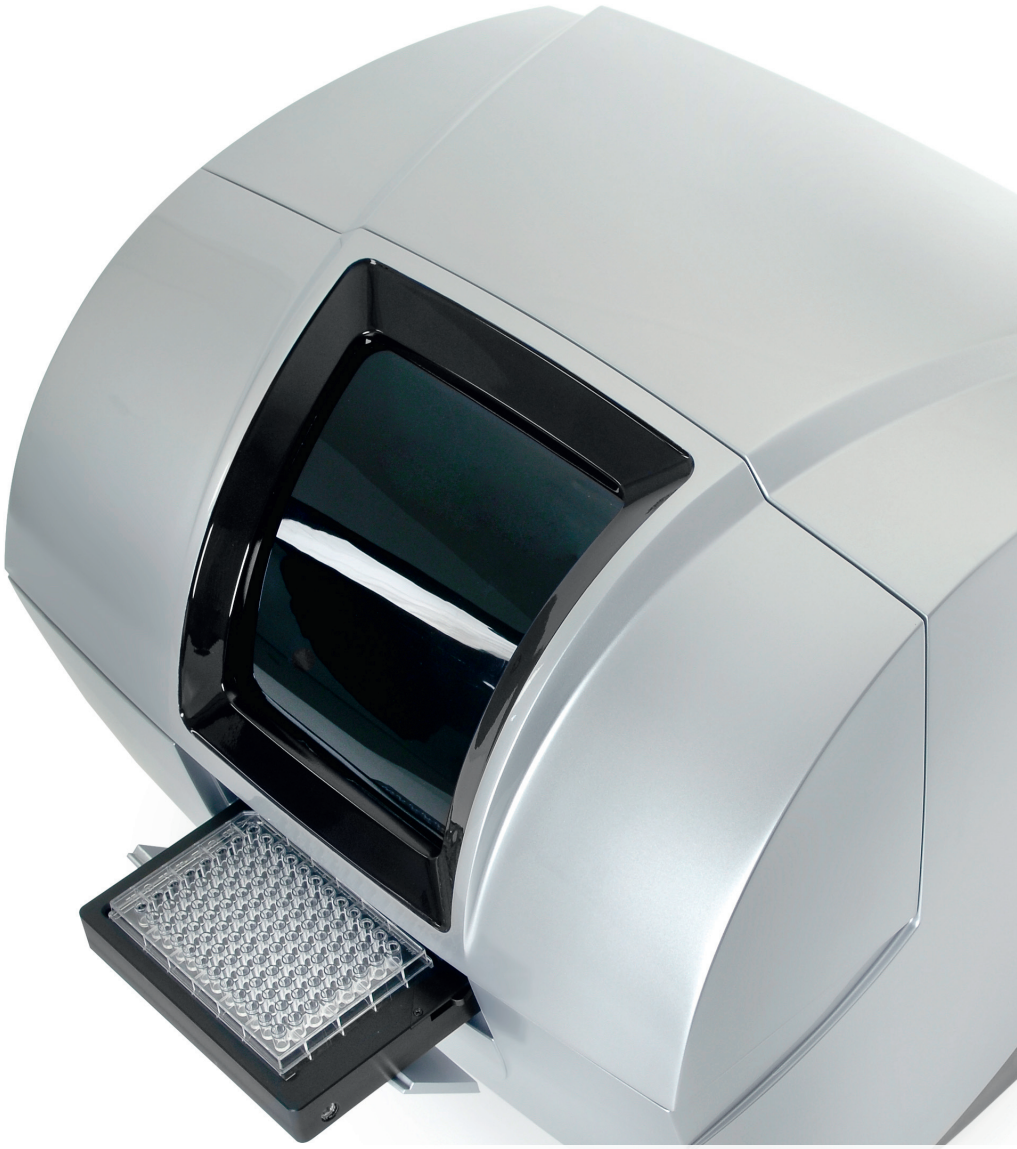
Malvern



PARTICLE SIZE

MOLECULAR SIZE

ZETASIZER **APS**
USER MANUAL

Zetasizer APS
User Manual

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Part 1 -
Operator's Guide

Introduction to this manual

Introduction

This manual covers the operation and maintenance of the Zetasizer APS particle size analyzer.

Note
The model, serial number, software and firmware version can be found by left-clicking the APS 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

Read this manual together with the **Zetasizer APS Basic Guide**, which describes Site Requirements, Health and Safety and Maintenance information.

Warning!
The instrument and the samples to be measured may be hazardous if misused. Users must read the **Health and Safety** information in the **Basic Guide** before operating the system.

We recommend reading both these manuals fully before starting the first measurement, though if more familiar with particle size analyzers, jump straight to **Chapter 4**. This chapter gives practical details on making measurements.

This manual is divided into three sections as follows.

Part 1 – Operator's guide

This guide and the **Basic Guide Manual** contain all the information required for the operator to use the Zetasizer APS instrument.

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

Part 2 – Supervisor's guide

The Supervisor's guide concentrates on the administration the Zetasizer APS and covers some of the more advanced features. It also provides a greater insight into the measurement procedures and expands on the analysis theories.

Topics covered are: security aspects, use of **S**tandard **O**perating **P**rocedures (**SOP**s), and organising the measurement files, and discussion on each of the analysis theories used.

It is recommended that the supervisor should also read Part 1 – Operator's guide.

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 main covers by unauthorised personnel, even a supervisor, will invalidate the warranty of the instrument. This excludes removal of the front cover, which gives access to the internal user-serviceable parts, when following the procedures described in the **Basic Guide**.

Supervisor

The supervisor is responsible for the management and safety of the instrument and its operation. The supervisor also trains the operators. They can perform all user maintenance routines identified in the **Basic Guide**.

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 the **Basic Guide**, 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 APS will either be referred to in full, as the Zetasizer, or as “the instrument”.

The combination of the Zetasizer APS instrument, the computer and Zetasizer software will be referred to as the “the system”.

Plates

384 or 96 well plates are generally referred to as a “plate” or sometimes “well plate”. In industry, they are also dubbed “assay plates” or “microplates”.

Solvents and dispersants

To reflect the intended protein analysis applications of the system, the term “solvent” is used throughout this manual to refer to any fluid used to disperse a sample’s particles for measurement. However, solvent can also be taken to mean “dispersant” where this is more applicable to the sample type.

Menu commands

Menu commands from the Zetasizer software are referred to in the form **main menu-menu item**. As an example, the command **Measure-Plate Scheduler** refers to selecting the **Plate Scheduler** item in the **Measure** menu. Menu commands are shown in bold text.

Where to get help

Manuals and Software Help

In addition to this manual and the **Zetasizer APS Basic Guide**, the software contains a complete help system. Most windows within the software have a Help button. Click this to display help topics specific to that window. Alternatively press **F1** on your keyboard.

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).
- 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. This help line is primarily English speaking.

Remote support

Malvern Instruments offers a remote support service over the Internet. 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 must be available to make use of this facility.

Malvern Website - www.malvern.com

The Malvern website offers a comprehensive range of particle characterization resources for use by customers 24 hours a day, seven days a week.

Resources include software downloads, frequently asked questions, a knowledge base and application notes, plus information on other particle characterization solutions that Malvern can provide.

2

What is the Zetasizer APS?

Introduction

This chapter gives a brief overview of the Zetasizer APS instrument and simple explanations about the measurement technique.

About the Zetasizer APS

The Zetasizer **A**uto **P**late **S**ampler automates particle size measurements from samples prepared in 96- or 384-well plates.

More specifically, the Zetasizer APS instrument provides the ability to measure size characteristics of particles or molecules in a liquid medium. This allows characterisation of the protein size distribution to determine the presence and proportion of aggregates as a function of solvent and buffer conditions, speeding development of the protein's optimum environmental conditions.

By using unique technology, particle size can be measured over a wide range of concentrations. The Zetasizer APS also has the ability to perform **Trend** measurements, including the determination of the **Protein aggregation point**.

The Zetasizer APS features pre-aligned optics and the precise temperature control necessary for reproducible, repeatable and accurate measurements.

Designed with simplicity in mind, the Zetasizer APS requires only a minimal amount of user interaction to achieve excellent results. The use of **S**tandard **O**perating **P**rocedures (SOPs) alleviate the need for constant attention.

What is Particle Size?

This section describes the basics of Particle Size and why it is important. Greater detail on the instrument's measurement techniques is given in **Chapter 12**.

What is Size?

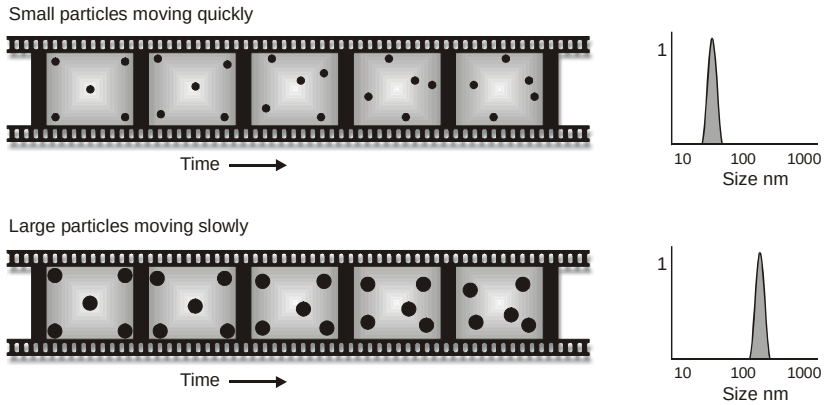
The Particle Size measured in a DLS instrument is the diameter or radius of the sphere that diffuses at the same speed as the particle being measured.

The Zetasizer APS determines the **size** by first measuring the **Brownian motion** of the particles in a sample using **D**ynamic **L**ight **S**cattering (**DLS**) and then interpreting a size from this using established theories - see **Chapter 12**.

Brownian motion is defined as:

"*The random movement of particles or molecules in a liquid due to the bombardment by the solvent 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.



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Small particles move quickly in a liquid and large particles move slowly. This movement is continuous, so if two 'pictures' of the sample are taken, separated by a short interval of time, for example 100μS, the range of movement of the particle can be measured which can be used to calculate its size.

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. A more detailed explanation is given in **Chapter 12**.

3

How does the Zetasizer APS work?

Introduction

This chapter introduces the Zetasizer APS hardware and software features as follows:

- The overall method employed by the system to measure a sample.
- An outline of the steps involved in making a measurement.
- Details on the major components of the system.
- An overview of the Zetasizer software.

The complete measurement process is described in later chapters.

Measurement overview

The Zetasizer APS extracts a sample directly from a plate well and then measures it within an enclosed measurement area. The sample is then either sent to waste or returned to the plate. A system of rinsing the extraction needle and tubes, before and after measurement, minimises the possibility of contamination. The sample measurement area of the Zetasizer APS is completely enclosed within the instrument and can control the temperature of the sample from 2°C to 90°C.

Optionally, a Plate Temperature Controller (PTC-1000) can be attached to the system to maintain the plate itself within the 4°C to 40°C range. This not only has the advantage of keeping the sample at the correct storage temperature, but also potentially reduces the amount of equilibration time needed to reach the required measurement temperature.

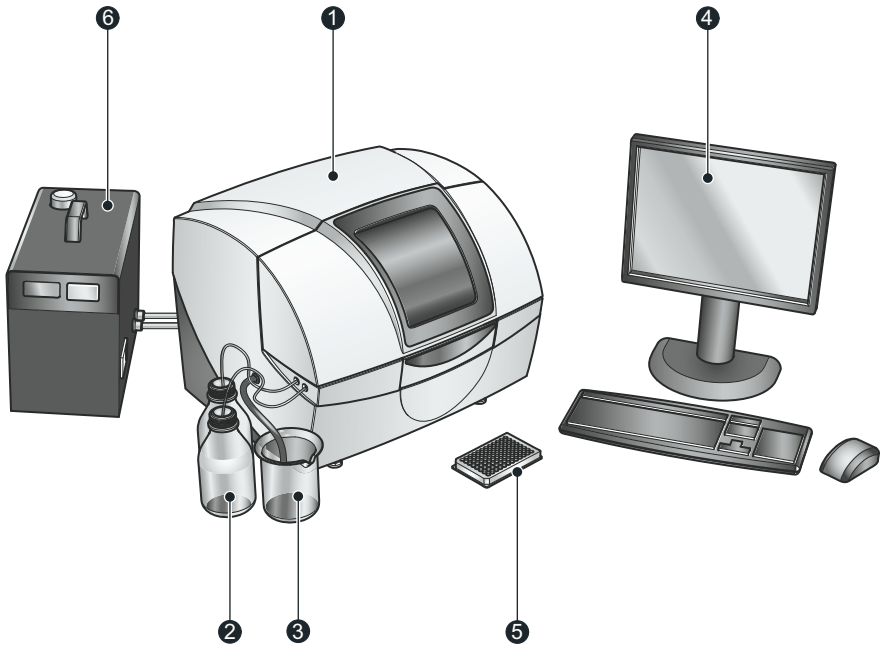
By using the Zetasizer software the user has full control over the measurement, including the ultimate destination of the sample and the rinsing cycle.

There are two halves to all Zetasizer APS measurements:

- **Standard Operating Procedures (SOPs) -** these are templates pre-defining all the measurement settings to ensure that measurements are made in a consistent way. SOPs not only remove the tedium of repetitive inputting of parameters, but also reduce the risk of making errors. SOPs can be created, saved and modified as required.
- **Plate schedule -** the Zetasizer APS uses a plate schedule each time it performs a measurement. This allows SOPs to be allocated to individual wells in the plate. It is possible to perform any SOP against any well in the plate as required.

Zetasizer APS hardware

A typical system, shown below, comprises the Zetasizer APS instrument ①, two rinse bottles - one solvent, one wash ②, a waste receptacle ③ and a computer ④ with the Zetasizer software installed. A plate ⑤ is filled with the sample and loaded into the instrument. The optional Plate Temperature Controller ⑥ maintains the plate at a pre-selected temperature.



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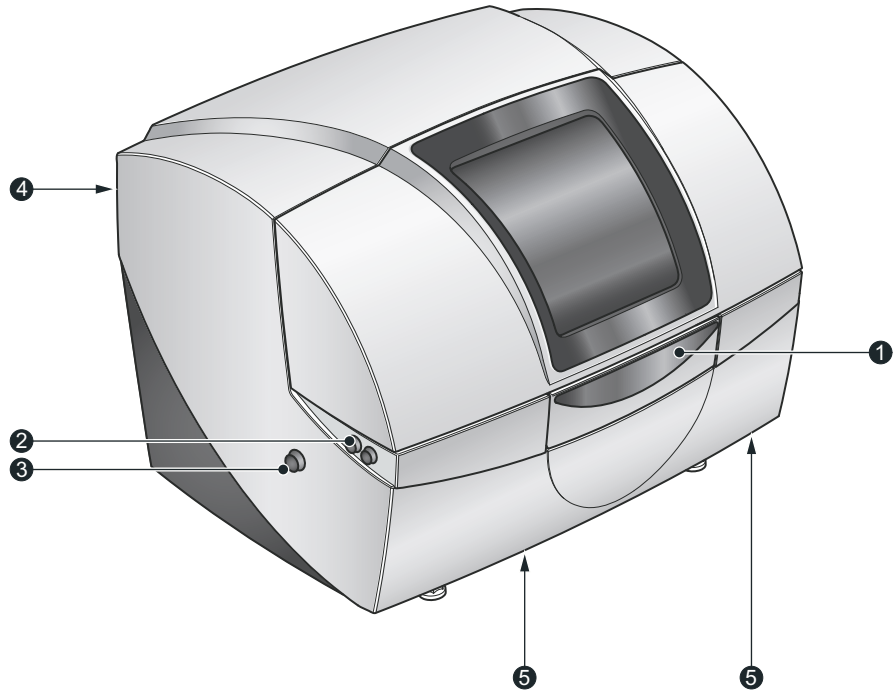
The software controls the Zetasizer APS and analyzes the data from the instrument to give the size for the sample measured. It is preferable that the computer is dedicated to just running the Zetasizer software.

The Zetasizer APS instrument



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① Plate holder opening

The plate holder is used to support a plate within the instrument during a measurement. With the optional Plate Temperature Controller fitted, the plate holder can control the plate temperature to within the range 4°C to 40°C. This is particularly useful if the system is running a long measurement cycle and then will be left unattended for a period of time with the plate still in the machine.

Note
When the Zetasizer APS is initially switched on, the cell measurement area (inside the machine) 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.

Plate holder cover flap
The flap will slowly lower as the plate holder ejects.

Measurements cannot be performed unless the plate holder is withdrawn into the instrument. To close the plate holder, use the software options described in **Chapter 4**.

Caution!
Never push the ejected plate holder into the machine as this could damage the precision motor drive.

Chemical compatibility

Apart from the wells of the plate, and the internal tubing and needle, the only part of the instrument that should come into contact with any sample is the plate holder area, and this only if spillage occurs. The plate holder area has been manufactured from materials that are considered to give the widest protection from chemical attack. However, it is important to check that any sample or solvent used is chemically compatible with the materials from which the plate holder is manufactured.

Refer to **Appendix A** for a full list of components that may come into contact with the sample.

② Rinse input x2

There are two rinse inputs, coloured black and red. These are used to provide two separately controlled rinses (a solvent connected to black and a wash connected to red) which aid with the process of reducing sample contamination. See **Chapter 8** for more information on using the software to specify the rinse configuration used by an SOP.

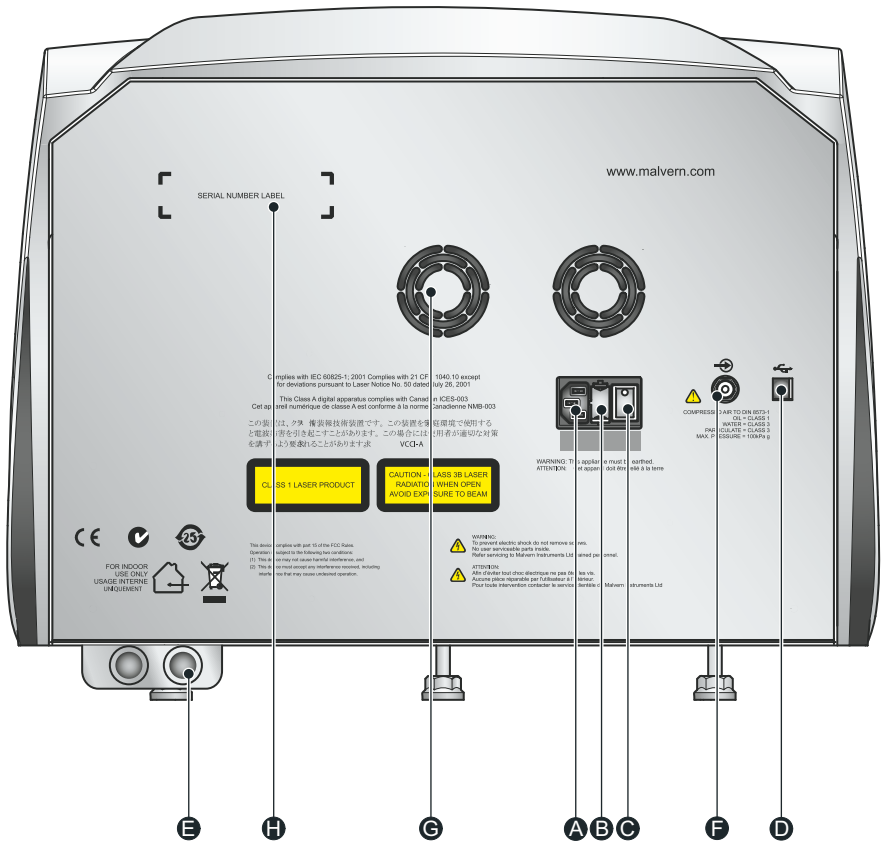
③ Waste output

The waste output is where both rinse and sample (if selected) are discharged by the system. Set up of any waste container and tubing should adhere to the following guidelines, to maintain drainage of sample/rinse waste fluid:

- Ensure any external waste tubing is routed with a continual drop from the instrument bulkhead connector to the waste container.
- The tubing should be cut to length such that it just fits into the neck of the waste container.
- The waste container should be routinely emptied **before** the liquid level reaches the waste tube, since this can sometimes cause airlocks in the sample waste line.

④ Rear Panel

The rear panel provides all the connections. These are identified in the following illustration.



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Ⓐ 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 the **Basic Guide**.

Ⓒ Power switch

The on/off power switch for the instrument.

Ⓓ Computer connection

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

Ⓔ Plate Temperature Controller connections
The Plate Temperature Controller is an optional unit that maintains the plate holder at a consistent temperature, which can be specified in the range 4°C- 40°C.

Ⓕ Purge connection
If measuring samples at low temperatures there is a risk of condensation occurring within the measurement area; this occurs when the measurement temperature is less than the 'dew point' of the ambient air surrounding the sample measurement area. 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 a dry-air supply - i.e. a supply with a dew point below that of the target temperature. This will remove any moisture in the air immediately surrounding the measurement area and prevent condensation.

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

- Compressed air to DIN 8573-1
- Oil = Class 1
- Water = Class 3
- Particulate = Class 3
- Pressure = 100 kPa

For connection purposes, the purge input uses a push-fit connector.

An optional **purge connection kit** is available from Malvern instruments if this facility is to be used.

When performing a purge measurement the following rules should be taken into account:

- The flow rate will be preset at >0.5 l/min at 1 bar before connection to the instrument.
- The instrument should be operated at a maximum of 30°C & 70% relative humidity when the requested temperature is below the dew point.
- When operating at a humidity higher than 50%, the measurement should allow a minimum delay of 4 minutes to allow the temperature to stabilize.

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.

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

Warning!
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 APS model and its serial number. Please quote all numbers in any correspondence with Malvern Instruments.

Note
The Zetasizer APS serial number can be found by double-clicking the APS icon in the right corner of the status bar.

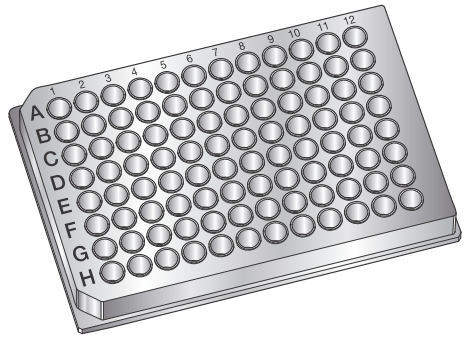
⑤ Drain ports x2

In case of spillage within either the plate area or the measurement area, there are basins and drains incorporated into the base of the machine.

Warning!
Any spillage will exit onto the bench area underneath the instrument.

Plates

A wide range of plates are available to use with the Zetasizer APS from suppliers such as Corning Costar and Whatman. The system is pre-configured for certain plates, but is compatible with most standard 96/384 well plates providing that the relevant parameters are set up in the SOP. An example of a 96 well plate is shown below:



Plates can be either transparent or opaque, come in a variety of colours and are manufactured from many materials. The Zetasizer APS is compatible with most ANSI standard types because all measurements are made externally from the well. Full details are given in **Chapter 4**.

Navigating the software

The Zetasizer software controls the system during a measurement and then processes the measurement data to produce a result. It displays the results and allows reports to be printed.

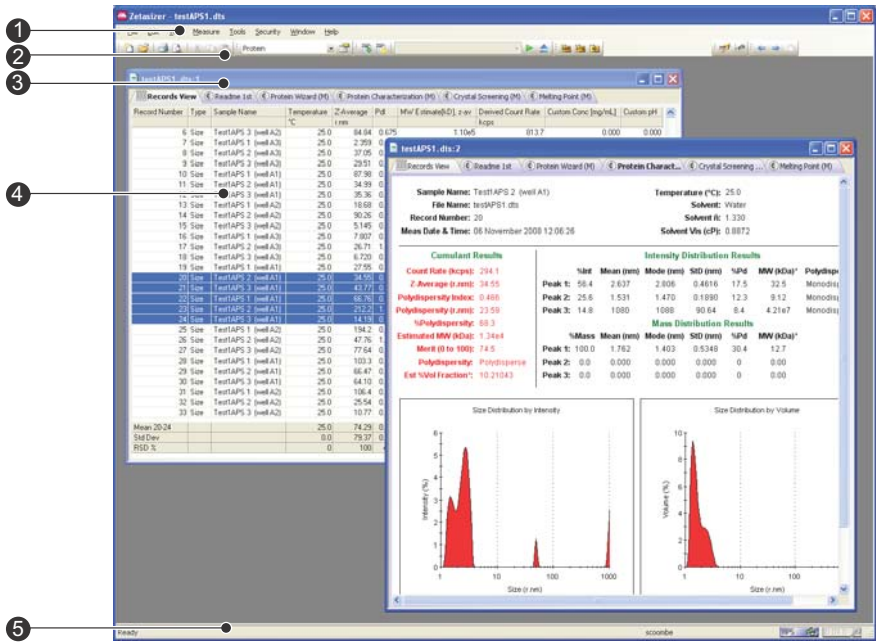
There are two software modules:

- The main Zetasizer application, which is described below.
- A secondary module called Report Designer that enables custom reports to be created to display the results. The features of Report Designer are detailed in **Chapter 10**.

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

The Zetasizer APS software application

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



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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 dialog boxes to appear. Similarly any items which end with an arrow (▶) will cause a second menu to appear.

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

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 the function of a tool, move the cursor over it - a tool tip displays a short description of its action in the status bar. If a tool or accessory is not available it will be shown "grayed out".

Standard	These tools perform the most popular File and Edit menu options functions.
Navigation	Use the arrow icons to scroll up and down the records list and the Plate Navigator to view records as they were measured on the plate.
Folders	Selecting either of these will open the required folder - SOP, Measurement Data or Export - in a new window. This window is separate from the main application allowing the files to be copied, moved or deleted.
SOP Toolbar	The **SOP toolbar** records the most recent SOP used. Also provided are options to quickly edit an existing SOP or create a new one. Refer to **Chapter 8** for a description of the SOP toolbar.
Workspace	Allows selection and configuration of the Workspaces. Refer to **Chapter 7**.

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

③ Title bar

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

④ 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 7**.

Measurement file workspace

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 them 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.

If selected using the **View** menu, a **Statistics bar** will be available at the bottom of the record view. This can display the Standard deviation, the minimum, the maximum or the mean for the records selected.

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

Selecting a **report tab** will display a predefined report as selected by the **Report pages** tab in the **workspace** dialogs. 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 5**.

⑤ Status bar and Status icons

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

On the right of the status bar, icons are shown to detail the operating state of the instrument and what additional features may be installed.

APS icon 

Double-click the APS icon to display the Zetasizer APS model, serial number, software and firmware version of the instrument (only if the instrument is connected and switched on). If the instrument is disconnected the icon is 'grayed' out: 

21 CFR 11 icon 

If the 2**1 CFR part 11** feature key is installed, the **21 CFR 11** icon will appear in blue on the right of the status bar.

If unavailable the icon will be 'grayed' out, like this: 

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.

Measurement display

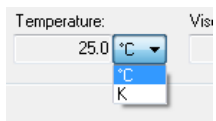
When a measurement is being performed a **measurement display** will appear.

