 12-11
Protein melting point measurements	4-21	Table tool	12-10
Purge		Text tool	12-4
Connection	3-6	Toolbox	12-2
Specification	3-7, C-3	Report tab	3-20, 5-2, 10-6

Reports (SOP)	9-3, 9-8	Shape model	5-9
Result calculation (SOP)	9-3, 9-16, 9-25	Site requirements	C-0
Results	5-5, 5-9, 5-11	Size	
Editing	4-23	Basic explanation	2-3
RS232 (IOIOI) Ports	3-6	Cell	4-4
Runs	4-21	Cuvette	4-4
		Filling the cell	4-9
		Inserting the cell	4-11
		Measurements	4-21
		Operation	13-1
		Range	2-2, 9-17, 9-26
			B-1
		Sample preparation	6-1
		SOP	9-10
		Theory	13-1
		What is...	2-3
		Slipping plane	2-6, 15-1
		Slow Field Reversal	15-8
		SLS	14-1
		Smoluchowski approximation	15-4
		Software	3-13
		Installing	E-2
		Software version	3-19
		Starting	4-3
		Version	1-5
		Solvent properties (Molecular weight)	9-19
		SOP	3-2
		Creating	9-2
		Distributing	9-30
		Existing	3-16
		Extracting	3-15, 9-1
		Extracting an...	9-29
		Measurement type	9-4
		Measurements	3-2, 4-14
		Melting point	9-27
		Modifying	9-1, 9-29
		Molecular weight	9-18
		New	3-16
		Open SOP	4-15
Safety	A-1		
Sample (SOP)	9-2, 9-10, 9-18		
	9-22		
Sample preparation	4-3		
Concentration	6-1		
Filtration	6-4		
Gelation	6-2		
Large particles	6-3		
Molecular weight	6-5		
Size	6-1		
Small particles	6-2		
Ultrasonics	6-4		
Zeta potential	6-6		
Sample volume			
Minimum	4-4, 4-5, 4-6		
	4-7, 4-9		
Save file	3-14		
Scattering intensity fluctuations	13-1		
Second Virial Coefficient	5-9, 14-1		
Security	3-18, 8-1		
Editing a user	8-5		
Editing a user group	8-4		
Enabling	8-3		
Login and logout	8-3		
Passwords	8-6		
Security menu	3-18		
Serial number	1-1, 1-5, 3-6		
Serial port	3-18		
Settings	4-17		
Settings button	4-15, 4-17		
SFR	15-8		
Shape correction model	9-21		



Size measurements 9-10

Starting 4-15

Trend 9-27

Zeta potential measurements 9-22

SOP dialogues

- Cell 9-2, 9-7
- Export 9-3, 9-9
- Instructions 9-2, 9-6
- Labels 9-2, 9-5
- Measurement 9-3, 9-13, 9-20
9-24
- Measurement type 9-4
- Melting point 9-27
- Reports 9-3, 9-8
- Result calculation 9-3, 9-16, 9-25
- Sample 9-2, 9-10, 9-18
9-22
- Trend 9-27

SOP measurement 9-2, 9-10, 9-18
9-22

Space required C-2

Specification B-1

- Computer C-4
- Purge air C-3

Spillage 3-11, 7-1

Standard 14-2

- Properties 9-19

Standard Operating Procedure 3-2

Start button 4-17

Starting the software 4-3

Static light scattering 14-1

Status bar 3-16, 3-21
4-18

Status indicator 3-7

Stokes-Einstein equation 13-2

Stop button 4-17

Stopper 4-10

Summary 10-2

Supervisor 1-2, 1-3

T

Tab dialogues 4-18

Tables 12-10

Temperature

- Equilibration 9-14, 9-28
- Operating C-1
- Range 3-8, B-1
- SOP settings 9-14, 9-21
9-24
- Storage C-1
- Warnings A-3

Theory of operation 14-1, 15-1

Thermal cap 3-10, 3-12

Thermal contact plates 3-12, 4-12

Thresholds 9-17, 9-26

Tip of the day 3-19

Title bar 3-21

Toluene 14-2

Toolbars 3-19

Tools menu 3-18

Trend (SOP) 9-3

Trend measurements 4-21

Turning on 4-2

Tutorials 3-19

U

Ultrasonics 6-4

Universal 'Dip' cell 2-3, 4-8, 4-9
4-13, 15-12

- Cleaning 7-2

Unpacking instructions D-0

Upper/Lower limits 5-6

USB E-1

User groups 8-3

Users 8-5

V

View menu 3-16

Viscosity 9-12

Volume distributions 13-5

W

Warning triangle	3-8, 3-10
Warnings	
Electrical	A-1
Sample handling	A-3
Temperature	A-3
Weight	B-1, C-1
What does the Zetasizer Nano do?	2-1
Workspace	3-20, 10-2

Z

Z-average	5-5
Zeta potential	
Basic explanation	2-5
Cell	4-7
Dip cell	4-8
Filling the cell	4-9
Huckel	15-5
Inserting the cell	4-12
M3	15-7
Measurements	4-22
Modulator	15-6
Operation	15-11
PALS	15-7
Sample preparation	6-6
Size range	2-2, B-1
Smoluchowski	15-4
SOP	9-22
Theory	15-1
What is...	2-5
Zetasizer Nano	3-1
Cleaning	7-1
Features	3-4
Model range	1-1

SECTION II: UCSB POLICIES, PROCEDURES AND RESOURCES

Introduction: UCSB Policies, Procedures and Resources	2
Emergency Preparedness and Response	
Emergency Response Procedures	3
Recommended Chemical Spill Cleanup Procedures	4
Fire Extinguishers, First-Aid Kits and Emergency Shower/Eyewashes	5
Personal Protective Equipment	
Campus Policy on Laboratory Personal Protective Equipment (PPE)	6
Laboratory PPE Use Poster	7
Personal Protective Equipment in UCSB Storerooms	8
Respiratory Protection Program	9
Selecting the Proper Gloves	10
Chemical Safety	
Exposure Limits for Chemicals	11
Safety Data Sheets (MSDS)	13
Chemical Labeling	14
Identification and Classification of Hazardous Chemicals	15
Carcinogen Safety Program	19
General Procedures for Working with Hazardous Chemicals	21
Fume Hood Usage Guide: Standard Hoods	26
Fume Hood Usage Guide: Variable Air Volume Hoods	27
Safe Storage of Chemicals	28
Refrigerators in Labs	30
Laboratory Safety Training	
Campus Policy on Laboratory Safety Training	31
Non-UCSB Online Laboratory Safety Training	32
Laboratory Inspection Program	
Laboratory Self-Inspection Checklist	33
Waste Disposal	
Chemical Waste Disposal	40
Disposal of Sharps	41

Introduction to Section II: UCSB Policies, Procedures and Resources:

Section II addresses the campus policies, procedures and resources which are core/universal and apply to all/most labs. In order to free lab supervisors from independently having to address these issues in their CHPs, they are provided herein. In Section I of this *Chemical Hygiene Plan* the *laboratory-specific* issues/Standard Operating Procedures for a particular lab must be addressed.

The issues summarized here are a formal part of the *UCSB Chemical Hygiene Plan*. Therefore, all lab personnel are responsible for being familiar with this information and following the prescriptions therein as they apply to their work. Virtually all of the issues addressed herein are based on current regulations and codes, such as those of the California Occupational Safety and Health Administration (Cal-OSHA); Cal-EPA; California Fire Code, etc.

An important aspect of the Cal-OSHA CHP safety standard is that it requires the addressing of *chemical* safety issues, but not other laboratory hazards. For example, biological and radiological hazards, electricity, high/low temperature and pressure, etc. Therefore, these issues are largely not addressed here. However, supervisors are free/encouraged to address these issues in their CHP. Additional information on these non-chemical safety topics can be found on the EH&S website:

<http://www.ehs.ucsb.edu/units/labsfty/labsafety.html>

Some of the terms used in this document and other safety documents such as Safety Data Sheets (formerly MSDS) may be unfamiliar to some. To aid in clarifying these terms, a **Glossary of Safety Terms** is provided in App. R of this document:

<http://www.ehs.ucla.edu/ChemicalHygienePlan.pdf>



Emergency Response Procedures

The primary informational tool for response to campus incidents is the *UCSB Emergency Information Flipchart* pictured below. This document **should already be posted** in, or near, every laboratory, as well as in many offices. If you want a copy for your work area, contact EH&S at x-4899. A Spanish language version of the flipchart is also available.

The last page (at right) should already be customized to include your *local* building information –such as the locations of the following: your building’s Emergency Assembly Point, fire extinguishers and fire alarm pull stations, first-aid kits, AEDs, etc. If it is not customized contact your local Department Safety Rep. Please familiarize yourself with the layout and general content of the flipchart. It can also be viewed online at:

http://www.ehs.ucsb.edu/homepage/hprsc/203655_Emerg_Flipchart.pdf

EMERGENCY

911
9-911
805-893-3446
888-488-UCSB
(888-488-9272)
888-SB-ROADS
(888-727-6237)
800-427-ROAD
(800-427-7623)
211
(800-400-1572)
1610 AM
(Within 3 miles of UCSB)
91.9 FM (KCSB)
www.ucsb.edu

Emergency
Emergency Campus Telephones
UCSB Police Dispatch (From cell phones)
Campus Emergency Information (Toll Free)
Santa Barbara Road Conditions (Toll Free)
California Road Conditions (Toll Free)
SB County Health & Human Services
(From cell phones)
Parking/Emergency Information
Campus Radio Station
UCSB Campus Web Site

UCSB
Environmental Health & Safety 2008

EMERGENCY MANAGEMENT AT UCSB
CIVIL DISTURBANCE OR DEMONSTRATION
EXPOSURE TO BLOOD OR OTHER POTENTIALLY INFECTIOUS MATERIALS
SHELTER-IN-PLACE
MEDICAL EMERGENCY
SEVERE WEATHER
FIRE
SUSPICIOUS PACKAGE/MAIL
RADIOACTIVE CONTAMINATION/SPILLS
CHEMICAL INCIDENTS
VIOLENCE/CRIME IN PROGRESS/ACTIVE SHOOTER
EARTHQUAKE / TSUNAMI
EVACUATION OF CAMPUS/BUILDINGS/PERSONS WITH DISABILITIES
BOMB THREATS
UTILITY FAILURE / POWER OUTAGES IN LABORATORIES
BUILDING-SPECIFIC EMERGENCY INFORMATION

BUILDING-SPECIFIC EMERGENCY INFORMATION

A copy of this completed document should be posted on departmental safety bulletin boards along with a building floor plan.

Building Name: _____ Building Number: _____
Building Exit Routes (Note the general locations of exits, e.g., exit stairwell is located on the north side): _____

Building Emergency Assembly Point
(View <http://ehs.ucsb.edu/emap> for the most recent list of the Emergency Assembly Points)

Primary Location: _____ Secondary Location: _____

Paste Photo Here
(optional)

Paste Photo Here
(optional)

Fire extinguishers and fire alarm pull station

Extinguishers are generally located at the **ends of exit hallways** and/or exit doors. Inside labs, they are located **near the exit door**.

Pull stations are generally located at regular hallway intervals and at the ends of exit hallways.

Type of fire alarm signal for the building
(Check all that apply. If in doubt, contact the EH&S Fire Safety Division.)

Bells
 Horns/Strobes
 Strobe Flashes

Department Safety Rep: _____ Phone: _____
Alternate Department Safety Rep: _____ Phone: _____

Building Resources

Location of First Aid Kits: _____
(Departmental and/or local work areas)

Location of Automated External Defibrillator (AED), if available: _____
(Device to restore normal heart rhythm to patients in cardiac arrest)

Location of other Emergency Resources (e.g., food, water, radios, flashlights, spill cleanup supplies, etc.) _____

For more information on emergency preparedness and fire prevention visit Environmental Health & Safety online <http://ehs.ucsb.edu>.
Date Prepared: _____ (An electronic version is on the EH&S Web site at <http://ehs.ucsb.edu>.)

BUILDING-SPECIFIC EMERGENCY INFORMATION

Recommended Chemical Spill Cleanup Procedures

You should NOT clean up a spill if:

- You don't know what the spilled material is
- You lack the necessary protection or equipment to do the job safely
- The spill is too large to contain
- The spilled material is highly toxic
- You feel any symptoms of exposure

Instead contact: **x3194** EH&S (24 hr line – after-hours may have to wait up to 30 min for response to campus). OR, if immediately health-threatening call **9-911** (campus phone)

Spill Response Scheme:

Evaluate and Notify

- Assess the toxicity, flammability, or other properties of material (see label & MSDS)
- For flammables, remove or turn off ignition sources such as motors, pumps, fridges.
- Determine if there is an immediate health threat to you or your neighbors. If so, alert neighbors, isolate the area and call for help using the phone numbers above.
- If spill is minor, begin cleanup following steps below

Containment/Cleanup

- Don appropriate gloves, eye protection, lab coat, etc.
- Contain and absorb spill using absorbents appropriate for the material*
- Protect floor drains with absorbents or barriers around them
- Package and label waste. Include contaminated clothes, rags, equipment, etc.
- Store temporarily in a fume hood if material is volatile

Followup

- Send [Hazardous Materials/ Waste Pickup Request](#) form to EH&S
- Reorder and restock cleanup materials used
- Inform EH&S if there were any personnel exposures, or release to the environment



*Self-help spill cleanup equipment are available using graduate master keys in some buildings: <http://www.ehs.ucsb.edu/units/labsfty/labrsc/emergency/spillcloset.htm>

Fire Extinguishers, First-Aid Kits and Emergency Showers/Eyewashes

Fire Extinguishers: Extinguisher(s) are in every lab – typically by the exit door and are the **ABC** variety (dry powder, ammonium phosphate). EH&S conducts hands-on fire extinguisher training for most individuals who attend the EH&S General Laboratory Safety class. There is also an online extinguisher tutorial/video that individuals can complete and receive credit. All campus individuals are strongly encouraged to complete this tutorial and view as refresher training when needed.

Online Fire Extinguisher Usage Tutorial:

<http://learningcenter.ucsb.edu/default.aspx>

Need “UCSB Net ID” to login. Then search on “fire extinguisher”

First-Aid Kits: Individual laboratories should have their own 1st aid kit nearby in a location known to all. Supplies should be checked regularly. Departmental kits may not be accessible after-hours.