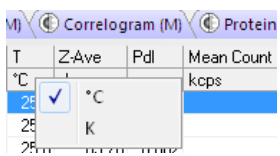
The Measurement display shows the progress of the measurement. The screen display shown changes depending on the type of measurement being performed and the view tab selected.

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.

Units Conversion





A number of parameters within the software have the option to change the units used to define the parameter, e.g. Temperature has the option to use either **°C** (Celsius) or **K** (Kelvin) in an SOP. You can also select alternative units in the **Records View** by clicking on a unit header.

For all parameters where this option applies a scroll down list will be available alongside the parameter.

The units can be selected in the SOP and other dialogs throughout the software.

The data is stored with a set of defaults, and is displayed with the selected units defined in the workspace chosen. This means that a particular record will be displayed with different units depending on the definition in the workspace used.

The Parameter unit chosen in the SOP dialogs will be displayed in all results and reports.

It is possible to subsequently alter the units used for the parameters in the Record and Report views - refer to **Chapter 5**.

4

A tutorial - making a measurement

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"** covers the basic steps and gives an overview of the measurement process. The rest of the chapter will focus on 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** in **Chapter 9**.

SOP measurements

The only measurement method of the Zetasizer APS is the **S**tandard **O**perating **P**rocedure (**SOP**).

An **SOP** measurement uses pre-set parameters to ensure that measurements are made in a consistent way. SOPs are also ideal if measuring the same sample in slightly different ways, reducing the need to re-input identical parameters each time a measurement is made, and thus minimizing error.

Chapter 8 gives details on creating and managing a user's SOPs.

Quick guide to making a measurement

This section gives a brief overview of the measurement process. More information on each stage can be found later in this chapter.

- **Turn on the instrument** and wait 30 minutes for the laser to stabilize.
- **Start the Zetasizer software**.
- **Prepare the sample** following the sample preparation guidelines.
- **Fill the plate well(s)** with the prepared sample(s).
- **Open or create a new measurement file**.
- Select **Measure-Plate Scheduler** from the Zetasizer software.
- Set up the **Schedule Settings**.
- **Allocate SOPs to wells** that contain samples.
- **Insert the plate** into the instrument.
- Click **Start**  and save the schedule if prompted. The measurement will be made, the results displayed and then 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

After switching on an **initialization routine** is performed that checks the instrument is functioning correctly. Additionally, an initial automatic rinse cycle is performed to ensure the system is clean and ready to receive the sample.

Switch on the power at the power socket and turn the power switch at the rear of the unit on.

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

Starting the Zetasizer APS software

Double click on the desktop icon to start the software.

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

Sample preparation

Before filling the plate 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 will involve specific preparation techniques depending on the substance being measured.

Consideration must be given to the physical properties of the sample, such as its particle or molecular size and 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).

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

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 solvent. As a guide, the scattering from water should give a count rate in excess of 10kcps.

Filtration

All liquids used to dilute the sample (namely 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 solvent.

In general, we recommend that samples are interfered with as little as is feasibly possible. In some cases, the process of filtration can cause sample loss through adsorption as well as the physical removal of larger contaminant particulates. Therefore, if it is possible to record a measurement for a sample without prior filtration then this is preferred. If poor or variable results are obtained or information about larger particles, such as agglomerates, is not of interest, then filtration of the sample will be necessary.

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 sample. Limits for ultrasonication intensity and application are strongly sample dependent. Some materials can even be forced to aggregate using ultrasound. **Emulsions and liposomes should not be ultrasonicated**.

About plates

The Zetasizer APS is compatible with standard 96 and 384 wells supplied by most major manufacturers. Choice of plate should be based solely upon its compatibility with the type of sample that will be measured as the Zetasizer removes the sample from the plate in order to make a measurement. If using a plate that is not pre-configured on the system, the **Well depth (mm)** setting may need to be adjusted in the **Plate Scheduler**.

Pierceable plate sealing films

Some plates employ a pierceable film or membrane to protect the sample. The Zetasizer APS is compatible with this type of plate **when using a piercing needle with an angled tip**. We only recommend the use of plates with a medium to tall flange height (6.10-7.62±0.38mm). Please refer to the **Basic Guide** for details on changing the needle.

Protective lids

Many plates are provided with a solid lid that protects the sample within the plate. Always remove any such lid before inserting the plate into the Zetasizer APS.

Plate composition

Always use a plate that is suited to the temperature range at which the sample should be maintained. Additionally, select a plate that is made from a material which is compatible with the sample being measured. Generally, disposable polystyrene plates can be used, however the following information should always be considered:

- Disposable polystyrene plates should never be used more than once.
- Disposable plates are not resistant to organic solvents - these can be measured in **quartz type plates** instead. However it must be noted that any organic solvent used must be verified as compatible with the tubing used in the system.

Inserting plates

This is explained later in this chapter. See “Inserting a plate” on page 14 for more details.

Filling plates

When filling well plates there are several considerations.

- Only **clean** plates should be used.
- The plate should be filled slowly to avoid air bubbles from being created.

A minimum sample volume must be provided, which is dependent on the plate type being used. Use a minimum of 20μL for a 384 well plate and 50μL for a 96 well plate.

Do not overfill the wells. Use no more than the manufacturer’s recommended maximum volume of sample, as this could result in cross contamination if spillage occurred.

Note
When filled, place a lid securely on the plate, but remember to remove the lid before inserting the plate into the instrument.

Making a measurement

The following sections, which assume that a plate has already been filled with sample, provide details of the complete measurement process as follows:

1. Open or create a measurement file.
2. Open the Plate Scheduler and create the Plate Schedule.
3. Add SOPs to the schedule.
4. Select wells and associate them with SOPs.
5. Specify the plate settings.
6. Run the measurement.

Chapter 8 gives all the detail required to create new SOPs.

Opening or creating a Measurement File.

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

- One measurement file containing all records (not recommended).
- Separate files for each sample type.
- 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 currently active measurement file. When the software starts it will automatically open the last measurement file used.

To open an existing measurement file:

1. Select **File-Open-Measurement File** or  .
2. Select the relevant file and click **Open**.

To create a new measurement file:

1. Select **File-New-Measurement File** or .
2. Name the file and specify where it should be saved, and then click **Save**.

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

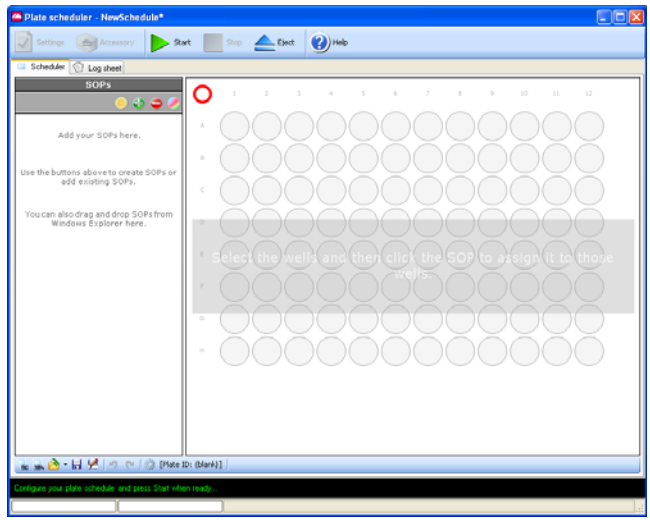
The Plate Scheduler

The Plate Scheduler provides a graphical representation of the well plate, enabling SOPs to be associated with specific wells and also the setting of other measurement parameters. It provides intuitive operation and offers flexible features to ensure that all variables of a plate measurement are both recordable and reproducible.

For each measurement a plate schedule must be created - these can be either set up ad hoc or previously created schedules can be opened and used.

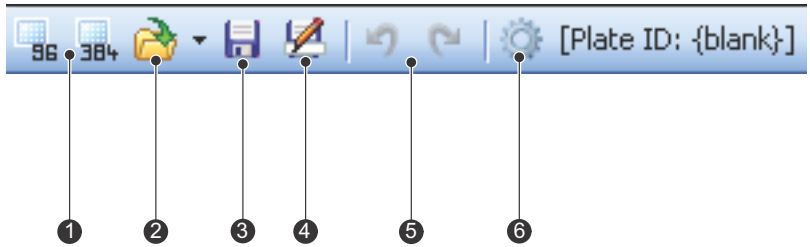
Note:
All plate measurements use SOPs - there is no manual measurement mode as used in other Zetasizer systems.

- Click **Measure–Plate Scheduler** or click the green **Start Measurement** button to display the Plate Scheduler.



Toolbars

The Plate Scheduler has two toolbars - upper and lower. The upper toolbar is described later in this chapter. The lower toolbar is illustrated below:



① **Create new 96 or 384 well plate schedule**.

② **Open existing plate schedule**.

③ **Save schedule** - saves the current plate schedule.

④ **Saves schedule as** - save schedule with another file name - useful if modifying an existing schedule.

⑤ **Undo/Redo** last operation.

⑥ **Schedule settings** - next to which is shown the current plate ID.

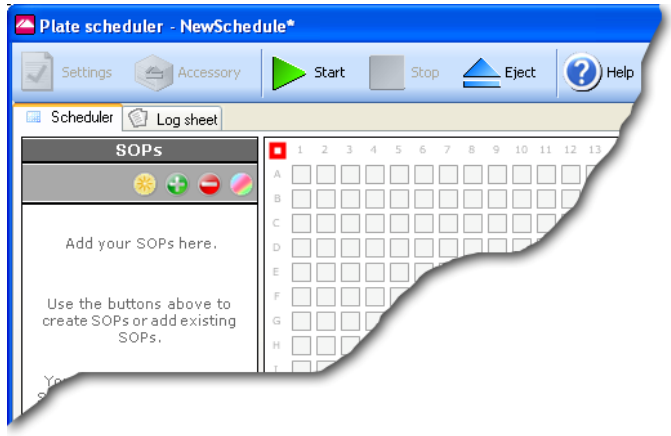
Creating the plate schedule

It is necessary to use a plate schedule for each measurement. This can be newly created each time a measurement is made, or a previously saved schedule can be used. The latter could be useful when measuring multiple plates using the same settings, or to repeat the measurement of a single plate.

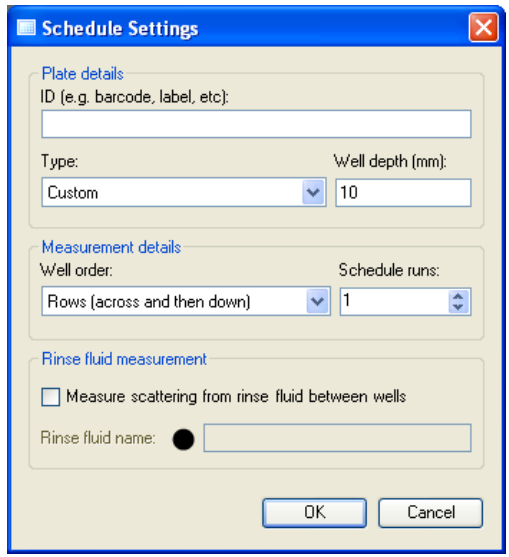
To create a new plate schedule:

1. By default the system selects a 96 well plate type – to change this to a 384 well plate, click the  icon on the lower toolbar.

Note:
This manual uses the example of a 96 well plate. The 384 well plate, shown below, differs from the 96 well plate by using squares to represent the wells instead of circles.



2. Click the **Schedule Settings** button on the lower toolbar to display the Schedule Settings dialog.



Enter any identifying text, such as barcode or plate label etc. in the **Plate ID** field. This is visible in the **Records View** as long as **Plate ID** has been enabled as a parameter for that view (listed under the **Measurement–Details** section – see **Chapter 7** for more details on configuring workspaces).

To determine the correct well depth the system needs to know the exact plate type in use. This is because well depths vary from plate to plate. Select one the standard plate types listed from the **Type** drop down menu. Alternatively, if

using a non-standard plate type, choose **Custom** and then specify a **Well depth** in mm.

In the **Measurement details** section, if required, choose a **Well order** of **Rows (across then down)** to force the sampling needle work from left to right across rows, starting at the top and working down. Alternatively select **Columns (down then across)** to ensure the sampling needle works from top to bottom, starting on the left column and working right.

Rinse fluid measurement provides the option for further analysis of the rinse fluid. This allows the user to ensure that the system is clean and that no residual sample remains. The results of the rinse fluid analysis are then shown as a separate figure within the measurement in the **Records View**.

Note:
This is the rinse fluid that is connected to the **black rinse input** on the instrument, which is normally the same solvent that is used within the prepared sample as a dispersant.

To use this feature, check the **Measure scattering from rinse fluid between wells** field and then enter a **Rinse fluid name**. Using this option will slow down the overall measurement, as the system is effectively appending another measurement - albeit of short duration.

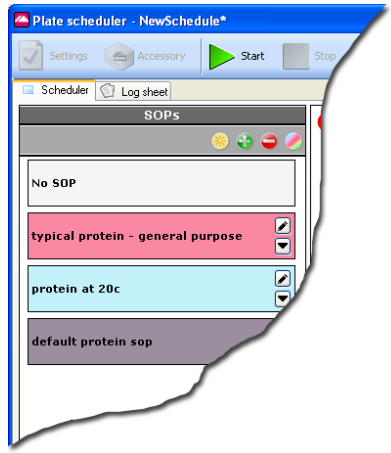
3. Click **OK** when the Schedule Settings dialog has been completed.

Adding the SOPs

Add all the SOPs that are required before selecting wells in the Plate Scheduler as follows.

1. Click the **Add existing SOPs**  button to display a folder browser window.
2. Browse to the folder containing the SOPs that are required.
3. Select an individual SOP, or if more than one of the required SOPs is located in the same folder, hold down the CTRL key and single-click items to multiple-select. Then click **Open** when all the required SOPs are selected.

The SOPs are now listed in the panel on the left side of the Plate Scheduler. Each SOP is allocated with a different colour by default.

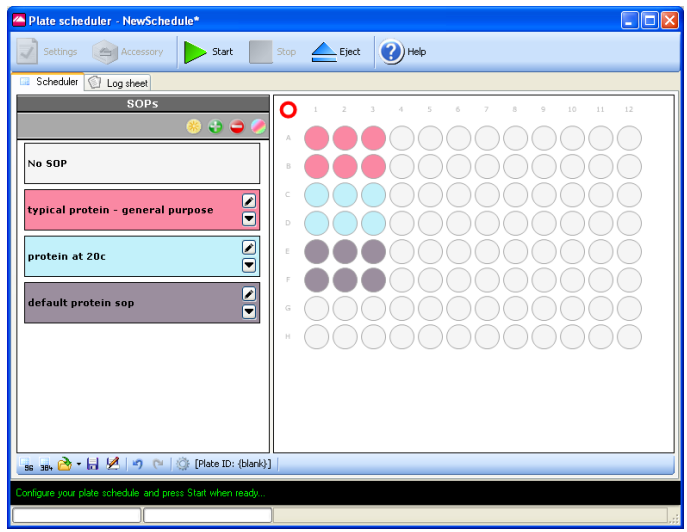


Alternatively, a new SOP can be set up from this screen by clicking the button. Follow the same steps detailed in **Chapter 8** if creating a new SOP.

To edit an SOP that is loaded into the Plate Scheduler, click the button on the SOP. To view more details about the SOPs listed in the left pane, click the **Show summary details button**. Click the button once again to hide the details.

Assigning wells to SOPs

To assign an individual well or set of wells to a particular SOP, highlight the well(s) and then click the required SOP in the left column. The colour of the well(s) changes to that of the SOP.

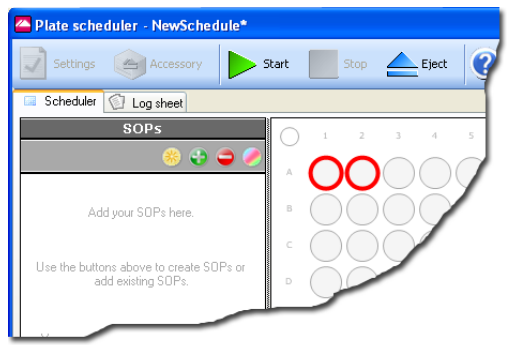


It is possible to select wells in a number of ways, which are described in the following sections. To remove any redundant SOPs that are not allocated to a well, click the **Remove unused SOPs** button.

Note:
If using multiple SOPs on the same plate, the system occasionally selects colours that are visually similar for some wells/SOPs, making observation difficult. To change the colour scheme used, click the **Change colours** button. Continue to click the button until the system selects a colour palette that provides easy distinction between the wells/SOPs.

Selecting individual wells

Select individual wells by clicking on them once – a red outline indicates that they have been selected. In the example below wells A1 and A2 are selected:



To deselect a well, click on it once again. Alternatively, click the circle in the top left of the plate representation - i.e. above the letter A and to the left of number 1. (If this is a 384 well plate, both the wells and top-left button will be squares rather than circles.) This top-left button also acts as a toggle to select all wells when it is highlighted red (i.e. when no wells are currently selected).

Selecting rows or columns

Click on the letter or number corresponding to the row or column respectively. To deselect the row or column, click on the same letter or number again.

Selecting a group

Click and hold the mouse button, and then drag a (red) marquee over the area of wells that are to be selected. Release the mouse button to finalise the selection.

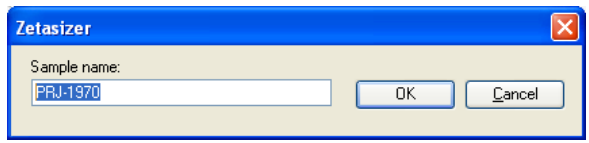
Clearing an SOP from a well

Any well associated with an SOP incorrectly can be disassociated by first highlighting the well and then clicking **No SOP** at the top of the SOP list in the left pane. The well can then be re-associated with another SOP if required.

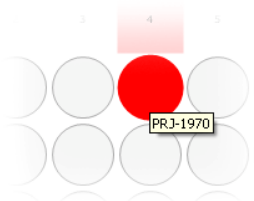
Identifying individual wells

The Plate Scheduler allows the user to identify each well with a separate **Sample name** if required. It is also possible to name groups of wells with the same name. By specifying a well's **Sample name** the SOP's own sample name is overridden.

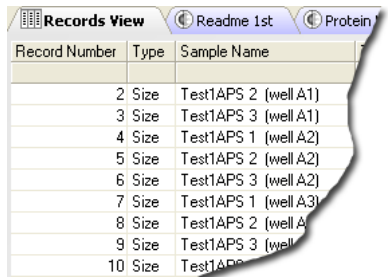
To name a well, select one (or a group of wells) and then right-click on it to show the **Sample nam**e dialog. Enter a name and click **OK**.



Move the mouse pointer over wells whose name has been allocated to view the name of the well.



If no **Sample name** is specified then the SOP's own **Sample name** is used to identify each well in the results (with the addition of the well coordinates in parenthesis).



Save the Schedule

Click the **Save schedule as** button  at the bottom of the Plate Scheduler window to display the standard Windows **Save As** dialog. Type a file name and click the **Save** button. Once the schedule has been saved initially, make further amendments to the file by just clicking the **Save** button  in the Plate Scheduler.

When an SOP has been allocated to each well required, it is time to start the measurement.

Initiating the measurement

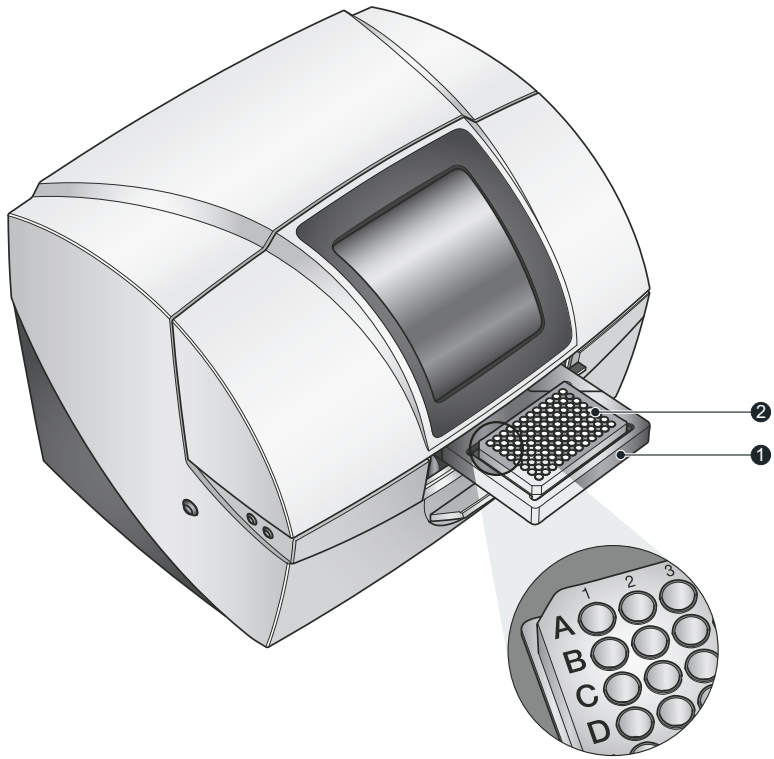
- If the plate holder is not already in the ejected position, click the **Eject** button 

on the Plate Scheduler toolbar to eject the plate holder.

Inserting a plate

Insert the plate into the holder at any point before clicking the **Start** or **Eject** button in the software. Once **Start** or **Eject** is clicked, the plate holder retracts into the machine.

Insert the plate ② gently into the plate holder ①, taking care not to spill any sample. There will be a small amount of play around the plate in the holder. This is normal as the holder clamps the plate more securely when it retracts into the instrument.



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Make sure that the well identification markings origin, which starts at the top left, is closest to the instrument as shown in the illustration. This is critical and ensures that the instrument measures the correct wells.

Starting the measurement

1. When the plate is in place, click the **Eject** button once more to retract the tray into the machine.

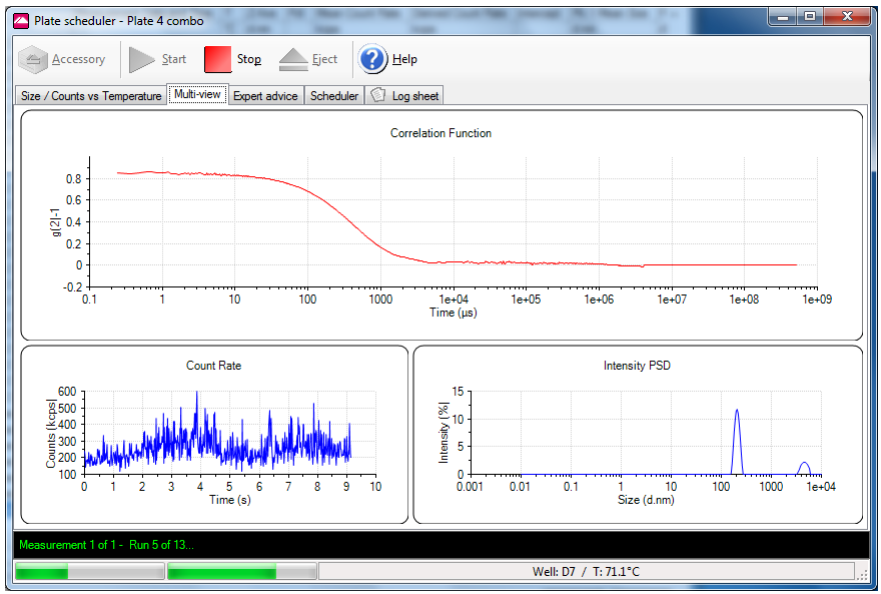
2. Click the **Start** button 

. If the plate schedule has not already been saved, the system asks whether it should save the active schedule.

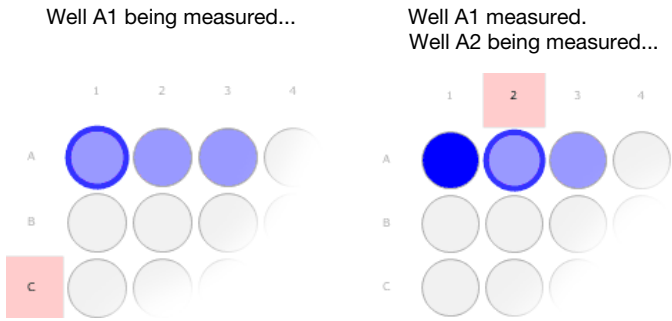
3. Click **Yes** and then give a file name to the active schedule to save it.

The plate holder retracts into instrument (if step 1 was missed) and the measurement procedure commences. The black status bar at the bottom of the window provides information on the process currently being undertaken.

Click on any of the tabs such as **Intensity PSD, Multiview, Expert Advice, Scheduler or Log sheet** to view live updates on the progress of the current measurement in more detail. An example of the **Multiview** tab during a measurement is shown below:



The **Scheduler** view shows a blue outline around the well currently being measured. When the well has been measured it is shaded in blue.



When the measurement is complete the well plate remains in the instrument and the view returns to the Plate Scheduler. (If using a Plate Temperature Controller - PTC-1000 - in combination with the Zetasizer APS, this will maintain the sample at the correct storage temperature if the unit needs to be left unattended for a long period after the measurement has completed.)

To remove the plate after the measurement has completed, click the Eject button 

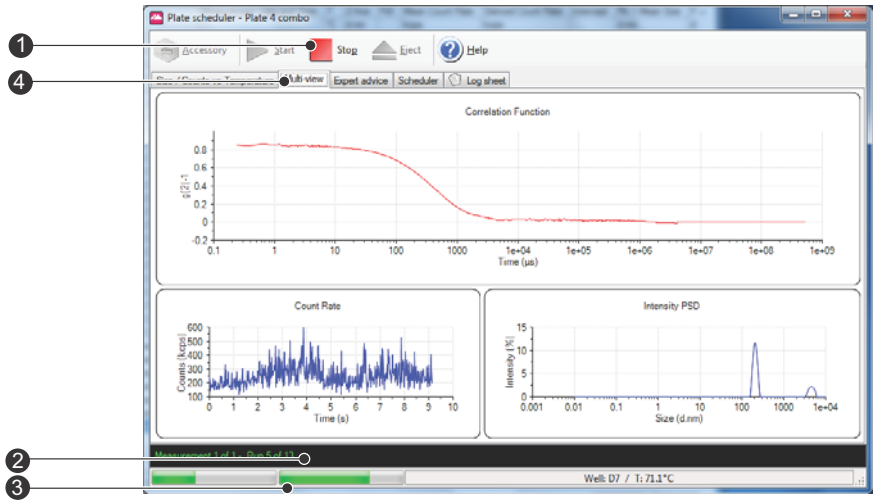
on the measurement window toolbar to eject the plate tray.

Close the Plate Scheduler.

The Measurement display in detail

When a measurement is started the Plate Scheduler expands to include the measurement display, showing the progress of the measurement.

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



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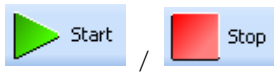
The features of the measurement display are:

① Button bar

The button bar provides the control for the measurement operation.

The **Settings** button (grayed out) - only used when a Zetasizer Nano instrument is connected.



These **Start** and **Stop** 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. When **Stop** is pressed, a warning box will appear asking "**Are you sure you wish to abort the measurement**".

Ejects the plate holder from the instrument. If the plate is already ejected, this button retracts the plate holder into the instrument.

Opens the **Help** file.

The **Accessory** button (grayed out) - only used when a Zetasizer Nano instrument is connected.

② Status bar

The status bar shows instructions and the current operation in the measurement sequence.

③ Progress meter

The progress meter shows how far the measurement has progressed plus the number of measurements performed and the measurement runs completed. Also shown are the temperature, measurement position and attenuator settings.

④ Tab views

The **Tab views** enable the progress and results of the measurement to be viewed. The **first** tab shows the results, and will change with respect to the measurement type and results view selected. In the above example, this tab is labelled **Intensity PSD** to identify that intensity results are being viewed. This tab shows different graph plots relevant to the measurement type selected. The other tabs - **Multi-view**, **Expert advice, Scheduler, Log sheet** and - are standard for each Measurement type.

- The views displayed in the **first** (result) tab, can be altered by right-clicking on the graph and selecting from the list displayed. A graph only, or a graph with a summary table will be shown depending upon the view chosen. The **Result** tab is named after the result view chosen.
- The **Multi-view** tab displays the results in three smaller windows. As with the **first** (result) tab, the view in each separate window can be altered by right-clicking in the window and selecting a different view from the list displayed. The size of each window can altered by moving the borders around.

The Tab views for each measurement type are described below.

Size measurements

Result tab (1st tab)

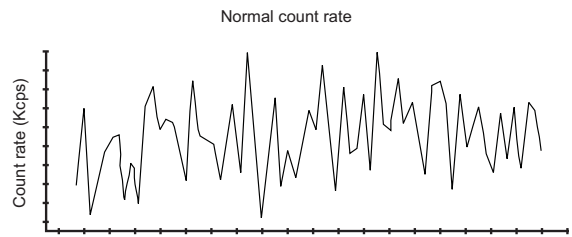
The **result** tab will show the result obtained as the measurement progresses. The result view will be updated after every run of the measurement. The result shown will be the sum of the acceptable data collected.

The **Result** tab is named after the result view chosen, the default result view shown is **Intensity PSD**. Different views can be selected by right-clicking on the graph and selecting from the list displayed. A graph only, or a graph with a summary table will be shown depending upon the view chosen.

The views available are: **Count Rate**, **Correlation Function**, **Intensity PSD**, **Volume PSD** and **Number PSD**. These are described below.

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

Normal count rate display.



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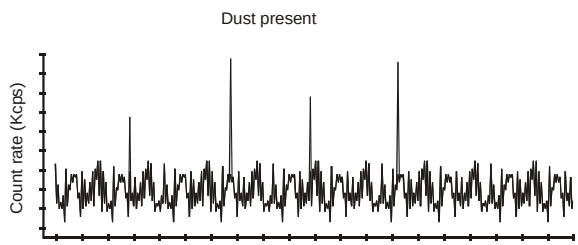
If dust is present then sharp spikes will be observed. Measurement runs with dust present will be removed from the final measurement calculation by a dust filtration algorithm.



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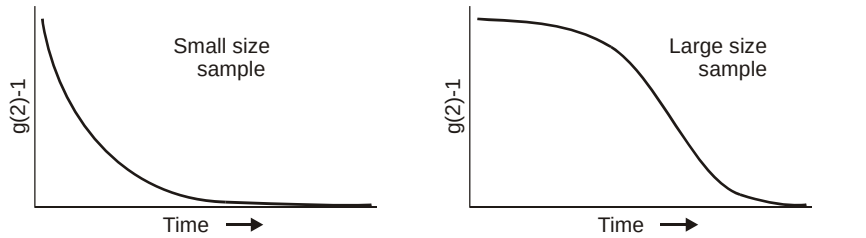
- **Correlation function**
  The correlation function helps the experienced user to interpret any problems with the sample.



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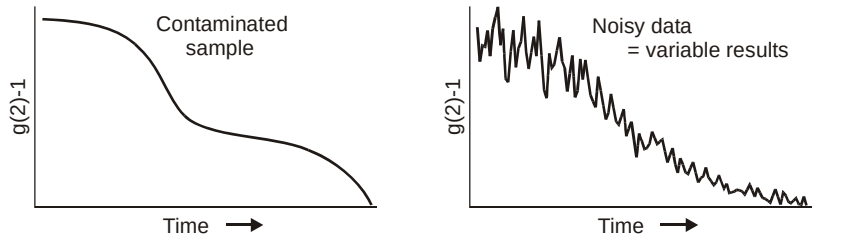

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- **Intensity PSD**
  Displays the result as an Intensity based Particle Size Distribution. A summary table will displayed alongside the graph.

- **Volume PSD**
  Displays the result as a Volume based Particle Size Distribution. A summary table will displayed alongside the graph.

- **Number PSD**
  Displays the result as a Number based Particle Size Distribution. A summary table will displayed alongside the graph.

Multi-view Tab

The Multi-view enables three different result views to be shown at the same time. The view in each separate window can be altered by right-clicking in the window and selecting a different view from the list displayed. The same views as used in the result tab are available.

Expert Advice Tab

The Expert Advice tab reports on how good the quality of the measurement is. This will show how good the measurements are and whether they display any unwanted attributes, such as aggregation.
Refer to the **Expert Advice** section in **Chapter 9** for a description.

Scheduler Tab

This shows the Plate Scheduler. Whilst a measurement is in progress this view shows which wells have been, and are currently being, measured.

Log sheet Tab
This displays the progress of the measurement. The log sheet can be saved by right-clicking the mouse on it, pressing the **Save to file...** button that appears and saving it as a **.txt** file.

Trend measurements

Trend dialogs are marked by the inclusion of an extra **Trend** graph view - this will show an evolving plot as the measurement progresses.
Aggregation Point measurements can be performed using the Trend measurement type.

Measurement sequence

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

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

The measurement will then continue with an optimization or initialisation stage where the attenuator settings for the 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 optimization 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 Plate 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' to allow data filtering. At the end of data collection the data quality of each run is assessed. Runs that contain poor data are rejected while the remaining runs are analyzed and used in the final measurement calculation.

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

5

Records and Reports - Viewing the Results

Introduction

Once a measurement has finished, the results will need to be reviewed. This chapter details how to display the final measurement result.

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

Displaying the results

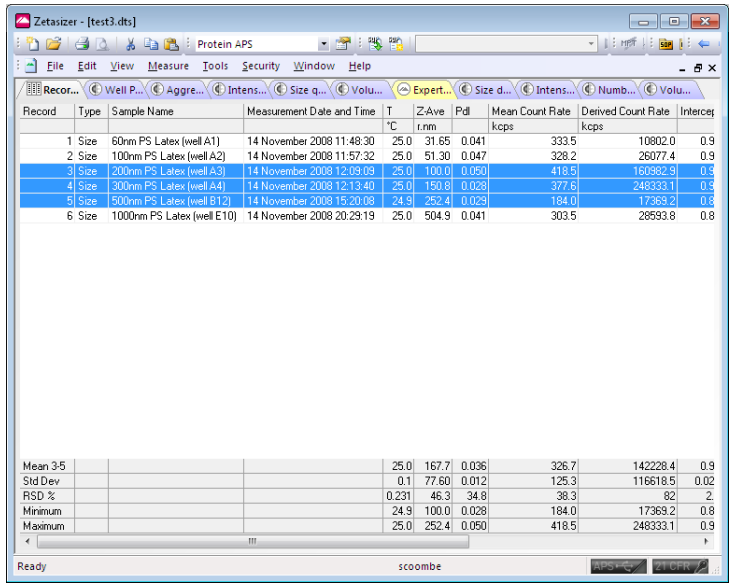
The results are displayed in two ways. A **Records view** that shows a list of the measurement records in a measurement file, and the **Report tabs** which show all measurement details of a selected measurement record.

Note
The **Records view** parameters and **Report** tabs that are displayed are dependent upon the **Workspace settings** selected- refer to **Chapter 7**.

Records view

Once the measurement is complete, a new measurement record will be added to 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.

The parameters shown will be those selected using the **Tools-Settings-Configure Workspaces** dialogs. The record view shown below depicts the **protein** workspace (see **Chapter 7**).



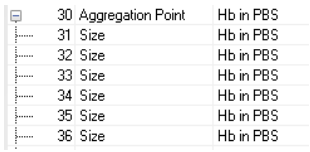
Under the **sample name** column, the individual well name (or identifying co-ordinates) is shown in parenthesis.

Aggregation point records

To view a sequence of Aggregation point records, select the first record in the sequence; all subsequent records are then automatically selected.

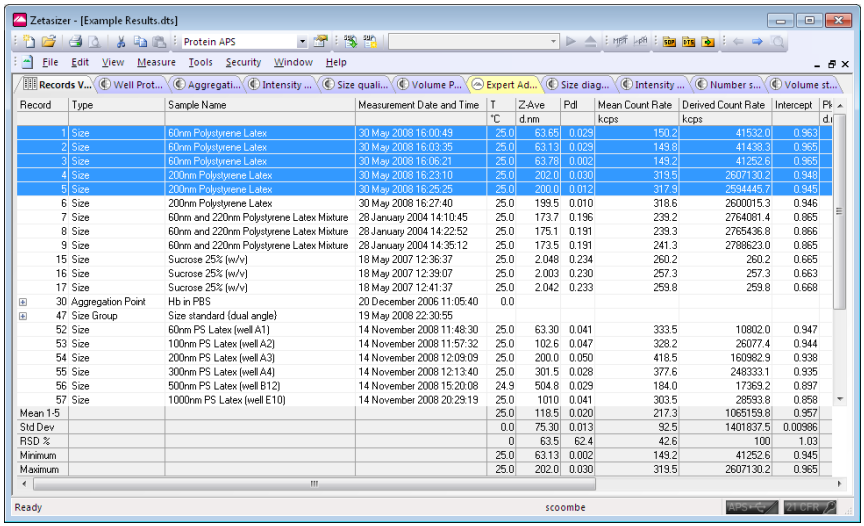
Outliners

For aggregation point records, **outliners** are employed. This enables the main 'parent' record to be shown without the 'child' records also being displayed. Selecting ⊞ will display the 'child' records, selecting ⊟ will hide them.



Statistics bar

Use this to display the Standard deviation, the Relative Standard deviation, the minimum, the maximum or the mean of a selected number of records. Select **View -Statistics bar** from the menu bar, and then the options to display. A bar will be added to the bottom of the record view displaying each of the parameters selected.



To display the statistics of a number or records, hold down **Shift** or **Ctrl** and then the records required in the normal Windows fashion; the statistics bar will be automatically updated as each record is chosen.

View-Statistic bars-Hide will remove the statistics bar from the record view.

Units conversion

Within the Records view, it is possible to change the units used to define some of the parameters. If the option is available, clicking on a parameter column will display a **parameter icon** enabling the units to be changed. A check mark (✓) will be displayed next to the currently selected units.

Note

Changing the units of a parameter will alter the units for each occurrence of the parameter in any report then displayed.

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.

To view a sequence of Aggregation point records, select the first record in the sequence; all subsequent records in that sequence will then automatically be selected.

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 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.

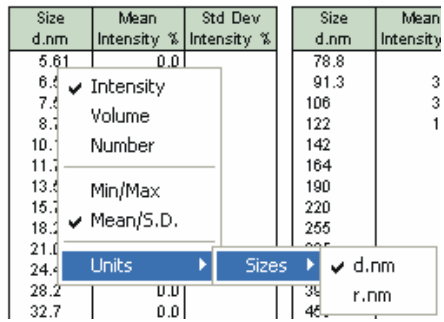
Each measurement type has a 'standard' report associated to it. The same result information is present in both the computer and printed versions, except for the diagnostic reports which contain extended information on the printed version.

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.

Units conversion

As in the **Records view**, within the results section of each report, it is possible to change the units used to define some of the parameters displayed. If the option is available, right-clicking on a parameter will display a **parameter icon** enabling the units to be changed. A check mark (✓) will be displayed next to the currently selected units.

If present, right-clicking on the table will also bring up the option to change the units displayed. For a table, select **units** from the displayed menu.

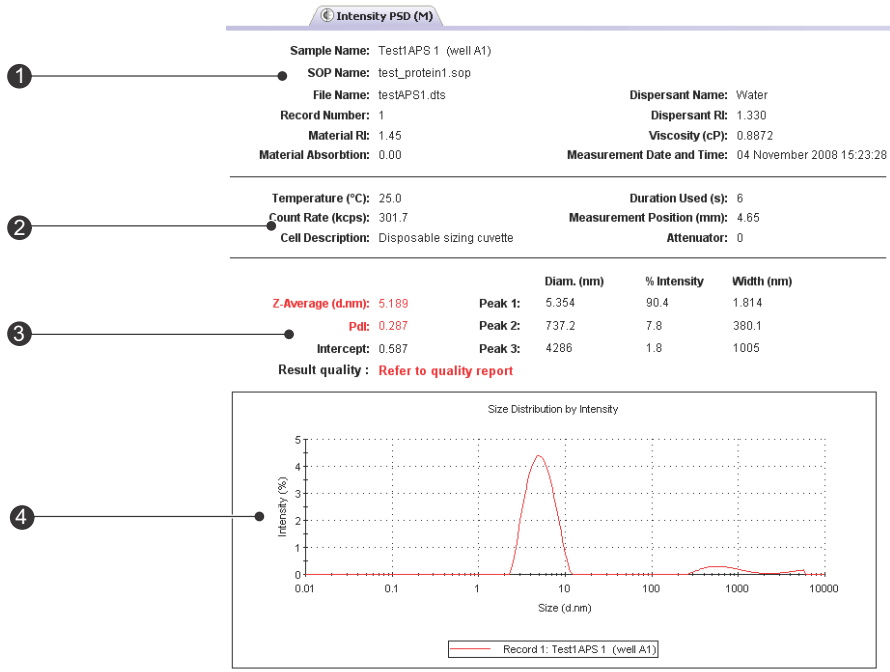


Note

Changing the units of a parameter in any field of the report, will alter the units for every other occurrence of the parameter in the report, and the parameter column in the **Records view**, however the default unit in the record is not changed.

Size measurements - standard report

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



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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/solvent refractive indices, viscosity, etc. The information shown is generally that which was entered by the user into the SOP measurement dialogs.

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.
- **Duration used(s)**
  Duration of measurement, used in the analysis of the result.

- **Cell type**
  This displays the cell type selected.

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. See **Chapter 12** for enhanced explanations.

- **Z-average Size** (also known as the "cumulants mean")
  In **D**ynamic **L**ight **S**cattering this is the most important and stable number produced by the technique. This is the required parameter for quality control purposes.

- **PdI**

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

- **Intercept**
  This is the amplitude of the G_1 correlation function at time 0.

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

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** dialog will appear. This dialog 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.

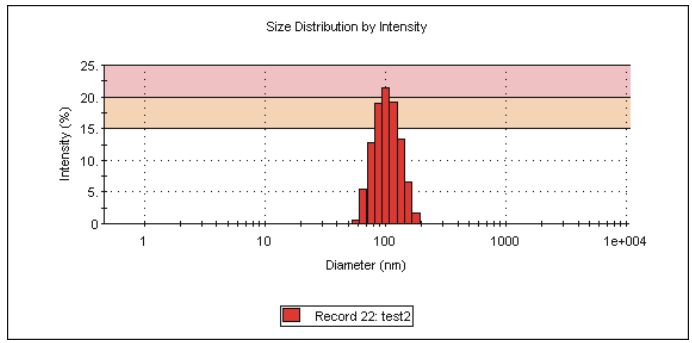
  The **Options** tab allows the graph key position to be chosen. A graph tips option can also be set, this allows the setting of pop up tips (flags showing data points on the graph) on the report itself.

- **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.

The graph below has been configured using the dialog 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.



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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.

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**.

Other Size reports

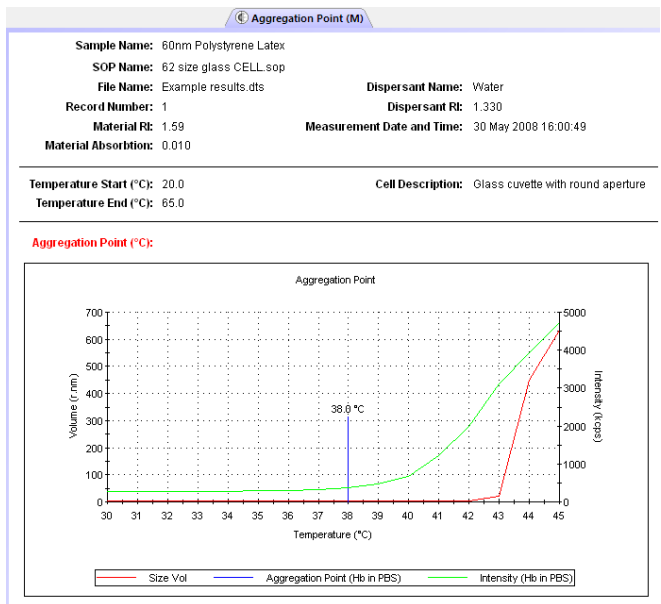
Other Malvern views available for size measurements include:

- **Intensity Stats Table (M)** - displays statistical data across repeat measurement data sets when multiple records are selected in the records view & 'intensity peak stats(M)' - displays statistical data for a single measurement data set.
- **Volume PSD (M)** - displays the volume-based particle size distribution derived from the Intensity size distribution result using a Mie Theory based calculation.
- **Volume Stats Table (M)** - displays statistical data across repeat measurement data sets when multiple records are selected in the records view.

- **Number PSD (M) -** displays a derived number-based size distribution.
- **Number Statistics (M) -** displays a derived number-based size distribution.
- **Size diagnostics report (M) -** displays multiple parameters and graphs aiding assessment of the quality of a dataset.
- **Size quality report (M) -** provides details on whether the result meets the Malvern Instruments quality criteria for a measurement.

Trend measurements (including Protein Aggregation point)

Trend measurements can be viewed using the reports: **Trend**, **Trend v Custom 1** and **Trend v Custom 2** reports. Aggregation point trend measurements can be viewed using the **Aggregation point (M)** report.



A trend report gives the same information as seen in a standard size report, plus a graph showing the trend progression.

Additionally a aggregation point report will show 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.

Aggregation point (°C)
The temperature at which the aggregation point is achieved.

Plate Navigator

Use the Plate Navigator to examine plate measurements in a graphical format showing their well position. User-definable upper and lower numeric settings allow results to be quickly screened as required. The Plate Navigator provides powerful features that show:

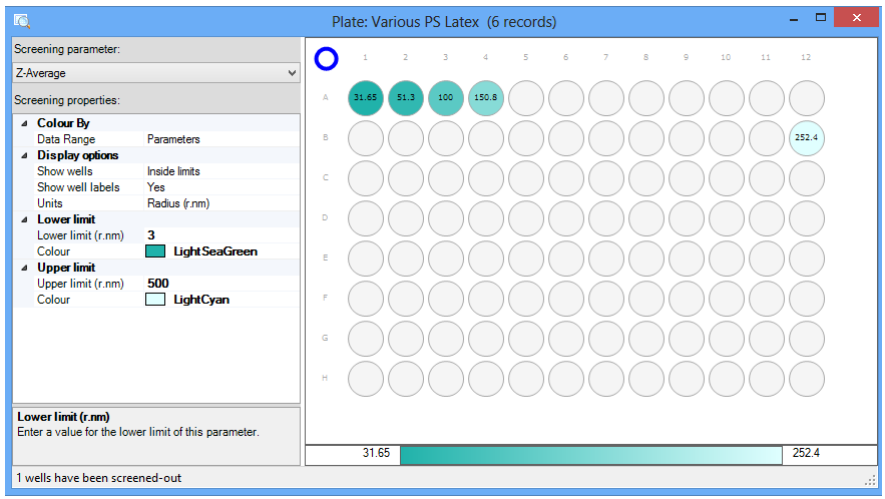
- Position of wells measured.
- Colour and numeric representation of the following measurement data:
  - **Z-Average** of wells measured
  - **PdI**
  - **Derived Count Rate**
  - **Intercept**

See **Chapter 12** for more explanation of these measurement types.

Plate Navigator basics

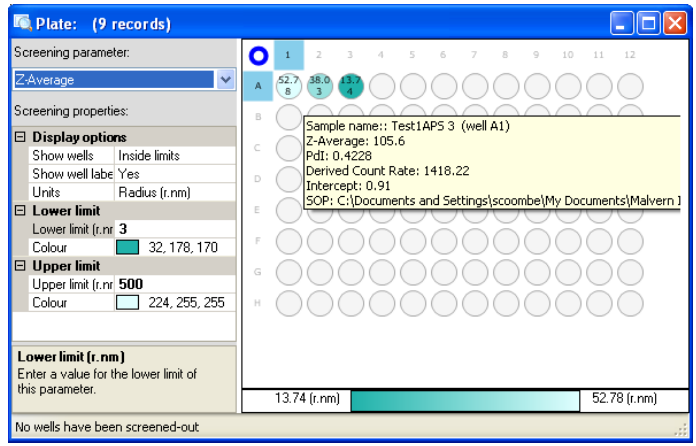
There are several ways to access the Plate Navigator - right-click a result from the Records View and select **Plate Navigator**, choose **View – Plate Navigator** or click  on the toolbar.

A window is displayed that presents a visual representation of the plate as originally measured.

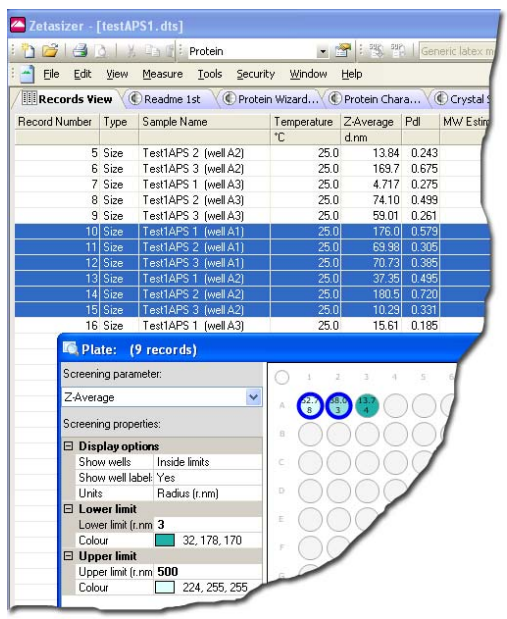


The Plate Navigator taskbar shows the name of the Plate ID as identified in the Schedule Settings dialog of the Plate Scheduler together with the total number of records in the measurement.

Move the mouse pointer over any of the wells (without clicking) to show all measurement information pertaining to that well in a pop-up window. If the well was not scanned in the original measurement no pop-up window is shown.



Click on individual wells to simultaneously highlight the measurement in the Records View. Select as many of the wells as required.



To deselect wells, click the circle (or square, if a 384 well plate was used) in the top left position. Select rows or columns by clicking on the letter or number corresponding the row or column respectively. To deselect the row or column, click on the same letter again.

To select only those wells currently shown, click the circle at the top left of the well display – click it once more to deselect the wells.

Setting screening parameters

By default, the system does not select any screening parameters, showing all measurements that were made on the plate (this is the **None (show all)** option). Set a **Screening Parameter** from the drop-down menu to show just those results that are of interest:

- Z-Average of wells measured
- Pdl
- Derived Count Rate
- Intercept

The software will select a different colour scheme depending on the screening parameter selected.

Display Options

Show wells – **All** shows all wells that were measured regardless of the values measured or the numeric limits set. **Inside limits** displays all wells that fall within the numerical limits set in the Upper and Lower limit fields.**Outside limits** displays wells that fall outside the Upper and Lower numeric limits.

Show well labels – the numeric data shown on the wells can be turned on or off by setting **Show well labels** to **Yes** or **No** respectively.

Units (Z-Average only) – figures used can be set as required to either **Radius (r.nm)**, the default setting, or **Diameter (d.nm)**.

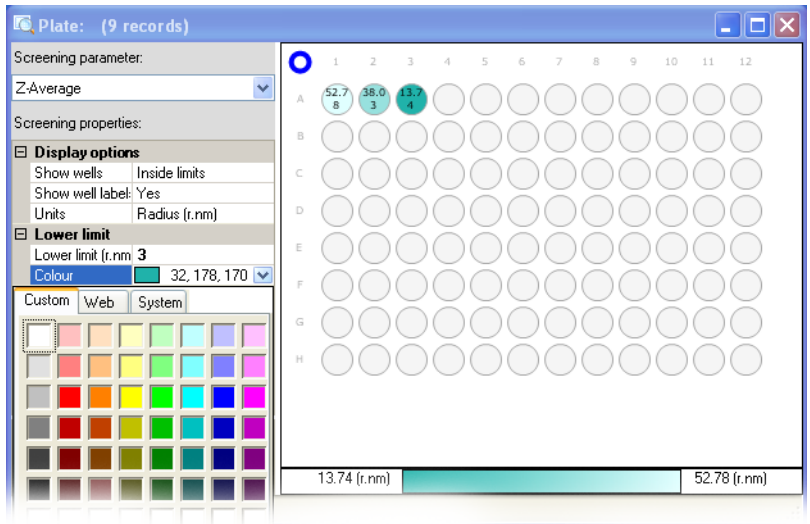
Lower and upper limit

By default, the system sets a range that captures all results measured.

Set a **Lower limit** to specify a figure below which information is not to be displayed, and also to associate a colour with that figure. Similarly, set an **Upper limit** to specify a figure above which information is not to be displayed, and also to associate a colour with that figure. Wells with measurement values falling above the **Upper limit** or below the **Lower limit** are excluded from the view altogether. To show these wells again, set the **Show wells** option to **Outside limits** or reset the Upper or Lower limits accordingly.

Colours

Colours form an important part of the Plate Navigator's graphical display, making it easy to see measurement information at a glance. The default colours can easily be modified to suit individual user requirements. Click the colour swatch next to **Colour** in either the Lower or Upper limit settings area to display the colour palettes.



Choose a colour from the **Custom**, **Web** or **System** palette. It is often easier to observe information when colours are selected that fall within the same tonal range, i.e. choosing a lighter and darker version of the same colour. In the Custom palette, for example, this means selecting two colours from any single column. If a colour is selected form the Custom palette, the RGB value of that colour is shown next to the colour swatch. Whenever colours are chosen from the other palettes, the name of the colour is shown next to the colour swatch.

The selected range of colours is also shown as a gradient strip along the bottom of the display for reference, together with the minimum and maximum values for the given screening parameters.

Part 2 - Supervisor's Guide

Security

Introduction

The system may be operated by users of varying skill levels. It is possible to limit user access so that certain functions such as editing SOPs, deleting records and editing results are 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 gray 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 just one user (Administrator) and one group (Administrators). This 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.

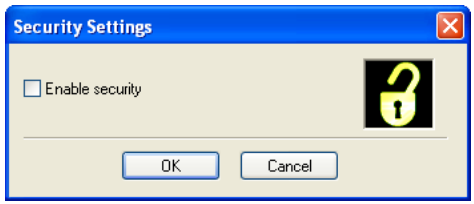
To secure the system, a password must first be specified for the administrator account (detailed later) and then the security enabled using the **Options-Security settings...** menu.