Emergency Showers and Eyewashes

- Know where your nearest unit is – they are typically within the lab, or in the corridor nearby. Units must be accessible always- no items should block access.
- In the case of chemical exposure to eyes or skin, flush the injury for a minimum of 15 minutes. Be sure to leave the eyes open under the water to flush them.
- Showers can also be used to extinguish a fire on an individual, or their clothing
- Consult the Safety Data Sheet (SDS) for material and show it to the doctor/nurse.
- Facilities periodically flushes emergency eyewash stations and showers. Lab personnel should also flush the eyewashes at least monthly as a precautionary measure. Call Facilities at x2661 if you have concerns regarding a specific unit.
- Eyewashes are plumbed with potable water - unlike the rest of the laboratory which is often on "industrial water"- and is considered safe to use on your body.
- Many eyewash/shower units are not equipped with a floor drain. This is because they are so infrequently used that they did not justify the cost of a drain when the building was constructed. Also, it is illegal to flush materials down the drain.



Campus Policy on Laboratory Personal Protective Equipment (PPE)

In July 2011 UCSB instituted a new policy on the use of personal protective equipment (PPE) in laboratories. The policy is intended to help protect lab workers from injury, meet regulatory requirements for worker protection and bring more consistency to PPE practices across the campus. All members of the lab community have responsibilities under the policy - particularly lab supervisors/faculty and lab workers. Full policy is at:




<http://www.policy.ucsb.edu/policies/policy-docs/lab-personal-protective-equip.pdf>

Key aspects of the policy are summarized on a wall poster which is mounted in all labs, or lab suites. A copy of the poster is presented on the following page. Other notable aspects of the policy are noted here, but the full policy should be consulted for details:

- The PPE requirements only apply when “handling” hazardous materials, not when an individual is simply in the same room with materials.
- Radiological and biological hazards are not covered by the policy. Instead, PPE for these is regulated by the Radiation and Biosafety Committees, respectively.
- Lab gloves must not be worn in public areas unless traveling to an adjacent lab space, or needed for protection while transporting materials between work areas – in which case clean gloves should be donned.
- Safety eyewear must meet American National Standards Institute (ANSI) requirements. This is denoted by the “Z87” stamp on the eyewear. Most reading glasses do **not** meet ANSI standards. Approved safety glasses can be worn over contact lenses, or splash goggles can be worn over reading glasses, or one can obtain prescription safety glasses.
- Contaminated lab coats should not be laundered at home. Some campus labs use a local vendor to provide routine lab coat laundering services. The service is inexpensive. See:
http://www.ehs.ucsb.edu/units/labsfty/labrsc/factsheets/Lab_Coats_FS35.pdf



Personal Protective Equipment (“PPE”) Required for UCSB Laboratory Workers

	CATEGORY 1: Significantly Hazardous Chemicals	CATEGORY 2: Hazardous Chemicals Not in Category 1	CATEGORY 3: Selected Physical Hazards
<p>This poster summarizes the key elements of the UCSB Laboratory Personal Protective Equipment Policy.</p> <p>The full Policy can be found at: http://www.policy.ucsb.edu/policies/az/ (click on "L") The full Policy is too detailed to be fully shown here and should be consulted as needed.</p> <p>MINIMUM PERSONAL PROTECTIVE EQUIPMENT REQUIRED</p>	<p>EXAMPLES^b:</p> <ul style="list-style-type: none"> Corrosives (above 5% by weight acids/bases)^d Flammables/Combustibles above one liter Materials Absorbed Through Skin Skin and Eye Irritants Highly Toxics and Neurotoxins Strong Air or Water Reactives, e.g. Pyrophorics^e Strong Oxidizing Agents Carcinogens and Reproductive Toxins 	<p>EXAMPLES^b:</p> <ul style="list-style-type: none"> Dilute Corrosives (below 5% by wt. acids/bases)^d Flammables/Combustibles less than one liter Liquid Cryogenics (LN₂, solvent/dry ice baths) 	<p>EXAMPLES^b:</p> <ul style="list-style-type: none"> Glassware under vacuum Vessels under pressure Mechanical hazards Hazardous chemicals not in Category 1,2
<p>“ANSI-Approved” Safety Eyewear^a</p> 	YES	YES	YES
<p>Appropriate Laboratory Gloves^c</p> 	YES	YES	RECOMMENDED
<p>Lab Coat, or Fire-Resistant Lab Coat</p> 	YES (for flammables use fire-resistant coat)	RECOMMENDED	RECOMMENDED
<p>Close-toed Shoes and Long Pants</p> 	YES	RECOMMENDED	RECOMMENDED

The Policy prescribes the minimum PPE required, but can't foresee all circumstances. So, in some cases additional PPE may be needed to adequately protect workers and meet regulatory requirements. These requirements only apply when an individual is directly "handling" a material in such a way as to have a reasonable possibility of being exposed. Questions or feedback are welcome and should be directed to UCSB Environmental Health & Safety, x-4699, or x-6243.

Footnotes

- Meet *American National Standards Institute* standards as indicated by "Z87" stamp on frame. Most "reading glasses" do not meet this standard. Eyewear in campus storerooms meet ANSI standards, as do prescription safety glasses from optometrists. Alternatively, safety goggles may be worn over glasses.
- Consult container label or MSDS to verify chemical hazard class(es). Definitions and specific chemical examples are provided in the full Policy.
- Consult container label, MSDS or a Glove Reference Chart to determine appropriate glove. Glove charts are available via the EH&S Website and other sites.
- Definition of "dilute corrosive" as below 5% by weight is only for purposes of this Policy. Corrosives can have other properties requiring gloves at any concentration, e.g., HF acid is particularly penetrative/damaging to skin.
- While handling flammable liquids (particularly pyrophorics), it is strongly recommended that synthetic clothing not be worn which is more ignitable than cotton.

Additional Policy Elements

- ⇒ Lab gloves should not be worn in public areas except where clearly needed for personal protection, e.g., during transportation of some particularly hazardous materials
- ⇒ PPE for infectious biological agents and radioactive materials are not covered by this Policy. They are regulated independently by the campus Biosafety and Radiation Safety Committees, respectively.

Campus Storerooms: Laboratory Personal Protective Equipment

You can buy supplies from any campus storeroom after establishing an account. The supplies noted below are only the basic PPE available. There are many more materials, styles and brands of PPE available thru safety products suppliers (e.g., *Fisher Scientific* and *Lab Safety Supply*)

Biological Sciences Storeroom, Bldg. 569, x-2537 or 3234

- **Gloves** - Nitrile; Latex (powdered); Vinyl examination; Wash gloves (Playtex); High/low temperature
- **Eyewear** - Goggles; Glasses; Goggles that fit over reading glasses
- **Lab coats** - Cotton (fire-resistant)
- **Sharps disposal** - Syringe/razor size; Broken glass box
- **Waste disposal bags** - Autoclaveable; Biohazard; Hazardous materials
- **First aid supplies** - Band-aids; Cotton

Chemistry Storeroom, room 1432, x-2563

- **Lab Coats** - Cotton (fire-resistant)
- **Gloves** - Nitrile; Latex; "Wash gloves"; Cleanroom glove (triple polymer)
- **Eyewear** – Safety Glasses; Goggles that fit over reading glasses
- **Sharps disposal** - Syringe/razor size
- **First aid supplies** - Bandages; Gauze pads; Burn cream

Physics Storeroom, Broida Hall, room 1301, x-2747

- **Lab Coats** - Cotton (fire-resistant)
- **Gloves** - Nitrile; Neoprene; Latex; Vinyl; Wash gloves (Playtex); Canvas; Leather; Nylon; Finger cots (latex)
- **Eyewear** - Goggles; Goggles that fit over reading glasses; Glasses w/ side shields; "Neon"-colored glasses; "Malibu" glasses (wraparound)
- **Respirator** - Dust mask
- **Ear plugs** - foam type
- **Sharps disposal** - Broken glass box
- **Waste disposal** - Glass and plastic 1-gallon containers
- **First aid** - Band-aids

University Center Bookstore, x-3271

- **Eyewear** - Goggles; Glasses
- **Lab coats** – Cotton/polyester



Respiratory Protection

Typically, respiratory protection is not needed in a laboratory to eliminate exposures, or keep exposure levels below OSHA Permissible Exposure Limits. Under most circumstances, safe work practices, small scale usage, and engineering controls (fume hoods, biosafety cabinets, and general ventilation) adequately protect laboratory workers from chemical and biological hazards. Under certain circumstances, however, respiratory protection may be needed as determined by EH&S. These can include:

- An accidental spill such as:
 - a chemical spill outside the fume hood
 - a spill of biohazardous material outside a biosafety cabinet
- Performance of an unusual operation that cannot be conducted under the fume hood or biosafety cabinet
- When weighing powdered chemicals or microbiological media outside a glove box or other protective enclosure. Disposable filtering face-piece respirators are generally recommended for nuisance dusts. If the chemicals are toxic, contact EH&S for additional evaluation
- When exposure monitoring indicates that exposures exist that cannot be controlled by engineering or administrative controls
- As required by a specific laboratory protocol or as defined by applicable regulations

Affected individuals must enroll in the *UCSB Respiratory Protection Program* which is designed to fully meet Cal-OSHA requirements (CCR, Title 8, 5144). Because there are numerous types of respirators available, and each has specific limitations and applications, respirator selection and use requires pre-approval by EH&S. For either required or voluntary use of a respirator, the worker must contact EH&S (x-8787, or x-3743). After an evaluation of the work situation, if a respirator is deemed necessary, EH&S will provide the required medical evaluation (questionnaire), training and quantitative fit-testing.



Selecting the Proper Gloves

The correct gloves protect the hands against chemicals; the wrong gloves enhance chemical contact. The type of glove used should be chosen to be compatible with the particular chemicals being used. **There is no universal glove that protects you from all chemicals. To choose the correct glove, go to a Glove Reference Chart.** (links below).

Disposable gloves provide minimal protection and should be used with this in mind. If using concentrated solvents, corrosives or toxics, more heavy-duty gloves should be worn. These provide more protection, but have the drawback of being more cumbersome. Note also that about 15% of the population is allergic to latex <http://www.cdc.gov/niosh/docs/97-135/> to some degree.

Check gloves before use for signs of wear or penetration. Disposable gloves can be inflated by mouth to check for pinholes. When removing gloves, be careful to avoid touching the outside of the gloves with your bare hands. Always remove gloves before leaving the lab.

All gloves are permeable, only the permeation rate varies, depending on the chemical, the glove material and thickness, temperature, concentration gradient, etc. However, once a material begins to permeate the glove, it will continue until an equilibrium is reached. You must, therefore, decide when it is appropriate to discard contaminated gloves.

Glove Reference Charts (No guarantees are made regarding the accuracy of these charts. Recommend cross-checking at least two sites.)

<http://www.microflex.com/Products/~-/media/Files/Literature/Microflex%20Chemical%20Resistance%20Guide.ashx> (Microflex)

<http://www.bestglove.com/site/chemrest/default.aspx> (Best Co.)

http://www.ansell-edmont.com/download/Ansell_7thEditionChemicalResistanceGuide.pdf

<http://www.mapaglove.com/ChemicalSearch.cfm?id=0> (MAPA Professionals)



Exposure Limits For Laboratory Chemicals

Below are airborne chemical concentration limits above which you can not legally be exposed at work. These limits are administered by the California Occupational Safety and Health Administration (Cal-OSHA). The materials listed here are a fraction of the total number (500+) of OSHA exposure limits – for the complete list, see: http://www.dir.ca.gov/Title8/5155table_ac1.html. If workers have reason to believe they might be exposed any hazardous materials, contact EH&S, x-4899, or x-8243. All workers should strive to maintain their chemical exposures to as low as reasonably achievable at all times, regardless of the legal exposure limits. Given the volatility of these materials, they should always be used in a properly functioning fume hood, or glove box, or in completely-sealed systems. The “S” notation indicates material is also readily skin absorbed.

Chemical Abstracts Registry Number ^(a)	Skin ^(b)	Name	Permissible Exposure Limit ^(d)			Short-term Exposure Limit ^(e)	
			ppm ^(e)	mg/M ^{3(f)}	Ceiling Limit ^(g)	ppm ^(e)	mg/M ^{3(f)}
64197		Acetic acid	10	25	40 ppm	15	37
67641		Acetone	500	1200	3000 ppm	750	1780
75058	S	Acetonitrile	40	70		60	105
79061	S	Acrylamide	--	0.03			
7664417		Ammonia	25	18		35	27
62533	S	Aniline	2	7.6			
7440371		Argon	(h)				
		Arsenic and inorganic arsenic compounds	--	0.01			
71432	S	Benzene	1			5	--
7726956		Bromine	0.1	0.7	C		
7440439		Cadmium metal dust, as Cd		0.005			
630080		Carbon monoxide	25	29	200 ppm		
7782505		Chlorine	0.5	1.5		1	3
67663		Chloroform; trichloromethane	2	9.78			
110827		Cyclohexane	300	1,050			
287923		Cyclopentane	600	1,720			
75343		1,1-Dichloroethane	100	400			
68122	S	Dimethylformamide; DMF	10	30			
74840		Ethane	(h)				
141786		Ethyl acetate	400	1,400			
64175		Ethyl alcohol; ethanol	1,000	1,900			
74851		Ethylene	(h)				
60297		Ethyl ether	400	1,200		500	1500
50000		Formaldehyde	0.75	--		2	--
75127	S	Formamide	10	18			
64186		Formic acid	5	9		10	19
7440597		Helium	(h)				
142825		n-Heptane	400	1,600		500	2000
110543	S	n-Hexane	50	180			

1333740		Hydrogen	(h)				
10035106		Hydrogen bromide	3	10	C		
7647010		Hydrogen chloride; muriatic acid	5	7	C		
7664393		Hydrogen fluoride, as F	3	2.5		6	--
7722841		Hydrogen peroxide, as H ₂ O ₂	1	1.4			
7783064		Hydrogen sulfide	10	14	50 ppm	15	21
26675467		Isoflurane	2	15			
67630		Isopropyl alcohol	400	980		500	1225
7439976	S	Mercury, metallic and inorganic compounds as Hg	--	0.025	0.1 mg/M ³		
67561	S	Methyl alcohol; methanol	200	260	1000 ppm	250	325
75092		Methylene chloride; dichloromethane	25	87		125	435
7697372		Nitric acid	2	5		4	10
10102439		Nitric oxide	25	30			
7727379		Nitrogen	(h)				
75525		Nitromethane	2	5			
1321126,	S	Nitrotoluene	2	11			
111659		Octane	300	1,450		375	1800
8012951		Oil (mineral) mist, particulate		5			
20816120		Osmium tetroxide, as Os	0.0002	0.002		0.0006	0.006
10028156		Ozone	0.1	0.2		0.3	0.6
109660		Pentane	600	1,800			
127184		Perchloroethylene	25	170	300 ppm	100	685
108952	S	Phenol	5	19			
7664382		Phosphoric acid	--	1		--	3
1310583		Potassium hydroxide; caustic potash	--	2	C		
110861		Pyridine	5	15			
61790532		Silica, amorphous, total dust	-	6			
61790532		Silica, crystalline; quartz total dust	-	0.3			
1310732		Sodium hydroxide; caustic soda	--	2	C		
7664939		Sulfuric acid	--	1		--	3
109999		Tetrahydrofuran	200	590		250	735
108883	S	Toluene; toluol	50	188	500 ppm	150	560
76039		Trichloroacetic acid	1	5			
1330207		Xylene; xylol; dimethylbenzene	100	435	300 ppm	150	655

Footnotes to Table. Footnotes have been edited for clarity. For the complete Cal-OSHA Table and complete footnotes, see: http://www.dir.ca.gov/Title8/5155table_ac1.html (b) The substances designated by "S" in the skin notation column indicates material may be absorbed into the bloodstream through the skin, the mucous membranes and/or the eye, and contribute to the overall exposure. Appropriate protective clothing shall be provided for and used by employees as necessary; (d) Permissible Exposure Limit (PEL) = the maximum permitted 8-hour time-weighted average concentration of an airborne contaminant; (e) Parts of gas or vapor per million parts of air by volume at 25°C and 760mm Hg pressure; (f) Milligrams of substance per cubic meter of air at 25°C and 760mm Hg pressure; (g) Ceiling Limit = the maximum concentration of an airborne contaminant to which an employee may be exposed at any time. A numerical entry in this column represents a ceiling value in addition to the PEL values. A "C" notation means the value given in the PEL columns are also ceiling values; (h) A number of gases and vapors, when present in high concentrations, act primarily as asphyxiants without other adverse effects. A concentration limit is not included for each material because the limiting factor is the available oxygen. (Several of these materials present fire or explosion hazards.); (o) Short-Term Exposure Limit = a 15-minute time-weighted average airborne exposure which is not to be exceeded at any time during a workday even if the 8-hour time-weighted average is below the PEL.