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 - adding users, changing of permissions, etc. The new changes must be saved (**User-Save**). A dialog will appear on exiting to give a reminder on saving changes.

Enabling security

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



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 grayed out.

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

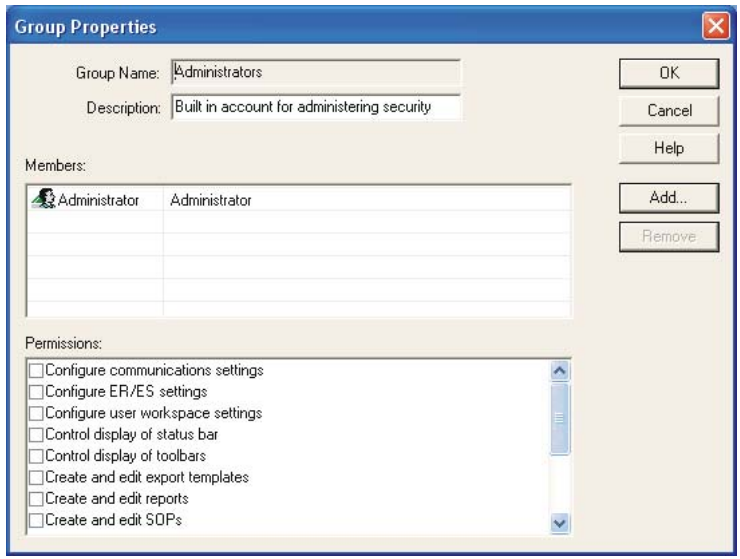
To change operators when the software is already open select **Security-Logout** and then **Security-Login** and enter the appropriate passwords.

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** dialog. Select **User-New Group...** to display the **Group Properties** dialog, alternatively double-click on an empty row. When editing double-click on an existing group to display the dialog.



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 dialog 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 dialog 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.

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** dialog. Select **User-New User...** to display the **User properties** dialog, alternatively double-click on an empty row. When editing double-click on an existing user to display the dialog.

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

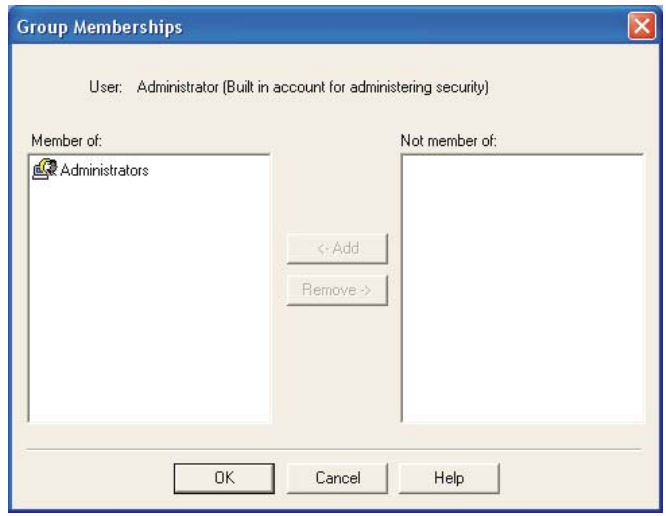
Username is commonly an abbreviated form of the individual name or a unique identifier such as an employee code.

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 user's name, and force the user to change their password the next time they log on using the password check boxes.

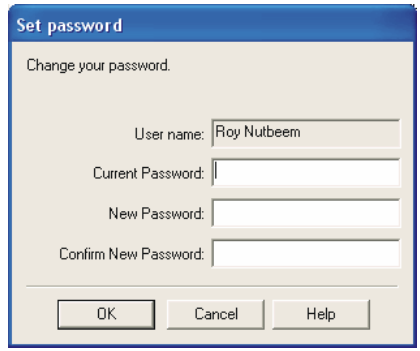
Press the **Groups** button to allocate the user to the required groups. Use the **Add** and **Remove** buttons to allocate the group(s) appropriate.



Password options

- **User must change password at next logon**
  The change password dialog 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 user's 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.

- **User can not change password**
  This option prevents a user from changing a password once it has been initially set.

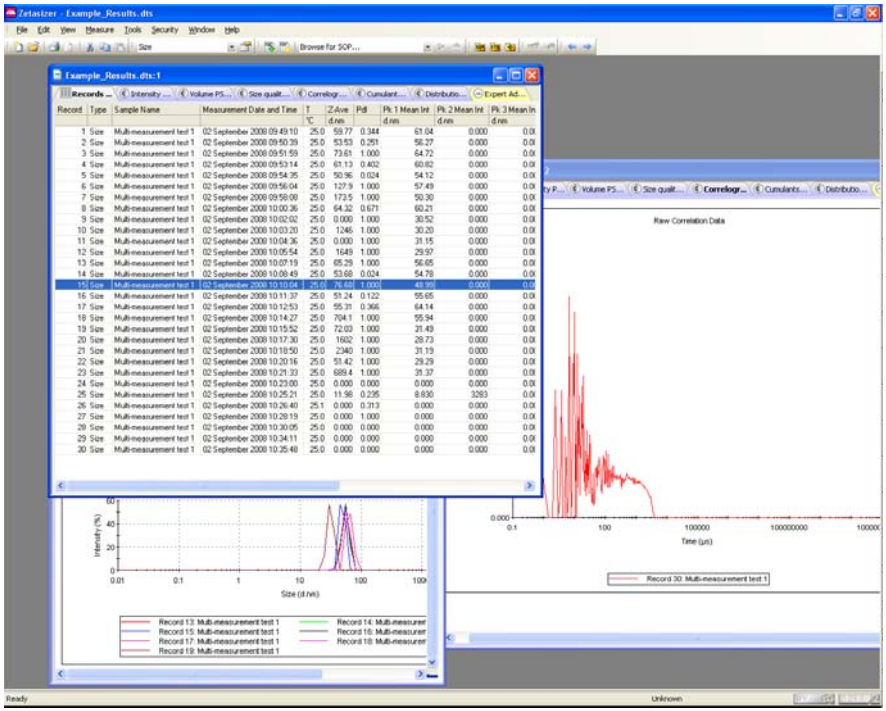
  For a secure system, it is advisable for users to regularly change their passwords to prevent unauthorised access. The only possible exception is the security administrator's account where forgetting the password to this account could prevent any further configuration of the security system.

7

Measurement file window - Workspaces

Introduction

The **Measurement File window** is where all results (or records), and reports for each measurement file can be viewed, moved and analyzed. Depending upon the **Window** options set, multiple measurement files can be viewed at the same time.



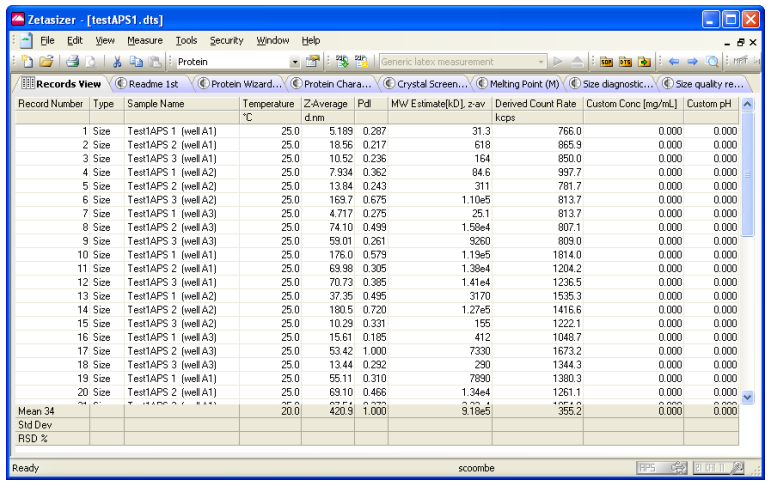
Once a result has been calculated, the data can be displayed as either a record or report - by selecting the appropriate tab.

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 10**.

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.



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.

  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.

- 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 grayed out if no measurement is selected.

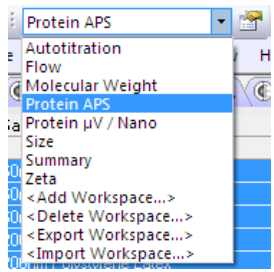
The measurement information presented in the **Report** and **Record** tabs is controlled by the **Workspace** selection dialogs (below). The tabs can be configured to display either all the measurement file records and associated reports (i.e. Summary), or only those that are relevant to one measurement type (e.g. Size).

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.

Workspaces

Instead of the measurement file windows showing every single measurement, it is possible to customize the window views so that, for example, only Trend measurements from that file are shown. Similarly a user can create a personalised workspace so that only parameters and reports relevant to them will be shown.

Initially only the workspaces shown in the **Workspace Toolbar** menu (below) are available, all with **Record view** parameters and **Report pages** already configured.



- Workspaces can be **selected** using the **View-Workspaces** menu option or the Workspace toolbar.
- Workspaces can be **managed** using the **Tools-Settings-Configure Workspace** menu option or the Workspace toolbar.
- The workspace toolbar can be turned on and off by selecting **View-Workspaces-Workspace Toolbar**.

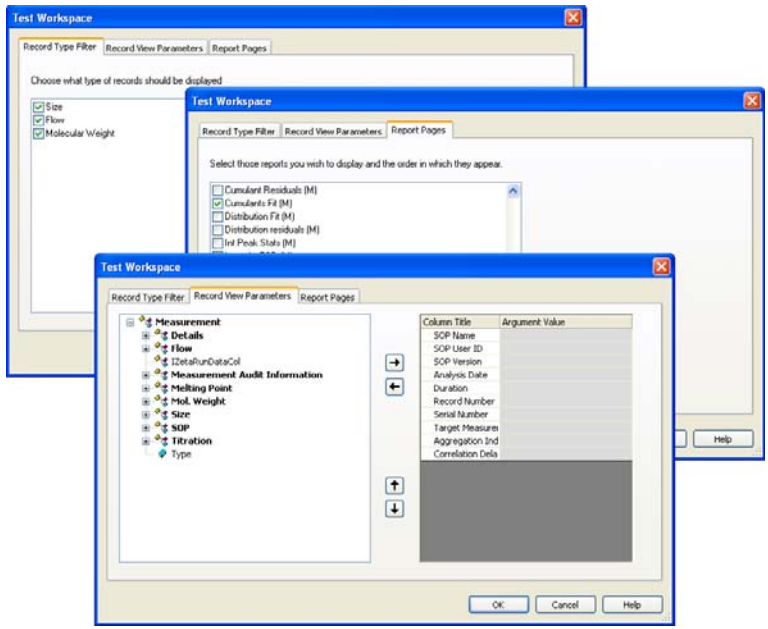
To Display a Workspace

To **display** an **existing** workspace window, select **View-Workspaces-** "..workspace choice.." or use the **Workspace** toolbar (above).

To Create a new Workspace

1. Select **Tools-Settings-Configure Workspaces-Add Workspace...** or select **<Add workspace>** from the toolbar.

2. Type in a **Name** for the new workspace and press **OK**. This will display the **Workspace settings** dialogs.



3. Use the **Workspace settings** dialog to display what is required.
4. Use the **Record type filter** to display only specific measurement types in the **Record View**.
5. Use the **Record view parameters** to specify what parameters will be viewed as the column headings.
6. **Report pages** allows selection of both Malvern created and customized reports.
7. When selected, click **OK**.

Once completed the new workspace can be selected using the **View** menu or **Workspace** toolbar.

To Modify a Workspace:

1. Select the workspace name from the toolbar drop-down list (or from the **Tools-Settings-Configure Workspaces** menu) and then click the **Configure** icon .
2. This will display the **Workspace Settings** dialog for the selected workspace.
3. Alter the settings as required and press OK; the new settings will be saved.

Deleting a Workspace

Select **<Delete workspace>** from the toolbar or the **Tools-Settings-Configure Workspace** menu.

From the list, select the workspace to be deleted and click **OK**.

Exporting and Importing Workspaces

Workspaces can be exported to, and imported from, folder locations by using the **<Export workspace>** and **<Import workspace>** options in the toolbar and **Tools** menu.

To export a workspace

First select the workspace to be exported, and then select **<Export workspace>**, In the dialog that appears, select the destination folder and save the workspace. The workspace will be saved as a .**zwrk** file.

To import a workspace

Select **<Import workspace>**, browse for the folder and select the workspace. Press **Open** and then **OK** to display the new workspace.

Record view

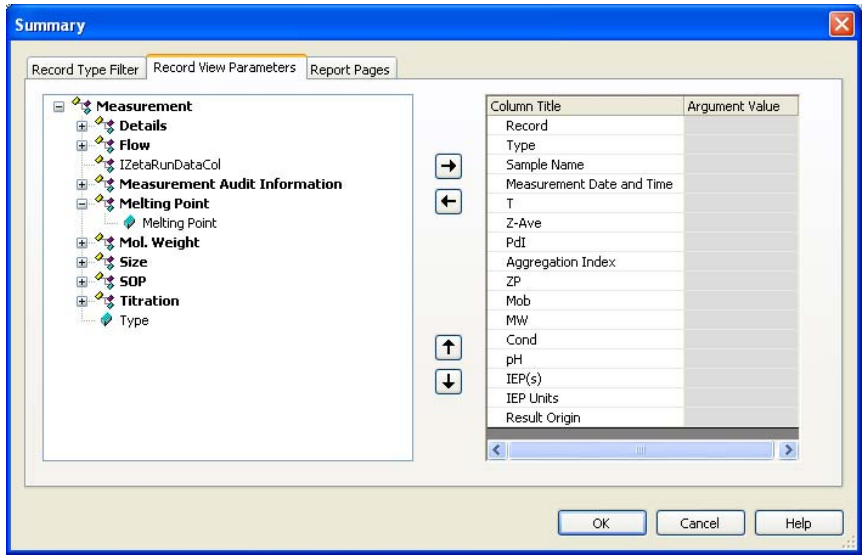
With the **Summary** workspace selected, the default parameters displayed when the software is first installed are: **Record**, **Type**, **Sample Name**, **Measurement Date and Time**, **T** (Temperature), **Z-Ave**, **PDI, Aggregation Index, ZP, Mob, MW, Cond, pH, IEP(s), IEP Units** and the **Result Origin**.

For each workspace the record view can be customized 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** dialog 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 → ← .

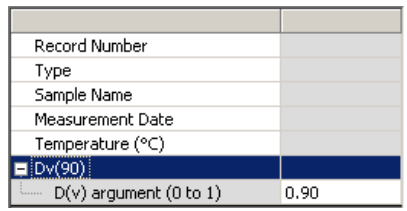
Change the order in which the parameters are displayed by selecting a parameter, and pressing the **Move up** or **Move Down** ↑ ↓ buttons.

Press **OK** to exit and return to the **Records view.**

Parameters with arguments

Certain parameters allow specific values (or arguments) to be added.

- A Dv(90) percentile can be viewed by adding **Measurement-Size-D(v)** and then typing 0.90 as the argument value.
- Column titles can be altered by double-clicking on them e.g. double-click on D(v) and alter to Dv(90) to reflect the particular percentile size viewed.



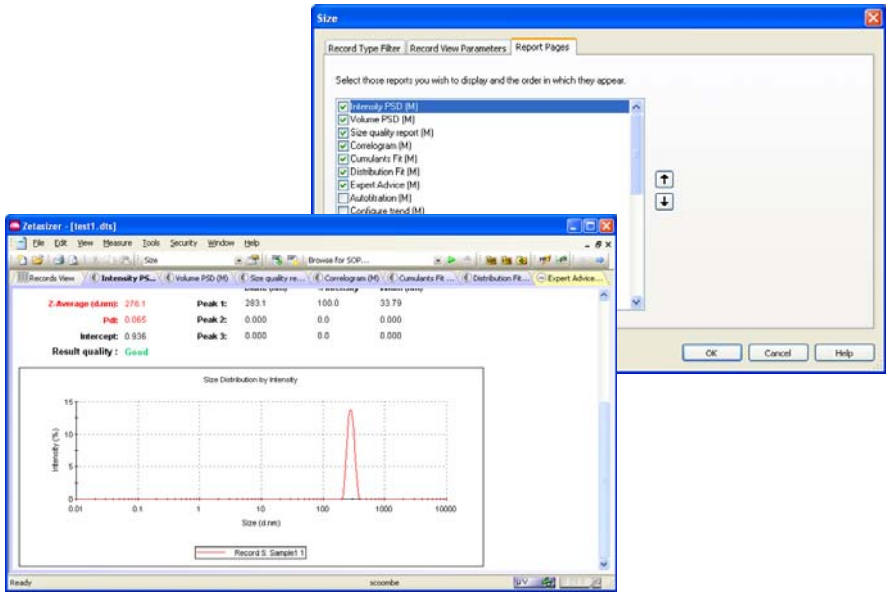
Note
If the software is subsequently updated with a new version, the changed parameter names will be preserved.

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)** and **Molecular weight (M)**. These and other reports can be added or removed using the workspace dialogs.

Selecting a report to view

Select **Tools-Settings-Configure Workspace-** "..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

The 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.

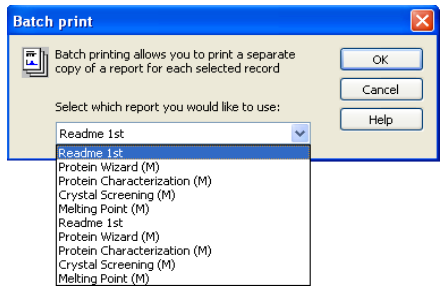
- A record can also be copied by selecting the item then selecting **Edit-Copy**. The item can then be pasted into another file (**Edit-Paste**).

- 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 **My Documents\Malvern Instruments\Zetasizer\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.

Batch printing

To immediately print reports from more than one record choose the required measurement records, and select **File-Batch print...** The **Batch print** dialog will appear, requesting which report template to print out.



Using SOPs

Introduction

SOPs use pre-set parameters to ensure that measurements are made in a consistent way. SOPs are integral to the operation of the Zetasizer APS - all measurements are controlled by SOPs. This provides a simple, repeatable workflow and saves having to set the same parameters each time a measurement is made. Not only does this save time, but also reduces the risk of introducing errors into the settings.

Chapter 4 gave instructions on how to use an SOP to make measurements, but this section 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 two 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.

This chapter focuses on how to create a new SOP and details all the measurement settings that are available.

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 the measurement record and selecting **Extract SOP**.

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

The SOP Editor

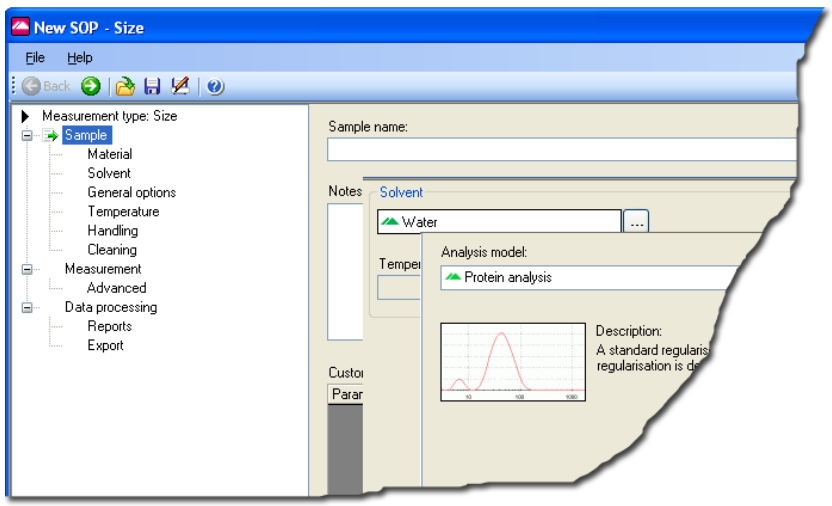
The **SOP Editor** is displayed if any of the following is performed:

- Selecting **File-New-SOP** from the Menu bar. This will open a new SOP.

- Selecting the **New SOP** icon from the toolbar. This will open a new SOP.
- Selecting **File-Open-SOP** from the Menu bar. This will open an existing SOP.
- Selecting the **Existing SOP** icon from the toolbar. This will open an existing SOP.
- Selecting a measurement record, then selecting **Edit-Extract SOP** from the Menu bar. This will open the SOP editor. (The title bar will show **Extracted SOP Settings**) so a measurement's SOP settings can be viewed.
  If altered, the new settings can be saved by using **File-Save** or pressing the **Save icon**.
- Right-clicking on a measurement record and selecting **Extract SOP.** (see the above description).
- Clicking the button on an SOP from within the **Plate Scheduler**.

Tree menu view

On opening the SOP editor the window below will be displayed; the exact list displayed will depend upon the **Measurement type** selected. The SOP dialogs available for each measurement type will be displayed down the left hand side of the window, with the respective highlighted SOP dialog shown alongside. The dialog will alter as each SOP dialog is selected.



Note
Always select the Measurement type first when editing an SOP otherwise any changes will not apply.

The dialogs can be stepped through by using the 

navigation buttons, the **up** / **down** keys on the keyboard, or selecting directly from the record list.

Most of the SOP dialogs are common to all measurement types; other SOP dialogs are **unique** to the **measurement type** selected and are discussed in individually.

Throughout the SOP dialogs some parameters will be available with an option to change the units used in their description. For example, Temperature can be set to **ºC** (Celsius) or **K** (Kelvin). For all parameters where this option applies a scroll down list will be available alongside the parameter. The Parameter unit chosen will be displayed in the final results and reports.

The SOP dialogs available are:

Measurement type

Allows selection of the type of measurement that the SOP will perform, i.e. **Size** or **Trend**.

Note
It is important that the **Measurement type** is the **first** parameter that is selected, as all subsequent settings will be related to this measurement type.

Sample

This dialog 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 dialog for **all** SOPs.

Sample - Material

This dialog allows the physical properties of the sample **material** to be set. The refractive index and absorption can be entered for the material used.

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

Sample - Solvent

This dialog allows the physical properties of the sample **solvent** to be set. The refractive index and viscosity can be entered for the solvent used.

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

Sample - General Options

This dialog allows additional physical properties of the sample that will be used to be entered.

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

Sample - Temperature

This enables selection of the temperature at which the measurement will be performed.

This is a common dialog for **all** SOPs **except** Trend and Protein aggregation point.

Sample - Handling

This option allows the sample loading and destination options to be specified.

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

Sample - Cleaning

Provides options that tell the system which cleaning sequence to follow, including which bottle (wash or solvent) to use.

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

Measurement

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

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

Measurement - Advanced

Use the **Advanced** dialog to alter the attenuator settings.

Data Processing

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

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

Data Processing - Reports

This dialog enables reports to be printed automatically 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 dialog for **all** SOPs**.**

Data Processing - 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 dialog for **all** SOPs**.**

Trend - Sequence

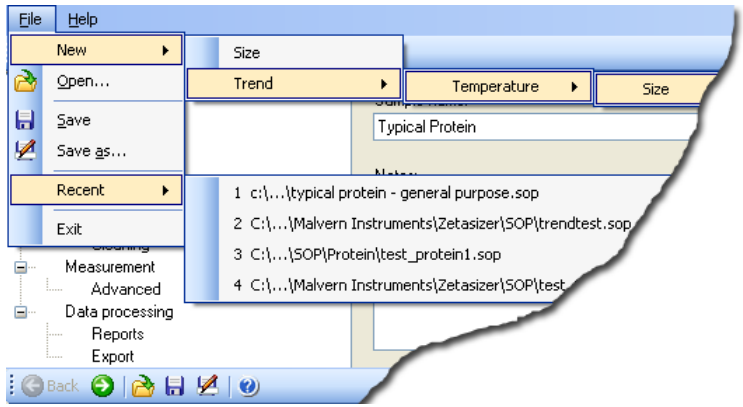
Enables the selection of the temperature trend parameters, i.e. start and end temperatures, and intervals between measurement steps. This is also where the option to check for aggregation point is set.

This is a dialog for **Size** trend measurements.

All SOPs are described in detail in the following sections.

SOP Editor Menu and Toolbar

SOP file management, saving and associated software functions are handled using either the Menu bar or the Toolbar.



The functions are described in the following table.

Menu item	Toolbar icon	Description of function
File-New		Opens a **new** SOP
File-Open...		Opens a **existing** SOP
File-Save		Saves the currently open SOP
File-Save as...		Saves the currently open SOP under a **new** name
File-Recent		Displays the three most recently used SOPs
File-Exit		Exits the SOP editor
Help Menu		Displays the Zetasizer software help menu
	Back	Use to move up and down the SOP tree menu. The SOP dialog will alter to match the selection

Creating an SOP

- Select **File-New-SOP**. This will open up the **SOP Editor**.
- Select the ▶ **Measurement type** required (see next section).
- Change the settings within the SOP dialogs. These are described in the following pages under the Measurement type selected (e.g. Size SOPs).

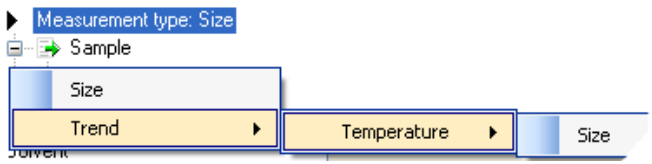
- When all the SOP settings have been completed, select **File-Save** or press the **Save icon** , and enter a name, and save the new SOP.

Measurement type selection

The measurement type must first be selected before entering the settings on the subsequent SOP dialogs.

To Select the Measurement type

- Left click on **Measurement type** and select the measurement type from the pull down menu.



- The Measurement types are:

  Size
  Trend - temperature against Size.

  Aggregation Point measurements can be performed using the Trend measurement type.

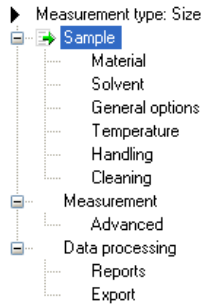
- When selected, the content of the SOP tree menu changes to match the measurement type.

Note
The following **Size SOP** section describes the SOP dialogs shown when a **Size Measurement type** is selected. This **includes** all sizing SOP dialogs **and all** dialogs that are common to **all** SOPs.

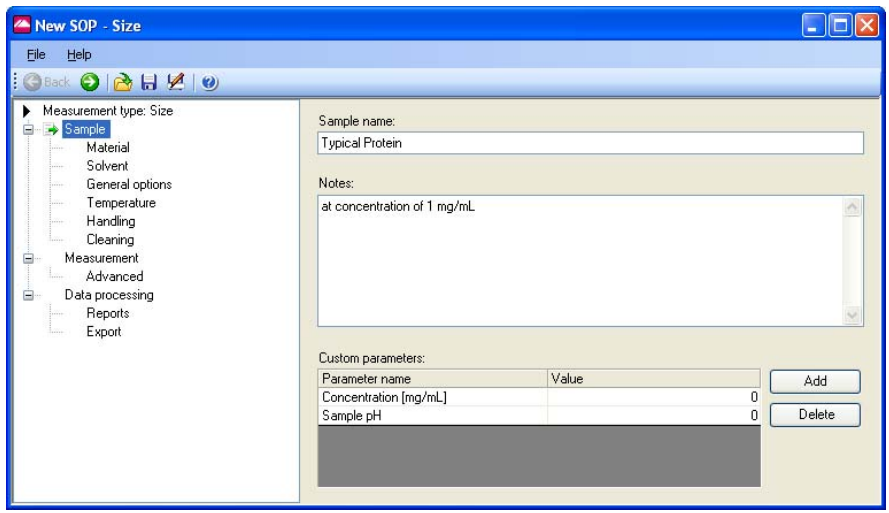
Size SOPs

Though the majority of the **Sample** and **Measurement** dialogs described below are **unique** to **Size** measurements; this section also includes SOP dialogs that are **common** to **all** SOPs.

The **Size SOP Editor** menu is:



Sample



The components of the dialog are:

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.

The name typed into the **Sample name** entry box will be displayed in the **SOP toolbar** browser. Ensure a suitable name is chosen that will identify the measurement the SOP has been created to perform.

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.

Custom parameters:

This function enables the user to manually enter a parameter at the beginning of a measurement, associated with the sample that is not measured by the system. This parameter is stored in the result record for subsequent use in result reports or for generating a trend plot using this parameter. For example the parameter could be the concentration of an additive which is varied in subsequent measurements, then plotted as a function of the size of the material, for example to study its effect.

Once in the record, the parameter will be available for selection for both the workspace and Records view, and for data export.

Adding a parameter (Add button)

To add each parameter, select **Add** and input the **customer parameter name**, i.e. Surfactant A. When the SOP is subsequently run, the SOP will request the addition of a value for the new parameter. By running the SOP a number of times with different input values, the resultant measurements can be subsequently plotted and any effects observed.

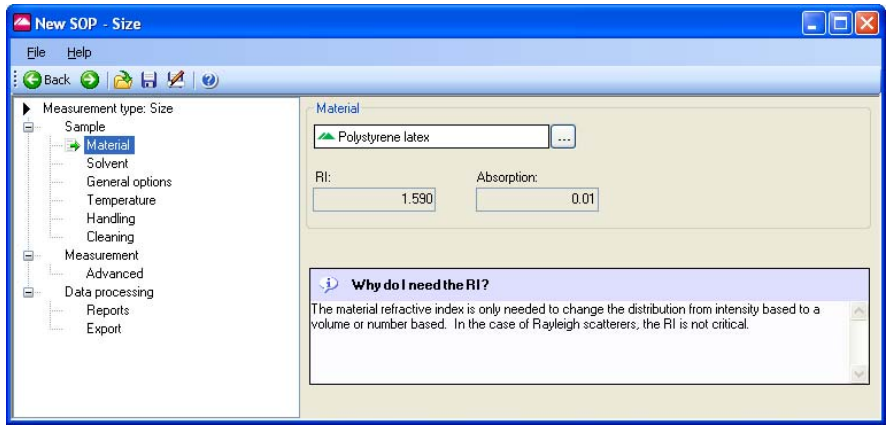
Show this page when the SOP is started…

With this check box selected the **Sample identification** dialog 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.

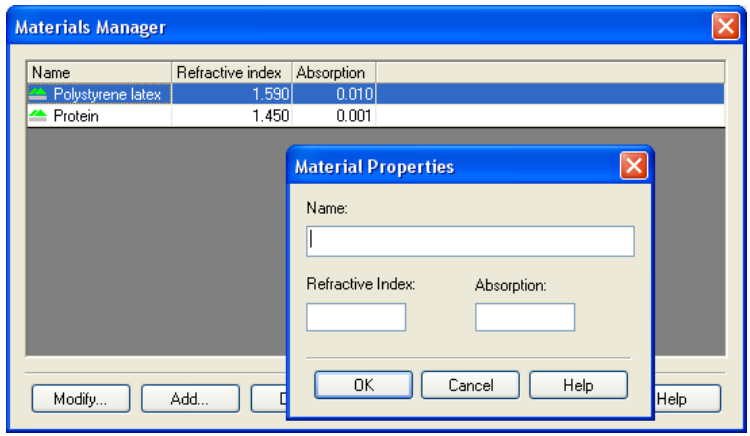
Sample - Material

The Zetasizer software requires certain information about the physical properties of the solvent that is used in the sample that is to be measured. Selecting the **Sample - Material** dialog and pressing the ... button, displays the **Material properties manager** where these properties can be defined.



Materials Manager

From the list displayed, an available material can be selected from the list for inclusion in the SOP; alternatively a material can be added, modified or deleted from the list.



To define a new material, press the **Add...** button to display the **Material Properties** dialog. Here, the material **name**, **refractive index** and **absorption** can all be specified.

The **Refractive index** and **Absorption** are only needed if the result is required as a volume distribution.

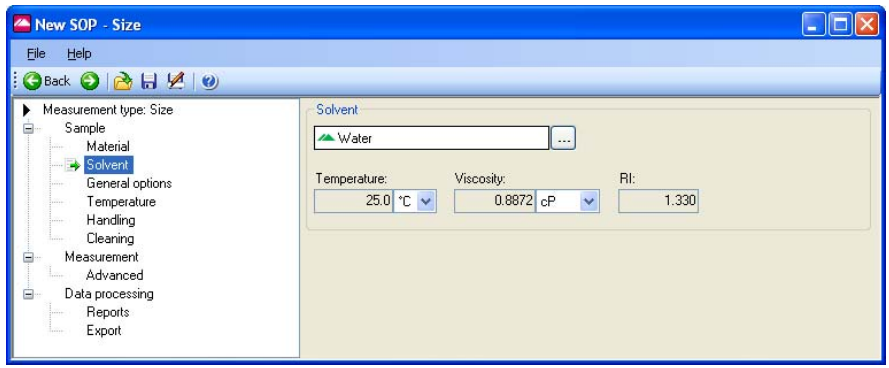
- The **Refractive index** can be obtained from a number of sources and is only required to two decimal places. Note that the refractive index is wavelength dependent.
- The **Absorption** is the quantity of light absorbed by the material when suspended in the solvent. Generally clear samples will have 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

Other materials such as gold and silver colloids can have absorbance values greater than 1, and refractive index values less than 1.

Sample - Solvent

The Zetasizer software requires certain information about the physical properties of the solvent that is used in the sample that is to be measured. Selecting the **Sample - Solvent** dialog and pressing the ... button, displays the **Dispersants properties manager** where these properties can be defined.



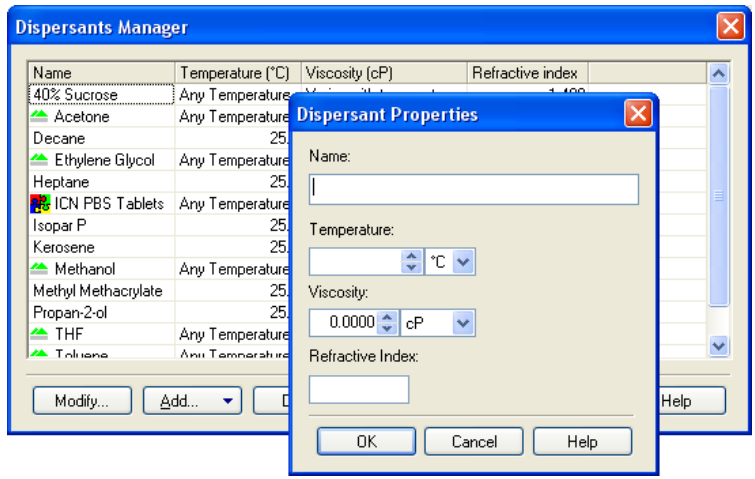
Dispersants Manager

From the list displayed, an available dispersant can be selected for inclusion into the SOP; alternatively a dispersant can be added, modified or deleted from the list.

To define a new dispersant, press the **Add...** button and choose from defining either a **Simple dispersant or solvent** or a **Complex solvent** - for details on

defining a Complex solvent please refer to the **Solvent builder** section in **Chapter 9**.

On selection of a **Simple dispersant or solvent** the **Dispersant Properties** dialog will appear, allowing new dispersants to be defined. The dispersant **name**, **refractive index** and **viscosity** can all be specified.



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.

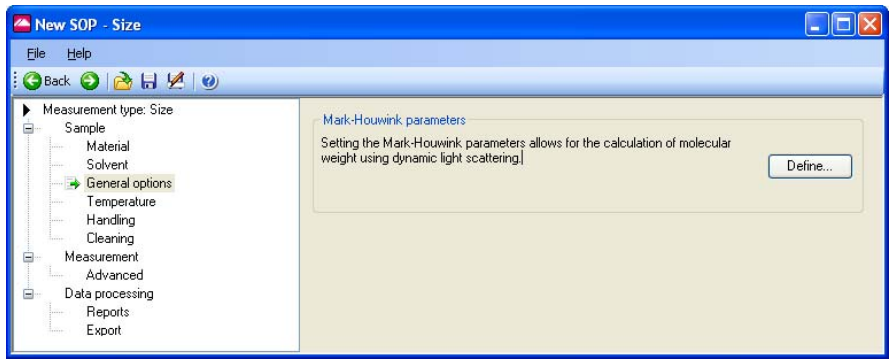
Note
Viscosity is temperature dependent. The Malvern defined dispersants include a built in viscosity calculation that determines the correct viscosity of the dispersant at any sample temperature.

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** dialog 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.

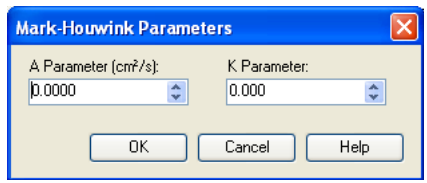
Note
Note that it is not possible to modify or delete the dispersants that were specified by Malvern.

Sample - General Options



Mark-Houwink parameters.

Selecting the **Define** button displays the **Mark-Houwink parameters** dialog.



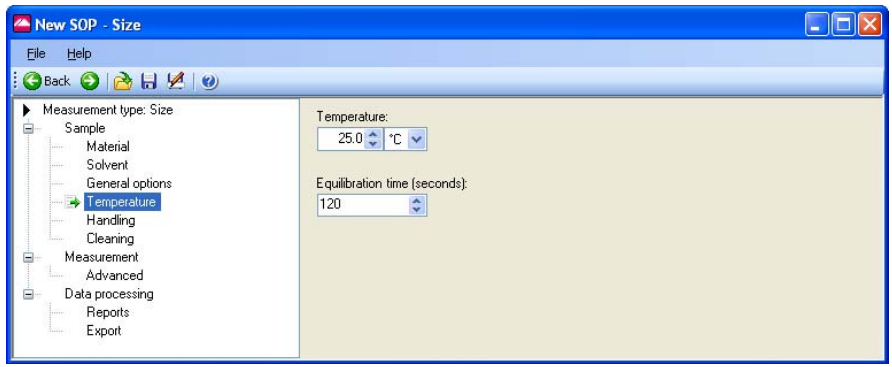
This allows the Mark-Houwink parameters to be entered to enable a Molecular weight to be calculated based on the dynamic light scattering data.

In the Mark-Houwink relationship: $D = kM^{-a}$

From knowledge of the Diffusion Coefficient *D* and th**e** *a* and *k* constants (both available in many reference books and the help file), the Molecular weight *M* can be determined.

Once entered, both a Molecular weight calculation and Size measurement will be available for display.

Sample - Temperature



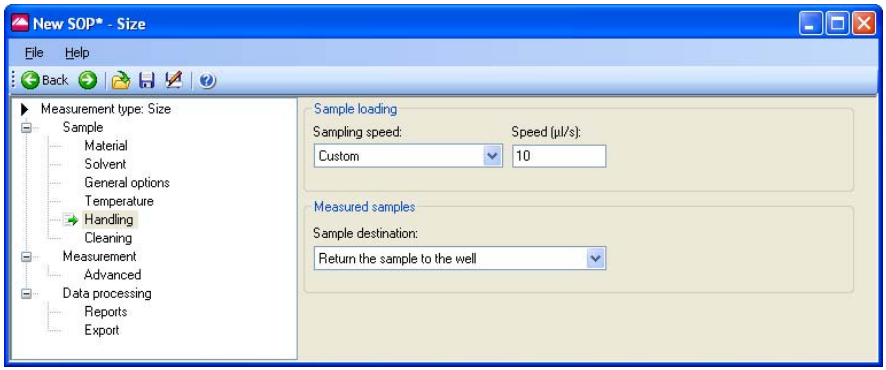
Note
The Zetasizer is capable of heating or cooling the sample to allow the measurement to be made at a specific temperature. The measurement will not start until the sample has reached the temperature specified.

Input the **Temperature** required, and how long the sample should be left 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 convection 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.

Sample - Handling



The **Handling** dialog allows the **Sampling speed** and **Sample destination** to be set. Either accept the default Sampling speed of 11.5 µl/s or select **Custom** from the menu and enter a value.

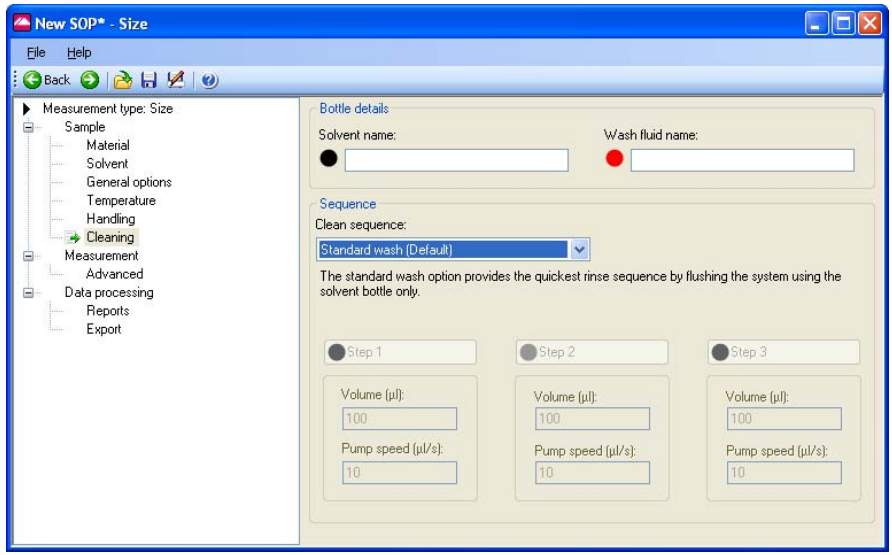
By default **Return sample to the well** is selected. Sending the sample to waste is slightly quicker, providing the sample is not needed again. This might be a useful option where, for example, a temperature modification has been made which denatures the sample, rendering it of no further use.

Sample - Cleaning

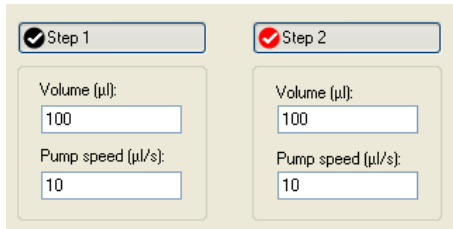
The **Cleaning** section of the SOP allows the user to name both the **Solvent** (i.e. the rinse solvent) and the **Wash**, together with setting details of the **Clean sequence**.

Note:
This screen relates to the red and black rinse inputs on the instrument. **Ensure that the solvent rinse is connected to the black input and the wash fluid is connected to the red input**. See **Chapter 3** for an illustration of the instrument connections.

There are two wash sequences: **Standard wash** (the default setting) and **Vigorous wash**. The **Standard wash** option provides the quickest rinse sequence, flushing the system using the solvent bottle only. The vigorous wash option provides the most comprehensive cleaning sequence, consisting of quick rinse, wash, and final rinse steps. The solvent bottle is used for the rinse steps and the wash bottle is used for the wash step.



Additionally, it is possible to tailor up to three steps in a wash sequence. To do this, choose **Custom** from the **Clean sequence** drop down menu. Then select **Steps 1-3** as required, entering a value for **Volume (µl)** and **Pump speed (µl/s)** if the default settings are not required. The maximum **Pump speed** allowed is 156µl/s - after this, there is a risk of valve leakage. When a step is selected, a check mark is shown:

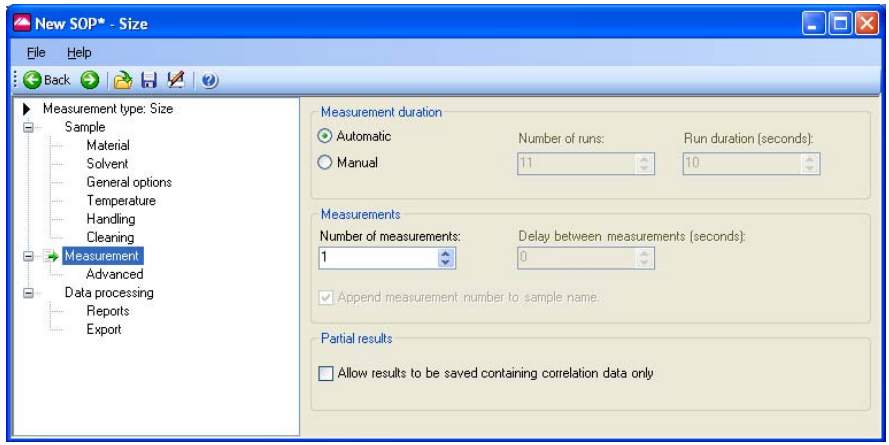


If no check mark is shown on the step, it will be omitted.

APS Wash Controls: as part of good practice for reducing contaminants in the system, it is advisable occasionally to clean the system without making a measurement. This has the effect of flushing the tubing and needle to ensure that any residual sample is completely removed. To do this, select **Tools-Instrument-APS-Wash Controls** from the main Zetasizer menu. Either select one of the clean sequences (such as **Standard wash**) included with the system or choose **Custom** to create an individual cleaning cycle. Click ▶ to execute the wash sequence.

Measurement

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



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 of a latex standard, or increased to improve the repeatability of the measurement of particularly polydisperse samples. Manual run durations can be set between 1 and 600 seconds, and 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.

Measurements

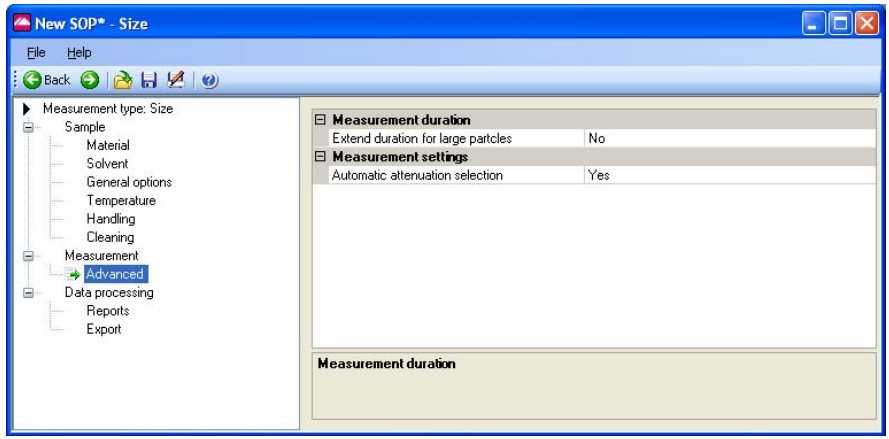
This option allows a sample to be measured more than once; to investigate the effect on Particle Size over time, or to prove repeatability. Set the **Number of measurements** to the required number. Add a delay between measurements in the **Delay between measurements** text box if required.

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.

Partial Results

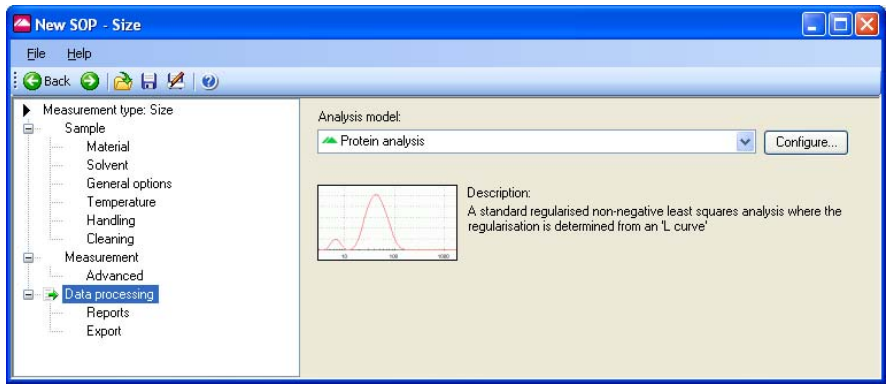
If it is likely that a measurement will not produce a correlation function that can be analyzed - for example, measurement of a surfactant solution at lower than the critical micelle concentration - then the data collected can still be saved by selecting the **Allow results to be saved containing correlation data only** check box within the **Partial Results** area.

Measurement - Advanced



The **Advanced** dialog contains functions to alter the attenuator level and also to extend the measurement duration when large particles are present in the sample.

Data Processing



If characteristics are already known about the sample being measured, this dialog will allow an appropriate analysis model to be applied and so optimize 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.

Three **Analysis Models** are available - **Protein Analysis**, **General Purpose** and **Multiple Narrow Modes**, plus a custom analysis model can be applied (**Load analysis settings...**).

Each **Analysis Model** can be altered using the **Configure** button.

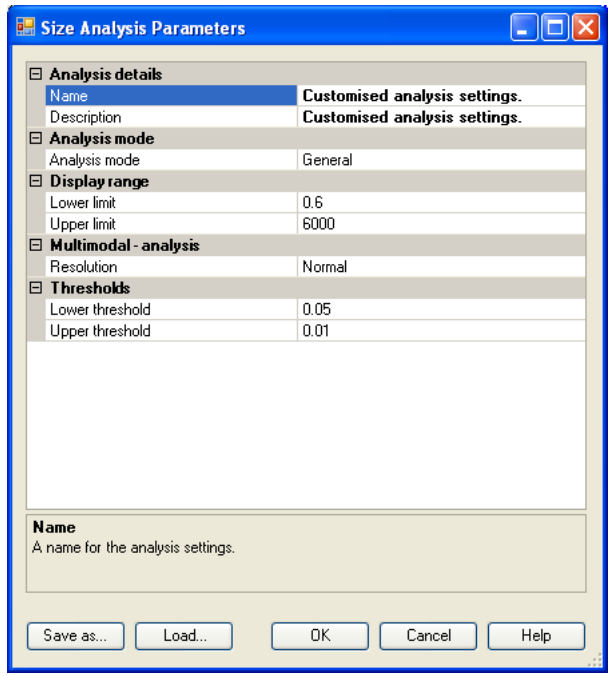
Please see the **Help** file for more information.

Analysis Models

- **Protein analysis**
  The default setting, select this model when undertaking a protein analysis.
- **General Purpose**
  Select this model if the characteristics of the sample to be measured are unknown.
- **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.
- **Load Analysis settings...**
  This enables a previously saved analysis model to be loaded and used for the data processing. These are saved as **.dass** files. (The last **s** indicates the analysis setting has been created for a **Size** or **Molecular weight** measurement).

Configure button

The **Configure** button dialog enables various attributes of the analysis model to be altered. These include the **Name**, the measured Size **Display range**, the **Analysis mode** and the measurement **thresholds**. The altered analysis model can then be saved. Analysis models are saved as **.dass** files.



If it is known that all particles within the sample will fall within a certain size range, then the size **Display 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.

Change the **Name** and **Description** fields in the **Analysis details** to reflect the new Analysis settings.

To **keep** the new analysis model, press the **Save as...** button, then press **OK** to exit the dialog and use the analysis model; the new analysis model name will now be displayed in the **Analysis model** entry box on the **Data Processing** dialog.

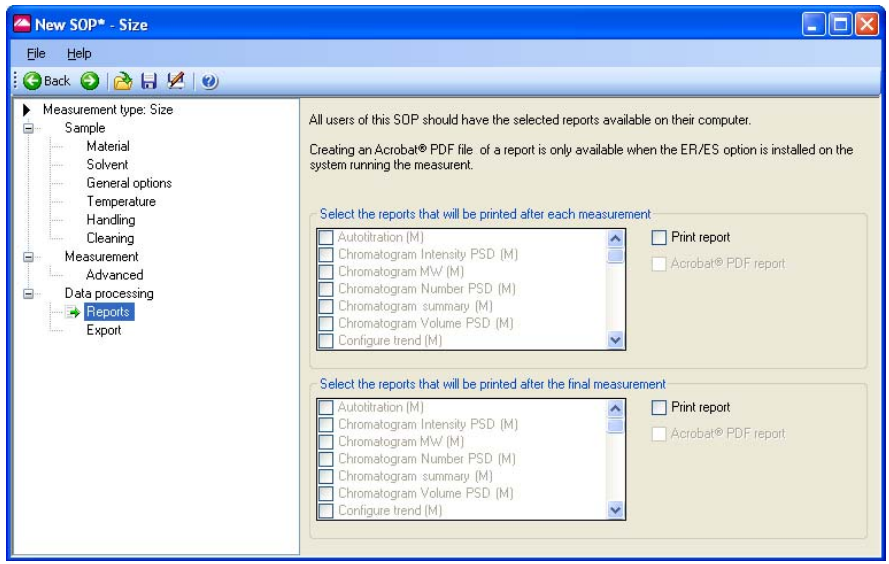
Note
If no name has been given, the analysis model will be displayed as **Customized analysis settings** in the **Analysis model** entry box.

Previously saved analysis models can be used, viewed and altered by pressing the **Load** button. Pressing **OK** will exit the dialog and add the model to the **Analysis model** entry box.

Note
The Analysis settings files can be saved and loaded independently of the SOP. Only the analysis settings in the file will be used in the SOP, the file itself is **not** attached. This means that any subsequent changes to the Analysis file will **not** change the SOP. The analysis settings file will have to be reloaded into the SOP for the changes to take place.

Data Processing - Reports

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



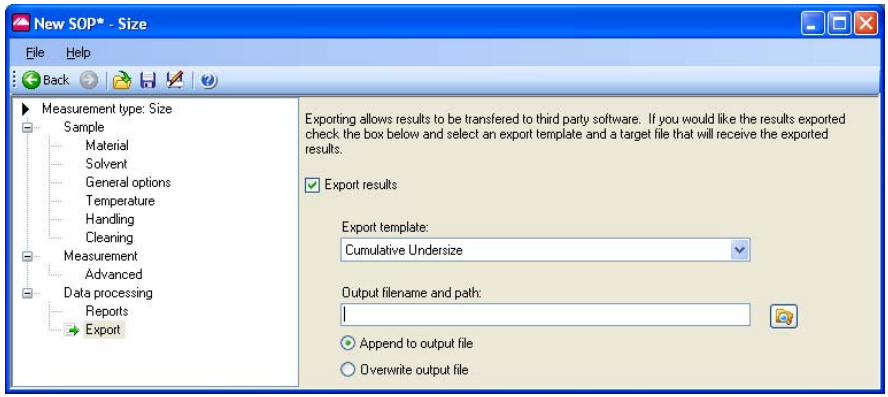
Two selection boxes are available. One to enable a report to be printed after **each** measurement is completed and the other to print out a report after **all** measurements have been completed. For each, select the **Print report** check box

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.

Data Processing - Export

The **Export** dialog enables the measurement results to be exported to third party software packages such as Excel or Wordpad.