Safety Data Sheets (formerly known as MSDS)

What is a Safety Data Sheet? SDS –formerly known as Material Safety Data Sheets - are a summary of the health hazards of a chemical material and associated recommended safe work practices. SDS are required by OSHA under the Lab Safety Standard and Hazard Communication Standard (CCR, Title 8, 5194) to be made readily available by chemical vendors to the purchasers of their chemicals. The use and relevance of SDS are covered in the mandatory EH&S Fundamentals of Laboratory Safety class. If you work in a lab, then OSHA says you should:

- be aware of what an SDS is and their relevance to your health and safety
- be aware of how to access SDS for your work area
- maintain SDSs that are received with incoming chemical shipments and ensure that they are readily accessible to lab employees during each work shift when they are in their work area(s). Electronic access per below is acceptable with a printer.

SDS Sources

[University of California-MSDS Database](#)

[Laboratory Chemical Safety Summaries](#) (not MSDS, but quality info aimed at labs)

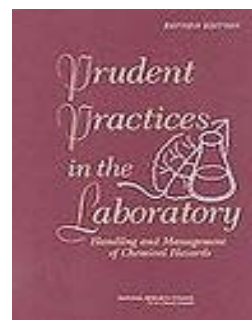
[Fisher Scientific MSDS](#)

[Vermont Safety Information Resources, Inc. \(SIRI\)](#)

[Sigma-Aldrich MSDS](#)

[MSDS Provider \(Manufacturer-direct access\)](#)

[Matheson's Gases](#)



Another Information Resource: The book entitled: [Prudent Practices in the Laboratory](#) by the National Research Council is widely considered to be a definitive reference. It can be purchased, but is also available **free** on-line in a searchable format. Strongly recommended that all lab workers have ready access to this important reference.

CHEMICAL LABELING

Under the Cal-OSHA Hazard Communication Standard (CCR, Title 8, 5194) all chemical containers must be properly labeled – unless a material is temporarily put into a new container for immediate use and is not going to be stored after that immediate use.

Labeling requirements for all hazardous substances are summarized as follows:

- All containers of hazardous materials must be labeled with the identity of the hazardous substance
- The label must contain all applicable hazard warning statements, e.g. flammable, carcinogen
- For commercial products the name and address of the chemical manufacturer or other responsible party must be present
- Manufacturer's product labels must remain on all containers, and must not be defaced in any way.
- Labels must be legible, in English, and prominently displayed
- Secondary containers (such as spray bottles) must be labeled with the identity of the substance and appropriate hazard warnings

New synthesized compounds, or commercial products that are repackaged, must be labeled with the appropriate hazard warnings based on the knowledge of the chemical and physical properties of that substance.



Identification & Classification of Hazardous Chemicals

Chemicals can be divided into different hazard classes. The class will help determine how these materials should be stored and handled and what special equipment and procedures are needed to use them safely. **See also “Safe Storage of Chemicals” section.**

It is essential that all laboratory workers understand the types of hazards, recognize the routes of exposure, and are familiar with the major hazard classes of chemicals. In many cases, the specific hazards associated with new compounds and mixtures will not be known, so it is recommended that all chemical compounds be treated as if they were potentially harmful and to use appropriate eye, inhalation and skin protection equipment.

FLAMMABILITY HAZARDS

A number of highly flammable substances are in common use in campus laboratories. Flammable liquids include those chemicals that have a flashpoint of less than 100 degrees Fahrenheit. These materials must be stored in flammable storage cabinets in aggregate quantities of 10 gallons or more. Flame-resistant laboratory coats must be worn when working with large quantities (1 liter or more) of flammable materials and/or with procedures where a significant fire risk is present (e.g., when working with open flame, etc.). These materials can constitute a significant immediate threat and should be treated with particular care, even though the use of these materials is fairly common in the laboratory setting. Particular attention should be given to preventing static electricity and sparks when handling flammable liquids, i.e., when pumping flammable liquids between containers.



REACTIVE HAZARDS

Reactive substances are materials that decompose, or cause decomposition of other materials, under conditions of mechanical shock, elevated temperature, or chemical action, and release of large volumes of gases and heat. These materials must be handled with utmost care and individuals must thoroughly understand their properties. Reactive hazards fall into a number of categories:

1. Oxidizers - A material that readily yields oxygen or other oxidizing gas, or that readily reacts to promote or initiate combustion of organic materials. Examples: nitric, chromic, and perchloric acids; nitrates; nitrites; hydrogen peroxide. Typically oxygen-rich materials. These are probably the most common laboratory reactive hazards. Oxidizers are generally not unstable themselves, but contact with combustible material (e.g., organic solvent) can result in violent reaction/fire.
2. Pyrophorics – a material that will spontaneously ignite in air below a temperature of 130°C. Examples: t-butyllithium, silane, diborane, trimethylaluminum

3. Water Reactives – explodes; violently reacts; produces flammable, toxic, or other hazardous gases; or evolves enough heat to cause self-ignition upon exposure to water. Examples: sodium or potassium metal, aluminum alkyls, diethylzinc
4. Unstable Reactive – will vigorously polymerize, decompose or condense, becomes self-reactive, or otherwise undergo a violent chemical change under conditions of shock, pressure or temperature. Examples: picric acid, hydrogen peroxide greater than 50 percent, hydrazine, dinitrobenzene, nitromethane, acrolein
5. Organic Peroxides- an organic compound that contains the R-O-O-R structure. Some can present an explosion hazard, or they can be shock-sensitive or temperature sensitive. Examples: will have the term “peroxide” or “peroxy” in the chemical name.
6. Peroxide-formers- a chemical that when exposed to air, will form potentially explosive peroxides which are shock, pressure and temperature sensitive. Examples: ethers are the most common examples, but includes other types of solvents and materials. Peroxide formation is a complex subject because the rate of peroxidation and instability of the resulting peroxide vary widely depending on the starting material. For more information see:
<http://www.cchem.berkeley.edu/cchasp/?q=node/19>

HEALTH HAZARDS

Cal/OSHA uses the following definition for health hazards:

The term ‘health hazard’ includes chemicals which are carcinogens, toxic or highly toxic agents, reproductive toxins, irritants, corrosives, sensitizers, hepatotoxins, nephrotoxins, neurotoxins, agents which act on the hematopoietic systems, and agents which damage the lungs, skin, eyes, or mucous membranes.



The major classes of “hazardous” and “particularly hazardous substances” and their related health and safety risks are detailed below.

Corrosive Substances

As a health hazard, corrosive substances cause destruction of, or alterations in, living tissue by chemical action at the site of contact. Major classes of corrosive substances:



- Strong acids – e.g., sulfuric, nitric, hydrochloric
- Strong bases – e.g., sodium hydroxide, potassium hydroxide and ammonium hydroxide
- Dehydrating agents – e.g., sulfuric acid, sodium hydroxide, phosphorus pentoxide and calcium oxide
- Oxidizing agents – e.g., hydrogen peroxide, chlorine and bromine.

Symptoms of exposure for inhalation include a burning sensation, coughing, wheezing, laryngitis, shortness of breath, nausea, and vomiting. For eyes, symptoms include pain, blood shot eyes, tearing, and blurring of vision. For skin, symptoms may include reddening, pain, inflammation, bleeding, blistering and burns. As a physical hazard, corrosive substances may corrode materials they come in contact with and may be highly reactive with other substances.

Irritants

Irritants are defined as non-corrosive chemicals that cause reversible inflammatory effects on living tissue by chemical action at the site of contact. A wide variety of organic and inorganic compounds, including many chemicals that are in a powder or crystalline form, are irritants. Consequently, eye and skin contact with all laboratory chemicals should always be avoided. Symptoms of exposure can include reddening or discomfort of the skin and irritation to respiratory systems.

Sensitizers

A sensitizer (allergen) is a substance that causes exposed people to develop an allergic reaction in normal tissue after repeated exposure to the substance. Examples of sensitizers include diazomethane, chromium, nickel, formaldehyde, isocyanates, arylhydrazines, benzylic and allylic halides, and many phenol derivatives. Sensitizer exposure can lead to all of the symptoms associated with allergic reactions, or can increase an individual's existing allergies.



Hazardous Substances with Toxic Effects on Specific Organs

- Hepatotoxins – i.e., substances that produce liver damage, such as nitrosamines and carbon tetrachloride
- Nephrotoxins – i.e., agents causing damage to the kidneys, such as certain halogenated hydrocarbons
- Neurotoxins – i.e., substances which produce their primary toxic effects on the nervous system, such as mercury, acrylamide and carbon disulfide
- Biotoxins – biologically-derived toxins, such as botulinum toxin, tetrodotoxin. Some of these are regulated under the U.S. Patriot Act as “select agents”
- Agents which act on the hematopoietic system – e.g., CO and cyanides which decrease hemoglobin function and deprive the body tissues of oxygen
- Agents which damage lung tissue – e.g., asbestos and silica.

Symptoms of exposure to these materials vary. Staff working with these materials should review the SDS for the specific material being used and should take special note of the associated symptoms of exposure.

Particularly Hazardous Substances

OSHA recognizes that some classes of chemical substances pose a greater health and safety risk than others. To differentiate this different risk characteristic, OSHA identifies two categories of hazardous chemicals:

1. **hazardous chemicals**
2. **particularly hazardous substances.**

Substances that pose such significant threats to human health are classified as "particularly hazardous substances" (PHSs). The OSHA Laboratory Standards requires that special provisions be established to prevent the harmful exposure of researchers to PHSs, including the establishment of designated areas for their use. In most cases the entire lab where the material is used would be the designated area per Standard Operating Procedure for that material(s). See Section I.

Particularly hazardous substances are divided into three primary types:

1. **Acute Toxins;**
2. **Reproductive Toxins;** and
3. **Carcinogens.**

Acute Toxins

Substances that have a high degree of acute toxicity are interpreted by OSHA as being substances that "may be fatal or cause damage to target organs as the result of a single exposure or exposures of short duration." These chemicals, associated chemical waste, and storage containers must be handled with care to prevent cross contamination of work areas and unexpected contact. These chemicals must be labeled as "Toxic." Empty containers of these substances must be packaged and disposed of as hazardous waste without rinsing trace amounts into the sanitary sewer system.

Reproductive Toxins

Reproductive toxins include any chemical that may affect the reproductive capabilities, including chromosomal damage (mutations) and effects on fetuses (teratogenesis). http://www.ehs.ucsb.edu/units/labsfty/labpsc/chemistry/2011_particularly_hazardous_list.pdf

Reproductive toxins can affect the reproductive health of both men and women. For women, exposure to reproductive toxins during pregnancy can cause adverse effects on the fetus; these effects include embryoletality (death of the fertilized egg, embryo or fetus), malformations (teratogenic effects), and postnatal functional defects. For men, exposure can lead to sterility.

Examples of embryotoxins include thalidomide and certain antibiotics such as tetracycline. Women of childbearing potential should note that embryotoxins have the greatest impact during the first trimester of pregnancy. Because a woman often does not know that she is pregnant during this period of high susceptibility, special caution is advised when working with all chemicals, especially those rapidly absorbed through the skin (e.g., formamide). Pregnant women and women intending to become pregnant should consult with their laboratory supervisor and EH&S before working with substances that are suspected to be reproductive toxins.

Carcinogens (see next section)

Carcinogen Safety Program

Carcinogens are chemical or physical agents that cause cancer. Generally they are chronically toxic substances; that is, they cause damage after repeated or long-duration exposure, and their effects may only become evident after a long latency period. Chronic toxins are particularly insidious because they may have no immediately apparent harmful effects. These materials are separated into two classes: **Select Carcinogens** and **Regulated Carcinogens**.

Select carcinogens are materials which have met certain criteria established by the National Toxicology Program (NTP) or the International Agency for Research on Cancer (IARC) regarding the risk of cancer. There are hundreds of materials in this category. It is important to recognize that some substances involved in research are new compounds and have not been subjected to testing for carcinogenicity. The following references (links provided) are used to determine which substances are select carcinogens by Cal/OSHA's classification:

- OSHA Carcinogen List (*see next page*)
- Annual Report on Carcinogens published by the NTP, including all of the substances listed as "known to be carcinogens" and some substances listed as "reasonably anticipated to be carcinogens"
http://www.ehs.ucsb.edu/units/labsfty/labrsc/chemistry/2011_particularly_hazardous_list.pdf
- IARC list including all of Group 1 "carcinogen to humans" materials; and some in Group 2A or 2B. See above link.

Regulated Carcinogens (next page) fall into a higher hazard class and have extensive additional OSHA requirements associated with them. The most common ones found in the lab are **formaldehyde, methylene chloride and benzene**. The use of these agents may require personal exposure sampling. When working with Regulated Carcinogens, it is particularly important to review and effectively apply engineering and administrative safety controls as the regulatory requirements for laboratories that may exceed threshold values for these chemicals are very extensive.

Managing Carcinogens

The safe use of all carcinogens should be addressed in the lab-specific *Chemical Hygiene Plan* (SOPs) of each lab using these materials – See Sec. I. EH&S does periodic reviews of labs using regulated carcinogens to determine if there is reason to believe that exposure levels exceed the Cal-OSHA limits. The review process is described in Sec. III.C.3. Typically, if these materials are used in a certified fume hood and proper PPE is utilized, there is little reason to believe exposure levels are a concern. In some circumstances, EH&S will do periodic exposure monitoring to verify this. In the unlikely event exposure limits are exceeded, additional steps must be taken under to eliminate the exposure, or protect the individual.

Regulated Carcinogens

The term “regulated carcinogen” means a recognized cancer causing substance, compound, mixture, or product regulated by Cal/OSHA sections 1529, 1532, 1532.2, 1535, 8358, 8359 or Article 110, sections 5200-5220.

- Acrylonitrile
- Arsenic metal and inorganic arsenic compounds
- Asbestos
- Benzene
- 1,3-butadiene
- Cadmium metal and cadmium compounds
- Chromium(VI) compounds
- Coke Oven Emissions
- 1,2-Dibromo-3-chloropropane (DBCP)
- Ethylene Dibromide (EDB)
- Ethylene Oxide (EtO)
- Formaldehyde gas and formaldehyde solutions
- Lead metal and inorganic lead compounds
- Methylene Chloride
- 4,4'-Methylene bis(2-chloroaniline) (MBOCA)
- Methylenedianiline (MDA)
- Vinyl Chloride
- **2-Acetylaminofluorene**
- **4-Aminodiphenyl**
- **Benzidine (and its salts)**
- **3,3'-Dichlorobenzidine (and its salts)**
- **4-Dimethylaminoazobenzene**
- **alpha-Naphthylamine**
- **beta-Naphthylamine**
- **4-Nitrobiphenyl**
- **N-Nitrosodimethylamine**
- **beta-Propiolactone**
- **bis-Chloromethyl ether**
- **Methyl chloromethyl ether**
- **Ethyleneimine**

Those materials marked in **bold** have the additional requirement of needing to be reported to Cal-OSHA as being in use at a workplace (CCR, Title 8, 5209). If you possess, or are going to order, any of these materials, please contact EH&S. These materials are rarely used in research labs.

GENERAL PROCEDURES FOR WORKING WITH HAZARDOUS CHEMICALS

(adapted from *Prudent Practices in the Laboratory*, National Academies Press, 2011)

Personal Behavior

Demonstrating prudent behavior within the laboratory is a critical part of a culture of safety. This includes following basic safety rules and policies, being cognizant of the hazards within the laboratory, and exhibiting professionalism with co-workers. Maintaining an awareness of the work being performed in nearby hoods and on neighboring benches and any risks posed by that work is also important.

Minimizing Exposure to Hazardous Chemicals

Take precautions to avoid exposure by the principal routes, that is, contact with skin and eyes, inhalation, and ingestion. The preferred methods for reducing chemical exposure are, in order of preference,

1. substitution of less hazardous materials or processes
2. engineering controls,
3. administrative controls, and
4. personal protective equipment (PPE)

See also the Occupational Safety and Health Administration's (OSHA) Safety and Health Management eTool, Hazard Prevention and Control module available at www.osha.gov. Before beginning work, review all proposed laboratory procedures thoroughly to determine potential health and safety hazards. Refer to the Safety Data Sheets (SDS) for guidance on exposure limits, health hazards and routes of entry into the body, and chemical storage, handling, and disposal. Avoid underestimating risk when handling hazardous materials.

Engineering Controls

Engineering controls are measures that eliminate, isolate, or reduce exposure to chemical or physical hazards through the use of various devices. Examples include laboratory chemical hoods and other ventilation systems, shields, barricades, and interlocks. Engineering controls must always be considered as the first and primary line of defense to protect personnel and property. When possible, PPE is not to be used as a first line of protection. For instance, a personal respirator should not be used to prevent inhalation of vapors when a laboratory chemical hood (formerly called fume hoods) is available.

Avoiding Ingestion of Hazardous Chemicals

Eating, drinking, smoking, gum chewing, applying cosmetics, and taking medicine in laboratories where hazardous chemicals are used or stored should be strictly prohibited. Food, beverages, cups, and other drinking and eating utensils should not be stored in areas where hazardous chemicals are handled or stored. Glassware used for laboratory operations should never be used to prepare or consume food or beverages. Laboratory

refrigerators, ice chests, cold rooms, and ovens should not be used for food storage or preparation. Laboratory water sources and deionized laboratory water should not be used as drinking water. Never wear gloves or laboratory coats outside the laboratory or into areas where food is stored and consumed, and always wash laboratory apparel separately from personal clothing.

Avoiding Inhalation of Hazardous Chemicals

Conduct all procedures involving volatile toxic substances and operations involving solid or liquid toxic substances that may result in the generation of aerosols in a laboratory chemical hood. Air-purifying respirators are required for use with some chemicals if engineering controls cannot control exposure. Significant training, along with a medical evaluation and respirator fit, are necessary for the use of respirators. Contact the campus Industrial Hygienist for more information about respirator fitting and use.

Laboratory chemical hoods should not be used for disposal of hazardous volatile materials by evaporation. Such materials should be treated as chemical waste and disposed of in appropriate containers according to institutional procedures and government regulations.

Avoiding Injection of Hazardous Chemicals

Solutions of chemicals are often transferred in syringes, which for many uses are fitted with sharp needles. The risk of inadvertent injection is significant, and vigilance is required to avoid an injury. Use special care when handling solutions of chemicals in syringes with needles. When accompanied by a cap, syringe needles should be placed onto syringes with the cap in place and remain capped until use. Do not recap needles, especially when they have been in contact with chemicals. Remove the needle and discard it immediately after use in the appropriate sharps containers. Blunt-tip needles, including low-cost disposable types, are available from a number of commercial sources and should be used unless a sharp needle is specifically required to puncture rubber septa or for subcutaneous injection.

Avoiding Skin Contact with Hazardous Chemicals

All laboratory operations must be conducted in accordance with the campus Personal Protective Equipment (PPE) policy. In general, a lab coat and safety glasses are the minimum required PPE when working in the laboratory. **Ordinary prescription glasses do not provide adequate protection against injury because they lack side shields and are not resistant to impact, but prescription safety glasses and chemical splash goggles are available.** Please see the section on PPE in this document for further information.

Housekeeping

A definite correlation exists between orderliness and the level of safety in the laboratory. In addition, a disorderly laboratory can hinder or endanger emergency response personnel. The following housekeeping rules should be adhered to:

- Never obstruct access to exits and emergency equipment such as fire extinguishers and safety showers. Comply with local fire codes for emergency exits, electrical panels, and minimum aisle width.
- Store coats, bags, and other personal items in the proper area, not on the benchtops or in the aisles.
- Do not use floors, stairways, and hallways as storage areas. Items stored in these areas can become hazards in the event of an emergency.
- Keep drawers and cabinets closed when not in use, to avoid accidents.
- Properly label in permanent marker and store all chemicals appropriately by compatibility.
- Label transfer vessels with the full chemical name, manufacturer's name, hazard class, and any other special warnings.
- Store chemical containers in order and neatly. Face labels outward for easy viewing. Containers themselves should be clean and free of dust. Containers and labels that have begun to degrade should be replaced, repackaged, or disposed of in the proper location. Do not store materials or chemicals on the floor because these may present trip and spill hazards.
- Keep chemical containers closed when not in use.
- Secure all compressed gas cylinders to walls or benches in accordance with regulation and the local requirements for seismic safety.
- Secure all water, gas, air, and electrical connections in a safe manner.
- Return all equipment and laboratory chemicals to their designated storage location at the end of the day.
- To reduce the chance of accidentally knocking containers to the floor, keep bottles, beakers, flasks, and the like at least 2 in. from the edge of benchtops.
- Keep work areas clean (including floors) and uncluttered.
- To avoid flooding, do not block the sink drains. Place rubber matting in the bottom of the sinks to prevent breakage of glassware and to avoid injuries.
- Do not pile up dirty glassware in the laboratory. Wash glassware carefully. Remember that dirty water can mask glassware fragments. Handle and store laboratory glassware with care. Discard cracked or chipped glassware promptly.

- Dispose of all waste chemicals properly and in accordance with organizational policies.
- Dispose of broken glass and in a specially labeled container for broken glass. Treat broken glassware contaminated with a hazardous substance as a hazardous substance.
- Dispose of sharps (e.g., needles and razor blades) in a specially labeled container for sharps. Treat sharps contaminated with a hazardous substance as hazardous substances.

Transport of Chemicals

When transporting chemicals outside the laboratory or between stockrooms and laboratories, use only break-resistant secondary containment. Commercially available secondary containment is made of rubber, metal, or plastic, with carrying handle(s), and is large enough to hold the contents of the chemical containers in the event of breakage. Resealable plastic bags serve as adequate secondary containment for small samples. When transporting cylinders of compressed gases, the cylinder must always be strapped in a cylinder cart and the valve protected with a cover cap. When cylinders must be transported between floors, passengers should not be in the elevator.

Working with Scaled-Up Reactions

Special care and planning is necessary to ensure safe scaled-up work. Scale-up of reactions from those producing a few milligrams or grams to those producing more than 100 g of a product may magnify risks by several orders. Although the procedures and controls for large-scale laboratory reactions may be the same as those for smaller-scale procedures, significant differences may exist in heat transfer, stirring effects, times for dissolution, and the effects of concentration—all of which need to be considered. When planning large-scale work, practice requires consulting with experienced workers and considering all possible risks. Although one cannot always predict whether a scaled-up reaction has increased risk, hazards should be evaluated if the following conditions exist:

- The starting material and intermediates contain functional groups that have a history of being explosive (e.g., N—N, N—O, N—halogen, O—O, and O—halogen bonds) or that could explode to give a large increase in pressure.
- A reactant or product is unstable near the reaction or workup temperature. A preliminary test to determine the temperature and mode of decomposition consists of heating a small sample in a melting-point tube.
- A reactant is capable of self-polymerization.
- A reaction is delayed; that is, an induction period is required.
- Gaseous byproducts are formed.

- A reaction is exothermic. What can be done to provide, or regain, control of the reaction if it begins to run away?
- A reaction requires a long reflux period. What will happen if solvent is lost owing to poor condenser cooling?
- A reaction requires temperatures less than 0 °C. What will happen if the reaction warms to room temperature?
- A reaction involves stirring a mixture of solid and liquid reagents. Will magnetic stirring be sufficient at large scale or will overhead mechanical stirring be required? What will happen if stirring efficiency is not maintained at large scale?

In addition, thermal phenomena that produce significant effects on a larger scale may not have been detected in smaller-scale reactions and therefore could be less obvious than toxic or environmental hazards. Thermal analytical techniques should be used to determine whether any process modifications are necessary. Consider scaling up the process in multiple small steps, evaluating the above issues at each step. Be sure to review the literature and other sources to fully understand the reactive properties of the reactants and solvents, which may not have been evident at a smaller scale.

Fume Hood Usage Guide: Standard Hoods

(Standard hoods do not have the “VAV control box” shown on the next page)

Per Cal-OSHA regulations, users of hoods must be trained on use of their fume hood. Attendance at one of the live or on-line lab safety orientations described below on the “Campus Policy on Laboratory Safety Training” page satisfies that requirement. The information on this page should also be read by all hood users and is posted on campus hoods for easy reference.

1. **Always work with the sash at the level of the red arrow sticker (picture on next pg.) and close it when not attended.** To adequately protect you, your hood should be producing a face velocity of about 100 ft/min. EH&S tests your hood and posts the red arrow stickers at the **proper sash level to:**
 - satisfy the required air flow and protect you (10 - 100 times more than full open sash) against airborne chemicals
 - protect you better from incidents within the hood
2. *All hoods on campus are equipped with an air flow monitor and/or alarm to warn you if the air velocity is too low – see examples pictured below. **If the alarm engages, lower the sash slightly until the alarm stops.** Do **NOT** disengage or over-ride the alarm. If your alarm sounds consistently this could indicate a real problem – call EH&S.
3. Always work at least 6 inches inside the hood to maximize capture efficiency.
4. Store only a minimum of equipment and chemicals in your hood because:
 - Excess materials will block the air flow into the intake slots at the back of the hood. Permanent equipment should be raised on a jack to allow the air to flow smoothly.
 - Most fires and explosions occur in the hood. Minimizing chemical volumes will reduce the chances of a small accident escalating into a large one.
5. Keep the lab windows closed. Drafts from open windows and doors can significantly affect your hood’s performance (100 ft/min is only a few miles/hr of air).



*“Magnihelic gauge” – note normal gauge position. Significant deviation may indicate condition of low air flow.



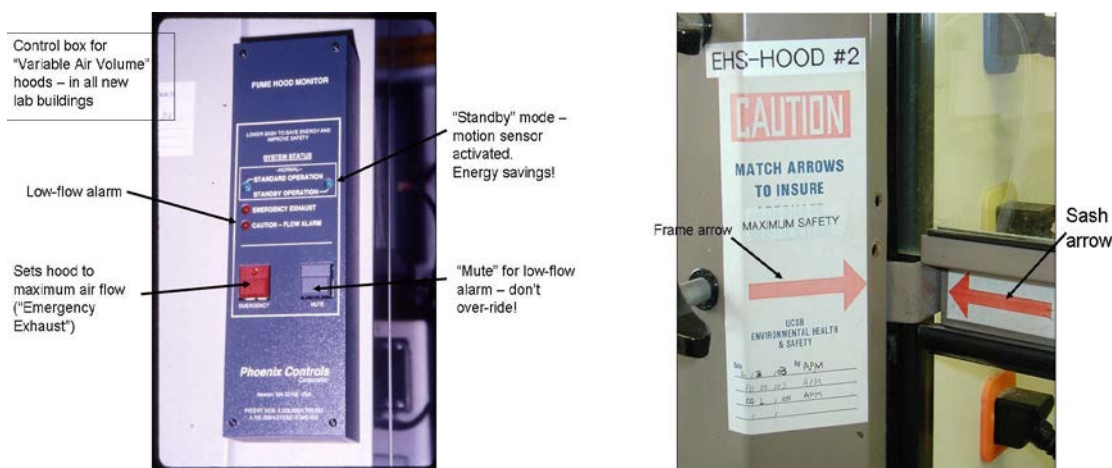
*Visible/audible alarm Sounds during low-flow

Fume Hood Usage Guide: Variable Air Volume Hoods ("Phoenix" system)

Per Cal-OSHA regulations, users of hoods must be trained on use of their fume hood. Attendance at one of the live or on-line lab safety orientations described below on the "Campus Policy on Laboratory Safety Training" page satisfies that requirement. The information on this page should also be read by all hood users and is posted on campus hoods for easy reference.