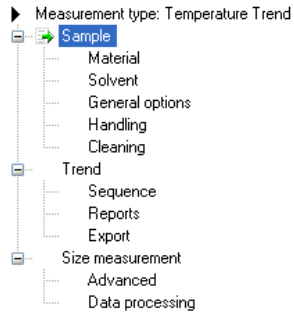
With the **Export results** check box selected measurement parameters can be defined and exported on completion of the measurement. Select the required **Export template** from the list; this will display the export dialogs described in **Chapter 9**. Refer to this chapter for full details on creating 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.

Trend SOPs

The **Trend SOP Editor** menu is:

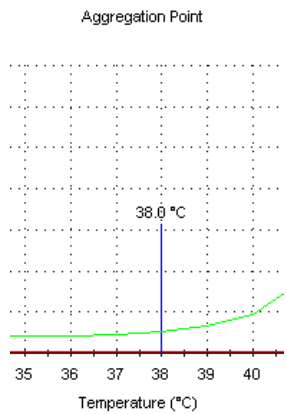


The **Trend** SOP allows size measurements to be performed and plotted as a function of temperature.

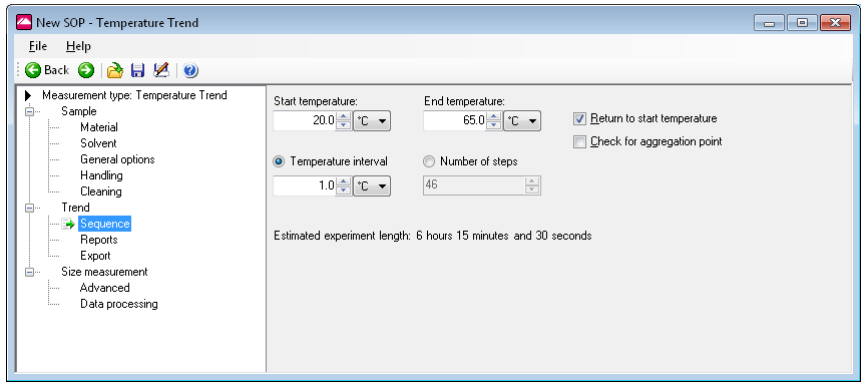
Trend SOPs are similar to the standard size SOPs, the differences being the inclusion of a trend and altered measurement dialogs.

Aggregation Point SOPs

Aggregation point measurements can be performed by selecting a check box on the **Trend - Sequence** dialog. This will enable the protein **denaturation** point to be determined. This measurement will be performed as a function of temperature.



Trend - Sequence



The **Trend - Sequence** dialog enables the selection of the temperature trend parameters, i.e. start and end temperatures, and intervals between measurement steps.

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.

Return to Start temperature
If this check box is selected, once the aggregation point has been determined the measurement will return to the original starting temperature defined above.

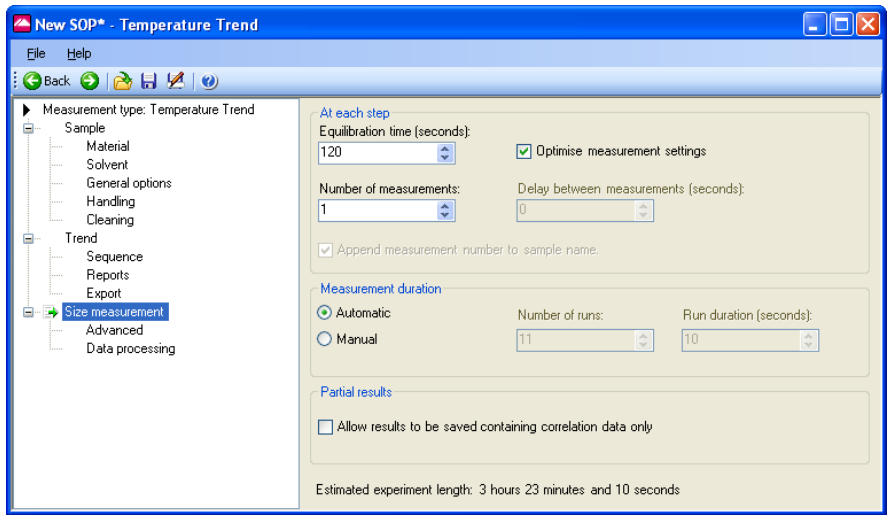
Temperature Interval / Number of steps
The measurement can be configured to change either in specific measurement intervals (e.g. every 5°C), or in a specific number of steps (e.g. 10 steps). Select the button to identify which is required.

Aggregation point measurements only

Check for aggregation point
If the protein **denaturation** point is to be determined as part of the measurement, select the aggregation point check box.

Stop when aggregation point found
If this check box is selected the measurement will stop once the aggregation point has been determined.

Size Measurement



The trend size measurement dialogs are generally the same as those used with either the standard size measurement SOP. The additional features are:

- **At Each step** gives the option to control the **Equilibration time** and **Number of measurements** performed.
- **Optimize Measurement settings** enables the cell position to be checked and verified before each individual measurement is performed. During aggregation point determination this setting is not used.

Please refer to the **Measurement** description in the size SOP sections for details of the **Measurement duration** and **Advanced** settings.

Extracting an SOP

The SOP used when performing a measurement can be viewed by right-clicking on the measurement record and selecting **Extract SOP**. This will display the SOP dialogs.

If required the SOP can be modified and saved under a new name.

Modifying an SOP

To modify an SOP, select **File-Open-SOP**. The SOP dialogs will be displayed, allowing any settings to be changed.

Alter the settings within the dialogs, and then save (select **File-Save** or **File-Save as...** as appropriate).

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 allow measurements of the same to be made 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 APS instrument so that measurements are consistent. Simply copy the .**sop** file to all computers running a Zetasizer APS system.

If the SOP has a non-standard solvent defined, 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 solvent to the dispersant database.
- If the solvent already exists, the software will ask if the existing solvent parameters should be overwritten or saved under a new name. It should be noted that if the parameters are overwritten, the new parameters will then be used for all SOPs that use that solvent.

Results created on another APS instrument will also have the associated well identification information included in the files. To see this data, use the **Plate Navigator** described in **Chapter 5**.

9

Advanced features

Introduction

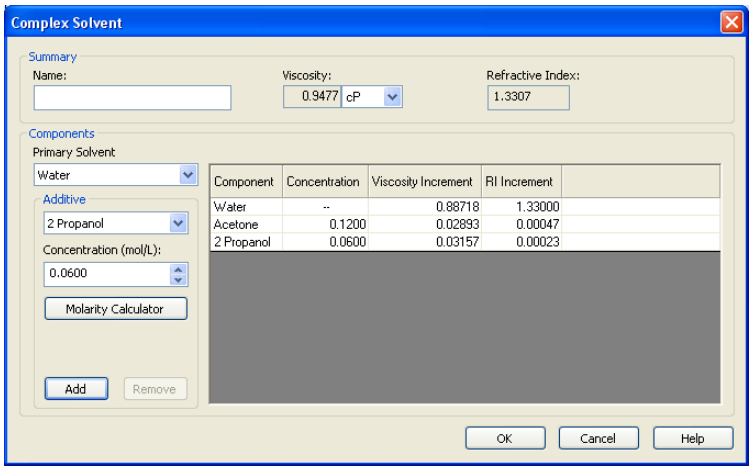
This chapter contains details about the following **advanced** features and capabilities of the Zetasizer APS:

- Solvent builder
- Averaging results
- Editing the result
- Exporting the results
- Options dialog
- Expert advice

Solvent Builder

The Solvent builder is a database of components that can be added in different quantities for estimating the properties of solutions. The database is used for the calculation of **Complex solvents**; generally necessary when performing measurements involving proteins.

The dialog below will appear when **Complex solvent** is selected when adding a dispersant or solvent in the **Sample** SOP dialogs.



The Solvent builder is used by SOPs for:

- Calculating the viscosity and refractive index for non-standard solvents.

The calculations operate using the assumption that changes in the base solvent properties will be additive.

- For example, if the addition of 10% glycerol increases the refractive index of water by 0.0118 and the addition of 0.5 M calcium chloride increases the refractive index of water by 0.0128, then the addition of both will increase the value by 0.0118 + 0.0128, in total 0.0246.

Note
The Additive effect on both viscosity and refractive index is determined using 3rd order polynomial fits of data compiled in the “65th Edition of the CRC Handbook for Chemistry & Physics”.

Building a Complex solvent

To generate a Complex solvent, a few sample parameters have to be entered into the builder dialog.

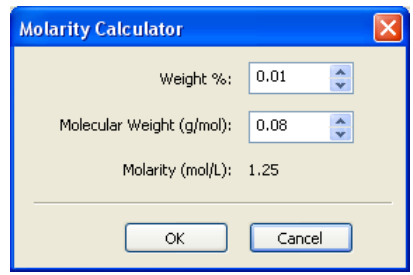
The **Summary** area details the chosen name for the new solvent, plus its calculated viscosity and refractive index. Do the following:

- Input the **Name** of the new solvent.
- Select the **Primary solvent** used for the new solvent (i.e. water).
- Select an **Additive** from the list and enter its **Concentration**, either directly or by using the **Molarity Calculator** button and associated dialog (below).

  Input the **weight** (%) and the **Molecular Weight** (g/mol) of the additive.

  The **Molarity** (mol/L) will be automatically calculated.

  Press the **OK** button to enter the value into the **Concentration** column of the solvent table, otherwise press **Cancel** to exit the dialog without updating.



- Press the **Add** button. The refractive index and viscosity increments will be added to the table, with the total solvent viscosity and refractive index, or dielectric constant, calculated and displayed in the **Summary** area.
- If any additive needs to be deleted from the solvent, highlight it and press the **Remove** button to delete the additive from the table.
- Press **OK** and the new solvent will be added to the Dispersants/Solvents manager.

To **modify** a dispersant, once created, select the dispersant from the list and press the **Modify...** button (or double-click the dispersant in the list). The **Complex Solvent** dialog will appear, allowing the parameters to be changed. The builder will only appear if the solvent was originally created using the builder and not the standard **Dispersant** properties dialog

Complex solvents have this icon displayed before the solvent or dispersant name in the **Solvents** or **Dispersants Manager** dialogs.

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

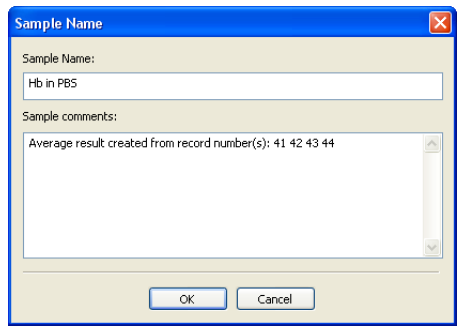
Note that it is not possible to modify or delete any dispersants or solvents that were specified by Malvern.

Result averaging

What is the average result of a selection of records?

Highlight the required records and either select **Edit-Create Average Result** or **right-click** on the records and select **Create Average Result** from the displayed menu. An averaged result record will then be generated and added to the end of the measurement file. The averaged result will display **Average** in the result's Origin column.

Before the averaged result is created a dialog will appear so comments can be added and the sample name changed, if required.



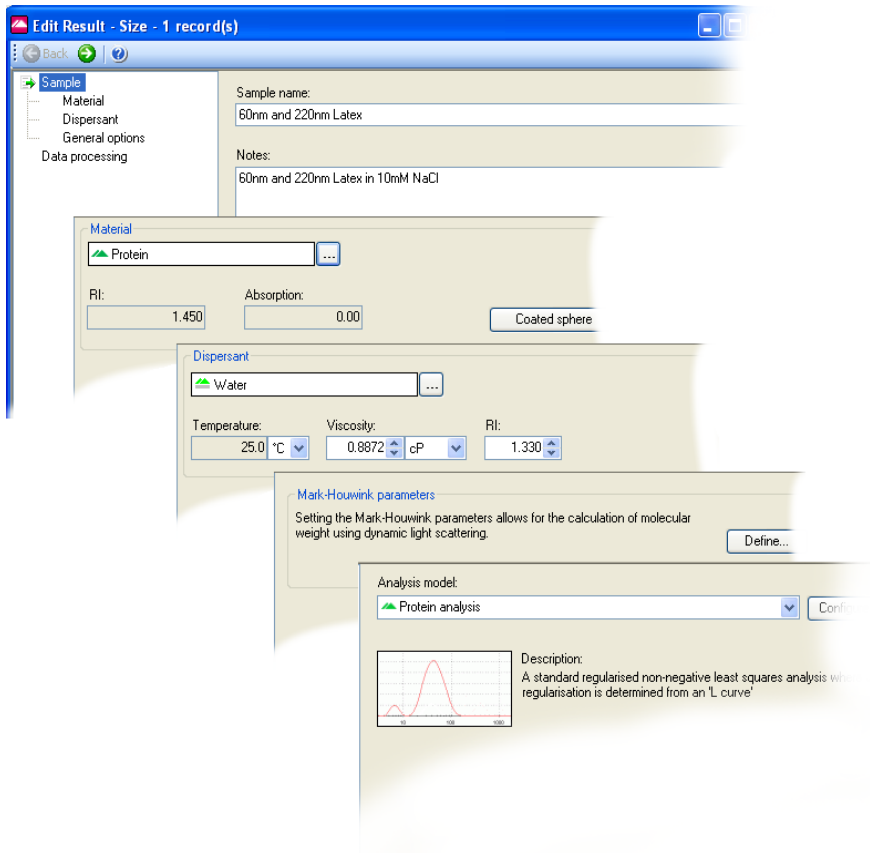
Note
When averaging, the result source must be the same. A previously averaged record cannot be averaged again.

Editing the result

It is possible to re-analyze a measurement record using different measurement parameters. The re-analyzed 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 re-analyzed without the need for the instrument to be connected.

Select the menu item **Edit-Edit result** or right-click on a measurement record and select **Edit Result**; The **Edit Result** dialogs will appear - these will be similar dialogs to those within the **SOP Editor**. As with the **SOP Editor**, the dialogs shown will differ, depending upon the measurement type selected. The example below shows the **Edit result dialogs** available when a **Size measurement** is selected.



Note
Each edit result dialog will be slightly different depending upon the measurement type originally performed. The picture above shows the size view.

Multiple results can be edited by selecting the records required before selecting the edit result dialog.

To Edit a result:

- **Right-click** on the required measurement record(s) and select **Edit Result**.
- Select the appropriate dialogs and alter the required parameters, then press **OK**. It is advisable to add the modified parameter to the records list so the altered records can be instantly identified.
- The result will instantly be re-analyzed and the result added to the **Record view**.

21 CFR Part 11

If the 21 CFR part 11 feature is installed, a **Reason for change** dialog 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** dialog (Select **Configure-Workspaces-"..workspace choice.."** and then the **Record view** parameters tab).

Exporting results

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 Notepad. 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. Note that the edit menu selections available will depend on the contents of the report.
- Any of the parameters from one or more records can 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 **Tools-Settings-Data export templates** dialog allows users to create and modify their own templates.
- A complete measurement file, or selection of records, can be exported using **File-Export...**. Refer to the following section for details.

Exporting using Drag and Drop

As the name suggests, exporting data by "drag and drop" is simply a matter of dragging the records to another application and then dropping them.

Select a record then move the cursor to the boundary or edge of the record until a small rectangle and cross attaches. Press and hold the mouse button 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**.

The same technique can be applied when exporting tables or graphs.

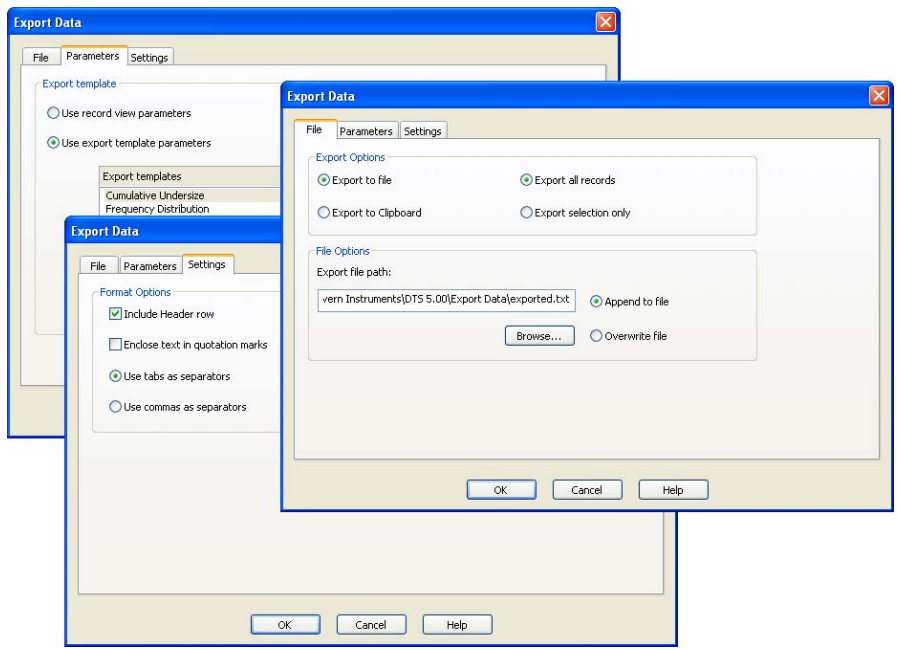
Exporting using Menu controls

To export a complete measurement file, or selection of records, first open the required file and select **File-Export...**

Use the tabs - **File**, **Parameter**, **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 grayed 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

This 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**.

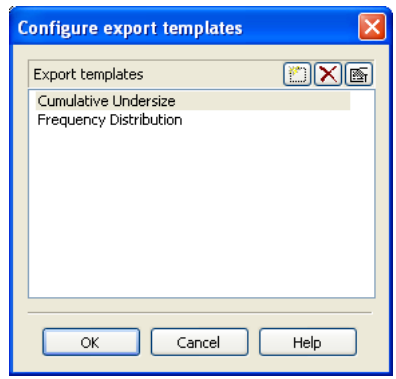
Settings tab

This tab determines how the data are 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.

Creating an export template

New export templates can be created (or existing ones modified) by selecting **Tools-Settings-Data exports templates**. and using the associated dialog, shown here.

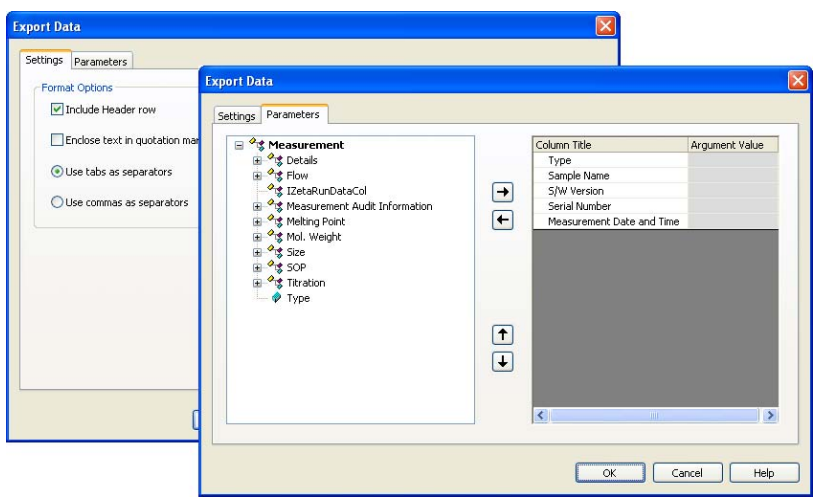


Two export templates are supplied as standard: **Cumulative Undersize** and **Frequency Distribution**.

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 **Export data** dialog below will appear: only **Settings** and **Parameters** tabs are available.



Settings tab

The settings tab works in the same way as previously described.

Parameters tab

- The list on the left displays all available parameters.
- The list on the right displays all parameters that will be included in the export template.

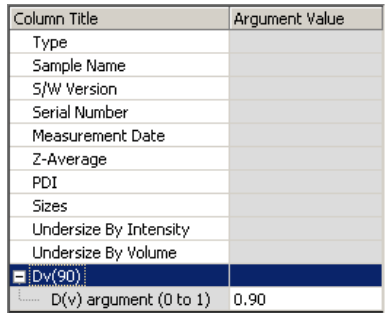
Parameters can be added or removed from this list by using the **Add** and **Remove** keys.

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 **Configure export templates** dialog. Press **OK** again to save the template.

Parameters with arguments

Certain parameters allow specific values (or arguments) to be added.



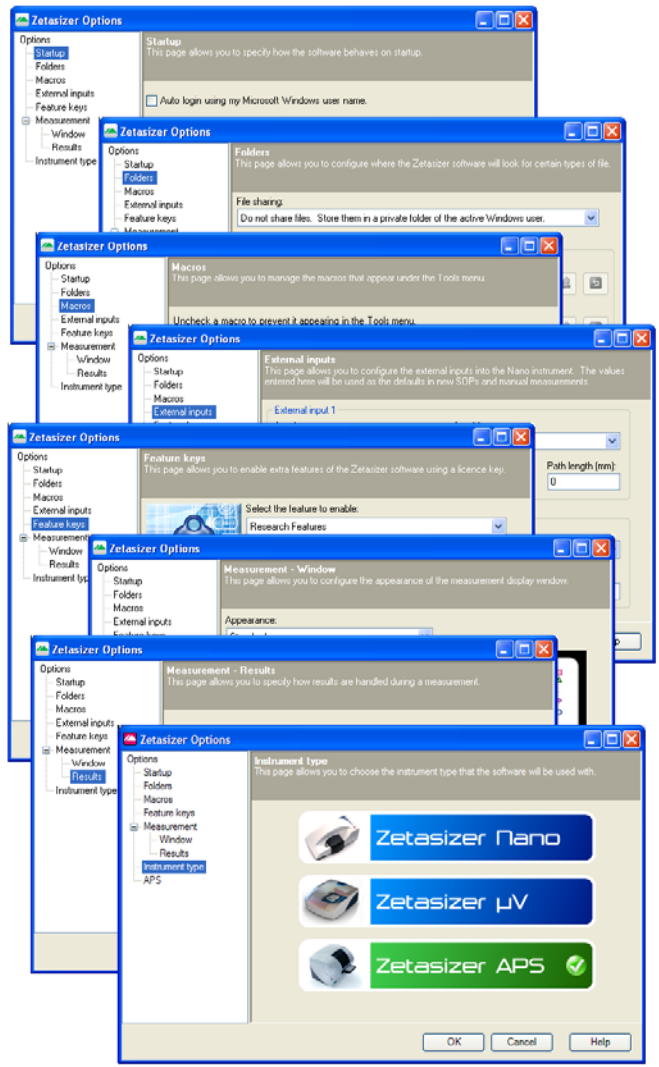
A Dv(90) percentile can be viewed by adding **Measurement-Size-D(v)** and then typing 0.90 as the argument value.

Column titles can be altered by double-clicking on them e.g. double-click on D(v) and alter to Dv(90) to reflect the particular percentile size viewed.

Note
The Export templates can also be selected and edited using the SOP creation procedures - **Export** dialog.

Options dialog

The **Options** dialog enables extra features and programs for the Zetasizer software to be installed, and to configure the default folder for saving of data.



The **Options** dialog enables extra features and programs for the Zetasizer software to be installed, and to configure the default folder for saving of data.

To open the Options dialog, select **Tools-Options...**

The features available from the menus are described below.

Options

Startup

The Startup option specifies how the software will behave on startup. Either using the Microsoft Windows password to logon with, or one specific to the application software.

Folders

The **Folders** dialog indicates and defines where the measurement data, SOPs and Export data are saved to. The Zetasizer software will look for certain types of file at these locations.

When the software is first installed, these file-paths will be configured to their **default** setting. This page allows the file-paths to be configured so the files can be saved to more appropriate locations.

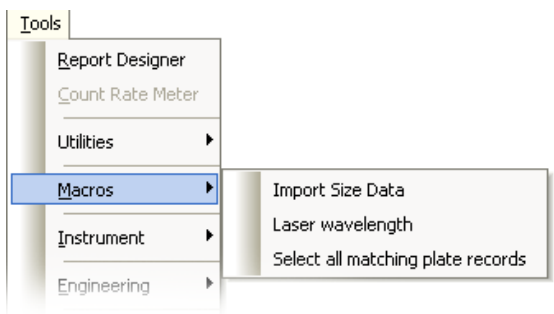
- Pressing the button allows another destination to be selected. This will open a **Browse for Folder** dialog; select the new destination directory and press **OK**.
- When the file-path used is different to the default, the second button alongside will turn blue ; pressing the button will revert the file-path to the default location.

Macros

Additional and Customized software programs can be added and installed by using the Macros option. This dialog allows new macros programs (.**zmac** files) to be installed and selected.

The Macro programs are written by Malvern to enable the system to operate in a mode required for a specific application. Highlighting the Macro in the left box will display the Macro details in the right box.

Any installed and selected macros will be seen in the **Tools-Macros** menu (the list below is shown only as an example).



- Press the **Install new macro...** button, select the macro from its location (CD, floppy disc, computer folder, etc.) and press **Open**. This will copy the macro to the **zetasizer/macros** directory and add the macro to the list of available programs.
- To **display** the Macro in the **Tools-Macros** menu, select the **check box** alongside the macro and press **OK**. When the **Tools-Macros** list is next viewed the macro will be available to use.
  Any un-checked macros will **not** be seen.
- **Uninstall Macro** will remove the macro from the computer. A warning message will appear to indicate the macro will be deleted: **"Are you sure you want to delete the selected macro?"**.

Feature keys

This page allows additional features of the Zetasizer software to be enabled using a licence key. Extra features available include **Research** software and **21 CFR Part 11** capability.

- Enabling is done by typing the licence key number supplied into the entry box and pressing the **Install Licence key** button.

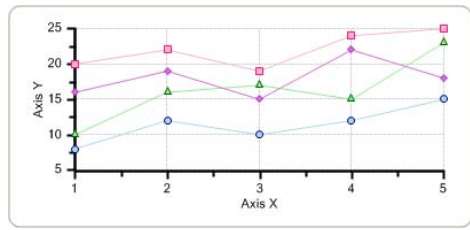
Full details on installation and enabling will be sent with each additional software package.

Measurement

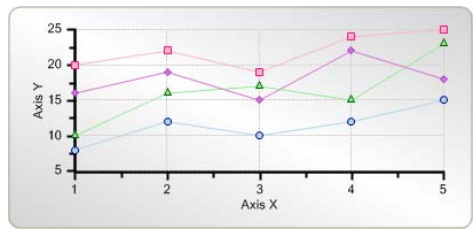
Window

This option allows the appearance of the measurement display to be changed and selected. The appearance choices are shown below, for each one an anti-aliasing view is also available.

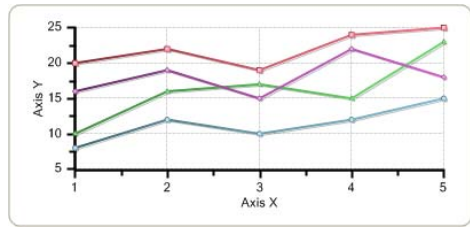
Standard



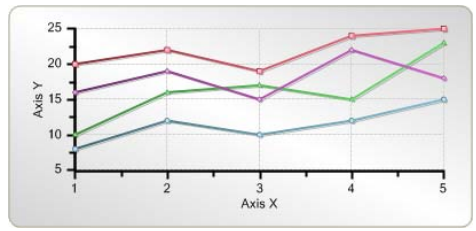
Style 1



Style 2



Style 3

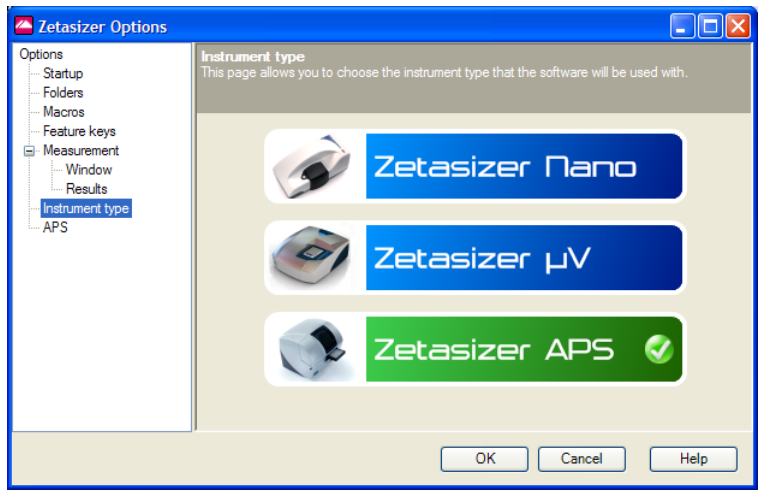


Results

This dialog contains a check box that will allow measurements results to be saved even if the measurement is aborted before the measurement has finished collecting and analysing the data.

Instrument type

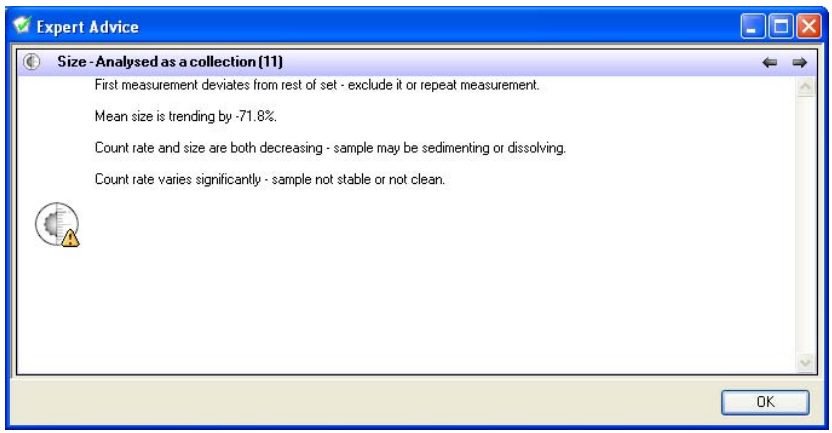
This dialog provides the option to select which instrument type is currently in use. The software remembers the selection and uses the same mode the next time it is initialised.



Expert Advice

Expert Advice enables a quality check to be performed on one or more completed measurement records. This will show how good the measurements are and whether they display any unwanted attributes, such as aggregation.

To enable the Expert System, choose **one or more** records, **right-click** on the **records** and select **Expert Advice**, or select **View-Expert Advice**. This will display the **Expert Advice** dialog indicating the quality of the measurement. Advice may be given about how to rectify a poor measurement.



Explanation

In the above example a Size measurement has been selected. The quantity of records selected is indicated in the measurement header line (i.e. **Size [11]**).

The quality of the records chosen is indicated by either a **green** circle - for a good measurement, or an **amber** triangle for a poor or unacceptable measurement. This is followed by a brief description of the measurement quality and **tips** for improvement.

The sentence shown in the measurement title lines above - **analyzed as a collection** - indicates that the records selected for the expert system to look have been analyzed as one. To see each measurement records advice individually, use the

10

Creating Custom Reports

Introduction

This chapter describes how to use the **Report Designer** to design custom reports. These can display parameters which are not on the default reports.

Note
The Malvern Instruments default reports described in **Chapter 5** will be sufficient for most users. These are identified with (M) appended to the name - e.g. Intensity PSD (M))

This chapter covers:

- An overview of screen and page layout views, what a report contains, and how to start the **Report Designer**.
- Opening an existing report.
- Creating a new report.
- Adding elements to the report.
- Setting up the report elements.
- Selecting elements.
- Aligning and sizing elements.
- Saving a report.
- Viewing the new report.
- Other information shown on a report.
- Protecting a report.

Overview

This section introduces the Report Designer.

Views

Two views must be created for the different aspect ratios of the printed page and computer screen:

-  **Screen Layout** – shows the computer screen version.
-  **Page Layout** – shows the printed version. This tailors the same contents to the dimensions of the printed page.

The two views do not have to be identical; for example, company logos can be added to the printed reports but not shown on the screen display. Both views are saved in the same **.pag** file.

To change either layout, right-click on the screen and select **Properties**. For the page layout this allows changes to paper size, portrait/landscape orientation and margins. For the screen layout the screen size can be changed.

Report contents

The report can contain one or more of any of the following elements:

- **Text** – user-defined text.
- **Picture** – graphics files can be added to Customize the report.
- **Frame** – used to separate parts of the report- inserts a border around an area.
- **Parameter** – any parameter stored in a record can be added to the report.
- **Calculation** – custom calculations using system parameters. These can be used to provide pass/fail decisions.
- **Well-plate** – a graphical representation of the Zetasizer APS well plate.
- **Graph** – data in a selected record can be added to the report in the form of a trend graph or distribution graph.
- **Table** – a table summarising a required parameter.

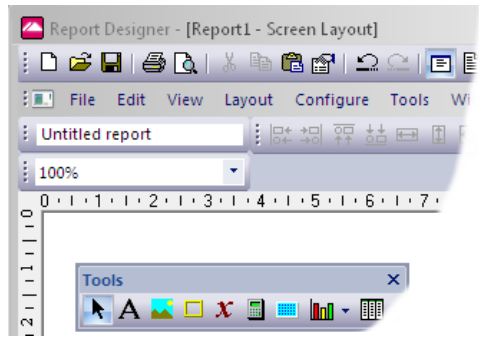
These are added using a **Control Palette** toolbar.

Note
During creation of a report data from the current record(s) is used. If no record is selected dummy data is used.

Getting started

To open the **Report Designer** select **Tools-Report Designer**. The **Report Designer** opens in a separate window as shown below.

In this example the **Control palette** has been dragged from the toolbar. This tool can be used in either position, depending on your preference.



The **Edit** menu commands are of general use:

- **Edit**, **Copy** and **Paste** – allow copying and pasting of text and graphics from other programs. The toolbar buttons have the same function.
- **Undo** ( ) and **Redo** ( ) – for undoing or repeating commands, respectively.

Opening an existing report

To open an existing report use the **Report Designer**'s menu command **File-Open...**. Report files have the extension **.pag**.

Creating a new report

A new report can be created in two ways:

- Selecting **File-New** from a blank page and adding the report contents.
- Opening an existing report, editing it, then saving it with a new name.

For both methods the procedure for designing a report and customising its content is the same.

Adding elements to the report

This section shows how to add elements to a report, select any of these elements and move or align them on the screen. Add all the required elements to the report then move them to the correct positions before going on to the next stage.

The **Control palette** is used to design reports:

The palette can be moved anywhere on the screen. It can be switched on and off using the **View-Control Palette** command on the **Report Designer** menu.

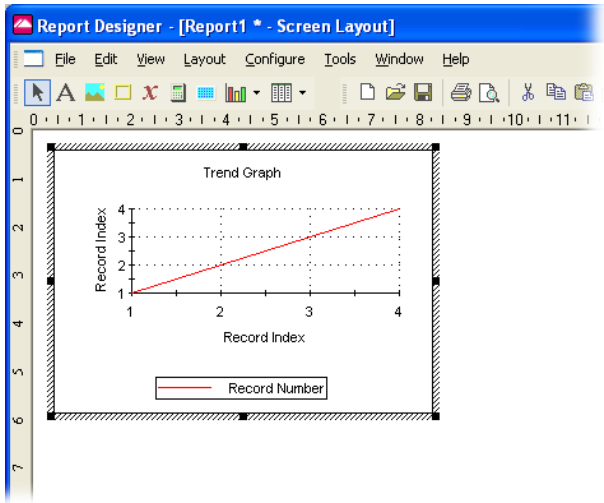
Use the following buttons on the palette to add the listed elements to a report:

Button	Use this to add:
	Pointer - select elements.
	Text.
	Picture.
	Frame – used to split the report into sections.
	Parameter.
	Calculation.
	Well-plate.
	Graph – use the list button to select a Trend graph, or a Distribution graph.
	Table – use the list button to select a Trend table, or a Distribution table.

To add an element:

1. Click one of the above buttons.
2. Move the cursor away from the palette. When the cursor changes to a "+" shape, hold the left mouse button down and drag the cursor to draw a rectangle on the report. This is called a **marquee**.

3. When the mouse button is released, the marquee receives the selected element. This will be marked initially by a hatched border like those shown below. This shows that the element is currently selected:



4. Use the cursor to drag the selected element or the black resize handles around its border to resize the element if necessary.

5. Right-click on the element and use the menu this displays to configure its properties, as described below.

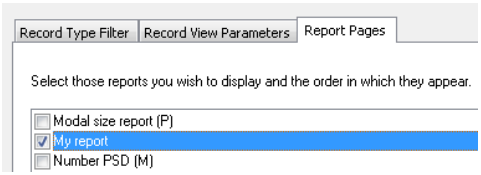
Setting up the report elements

Set the properties of any added element by selecting it and double-clicking to open the **Properties** dialog box. The on-line help in these dialogs details how to use each one. The sections below show the properties each type of element has.

Note
This dialog can also be opened using **Edit-Properties**, by right-clicking and selecting **Properties**, or by typing **Alt-enter** on the keyboard.

Report name 

In the **Report name** toolbar, enter a name for your report. This identifies the report in the **Report Pages** tab of the Workspace configuration window, allowing it to be selected for display in the workspace.



Text 

Use the **Text** tool to add general text such as a report name to the report.

With the element selected, use its **Properties** dialog to edit the text and its style (including its alignment), colour and size. Edit text or type new text using the **Caption:** box on the **General** tab.

Picture 

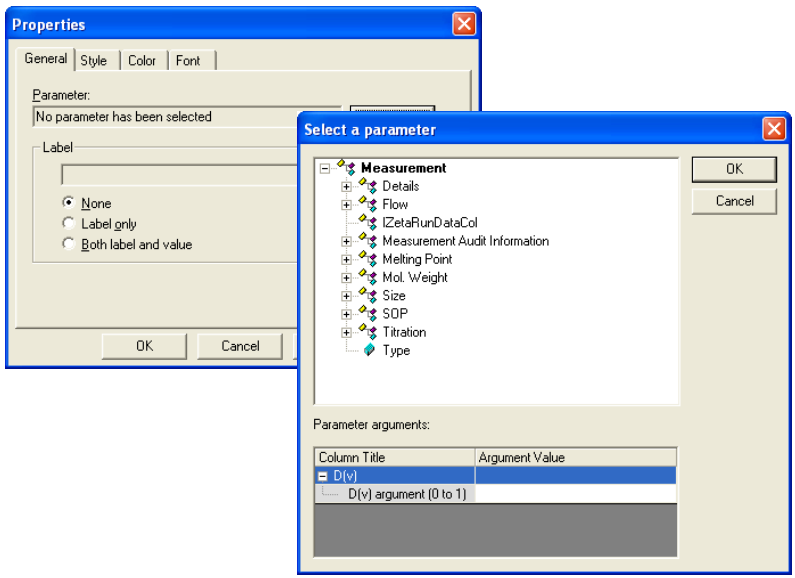
The **Picture** tool is generally used for company logos, etc. All the main graphics file formats are supported.

With the element selected, use its **Properties** dialog to browse for the picture to insert. Use the **Keep Aspect ratio** check box if required to maintain the aspect ratio of the original image. Use the **Link to file** check box to link to rather than embed the file; this means that when the file is updated the contents of the report change too.

Parameter

Use the **Parameter** tool to display measurement variables such as **Dv90**, **SOP Name**, etc.

The parameter **Properties** window has the same tabs as the text dialog, plus a **General** tab to specify what the parameter is, as shown below:



On the **General** tab click the **Select** button. In the **Select a parameter** dialog select the appropriate parameter (see below) and click **OK**.

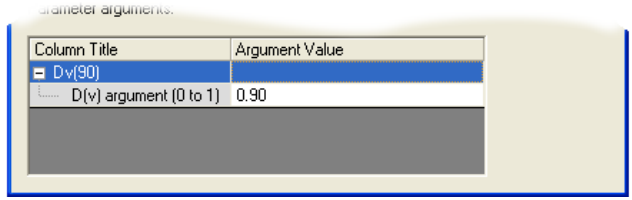
Use the **Label** selection area to add a parameter label alongside the parameter value. The style, colour and font of the label and value can also be defined here.

Parameters with arguments

Certain parameters allow specific values (or arguments) to be added.

A Dv(90) percentile can be viewed by double-clicking the cursor on **Measurement-Size-D(v)** and then typing 0.90 as the **Argument value**.

Parameter titles can be altered by double-clicking on them e.g. double-click on D(v) and alter to Dv(90) to reflect the particular percentile size viewed.



The **Label** selection area enables the parameter name to be added alongside the parameter value.

Frame

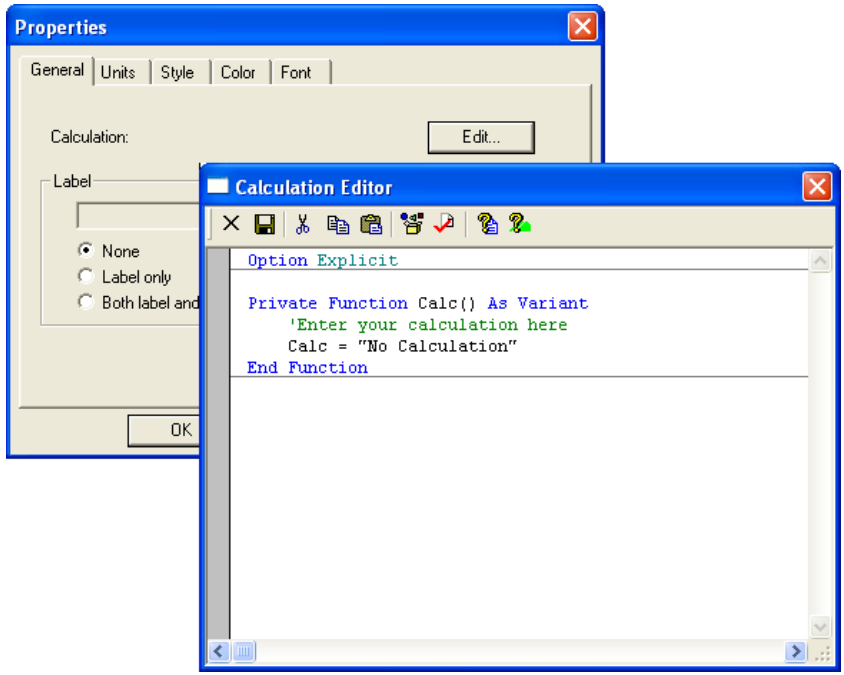
Use the **Frame** tool to split the report into relevant sections.
With the element selected, use its **Properties** dialog to select the frame style – etched, raised, sunken, etc. – or change its colour.
Horizontal and vertical lines can be inserted into the report to divide sections.

To stop a frame overlaying other elements, right-click on it and select the **Send to back** option.

Calculation

In addition to the defined parameters described in the parameters list, custom calculations can be entered. This is useful for displaying "Pass" and "Fail" criteria for measurement parameters. New "derived" parameters can also be created. With the newly created element selected, use its **Properties** dialog to configure the calculation.

The **Properties** dialog is similar to that for a text element, but with the addition of a **General** calculation tab that enables editing of the calculation. Press the **Edit** button to open the **Calculation Editor**.



Calculation Editor

Use the **Calculation Editor** to load an existing calculation for editing or to create a new calculation. The language used, Sax Basic, is similar to Microsoft Visual Basic for Applications (VBA).

Select 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.

More information is available from the **Calculation Editor** help buttons:

Button	Function
	Describes the Sax Basic language.
	Describes how to use the **Calculation editor**.

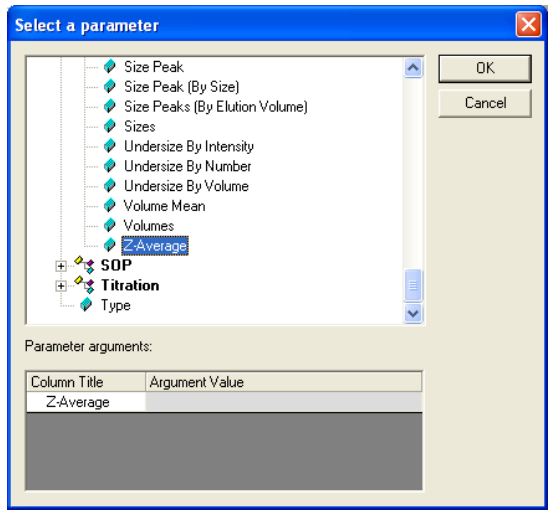
Well-plate

The **Well-plate** provides a graphical representation of the Zetasizer APS well plate on a report. It can be thought of as a reporting version of the Plate Navigator.

Click the **Well-plate mimic** button and then drag out an area on the report to display the well plate image. The system automatically displays the correct well plate (96 or 384 well) on the actual report despite showing a 384 plate well image in Report Designer. Double click on the image of the plate to set show the **Properties** dialog.

Setting Parameters

1. Select the **Parameter** tab and click **Select...**
2. Choose the required parameter, for example Z-Average from the **Size** group, and then click **OK**.



3. Ensure that **Show values in wells** is selected if numeric data is required on the wells.

Setting Colours

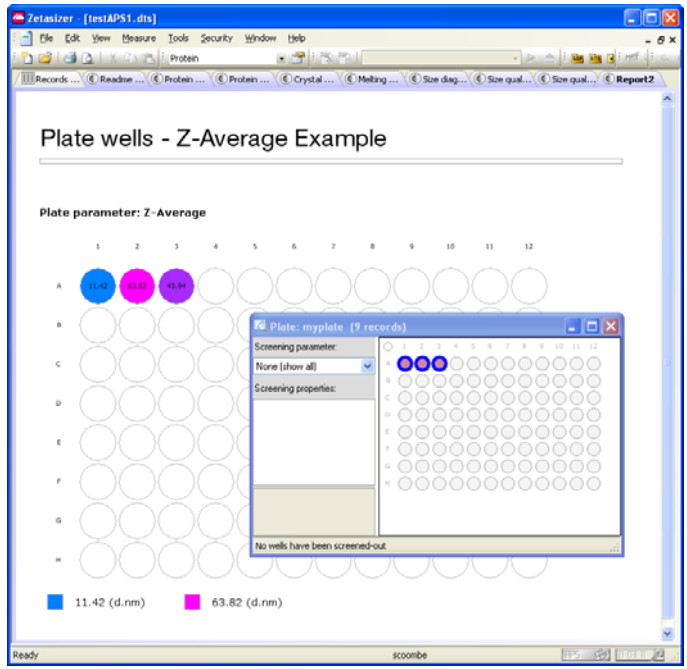
Choose the **Color** tab to set the high and low value colours - these are shown automatically on plate as legend indicators.

Using a report with a well plate display

When the report has been saved, it can be added to the workspace view (see **page 18** for more details). The report shows only those measurements that are currently selected - if no results are selected, the wells will be empty.

An easy way to navigate data that has been amassed through the measurement of a plate is to use the **Plate Navigator**, rather than the Records View. To do this, open the Plate Navigator at the same time as displaying the report containing the plate well. Then set screening parameters and range limits as required in the Plate Navigator (**refer to Chapter 5** for full information). Finally, select the wells needed on the report in the Plate Navigator.

In the example below, all three results have been selected in the Plate Navigator, and consequently they are displayed in the on-screen report.



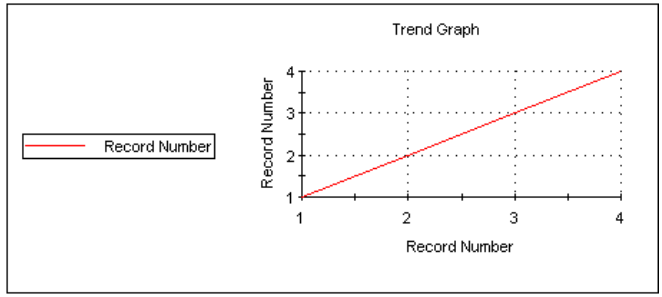
Graphs

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**, **Titration, Size** and **Molecular weight** with accompanying graph choices.

Trend graphs

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** dialog.

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.

Aggregation point
Displays the temperature at which the aggregation point is achieved.

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.

Graph properties dialog - general

This dialog 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** dialog.

  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).

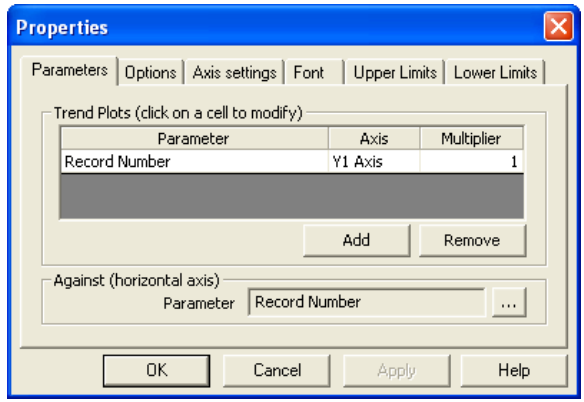
- **Axis settings** – Allows both the X and Y axis settings to be defined. Whether **logarithmic** or **linear** axis are required and the axis scales - defined or auto-scaling. **Graticule** (or grid) lines can also be shown on the graphs.

- **Font** – 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

For a **Trend** graph the **Properties** dialog has a **Properties** tab for selecting the parameters to view:



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 to select 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** dialog.

Use the **Against** area to select the X axis parameter.

Line styles

Select **Configure-Line styles** to display a line styles dialog. 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 use the **Symbols** tab to alter the symbols used for data points 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, Titration, Size** and **Molecular weight** 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** dialog.

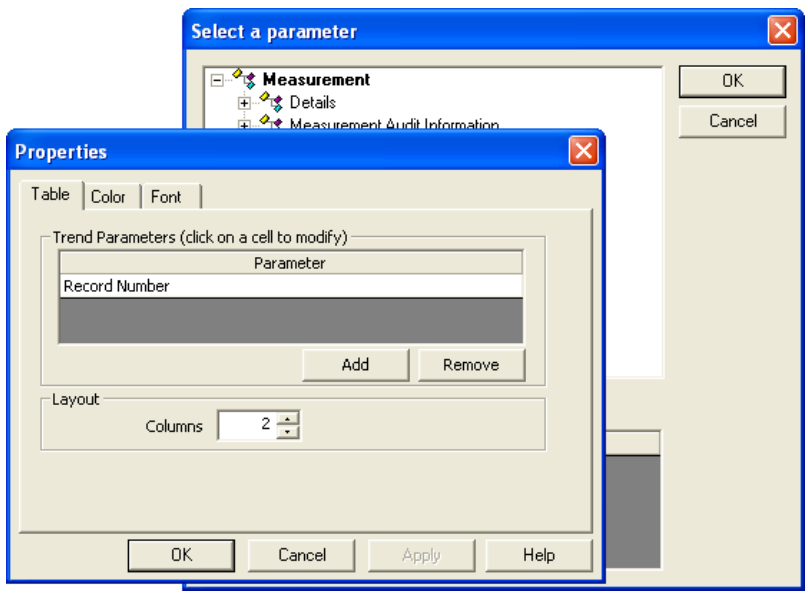
Molecular weight tables

The following Molecular weight table can inserted.

Debye
Shows the data used to generate a Debye plot in a tabular format.

Table properties dialog

This dialog is the same as that for the **text** dialog, plus a **Table** tab that defines the type of table displayed and how many columns to use. The **Trend** table properties dialog 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 dialog** 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.

Selecting elements

When selecting elements:

- To select one element, click on it.
- To select multiple elements, hold down the **Shift** or **Ctrl** key and click the mouse button on each element in turn. Alternatively, hold the left mouse button down and drag a box (**marquee**) over a group of elements.
- If one element is overlaid by another and cannot be selected, select any element and use the **Tab** key to step through all the elements one by one until the correct element is selected.

Aligning and sizing elements

Select two or more elements and use the following options of the **Layout** toolbar or menu to align/size them:

Button	Function	Button	Function
	**Layout-Align-Align left**		**Layout-Make Same Width**
	**Layout-Align-Align right**		**Layout-Make Same Height**
	**Layout-Align-Align top**		**Layout-Make Same Size**
	**Layout-Align-Align bottom**		

The **last** element selected, highlighted with **eight dark squares**, provides the reference position for alignment. The **Layout** menu also has commands to:

- **Space Evenly** several elements and **Centre** an element.
- **Send to front** or **Send to back** the selected element (when two or more elements are overlaid).

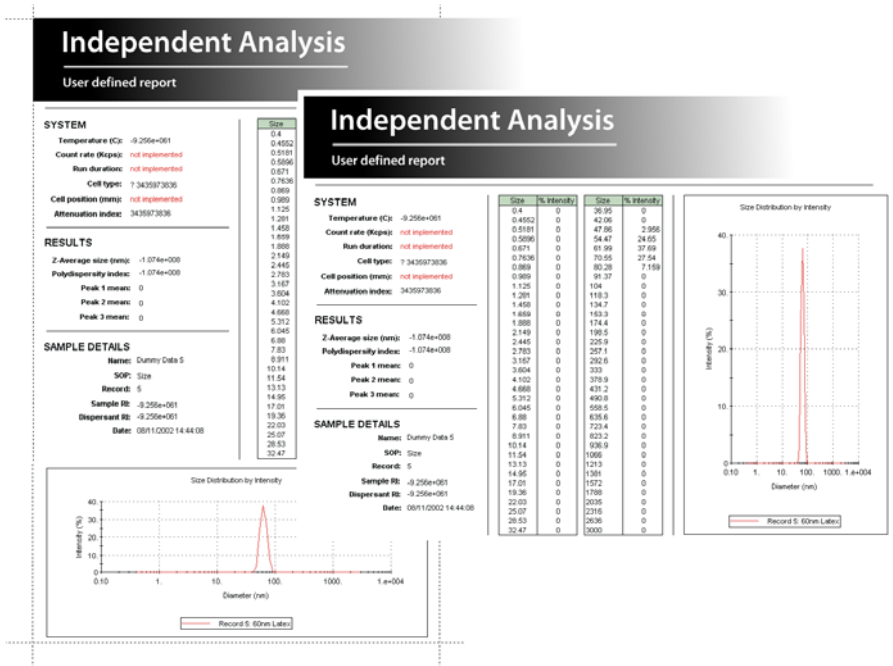
Note
Right-clicking on a selected element also brings up the layout options.