Variable Air Volume (VAV) hoods — unlike a standard hood above — automatically adjust the face velocity to stay within recommended safe work levels (~ 100 ft./min). A VAV hood is easily distinguished by the gray control box on the hood — pictured below.

1. **If the low-flow alarm engages, lower the sash until the alarm stops.** DO NOT over-ride the safety alarm by permanently engaging the "Mute" or "Emergency" button (e.g., with tape). If your hood is consistently alarming call EH&S (x-4899).
2. Always work with the sash at or below the level of the **red arrow sticker** (below), because:
 - If most bldg. sashes are raised, this will generate a hood alarm, and at your neighbor's hood, due to the limited capacity of your building's ventilation
 - A lowered sash protects you against airborne chemicals and incidents up to 100 times more than at sash full open.
 - The lower the sash, the greater the **energy conservation** – lower sash when not in use
3. Store only the minimum of equipment and chemicals in your hood because:
 - Excess materials block air flow into the slots at back of the hood. Permanent equipment should be raised on a stand to allow the air flow into the lower slot.
 - Most lab fires/explosions occur in hoods. Minimizing chemical volumes will reduce the chances of a small accident escalating into a large one.
4. Always work at least 6 inches inside the hood to maximize hood capture efficiency.



Safe Storage of Chemicals

INTRODUCTION: If incompatible chemicals are mixed a fire, explosion, or toxic release can occur. In earthquake-prone areas like UCSB, it is vital that chemicals be stored safely. Note however, that chemicals can often fall into more than one hazard category and therefore the chemical label and/or Safety Data Sheet (MSDS) should be reviewed for storage requirements. Separate chemicals by adequate distance, or preferably by using physical barriers (e.g. storage cabinets). Avoid using the fume hood for chemical storage - this practice may interfere with the proper air flow of the hood. For especially dangerous materials, use a secondary container (e.g. plastic tub) large enough to contain a spill of the largest container. This chart indicates the most obvious chemical incompatibilities, and provides a basic segregation plan.

ACIDS: Examples: Acetic Acid; Chromic Acid*; Hydrochloric Acid; Hydrofluoric Acid; Nitric Acid*; Perchloric Acid*; Phosphoric Acid; Sulfuric Acid (* Indicates strong oxidizing acids, store per oxidizers section)

Storage Precautions:

- Store bottles on low shelf areas, or in acid cabinets.
- Segregate oxidizing acids from organic acids, **and** flammable materials.
- Segregate acids from bases, **and** from active metals such as sodium, potassium, etc.
- Segregate from chemicals which could generate toxic gases such as NaCN, iron sulfide, etc.

BASES: Examples: Ammonium Hydroxide; Potassium Hydroxide; Sodium Hydroxide

Storage Precautions:

- Separate bases from acids.
- Store bottles on low shelf areas, or in acid cabinets.

FLAMMABLES-fuels are reducing agents, examples:

Acetone	Ethyl Ether	Propanol
Benzene	Gasoline	Tetrahydrofuran
Cyclohexane	Hexane	Toluene
Ethanol	Isopropyl Alcohol	Xylene
Ethyl Acetate	Methanol	

Storage Precautions:

- Store in approved flammable storage cabinet(s) (required if there is > 10 gallons in the lab).
- Separate from oxidizing acids and oxidizers.
- Keep away from any source of ignition (flames, localized heat or sparks).
- Use only "flammable storage" (desparked) refrigerator



OXIDIZERS-react violently with organics (solvents,paper, wood, etc.

Example Solids

Iodine
Nitrates, Salts of
Peroxides, Salts of
Potassium Ferricyanide
Sodium Nitrite

Liquids

Bromine
Hydrogen Peroxide
Nitric Acid
Perchloric Acid
Chromic Acid

Storage Precautions:

- Keep away from organic solvents, and other combustible materials (i.e. paper).
- Keep away from reducing agents.

PEROXIDE-FORMING CHEMICALS-peroxides can be explosive and shock-sensitive.

Examples: Ethers and acetals with alpha-hydrogen (e.g. ethyl ether, THF); Alkenes with allylic hydrogen (e.g. cyclohexene). Store tightly sealed to exclude oxygen. Label with date of receiving/opening. Dispose within recommended guidelines – usually 6 months for ethers.

PYROPHORIC SUBSTANCES-spontaneously ignite in air.

Examples: Some finely divided metals; Some organoaluminum compounds (LiAlH_4 , $\text{Al}(\text{CH}_3)_3$); Silane; phosphorus, yellow (should be stored and cut under water)

Storage Precautions:

- rigorously exclude air and water from container.
- store away from flammables.

WATER REACTIVES -react violently with water to yield flammable or toxic gases.

Solids: Calcium carbide, magnesium, lithium, potassium, sodium

Liquids: phosphorous trichloride, thionyl chloride

Storage Precautions:

- Rigorously avoid exposure to water and air
- Store away from flammables
- Lithium, Potassium and sodium should be stored under kerosene or mineral oil

HIGHLY TOXICS, CARCINOGENS, REPRODUCTIVE TOXIN These chemicals can be very hazardous by themselves, or in combination with other chemicals. If they are easily inhaled, (gases and volatile liquids) then they are particularly hazardous.

Liquids - Seal tightly and store in a ventilated cabinet apart from incompatibles. Use secondary containment (e.g. plastic tub) to contain any spills.

Examples: Formaldehyde; Carbon disulfide; Mercury; Nickel carbonyl; Cyanide solutions

Gases - Store in a gas cabinet or other ventilated cabinet

Examples: Chlorine; Fluorine; Hydrogen chloride; Nitric Oxide; Hydrogen Cyanide

Solids -Store away from incompatibles (usually acids) that would release toxic gas upon contact.

Examples: Cyanides, Salts of; Sulfides, Salts of



Refrigerators and Freezers in Labs

Certain refrigerator/freezer units are designed specifically for the storage of flammable materials, and to prevent potentially injurious explosions in your lab. These units have special protections to prevent ignition of flammable vapors. For example, the light switch, defrost feature, and thermostat inside the storage compartment have been removed or relocated. This is critical, since flammable vapors coupled with an ignition source could result in an explosion. In other words, a normal kitchen refrigerator is not safe for the storage of chemicals.

Before purchasing a new refrigerator/freezer, or using an existing one, consider whether chemicals will be used for storage in this unit. Note that many lab refrigerators will be around for decades and therefore one can not guarantee that a normal unit will never be used for flammables storage. There are two types of refrigerator/freezer models that should be considered, depending on the type of hazardous material the unit will store.

I. FLAMMABLE MATERIAL STORAGE REFRIGERATORS/FREEZERS:

These have no internal electrical components which could trigger an explosion inside the unit. These must always be used for storage of volatile materials.

II. EXPLOSION-PROOF REFRIGERATORS/FREEZERS :

These units prevent triggering of interior or exterior explosions in a hazardous environment. Every motor and thermostat is designed to prevent arcing and possible ignition. Used for storage of volatile materials in areas with explosive atmospheres. These are rarely necessary in lab environments.

All refrigerator/freezer purchases and modifications on campus **must be pre-approved** by EH&S at X8243. In addition, all approved refrigerator/freezer units storing flammable materials must be labeled with signage reading, "Approved For Chemical Storage, No Food Storage". All refrigerator/freezer units not approved for storage of flammable materials must be affixed with signage reading, "Explosion Hazard". Please feel free to contact EH&S for your free labels.



This picture shows a UCI lab refrigerator which exploded when chemicals were inappropriately stored in a unit which was not designed for flammables storage.

Campus Policy on Laboratory Safety Training

Documentation of occupationally-related safety training is a legal requirement under Cal-OSHA. Accordingly, as of July 2010, UCSB instituted a policy regarding *baseline* safety training of all “laboratory workers”. The training covers the core/universal issues common to most/all labs and addresses some (not all) specific regulatory requirements for documented training. In short, the policy requires that **all** lab workers complete one of the EH&S general lab safety orientations as outlined below before being issued access to their lab.

Note that the trainings below are generic and do not address the specific hazards, procedures and practices for a particular laboratory, or individual. Lab supervisors/Pis are still responsible under the law for ensuring this has been provided.

All members of the campus lab community have responsibilities under the policy - particularly the individual’s home department which is responsible for ensuring new lab workers are identified and guided into the appropriate training session. The full policy can be found at:

<http://www.policy.ucsb.edu/policies/policy-docs/lab-safety-training.pdf>

Summary of Required General Laboratory Safety Orientations

1. **Required for Graduate Students:** A live 3-hour [instructor-led general laboratory safety orientation \(LS01\)](#) is offered regularly - generally twice per quarter. This training is more in-depth than those listed below and generally includes hands-on fire extinguisher training. This training is open to *any* laboratory worker and all are encouraged to take this course.
2. **Required for Other (Non-Graduate Student) Laboratory Workers,** (e.g., postdoctoral scholars, undergraduates working in research labs, visitors, etc.). Complete the appropriate one of the two online modules below. Alternatively, a PI/supervisor may, at their discretion, choose to send a worker to the more in-depth live training in #1.
 - [Laboratory Safety Orientation for Chemical Users \(LS40\)](#)
 - [Laboratory Safety Orientation for Non-Chemical Users \(LS41\)](#)



Non-UCSB Online Laboratory Safety Training

Documented safety training is a legal requirement of Cal-OSHA and ideally also helps prevent accidents. Within the UC system are a number of online safety training modules as listed below - the link below will lead to the trainings. However, as someone accessing these modules from outside that campus the training will not be documented. It is therefore recommended that the supervisor have the training documented at the time of completion.

Link to UC (Non-UCSB) Online Laboratory Safety Training:

<http://ehs.ucsb.edu/training/lsvideo1.html>

UC Online Training Catalog:

(campuses may have their own version/title for the same subject matter)

- Carcinogen Handling Safety
- Centrifugation Hazards
- Chemical Storage
- Compressed Gas & Cryogen Safety
- Day in the Life of the Lab (A PIs Perspective)
- Earthquake Safety
- Pressure Safety
- Cryogenics Safety
- Electrical Safety
- Ergonomics for the Laboratory
- Fire Safety in the Laboratory
- Fire Extinguishers Usage
- Formaldehyde Safety
- Fume Hoods
- Hazardous Materials Spill Response
- Hazardous Materials Shipping
- Material Safety Data Sheets
- Nanomaterials Handling
- Practicing Safe Science
- Pyrophoric Reagents Safety



Laboratory Self-Inspection Checklist

EH&S inspects all labs on campus at least annually. However, **lab supervisors should initiate regular self-inspections** (recommend minimum of twice-a-year) for the following reasons:

- By means of understanding between UCSB, SB County Fire and the State Fire Marshal, EH&S is allowed to perform safety surveys on their behalves. Without these agreements, these agencies would conduct their own inspections. However, to continue these agreements, their expectations are that regular self-inspections and corrections are done by every lab.
- Under California law (OSHA), supervisors (PIs) are required to: "... *include procedures for identifying and evaluating work place hazards including scheduled periodic inspections to identify unsafe conditions and work practices.*"
- Beyond any regulatory requirements, doing regular selfinspections will clearly increase the level of safety in your area.

To aid you in your surveys, a Self-Inspection Checklist follows, this is not a list of every possible safety issue, but are guidelines. Most items are based on applicable regulations or campus policy. Radiation and biohazard issues are not addressed here because they are highly specialized and these labs receive targeted EH&S visits. More information is also available at <http://ehs.ucsb.edu>. The links (underlined) noted below lead to further information.

Hazardous Waste

1. Are personnel generating chemical waste trained with waste disposal procedures? Individuals who have not taken the UCSB Lab Safety course (live or on the EH&S website) must take this course before generating hazardous waste for disposal [Online Hazardous Waste Course*](#) (EH09)
(*This course meets the waste management training requirements enforced by Santa Barbara County Fire Department)
2. Is the illegal disposal of hazardous substances down the drain prevented?
3. Are all hazardous waste containers labeled with the official UCSB Hazardous Waste label?
 - Is there a supply of UCSB waste labels handy (available in all campus storerooms)?
 - Are [labels](#) attached when the **first drop** of waste goes into the container?
 - Are all constituents in mixtures identified, as well as their concentrations?
Do not use generic names like "*Waste or Organic waste*" instead use proper chemical name(s).
 - Are chemically incompatible wastes segregated?
 - Is there a designated area for storage of hazardous waste and [labeled as such](#)?
4. Are lab personnel instructed not to dispose of chemicals by fume hood evaporation?
By law, waste containers must be capped when not in use.
5. Is chemical waste disposed of within **9 months** of their accumulation, *regardless how much material remains inside the container*? Contact [EH&S for waste disposal](#).

6. Are all “[sharps](#)” (syringes, razor blades, etc.) disposed in puncture resistant, leak-resistant containers and sealed tightly to preclude loss of contents? Is there a designated glass disposal container in the lab? Lab personnel are designated to empty these into their bldg. dumpster – custodial staff will not do so.

Chemical Safety

1. Is your lab’s legally-required (Cal-OSHA) Lab-specific [Chemical Hygiene Plan](#) (CHP) completed and shared with all workers? Does your CHP address your use of OSHA [Particularly Hazardous Substances](#) (human carcinogens, acute toxins, reproductive toxins, and pyrophorics)?
2. Are [Cal-OSHA regulated carcinogens](#) such as [formaldehyde/formalin](#), [dichloromethane](#), and [benzene](#) always used in a fume hood and with appropriate gloves/eyewear?
3. Are chemical containers properly labeled with chemical name and hazard type of the material? (e.g., repackaged materials and lab-synthesized materials)
4. Are stored chemicals segregated according to hazard classification/compatibility (acids, bases, flammables, oxidizers, water reactives, etc.)? [Compatibility Chemical Storage Chart](#)
5. Are all containers of [peroxide-forming chemicals](#) (e.g., ethers) dated upon receipt and disposed of within the prescribed time period (contact EH&S for prompt disposal)? Peroxides can be explosively unstable.
6. Are [flammable liquids](#) kept inside approved flammable storage cabinets whenever possible?
 - Are flammable liquids always stored in approved flammable cabinets when in excess of 10 gallons?
 - Do you have large volumes of flammable solvents (e.g., multiple cases or drums) in storage that are above what is reasonably needed? The quantities of flammables that can legally be stored are regulated by CA Fire Code. Please don’t stockpile large quantities of these materials.
 - Are flammable liquids stored away from sources of heat, ignition, electrical equipment or sources of static electricity?
 - Static Electricity** - Electrically ground and bond all containers/equipment involved in pumping flammable liquids to prevent buildup of static electricity as an ignition source.
7. Are acid volumes greater than 10 gallons stored in an approved storage cabinet?
8. Is there a catch pan beneath manometers, barometers, etc. which contain large quantities of mercury?
9. It is highly recommended chemical [spill cleanup materials](#) be available.
Are all lab workers familiar with the location of spill cleanup kits?
Note: Some [lab buildings](#) have a designated “spill closet” – generally keyed to graduate master key.

Laboratory Equipment

1. Are the [eyewash and emergency shower](#) stations free of any obstructions which would prevent ready access? These units are tested by FM regularly. It is recommended that labs run their eyewash units monthly to maintain clean water in the lines.
2. Have [fume hoods](#) been EH&S tested within the year (check label)?
 - Is **air flow indicator** present and operational?
 - Is lab **equipment or chemicals** within the hood minimized? **Keep only items in use.**
 - Are air entry slots at back of hood kept clear of obstructions? **Cluttered hoods interfere with proper air flow.**
 - Is front **sash lowered** to the appropriate level "**red arrow mark**" when hood is in use? If the low flow alarm engages, lower the sash until the alarm stops. If the alarm continues when the sash is lowered to the "red arrow mark" please contact EH&S at x3743. **DO NOT** over-ride the safety alarm by permanently engaging the "Mute" or "Emergency" button (e.g., with tape, paper clips, etc.).
 - Has everyone using a fume hood been properly trained to use their fume hood? *General fume hood use is covered in the Lab Safety training course. The training however, does not cover lab specific hood use. Ensure lab members have documented their fume hood training.*
3. Are biological safety cabinets certified annually or when moved (check sticker) and are they the proper types for the work being conducted?
4. Do labs using non-ionizing radiation equipment, such as [lasers](#), microwaves, and ultraviolet light sources, have properly posted warning signs and shielded work areas?
5. [Compressed gas cylinders](#)
 - Are cylinders dated upon arrival and contents clearly identified?
 - Inspect regularly for defects, i.e., excessive rust, dents, bulging, corrosion, etc.
 - Unidentified cylinders should be marked, "CONTENTS UNKNOWN" and returned to the manufacturer.
 - Non-lecture bottles ≥ 5 years old must be returned to the manufacturer to ensure they are safety/pressure tested as required by law ("[hydrostatic testing](#)") [Check stamped date on cylinder when it was last tested.](#)
 - Corrosive gases (e.g. **HF, HBr, HCl, H₂S**) can degrade the cylinder over time and/or produce dangerously high pressures of hydrogen. Dispose of within 2 years.
 - Are cylinders secured upright with welded chains and brackets bolted to a wall, bench or other secure object (no type C-clamps)?
 - Are protective caps in place while cylinders are not in use?
 - Flammable gases (e.g. hydrogen, methane) tubing should be equipped with a **flash arrestor** to prevent flame flashback to cylinder. Available from gas vendors.
 - Use of large cylinders of [highly toxic gases](#) must be reviewed/approved (EH&S, x-4899)
 - Highly toxic gas cylinders should be equipped with a **reduced flow orifice** (RFO) connection to prevent rapid discharge of cylinder contents. Available from gas vendors.
 - Gas cabinets with toxic or flammable gas delivery manifolds often have an **excessive flow detection and auto-shutoff valve** built-in. Verify that this safety feature is functional.
6. Are [refrigerators](#) for storing flammables clearly posted with signage indicating they are safe for such storage? (e.g. "desparked", "lab-safe", "explosion-proof", "flammable storage").

- Are refrigerators that are **NOT** designed for flammables storage clearly marked as such? (this is very important to prevent a potential explosion)
 - Are all chemical storage refrigerators marked with “No Food” labels?
 - Refrigerators in labs utilized for food or drinks should be marked “Food Only/No Chemicals?”
 - Laboratory refrigerators/freezers and other sensitive equipment, preferably should be connected to emergency back-up power.
7. Is the location of manuals/instructions for each piece of equipment known?
 8. Are the belt guards in place on all pumps, etc?
 9. **Solvent stills with water-reactive drying agents**
 - Are solvent stills clearly labeled with the solvent name and drying reagent?
 - Ensure water-flow monitor are installed that would automatically shut off the heating mantles in the event of cooling water loss (pic with arrow). Periodically test monitors by shutting down the water flow to verify the system is functioning properly. They are available commercially. We strongly recommend this important safety device be adopted. Fires associated with stills are not uncommon, including the \$3M fire at UCI in 2001.



- Ensure secondary containment pans are beneath the stills. In the event of a system leak this should capture any leakage and prevent the solvent from spreading out and finding an ignition source.
- **Quenching Solvent Stills** -The quenching of used still-pots is potentially dangerous but can be done safely if appropriate precautions are taken. "See [EH&S Fact Sheet](#) on still quenching"

Pressurized Systems - Inspect and test all high pressure vessels regularly per the owner’s manual requirements. Each vessel should have a use-log of: experiment conditions, dates of runs, testing/maintenance history, etc. in order to track the vessel’s life-expectancy. Pressure vessels must include a functional over-pressurization rupture disk to prevent a catastrophic vessel failure.

General Safety Concerns

1. Has EH&S posted outside the lab an [emergency information contact sign](#), indicating the hazards within, responsible persons and phone numbers? Is the information correct? Call EH&S to update (x-8243).
2. Has the [UCSB Campus Emergency Flip Chart](#) been posted in the work area? Has the, *Building-Specific Emergency Information* section page has been completed?



3. Are rooms containing regulated hazardous substances, such as infectious and radioactive materials, posted with warning/caution signs and appropriate authorizations?
4. Are aisles free of obstructions? Minimum clearance for lab aisles is 2 ft.
5. Do work areas have adequate ventilation and illumination? To prevent suffocation, verify that fresh air is supplied to cold/hot rooms that are used as work areas. Check emergency door release and alarm mechanisms.
6. Are [fire extinguishers](#) functional (plastic seal and pin intact and show pressure)?
Are the extinguishers located on their wall hooks?
Is the area in front of the extinguishers accessible?
7. Are food and beverages kept out of chemical work areas and out of laboratory refrigerators?
8. Is the [lab poster](#) summarizing the [UCSB Laboratory Personal Protective Equipment \(PPE\) Policy](#) in place and are all affected individuals following its provisions? Do all individuals understand the different PPE requirements for Category 1, 2 and 3 hazardous materials?

For more PPE, including glove reference charts, click [link](#).

9. Have all respirator users been certified through the [Respiratory Protection Program](#) as run by EH&S?
10. Is the level of [housekeeping](#) in the lab satisfactory?
 - No hazardous materials stored on floor
 - Aisles and corridors kept clear
 - Lack of clutter
 - Glassware that is scattered on benchtops and out in the open clutters working areas, is easily broken, will not stay clean, and, if dirty, may be confused for clean glassware and potentially negate any viable research.
11. Lab doors are fire-rated and therefore can not be propped open with a wedge or other device. Discontinue use of these, or SB County Fire may confiscate them and cite the University.
12. Secure your highly hazardous materials, e.g. highly toxic gas, radiation, select biological agents. Ensure the lab door(s), freezers, refrigerators, storage cabinets, etc. with these materials are locked whenever the lab is left unattended.

Electrical Safety

1. Check electrical equipment and inspect for frayed cords and damaged connections? Electrical tape is prohibited.
2. Multiple outlet strips plugged directly into a wall outlet? Does the power strip have a circuit breaker? Extension cords are not to be permanently used with power strips.



3. Are employees instructed **not** to use extension cords in place of permanent wiring (use allowed if only on a temporary, immediate, basis)? Have permanent receptacles installed for long-term electricity needs.
 - Ensure extension cords are 14-gauge (heavy duty) at a minimum, and **temporarily** servicing only one appliance or fixture?
 - Ensure extension cord is plugged directly into receptacle. Extension cords should never be used plugged end-to-end; use the proper length cord.
 - If extension cords are used, ensure cords are not running through walls, ceiling or doors?



4. Are cord guards provided across an aisle or other passageway to prevent tripping?
5. Is all electrical equipment grounded (three-prong plugs) or double insulated?
 - Are 3-prong plugs only used for 3-prong receptacles, and never altered to fit into an outlet?
6. Are Ground Fault Circuit Interrupters in place where electrical outlets are in use within 6 feet of water? Ensure GFCI's are working properly by using the "test" button.
7. Are all electrical boxes, panels and receptacles covered to protect against electrocution?
8. Are control switches, circuit breakers and electrical panels free of obstructions? These items must be accessible at all times.
9. Are high voltage control panels and access doors posted?

Seismic Safety

1. Do shelves used for chemical storage have seismic restraining devices (e.g. lip, wire or bungee cord) installed to prevent chemicals from falling? Is all valuable or hazardous equipment seismically anchored?

Visit web links for securing lab instruments & appliances:

[Seismic Protection Methods for Lab Instruments and Appliances](#)

[Earthquake Restraint System for Optical Tables](#)

[Securing Your Stuff](#)

2. Are cabinets, chemical shelves and furniture over 42 inches in height braced against walls to prevent their falling over in the event of an earthquake?
3. Is overhead storage of heavy objects minimized and restrained?

Administrative

(Note: these training requirements must be met by supervisors to satisfy their personal regulatory obligations)

1. All lab workers are required to complete the UCSB Lab Safety Orientation to satisfy numerous regulatory training requirements. Verify everyone has attended either the live class for [grad students & staff](#) (LS01), or completed the appropriate online course for [undergrads and postdocs](#) (LS04). Visit our [training history](#) link, to view personnel that have completed the course.
2. Are safety training records generated from the class maintained ([Lab Safety Training Checklist](#)) and available for review by employees, EH&S and outside agencies?
3. Are all employees aware of the following:
 - How to access [Material Safety Data Sheets](#) (now known as Safety Data Sheets)?
 - UCSB [Laboratory Personal Protective Equipment](#) policy/poster?
 - Know the location of the emergency eyewash/shower station?
 - The [Emergency Assembly Point](#) for your building?
 - The location of the nearest fire alarm pull station?
 - The three basic types of [fire extinguishers](#) and their applicability?
 - The location/availability of [first aid kits](#) within the building?
 - The location of the Automated External Defibrillator (AED), available in some departments?
 - The location of the circuit breaker box?
 - The location/purpose of your building's Safety Corner bulletin board?
 - The identity of your [Department Safety Rep](#)?
 - The availability/purpose of the UCSB [Hazard Reporting Form](#)

Chemical Waste Disposal

REGULATIONS: Hazardous waste regulations are stringent and penalties for violations can be severe. Santa Barbara County inspects UCSB labs for compliance on a regular basis.

STORAGE

- ♦ Store chemical waste in a designated area. Label as, "**HAZARDOUS WASTE STORAGE AREA**"
- ♦ Store chemicals in containers compatible with, and durable enough for, the waste.
- Liquid waste must be in screw-top containers. Do not overfill - allow for expansion.

LABELING

- ♦ Identify waste by proper chemical name (no abbreviations or chemical structures). List chemical names of hazardous components in that mixture by percent weight.
- ♦ Deface existing labels when reusing containers.
- ♦ Label and date container(s) when the first drop of waste is added. Hazardous waste shall be given to EH&S for disposal within **nine months** of start date.
- ♦ Use **UCSB HAZARDOUS WASTE** label on all hazardous waste containers. All portions of the label must be completed. Labels are available for free in all science storerooms –see below.

SEGREGATION: group waste into the following categories:

- | | |
|--|---|
| ♦ halogenated organics (<i>dichloromethane, chloroform</i>) | -non-halogenated organics (acetone, methanol, xylene) |
| ♦ acids with pH 2 or less (<i>HCL, sulfuric acid</i>) | -alkaline solutions of pH 12.5 or greater (sodium hydroxide) |
| ♦ alkali metals and other water reactives (<i>sodium, lithium</i>) | -heavy metal solutions and salts (<i>mercury, silver, zinc</i>) |
| ♦ strong oxidizers (<i>nitric acid, chlorates, permanganates</i>) | -cyanides (potassium cyanide) |
| ♦ unstable chemicals | |

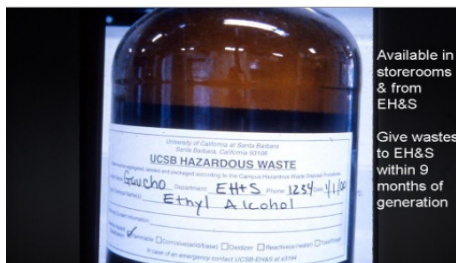
DISPOSAL

- ♦ Chemicals may not be disposed in regular trash, sink disposal, or allowed to evaporate.
- ♦ Complete a *UCSB Waste Pick-up Request Form*. Send by campus mail or fax(X8659). Also available on EH&S website for electronic submission:

<http://www.ehs.ucsb.edu/units/hw/hw.html>

- ♦ *EH&S cannot accept responsibility for improperly labeled, packaged, and/or segregated chemicals, and will not pick them up.*
- ♦ *Transferring waste into appropriate containers is the generators responsibility.*
- ♦ *Waste containers become the property of EH&S and will not be returned*

For more information see: <http://www.ehs.ucsb.edu/units/hw/hw.html>



UCSB Hazardous Waste Label

Disposal of Sharps

Sharps are defined as any object having acute corners, edges or protuberances capable of cutting or piercing, e.g. **needles, razor blades, glass, etc.** These items **can NOT be disposed of in the normal lab trash**. Every year custodians are injured by sharps in trash cans.

1. Lab Glassware Not Contaminated by Hazardous Materials (e.g. Pasteur pipettes)

Place glass into a labeled “*Sharps Only*” trash box or other sturdy container. Cardboard boxes specifically made and labeled for this purpose are available from scientific supply companies, or in campus storerooms. When full, dispose into the dumpster for your building - custodial staff will **not** empty.

Glass disposal box – available in lab storerooms



2. Needles, Syringes, Scalpels Not Contaminated by Hazardous Materials

These are particularly dangerous and require precautions beyond those of glassware:

- Label a **rigid puncture-proof container** with the words “*Sharps Waste*” (example pictured, note cardboard is not acceptable)
- Place all sharps into sharps container as they are generated
- When full, tape container closed or tightly lid containers prior to disposal
- Place sealed sharps containers directly into bldg. dumpster, or into the glass container noted above

Puncture-proof container for disposal of razor blades and syringes. Available in lab storerooms.



3. Sharps Contaminated by Hazardous Materials

If sharps are *uncontaminated* use the procedures above. For contaminated sharps do this:

- Sharps contaminated with chemicals** - A sharps container as described above should be labeled as “*Sharps contaminated with (chemical name)*”. Give to EH&S for disposal with other chemical wastes.
- Sharps contaminated with radioactive materials**- A sharps container as described above should be properly labeled as “*Sharps contaminated with (isotope name)*”. Dispose via EH&S.
- Sharps contaminated with biohazardous (infectious) materials**- must be placed in approved red plastic sharps container (picture above, available in storerooms). The sealed containers must be autoclaved before disposal and within 7 days of becoming filled. Place autoclaved container in an opaque bag and place into dumpster, or glass disposal container noted above.

Section III: REGULATORY FRAMEWORK

A. THE LABORATORY SAFETY STANDARD

1. Background	2
2. Applicability	2
3. Summary of Standard	3

B. THE LABORATORY SAFETY STANDARD AT UCSB

1. Introduction	4
2. Responsibilities	4
3. Standard Operating Procedures	6

C. CHEMICAL USE POLICIES

1. Employee Information and Training	8
2. Hazard Identification	9
3. Criteria for Establishing Exposure Monitoring	10
4. Medical Consultation and Examination	10
5. Criteria for Determination and Implementation of Control Measures	11
6. Particularly Hazardous Substances	12
7. Prior Approval	13
8. Maintenance of Engineering Controls and Emergency Equipment	13

A. THE LABORATORY SAFETY STANDARD

1. Background

The Standard for *Occupational Exposure to Hazardous Chemicals in Laboratories* (commonly known as the “Laboratory Safety Standard”, or “Chemical Hygiene Plan”) was adopted by the California Occupational Health & Safety Administration (Cal/OSHA) Standards Board on February 21, 1991. The Laboratory Standard is summarized on the following page and the [complete text](http://www.dir.ca.gov/Title8/5191.html) of the Laboratory Standard is available online. <http://www.dir.ca.gov/Title8/5191.html>

The intent of the Laboratory Standard is to protect laboratory employees from harm due to chemicals. The design of the Laboratory Standard is based on a recognition by OSHA, and other health and safety professionals, that laboratory work is typically different in character from industrial operations in their use and handling of chemicals. In contrast to many industrial operations, laboratory chemical work often involves a relatively large number of chemicals in relatively small scale procedures. In many labs, particularly those involved in research, the character of chemical usage can also change significantly over time to reflect evolving research conditions.

The Laboratory Standard is a performance standard. That is, there are few specific requirements to carry out procedures in a certain way. This allows the University administration and lab supervisors to make their own decisions, within the spirit (and limitations) of the Laboratory Standard, to determine the means for worker protection. These policy and procedure decisions are formalized within this written Chemical Hygiene Plan and within the lab-specific variations on campus.

2. Applicability

The Laboratory Standard does not apply to all places where hazardous chemicals are used in experiments and investigations. Labs meeting the following four criteria are subject to the Laboratory Standard:

- Chemical manipulations are carried out on a laboratory scale. That is, chemical containers are of a size that could be easily and safely manipulated by one person.
- Multiple chemical procedures are used.
- The procedures involved are not part of a production process, nor in any way simulate a production process.
- Protective laboratory practices and equipment are available and commonly used.

Clearly, most research and teaching laboratories here at UCSB, meet these criteria and therefore fall under the scope of the Laboratory Standard.

Students in campus teaching laboratories are not employees of the University and therefore do not fall under the provisions of the Laboratory Standard. Although not legally required to be given the same consideration as employees, it is the judgment of the University, that it is obligated to develop policies, procedures and course lab manuals which attempt to provide the same level of protection for students. It should also be noted that teaching assistants (TAs), faculty and staff who teach in instructional labs are covered by the Lab Standard and therefore need to be included in a Chemical Hygiene Plan.

3. Summary of the Laboratory Safety Standard

The [Laboratory Standard](#) contains the following elements. Each of these is addressed within the sections of this manual as noted

- **Chemical Hygiene Plan (CHP)**— A written plan must be developed to control and minimize chemical exposure in laboratories. The CHP must be readily available to affected employees, who need to be oriented to its provisions and relevance to their health and safety. [*this document*]
- **Responsibilities**— Personnel responsible for implementation of the CHP must be designated, including the appointment of a Chemical Hygiene Officer. [*Sec. III.B.2*]
- **Employee Information and Training**— The employer shall provide employees with information and training to ensure that they are informed of the hazards in their work area and their avoidance. [*Sec. III.C.1*]
- **Exposure Limits**— Occupational exposures to chemicals must not exceed specified Cal-OSHA levels. [*Sec. III.C.3*]
- **Employee Exposure Determination**— As appropriate, measurements must be taken to verify that exposure limits are not exceeded. [*Sec. III.C.3*]
- **Medical Consultation and Examinations**— Employees are entitled to medical attention when a significant chemical exposure is suspected. [*Sec. III.C.4*]
- **Hazard Identification**— Safety Data Sheets (formerly known as MSDS) and other reference materials need to be available. Labeling of chemicals is strictly regulated. [*Sec. III.C.2.*]
- **Control Measures**— Criteria must be established that the employer will use to determine, implement and adequately maintain control measures to reduce employee exposures, including lab ventilation, personal protective equipment, etc. [*Sec. III.C.5*]
- **Standard Operating Procedures**— SOPs must be developed, as needed, relevant to safety and health considerations when lab work involves the use of hazardous chemicals. [*Secs. I and III.B.3*]
- **Prior Approval**— Circumstances must be stipulated under which a particular laboratory operation, procedure or activity requires prior approval from the lab supervisor. [*Sec. III.C.7*]
- **Particularly Hazardous Substances**— Provisions must be specified for additional employee protection for work with substances such as "select carcinogens", high acute toxicity substances and reproductive toxins. [*Secs. I and III.C.6*]
- **Maintenance of Engineering Controls and Emergency Equipment** – fume hoods comply with section 5154.1 (Title 8, CCR) and that protective equipment function properly. [*Sec. III.C.8*]

B. THE LABORATORY SAFETY STANDARD AT UCSB

1. Introduction

The UCSB *Policy on Environmental Health and Safety* (Policy P-5400) states:

The University shall maintain as safe and healthy an environment as is reasonably feasible for its students, faculty, staff and visitors by:

- Conducting its operations and activities in a safe manner to minimize the risks of injury to people and minimize property damage at all locations where University operations and/or activities occur.
- Complying with applicable regulations, safety and health consensus standards, and practices generally accepted by experts in the field.

Therefore the intent of the University is to abide by the provisions of the Laboratory Standard to as great an extent as is reasonably feasible.

2. Responsibilities

a. Management

Department heads, deans, supervisors, vice-chancellors and the chancellor are responsible for ensuring that individuals under their management have the training and authority to implement appropriate health and safety policies and practices relative to the Laboratory Standard and per campus policy #5400.

b. Laboratory Supervisors/Principal Investigators

The term “supervisor” at UCSB refers to anyone having direct supervisory authority, and includes staff administrators, class instructors, research assistants, managers, and faculty. Under Cal/OSHA’s Injury and Illness Prevention Program, supervisors are responsible for protecting the health and safety of employees and students under their supervision. A [helpful guideline](#) to many common specific tasks of a lab supervisor can be found online. Supervisors can delegate specific tasks, but can not delegate their overall responsibility. General responsibilities include the following:

- Ensure that all employees under their supervision (staff and students), are aware of potentially hazardous operations (materials, processes, equipment) that could be encountered in their lab.
- Ensure training requirements are fulfilled and records are maintained – see Sec III.C.1
- Enforce safety rules within the lab, such as the wearing of personal protective equipment (Sec. III.5.D)
- Monitor the proper procurement, use and disposal of chemicals used in laboratory operations.
- Seek ways to improve chemical hygiene and laboratory safety within their operation.
- Implement policies and procedures described in this CHP, including:
 - the development of written Standard Operating Procedures (Sec.I) for hazardous lab operations, which involves:
 - determination/implementation of appropriate control measures (Sec. III.C.5),
 - development/implementation of provisions for additional employee protection from Particularly Hazardous Substances (Sec. III.C.6), and

- establishing criteria for any needed prior approval processes (Sec. III.C.7).

c. Environmental Health & Safety (EH&S)

Develop safety education and monitoring programs to maintain a safe and healthy environment for faculty, staff, students and visitors in order to facilitate the research and teaching functions of the University. Support research and instructional activities by developing legally mandated programs, providing technical guidance and consulting with and assisting departments in program implementation and maintenance. Make every effort to keep operations functioning smoothly in labs while meeting health, safety and environmental obligations.

d. Chemical Hygiene Officer

The Lab Standard specifically calls for the appointment of a Chemical Hygiene Officer by the employer: *“An employee who is designated by the employer, and who is qualified by training or experience, to provide technical guidance in the development and implementation of the provisions of the Chemical Hygiene Plan.”*

This role at UCSB is assigned to the EH&S Laboratory Safety Program manager. Duties: develop and distribute the *UCSB Chemical Hygiene Plan* to laboratory supervisors. Assist and advise faculty and staff in the customization and implementation of their CHP as requested/needed. Describe the provisions of the CHP to those attending EH&S lab safety trainings and orientations. Monitor and evaluate the effectiveness of the CHP. In cooperation with, and as a member of, the Laboratory Safety Committee, advise campus management on the effectiveness of CHP implementation and make recommendations for upgrades to the program.

e. Chemical Safety Committee and Laboratory Safety Committee

The Chemical Safety Committee reviewed and approved earlier versions of the *UCSB Chemical Hygiene Plan* as drafted by EH&S. In 2010 the committee was superseded by the Laboratory Safety Committee (LSC). The LSC assumed responsibility for overview and periodic review of the campus Chemical Hygiene Program.

f. Departments and Organized Research Units

- Under the campus *Injury and Illness Prevention Program* (Cal-OSHA requirement), and the associated [UCSB written program](#), the **department/unit Head or Chair** is identified as the individual with the authority and responsibility to implement the IIPP. The IIPP is the umbrella OSHA regulation, under which all worker safety programs - including laboratory safety - exist.
- To assist the Chair/Unit Head, each department has a *Department Safety Representative (DSR)* who coordinates health and safety program elements in the department and serves as a liaison with EH&S. The department may also have an Management Services Officer, safety committee, or lab manager.
- Responsible for maintaining a safe environment—including general oversight of department operations and communicating with supervisors/personnel any relevant safety issues, problem solving, and preplanning for emergencies.
- Per campus policy, departments are responsible for ensuring that all new lab workers attend one of the EH&S General Laboratory Safety orientations before access to departmental labs is granted - see also Sec. III.C.1.
- Ensure that all operations under departmental control develop and implement lab-specific CHPs. While individual lab supervisors have the primary responsibility for CHP development, department administrations need to coordinate and support these efforts.

g. Laboratory Workers (non-supervisors: graduate students, undergraduates, postdoctoral staff; permanent staff, visiting researchers, etc.)

General responsibilities are below - a more [complete list](#) is online.

- Follow the established work policies, procedures and safety guidelines in their area including those delineated in the campus and lab-specific Chemical Hygiene Plans.
- Attend and actively participate in appropriate safety training.
- Notify the laboratory supervisor or EH&S of any unsafe or potentially unsafe condition.

Workers also have rights under the law - See also the *UCSB Injury & Illness Prevention Program*

- to be informed of the hazards in their workplace
- to be properly trained on safe work practices
- to be provided appropriate personal protective equipment needed for the job at no cost to them
- to file a complaint with Cal-OSHA if they feel they are being exposed to unsafe conditions and no reprisals can be taken against them. Workers are encouraged to report any safety concerns to EH&S – anonymously if they wish.

h. Facilities Management

(see also Section III: C(8))

- Ensure the timely repair of malfunctioning physical plant equipment such as fume hoods, eye washes, safety showers, alarms, etc.
- Notify lab personnel and/or EH&S as appropriate when equipment is inoperable or malfunctioning and may affect the safe operation of University laboratories.

3. *Standard Operating Procedures*

As touched on in the previous section, the incorporation of Standard Operating Procedures (SOP) into our Chemical Hygiene Plan is a requirement. Given the diverse nature of laboratory work at UCSB, it is **incumbent on individual lab supervisors to develop lab-specific SOPs for operations which involve the use of a “hazardous chemical.”** Under the OSHA Standard, a “hazardous chemical” is one for which:

“...there is statistically significant evidence based on at least one study conducted in accordance with established scientific principles that acute or chronic health effects may occur in exposed employees.”

Standard Operating Procedures (SOPs) are written instructions that detail the steps that will be performed during a given experimental procedure and include information about potential hazards and how these hazards will be mitigated. SOPs should be written by laboratory personnel who are most knowledgeable and involved with the experimental process. The development and implementation of SOPs is a core component of promoting a strong safety culture in the laboratory and helps ensure a safe work environment. PIs/Laboratory Supervisors are required to develop and implement laboratory-specific SOPs for certain hazardous chemicals and “particularly hazardous substances” (PHS) that are used in their laboratories. For certain hazardous chemicals, PHS, or specialized practices, consideration must be given to whether additional consultation with safety professionals is warranted or required. Circumstances requiring prior approval from the PI/Laboratory Supervisor must also be addressed in laboratory-specific SOPs. SOPs should be kept in the Laboratory Safety Manual under the appropriate tab.

Guidance on how to prepare SOPs are given in Section I. In some UCSB departments “communal SOPs” have been developed for sharing amongst researchers who do similar operations. Also, at the time of this writing, “UC communal SOPs” are being developed for sharing - beginning with UC departments of Chemistry & Biochemistry. However, to adequately meet regulatory requirements, it is necessary that individual PIs/lab supervisors review and customize any communal SOP to meet their specific need.

In addition to the many chemical hazards in laboratories there are, of course, many associated *physical and biological hazards* such as electricity, radiation, high and low temperature operations, infectious organisms, etc. The Laboratory Standard does not stipulate that these be addressed within a Chemical Hygiene Plan. However, where deemed appropriate, it is strongly recommended that these issues are also addressed by an SOP. As noted above for chemical hazards, communal SOPs for some other types of hazards are available, or being developed.

C. CHEMICAL-USE POLICIES

1. Employee Information and Training

One of the major provisions of the Laboratory Standard - as well as the OSHA *Injury and Illness Prevention Program* - is a requirement for employee information and training. The employer must convey information to the employee regarding the general and specific occupational hazards identified in the workplace. In general, training is required for:

- All new employees
- Employees given new job assignments involving exposure situations for which training has not previously been received
- Whenever the employer is made aware of a new or previously unrecognized hazard for which training has not previously been received

Regarding training specific to the Laboratory Safety Standard, an individual's supervisor has responsibility for ensuring that personnel have had training on the elements listed below as stipulated in the Standard and any other required safety training. They are also responsible for ensuring that training records are maintained.

Laboratory Safety Training Policy

As of 2010, the UCSB policy titled: [Laboratory Safety Training - General](#) requires that all “lab workers” complete an EH&S fundamentals of laboratory safety training in order to be given access to their lab(s) by their department. See Sec. II: “*Campus Policy on Laboratory Safety Training*”. EH&S regularly offers this fundamentals training via a 3-hour live training, or Web-based version for certain audiences. Graduate students are required to take the live training.

Participation in the EH&S class goes a long way toward satisfying training requirements and thereby reducing potential liability. However, this class is general and does not cover the specific hazards and associated safe work practices for a particular lab. The lab supervisor is still responsible for ensuring that their employees are trained on the **specific** hazards and work practices appropriate for their laboratory. The EH&S training does cover the following elements of the OSHA Laboratory Safety Standard:

Safety Training Required by the Laboratory Safety Standard

- Employee rights and responsibilities under the Cal/OSHA Laboratory Standard and other regulations. *[Covered in EH&S Lab Safety Class]*
- The contents of the generic portions of the *UCSB Chemical Hygiene Plan* and its relation to the Laboratory Standard. *[Covered in EH&S Lab Safety Class]*
- The contents of the lab-specific Chemical Hygiene Plan, including any Standard Operating Procedures (SOPs) that have been developed. *[Lab supervisor training responsibility]*
- The physical and health hazards of hazardous materials including signs and symptoms of overexposure, particularly those defined as Particularly Hazardous Substances (see Sec. I and Sec. III: C(6)). As appropriate, training can address entire classes of hazardous materials (e.g., carcinogens) rather than individual substances. *[Major classes of chemical hazards are covered in EH&S Lab Safety Class]*
- Appropriate use and maintenance of control measures including engineering controls, personal protective equipment, and work practices. *[Generic control measures covered in EH&S Lab Safety Class]*
- The Permissible Exposure Limits for OSHA-regulated substances. *[PEL concept and access to PELs covered in EH&S Lab Safety Class]*
- Hazardous materials labeling, storage, and signage requirements. *[Covered in EH&S Lab Safety Class]*
- Relevance and access to SDSs (MSDS) and other informational references and resources pertinent to the lab. *[Use of, and general access to, SDSs covered in EH&S Lab Safety Class. Access to lab-specific SDSs is the responsibility of the lab supervisor]*
- Hazardous waste minimization and disposal practices. *[Covered in EH&S Lab Safety Class]*
- Spill response and emergency procedures. *[Covered in EH&S Lab Safety Class]*

EH&S Training Records

Currently, there is a [Web interface](#) which allows EH&S training records to be searched by worker name, supervisor name, or department for the period from July 2000 to approximately July 2012. EH&S-performed or online provided training which is completed after July 2012 is documented in the [UC Learning Center](#). Individuals can access the Learning Center using their UCSB NetID and password to view EH&S trainings and their training history. Likewise, upon request, departments can have designated administrator(s) provided access to all the training records for individuals associated with their department.

Other EH&S Training Programs

Enrollment in regularly-offered EH&S training courses, and links to trainings at other universities are through the [EH&S website](#).

2. Hazard Identification

University policies on hazard identification with respect to labeling and Material Safety Data Sheets are as follows:

- Labels on incoming containers of hazardous chemicals are not to be removed or defaced.

- The primary campus access to Safety Data Sheets (formerly known as MSDS) is through the internet, particularly the UC SDS search engine. See EH&S website. Individual labs are encouraged to maintain their own hardcopy files as well.
- For chemical substances developed in University laboratories, the provisions for hazard determination, training and labeling shall be those stated in the Laboratory Standard.

3. *Criteria for Establishing Exposure Monitoring*

The legally-acceptable levels for occupational exposure to approximately 500 chemicals which are toxic by inhalation, or skin contact, are codified by OSHA in so-called [Permissible Exposure Limit \(PEL\)](#) values. These values are generally expressed in parts per million (or mg/m³) and can relate to short-term (15 min.), long-term (8 hour), or ceiling level exposures. In Sec. II is a list of PELs for some common laboratory chemicals (*Exposure Limits for Laboratory Chemicals*).

The University policies on dealing with employee exposure determination and exposure monitoring shall be those expressed in the Laboratory Standard (5191(d)). Initial exposure monitoring for any substance regulated by an OSHA standard will be performed if there is reason to believe that exposure levels exceed the action level, or Permissible Exposure Limit. The tasks of determining if exposure monitoring is required, performing or supervising exposure monitoring and other related duties shall be the sole responsibility of the EH&S Industrial Hygiene and Laboratory Safety Divisions. All suspected chemical exposures on campus shall be reported in a timely manner to EH&S.

For regulated carcinogens (See Sec. II) EH&S does periodic reviews of usage practices to establish if there is reason to suspect there are significant exposures. The review process typically includes: a) evaluation of the chemical inventory questionnaire for each lab; b) distribution and evaluation of a carcinogen use questionnaire if a regulated carcinogen appears on the inventory; c) exposure monitoring if usage practices suggest possible routine exposure, e.g., a material is used in larger quantities, or frequently, or outside of a fume hood.

4. *Medical Consultation and Examination*

The Laboratory Standard states that: ***“The employer shall provide all employees who work with hazardous chemicals an opportunity to receive medical attention, including any follow-up examinations which the examining physician determines to be necessary, under the following circumstances”:***

- When an employee develops signs or symptoms associated with a hazardous chemical to which that employee may have been exposed
- Where exposure monitoring reveals an exposure level routinely above the action level or PEL for an OSHA-regulated substance
- Whenever an event takes place in the work area such as a spill, leak, or explosion resulting in the likelihood of a hazardous exposure”

The University has established the following policies and procedures, when it is known or suspected that an employee (or student) has been exposed to a hazardous chemical(s) or is otherwise injured on campus.

- All employees suffering from chemical exposure or other work-related injury incurred at UCSB shall be evaluated/examined at University expense. Non-employees such as students would be covered by their required medical insurance.

- If the injured/exposed person is safe and able to be transported, escort them to either Student Health Services on campus, Goleta Valley Community Hospital, or their primary physician for evaluation and examination. Contact EH&S at x3194 immediately.
- In some cases a work-related chemical exposure may be suspected but not certain. For example, some low-level but chronic exposures may be difficult to identify or relate to specific symptoms. In these instances, contact the Chemical Hygiene Officer at x4899 to arrange a review of the circumstances of the suspected exposure.
- The Laboratory Standard includes specific provisions regarding the employer's exchange of information with the examining physician. The provisions of the Laboratory Standard be followed as stipulated in that section.

5. *Criteria for Determination and Implementation of Control Measures*

The Laboratory Standard states that the CHP "... *shall include criteria that the employer will use to determine and implement control measures to reduce employee exposure to hazardous chemicals*"

Hazard controls are generally classified into three broad groups: engineering controls, administrative procedures and personal protective equipment. The University policies on determination and implementation of control measures are delineated here.

A. General

- Appropriate control measures shall be implemented in University laboratories to reduce chemical exposures and physical hazards to as low a degree as reasonably achievable.
- Lab supervisors shall determine and implement appropriate control measures, but in accordance with regulations and campus policy
- Environmental Health & Safety shall be responsible for assisting the above in determining and implementing these control measures. EH&S may do periodic evaluations of control measures on campus as deemed necessary and notify lab supervisors of their results and recommendations.

B. Engineering Controls—Criteria for Implementation

• **FUME HOODS, WET BENCHES, GAS CABINETS AND OTHER LOCAL EXHAUST VENTILATION**

- When using volatile substances that present a significant inhalation hazard
- When necessary to keep exposure levels below OSHA Permissible Exposure Limits, or action levels
- When using toxic gases above exempt amounts as defined by CA Fire Code
- When indicated in Standard Operating Procedures
- As indicated in MSDSs

• **BIOSAFETY CABINETS**

- With laboratory operations involving biohazardous material as directed by National Institutes of Health (NIH) and Centers for Disease Control (CDC) guidelines and the OSHA Bloodborne Pathogens Standard

- When stipulated by the Biosafety Authorization issued by the campus Biosafety Committee
- When indicated in Standard Operating Procedures
- **GLOVE BOXES**
 - When indicated in Standard Operating Procedures
- **APPROVED HAZARDOUS MATERIALS STORAGE CABINETS AND SAFETY CANS**
 - When CA fire code mandated volume limits for flammable liquids are exceeded
 - When indicated in Standard Operating Procedures
- **FLAMMABLE STORAGE REFRIGERATORS (APPROVED-TYPE)**
 - When refrigerated storage of flammable materials is needed

C. Administrative Controls—Criteria for Implementation

The variety of possible administrative controls to reduce hazard levels in laboratories is large. The controls instituted by a given laboratory shall be determined by the lab supervisor in consultation with EH&S, as required. In general, measures shall be implemented:

- As indicated in Standard Operating Procedures
- As necessary to reduce hazards as mandated by current health and safety regulations or as called for by accepted good practice

D. Personal Protective Equipment—Criteria for Implementation

The appropriate personal protective equipment (PPE) is stipulated in the UCSB policy: [Laboratory Personal Protective Equipment](#) (June 2011). The key provisions of the policy are summarized in Sec. II of this manual (*Campus Policy on Laboratory Personal Protective Equipment (PPE)*), including an educational poster that is mounted in all campus laboratories.

6. Particularly Hazardous Substances (PHS)

The Laboratory Standard states that: *“The Chemical Hygiene Plan shall include... provisions for additional employee protection for work with hazardous substances, including “select carcinogens,” reproductive toxins and substances which have a high degree of acute toxicity*

SELECT CARCINOGENS— Includes carcinogens as listed by the following organizations: OSHA; the National Toxicology Program; the International Agency for Research on Cancer.

REPRODUCTIVE TOXINS— A chemical which affects human reproductive capabilities, including chromosomal damage (mutations) and effects on fetuses (teratogenesis).

HIGH ACUTE TOXICITY SUBSTANCES— Substances such as hydrogen cyanide, hydrogen sulfide, and nitrogen

dioxide which may be fatal or cause damage to target organs as a result of a single exposure or exposures of short duration.

It is the responsibility of individual laboratory supervisors to institute Standard Operating Procedure(s) for using a specific Particularly Hazardous Substance in their laboratories. See Section I for template forms and instructions, etc. Also, see the SOP section herein (Sec. III.B.3)

Some recognized *general* safe work practices for the use of PHS are provided in this manual. A generic PHS Standard Operating Procedure is provided in the lab-specific CHP section of this manual. Due to its broad application, the information therein is very basic and common sense. However, again, lab supervisors are encouraged to expand upon this basic protocol as needed. These general procedures were approved by the campus Chemical Safety Committee as reasonable Standard Operating Procedures by which all labs on campus should abide.

The PHS section of the Lab Standard goes on to say: “*Specific consideration should be given to the following provisions which shall be incorporated where appropriate:*”

1. “*Establishment of a designated area*” (an area where all PHS should occur, unless another area is designated by the supervisor, the default designated area is the entire laboratory)
2. “*Use of containment devices such as fume hoods or glove boxes*” (See Sec. III.C.5)
3. “*Procedures for safe removal of contaminated waste*” (covered in Sec. II and individual SOPs)
4. “*Decontamination procedures*” (cover in individual SOPs)

7. *Prior Approval*

Another provision of the Laboratory Standard is for incorporating policies into the CHP on: “*The circumstances under which a particular laboratory operation, procedure or activity shall require prior approval from the employer or the employer’s designee before implementation.*”

Given the large diversity of chemical work performed in campus laboratories, it is impossible to specify the particular laboratory operations which would require prior approval. **It is therefore the responsibility of individual lab supervisors to establish these criteria for their operations. Establishment of prior approval criteria, if any, is solely the prerogative of the lab supervisor.** These criteria should be incorporated into the Standard Operating Procedures portion of their Lab-specific Chemical Hygiene Plan. In Section I of this document are provided template forms that lab supervisors can use for this purpose.

8. *Maintenance of Engineering Controls and Emergency Equipment*

As stated in the Laboratory Standard, provisions for maintenance of both control measures and emergency equipment must be included in the CHP: “*.....a requirement that fume hoods comply with section 5154.1 (Title 8, CCR), and that all protective equipment shall function properly and that specific measures shall be taken to ensure proper and adequate performance of such equipment....*”

It is the policy of the University that all control measures designed to reduce chemical or physical hazards to employees shall be maintained in proper working order. If not functioning properly, or inadequate for the task, it is the responsibility of the campus entity responsible for the equipment to immediately inform affected workers of its status. Such equipment shall be repaired/replaced on a timely basis.

General Responsibilities:

FACILITIES MANAGEMENT (FM): Responsible for routine maintenance, replacement and installation of University-owned building emergency systems and environmental controls.

ENVIRONMENTAL HEALTH & SAFETY: Responsible for evaluating effectiveness of engineering control measures and emergency equipment used on campus. Will make recommendations to FM and users on implementation of appropriate equipment and control measures.

LAB SUPERVISOR/LAB PERSONNEL: Responsible for monitoring status and effectiveness of equipment and control measures. Responsible for reporting to appropriate campus entity if equipment is not functioning properly or if new procedures require changes or additions to existing systems.

Specific Responsibilities:

FUME HOODS/GAS CABINETS (per CCR, Title 8, 5154.1)

Maintenance: Facilities Management
Annual certification: EH&S

BIOSAFETY CABINETS (per CCR, Title 8, 5154.2)

Maintenance: Owner (generally lab supervisors)
Annual certification: Generally, owner covers cost of outside vendor certification. Biosafety Committee typically requires cabinet use as part of their authorization

OTHER LOCAL EXHAUST VENTILATION

Maintenance: Facilities Management

EMERGENCY SHOWERS AND EYEWASHES (per CCR, Title 8, 5162)

Maintenance & testing: Facilities shall check on a regular basis by running water through them until the water runs clear and repair as needed and keep records.

RESPIRATORS (per CCR, Title 8, 5144)

Administration: EH&S has sole responsibility for approval, fit-testing and issuance. Lab supervisors are responsible for identifying and directing individuals to EH&S who may require respirators in their work.
Maintenance: Lab supervisor or designee

OTHER PERSONAL PROTECTIVE EQUIPMENT (per CCR, Title 8, 3380-3387)

Maintenance: Generally provided by the lab supervisor -- maintenance/replacement are the responsibility of the supervisor and lab personnel

FIRE EXTINGUISHERS, DETECTORS, ALARMS, SUPPRESSION SYSTEMS (per CA Fire Code and Building Code)

Administration: The campus Fire Marshal 's office is responsible for evaluation and approval of these systems
Maintenance: Facilities Management

GAS DETECTION/ALARM SYSTEMS (per CA Fire Code and Building Code)

Administration: EH&S has responsibility for evaluation and approval of code-required gas detection/alarm systems
Maintenance: Facilities Management/Cleanroom staff

EMERGENCY PHONES

Maintenance: Communications Services

HAZARDOUS MATERIALS SPILL RESPONSE EQUIPMENT (per CCR, Title 8, 5192)

Maintenance: EH&S is responsible for equipping, maintaining and using the primary hazardous materials response equipment stores for the campus. Individual labs or departments may have local supplies which they maintain.