Saving a report

To save a report select **File-Save...** and save it in the **Report Pages** directory. It must be in this location if it is to be available for inclusion in a workspace. Reports are saved with the default file extension **.pag**.

If the report was designed solely in **Screen Layout** view, a prompt appears, advising that the **Print Layout** version is blank. In **Screen Layout** view select **Edit-Select All** and then **Edit-Copy**. Use **View-Page Layout** and then **Edit-Paste** to copy the content into the printed report page. If the printed layout is to be different from the **Screen Layout** version, make the changes here. All that is then required is some rearranging and formatting to line up all the elements.

A finished report



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Using the tools described above a custom report can be created. For example a printed **Page layout** is shown above 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 **..\Zetasizer\pages** directory.

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; Some rearranging and formatting to line up all the elements may then be required.

Viewing the new report

To view a newly saved report in the main Zetasizer APS window:

1. Select **Tools-Settings-Configure Workspace** and select the workspace to associate the new report with.
2. In the **Report Pages** tab select the check box for the newly created report. Click **OK**.
3. When the appropriate workspace is selected, the report will be shown as a tab in the measurement file window. Click the tab to see the report.

Note
If a report that is currently displayed by the main application is edited, saving the edited report immediately refreshes the view in the main window. Any changes made are shown immediately.

Other information shown on a report

The printed copy of a report shows the software version number and instrument serial number at the bottom of the page. This is important information if a call is made to the Malvern Instruments help desk. The name of the measurement file the report is based on is also shown.

Protecting a report

A report can be locked with a password to prevent unauthorised changes.

To password-protect a report:

1. Open the report in the **Report Designer** and select **Tools-Protection-Protect report...**
2. A dialog appears asking for a password to be entered and confirmed. Write the password down in a safe place as it will not be possible to edit the report without supplying it.
3. Click **OK**.

Calculators

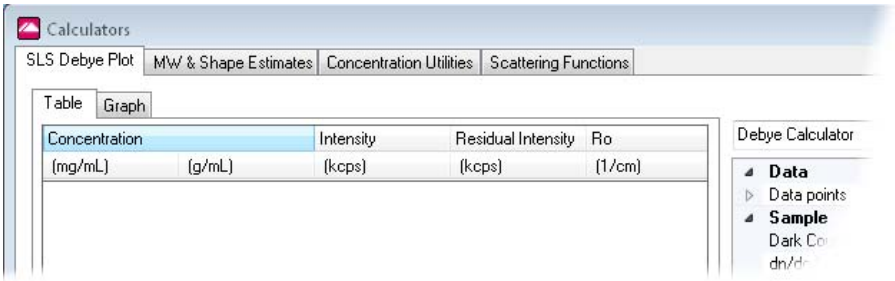
Introduction

By measuring the sample scattering intensity over a range of concentrations and entering the necessary sample parameters, the Molecular weight can be determined.

If the hydrodynamic diameter is also measured from one of these concentrations, the molecule shape or conformation can also be estimated.

The **Calculators** feature enables the calculation of the Molecular weight and also provides other calculation tools.

To access this feature select **Tools-Calculators**. The following tab selections are available:



- **SLS Debye Plot**. Enables a **Debye plot** to be constructed from freely available information, or from a record generated from actual data.
- **Molecular Weight Calculations**. A 'what if' calculation can be performed. If any two parameters from the **Molecular weight**, hydrodynamic size and conformation (**shape**) are known, then the third - either a Shape, Hydrodynamic diameter or Molecular weight parameter - can be estimated.
- **Concentration Utilities**. This dialog contains features to establish the concentration and scattering levels that may be observed.

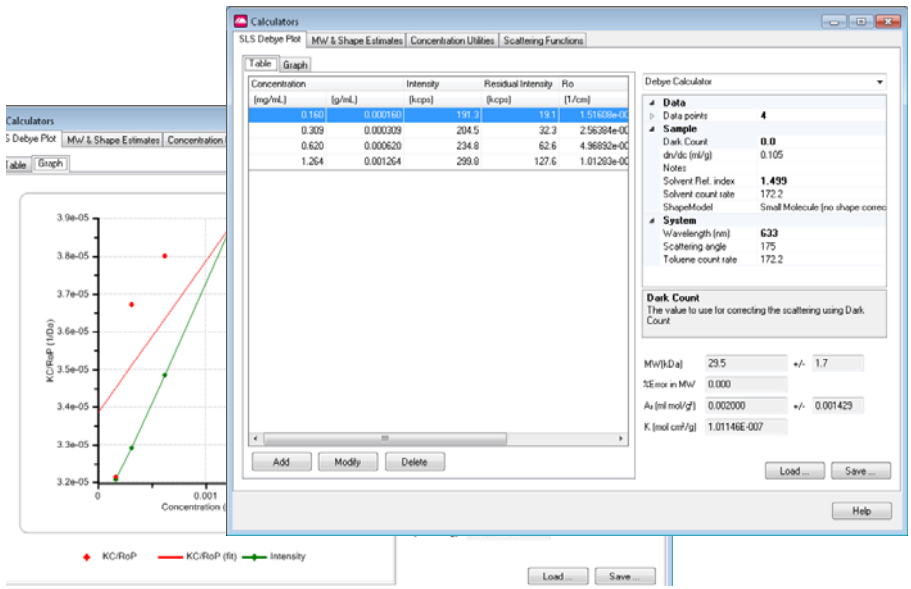
- **Scattering Functions**. A plot can be generated from entering the measurement data.

In each case the effect of changing any of the input parameters can be seen instantly in all of the derived parameters.

In this way it is possible to see directly the sensitivity of all the parameters required for the calculation on the result. This in turn will determine the accuracy of the instrumentation required to measure each parameter, for example the sample refractive index required to calculate the dn/dc value.

SLS Debye plot

The Calculators feature includes the ability to generate a Debye plot using inputted rather than measured data.



This feature can be useful for various reasons, for example:

- By combining individual measurements one single Debye plot can be generated
- A Debye plot can be created by entering the concentration points from an existing measurement, then adding additional concentration points.
- Any of the parameters in the dialog box can be changed; the other parameters will be instantly recalculated. This can be used to investigate the sensitivity of the result to changes in any parameter.

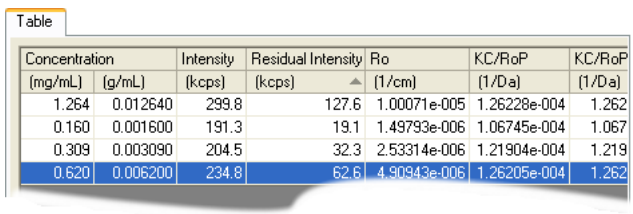
For example, by first entering the concentration points from an existing measurement, a sample parameter - e.g. sample temperature - can be altered and the effect immediately observed on the SLS Debye plot. This saves time in performing the original measurement again at the different temperature.

The format of the plot can be altered in the same way as the graphs in the main application, by moving the cursor over the graph and right clicking the mouse. The **Graph properties** dialog will appear; refer to the **Size measurements - Graph** section in **Chapter 5** for details in altering the attributes.

Adding/editing sample parameters & table data

To generate a Debye plot, the sample parameters and table concentrations have to be entered.

To access the **Debye plot** select **Tools-Calculators** and then the **SLS Debye plot** tab. Select the **Table** tab to begin entering data into the table.

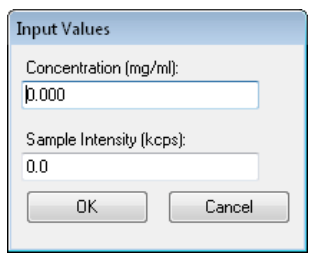


Adding data to, and editing the table

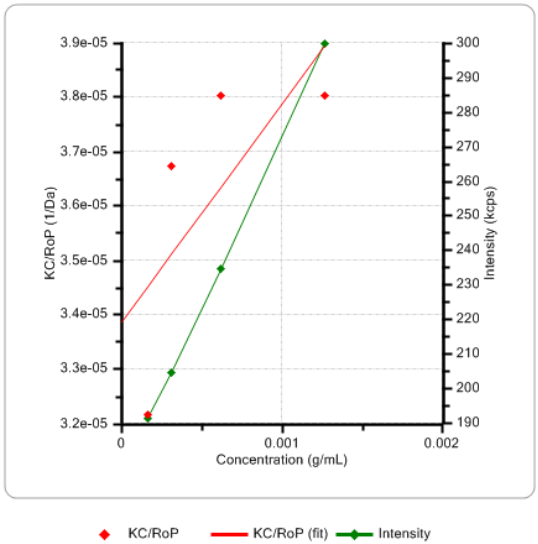
To access the **SLS Debye plot** select **Tools-Calculators** and then the **SLS Debye plot** tab. Select the **Table** tab to begin entering data into the table.

1. To **define a new** concentration, press **Add...** to display the **Input valueswindow**.

   Specify the **Concentration** and **Sample intensity** values - either new values or those taken from an existing measurement.



2. To **modify** a concentration, select it from the list and press the **Modify...** button. The **Input values** dialog will appear, allowing the parameters to be changed.
3. A concentration can be **deleted** by selecting the concentration from the list and pressing the **Delete...** button.
4. Select the **Graph** tab to see the resultant **Debye plot**.



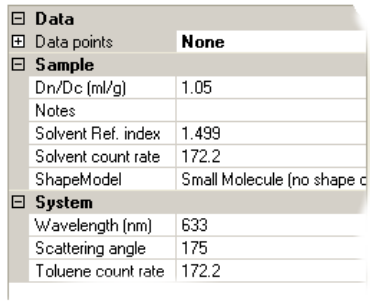
5. The table values and graph plot can be subsequently altered by changing the **Sample**, **Data** and **System** parameters in the measurement parameters table on the right of the dialog. These parameters are described in the following section.

Measurement parameters table

Once all the concentration values have been added into the table, the measurement parameters table can be used to alter the result and Debye plot.

To view and alter each parameter setting, click on the plus ⊞ sign next to each parameter group to open the list (Click on the minus ⊟ symbol to close the list).

Default parameters are in “normal” type, altered parameters will be made **bold**.



The parameters are described below.

Data

This indicates the data entries that have been entered into the debye plot table, see below.

Sample

Sample dn/dc (ml/g)

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

Experiment notes

Used to record specific details about the experiment or calculation performed.

Hydrodynamic radius (nm)

The radius as measured using dynamic light scattering.

Solvent Ref. Index (Refractive index)

The refractive index of the solvent used.

Solvent count rate (Kcounts)

The count rate used to calculate the Rayleigh ratio (R_θ) is the 'residual' count rate, which is derived by subtracting the solvent count rate from the sample count rate.

Shape Model

The shape model that is used to estimate the radius of gyration from the hydrodynamic radius, and therefore calculate the angle dependent effects on KC/RoP for particles of sizes outside the Rayleigh region (Diameter > ~50nm).

System

Wavelength (nm)

The wavelength of the laser used. Zetasizer APS instruments use an 830nm wavelength.

Scattering angle (degrees)

The angle at which the measurement will be performed. The Zetasizer APS uses a 90° optics arrangement.

Toluene count rate (Kcounts)
The scattering count rate of the toluene reference.

Saving the Debye plot

The parameters and data inputted to produce the plot can be saved by pressing the **Save** button, and then reviewed at a later stage by pressing the **Load** button.

Copying the Debye plot

The graph can be pasted into another application (such as Microsoft Word or Excel) by selecting the **Copy** button.

Results area

With both the table data and sample parameters entered the results will be automatically calculated and shown alongside the graph. The results displayed are:

MW (kDa) - Molecular weight
Shows the measured weight of a molecule within the sample expressed in atomic mass units; indicated in kiloDaltons. It is calculated from the intercept of the KC/RoP vs concentration Debye plot.

+/-
The expected error in the Molecular weight, derived from the scatter in the data about the least squares best fit line.

% Error in MW
The error in the calculated Molecular weight arising from the use of only a single angle. For isotropic scattering particles (diameter < ~50nm), this error should be negligible.

A_2 (ml mol/g^2) - Second virial coefficient
A property describing the interaction strength between the molecule and the solvent. This is calculated from the slope of the plot

K (Mol cm^2/g)
The instrument optical constant, as defined in **Chapter 12**.

Molecular weight calculations

The hydrodynamic size measured by Dynamic Light Scattering (DLS) is defined as "the size of a hypothetical hard sphere that diffuses in the same fashion as that of the particle being measured". In practice though, macromolecules in solution are non-spherical, dynamic (tumbling), and solvated. Because of this, the diameter calculated from the diffusional properties of the particle will be indicative of the apparent size of the dynamic hydrated/solvated particle. Hence the terminology, **'Hydrodynamic' diameter**.

If the **Molecular weight** (or mass) and the partial **Specific volume** (inverse density) for the particle being measured are known, then a mass equivalent spherical size can be calculated. The closer the particle is to being spherical, the closer the mass equivalent spherical diameter will be to the DLS measured hydrodynamic diameter. In fact, it is the difference in these two values, coupled with **Perrin** theory (below), that allows **particle shape** information to be extracted from DLS measurements.

Once the **Molecular weight** - either measured or estimated - and the **specific volume** are known, the **particle shape** information can be estimated by using the **Calculators** tool. The Shape estimate calculator takes the entered data and then applies two equations - the **Stokes-Einstein** and the **Perrin** factor.

Stokes-Einstein equation

The measured data in a DLS experiment is the correlation curve. Embodied within this curve is all of the information regarding the diffusion of particles within the sample that has been measured.

By fitting the correlation curve to an exponential function, the diffusion coefficient (D) can be calculated (D is proportional to the lifetime of the exponential decay).

With the diffusion coefficient (D) now known, the **Hydrodynamic diameter** can be calculated by using a variation of the Stokes-Einstein equation.

The **Stokes-Einstein** equation for the Hydrodynamic diameter is :

$$D_H = \frac{kT}{f} = \frac{kT}{3\pi\eta D}$$

- D_H : Hydrodynamic diameter.
- k : Boltzmann constant.
- f : Particle frictional coefficient.
- η : Solvent viscosity.
- T : Absolute temperature.
- D : Diffusion coefficient.

The Stokes-Einstein equation was developed using the assumption of hypothetical hard spheres.

Perrin factor

For non-spherical particles, the **Perrin** or shape factor (*F*) can be used to estimate particle shape.

The Perrin factor is used to calculate the prolate and oblate axial ratios for ellipsoids with the same Perrin factor value.

The Perrin factor is defined as the ratio of the frictional coefficient for a sphere with the same volume as the particle being measured, to the frictional coefficient for a sphere with the same mass as the particle being measured.

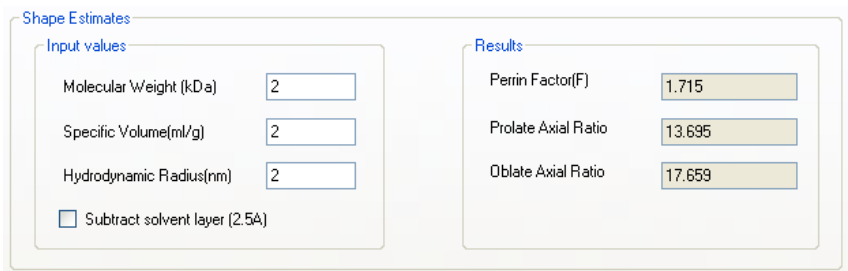
The **Perrin** factor (*F*) is : $$F = \frac{f_{Vol}}{f_{Mass}} = \frac{6\pi\eta D_{Vol}}{6\pi\eta D_{Mass}} = \frac{D_{Vol}}{D_{Mass}} = \frac{D_H}{D_{Mass}}$$

- D_H : Hydrodynamic diameter. The diameter as measured via DLS.
- D_{Mass} : The diameter by mass. This is calculated from the known Molecular weight and the specific volume of the particle.
- f : Particle frictional coefficient.
- η : Solvent viscosity.
- T : Absolute temperature.

Shape estimate calculation

Enter the **Molecular Weight** result, **Specific Volume** and **Hydrodynamic Radius** (measured using dynamic light scattering) into the appropriate text boxes.

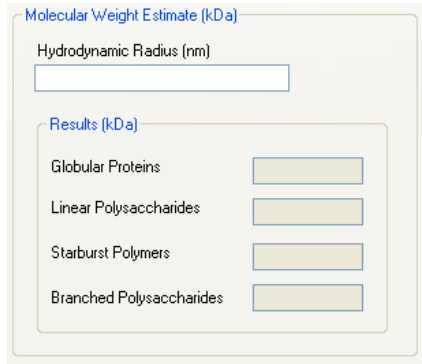
The Perrin (shape) factor (*F*), plus the Prolate and Oblate axial ratio will be automatically calculated and displayed in the results area.



If required, a solvent layer can be subtracted from the hydrodynamic radius when calculating the Perrin factor. To do this select the **Subtract solvent layer** check box.

Molecular weight estimate

Whilst the preferred method of measuring absolute Molecular weight is by performing concentration dependent light scattering measurements, this can be very time consuming, from the point of view of the sample preparation. Providing that only an estimate of the Molecular weight is required then it can by derived by utilising a relationship between the Hydrodynamic radius and the molecule conformation.

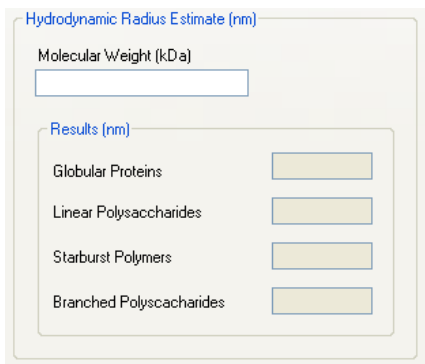


To find the Molecular weight estimate, enter the measured **Hydrodynamic radius** value into the text box and the estimated Molecular weight will automatically be calculated. The Molecular weight is displayed in four ways that are representative of the Molecular weight families as follows:

Globular Proteins	Linear polysaccharides	Branched poly-saccharides	Starburst polymers

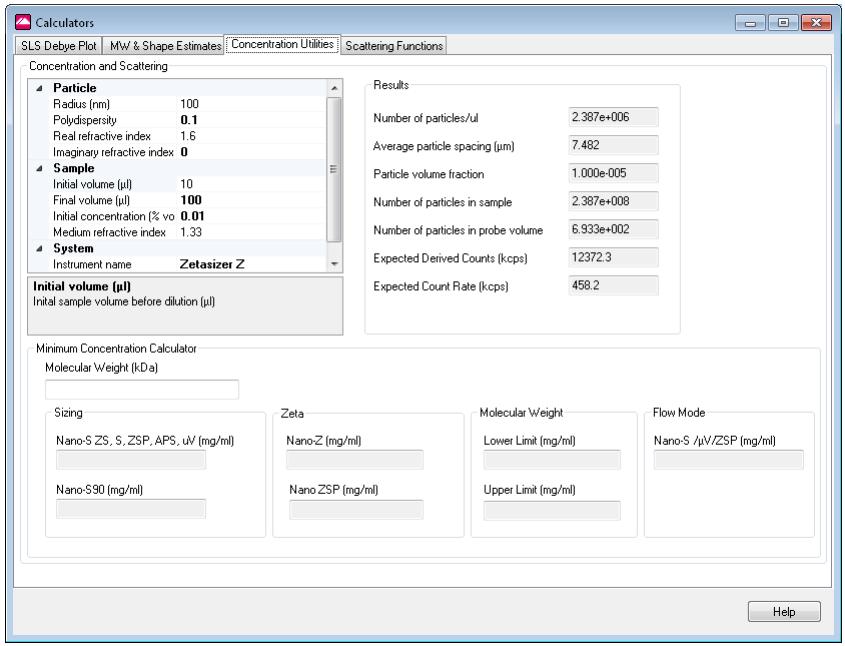
Hydrodynamic radius estimate

This works in the same way as above, except the **Molecular weight** (in KDaltons) is entered to find the Hydrodynamic radius instead.



Concentration utilities

Select the **Concentration Utilities** tab to view concentration and scattering parameters.



Concentration and Scattering

This area of the dialog contains features to establish the concentration and scattering levels that may be observed from the sample.

Enter the values from the measurement into the table. On entering each value, press the return key afterwards and the results table will be updated.

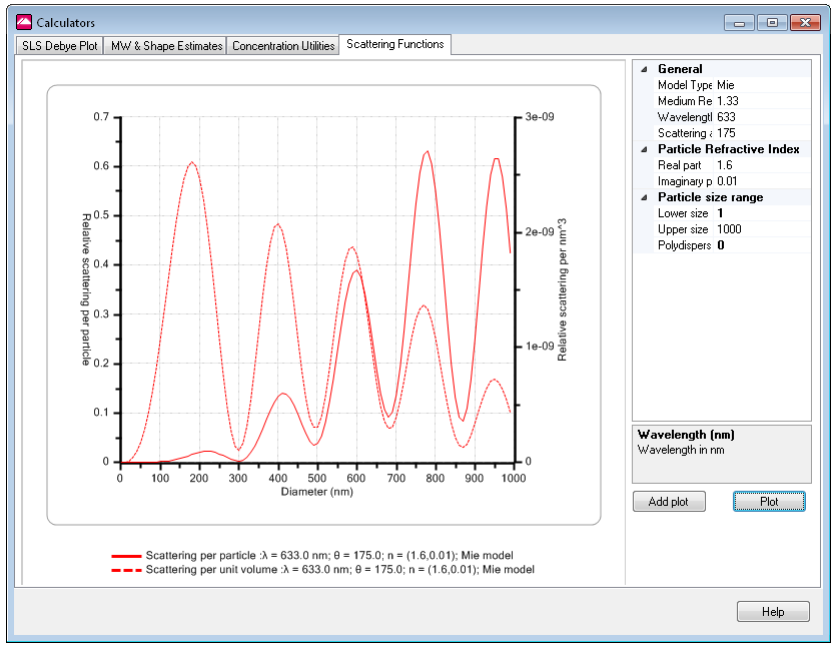
The table can be displayed in a categorised view or alphabetically .

Minimum Concentration Calculator

By entering only the Molecular weight the Sample concentration (mg/ml) values required for performing a measurement can be calculated - Zetasizer Nano instruments only.

Scattering functions

A scattering function plot can be generated by inputting the measurement data in the list on the right, in this dialog:



Enter the values from the measurement into the table and press **Plot** - the graph will update to show the values entered.

To see the result of changing a value, change the required value and press **Add plot** - a new plot line will be added to the graph.

Size theory

Introduction

The aim of this chapter is to describe the basic **Size** principles behind the Zetasizer APS. 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 APS - 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?

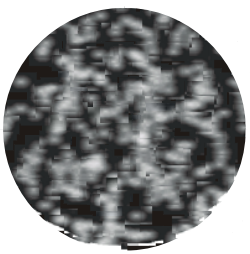
The Zetasizer APS series performs size measurements using a process called **D**ynamic **L**ight **S**cattering (**DLS**).

Dynamic Light Scattering (also known as **PCS - P**hoton **C**orrelation **S**pectroscopy) 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 alongside.



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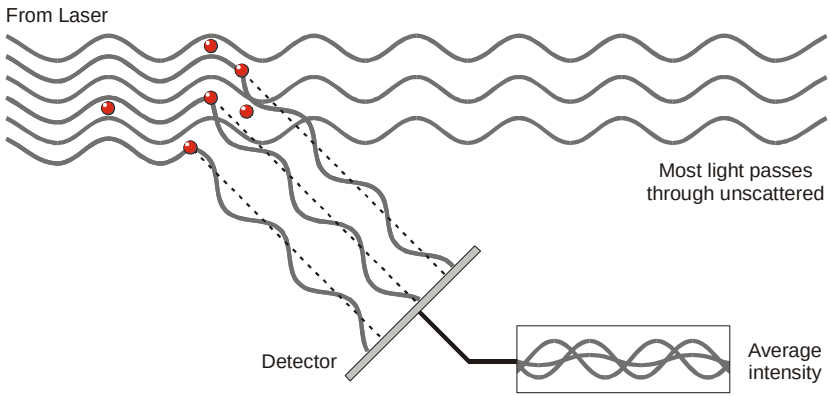
The speckle pattern will consist of areas of bright light and dark areas where no light is detected.

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 arrives 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.

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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 APS 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.

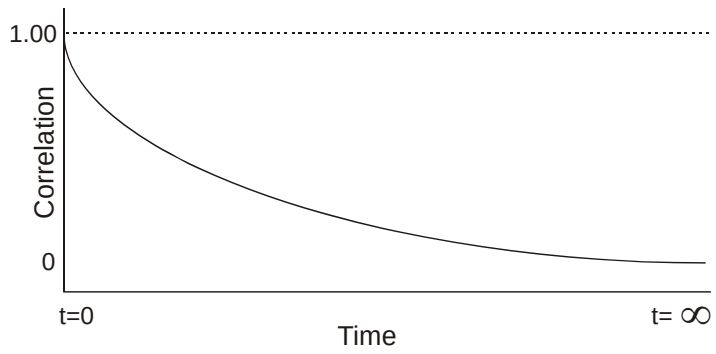
If we compared the intensity signal of a particular part of the speckle pattern at one point in time (say time = t) to the intensity signal a very short time later ($t+\delta t$) we would see that the two signals are very similar - or strongly correlated. If we then compared the original signal a little 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 ten'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 against time is shown below.



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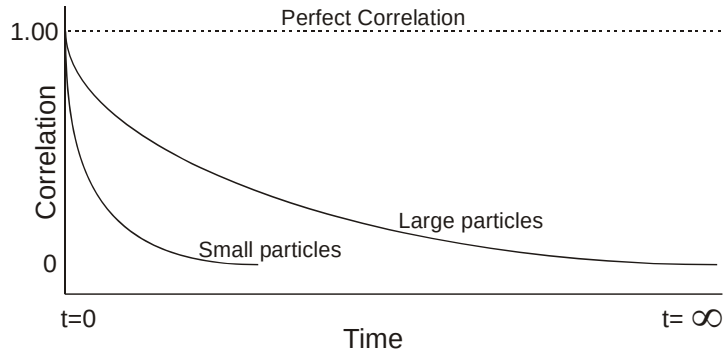
Using the correlation function

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 the 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.



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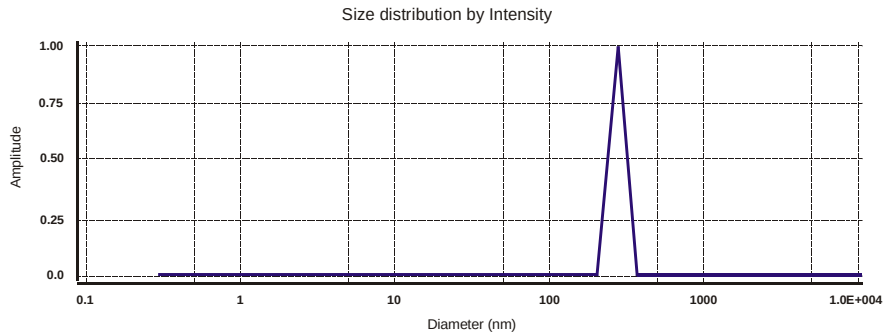
After the correlation function has been measured this information can then be used to calculate the size distribution. The Zetasizer software uses algorithms to extract the decay rates for a number of size classes to produce a size distribution.

A typical size distribution graph is shown below.



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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.

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

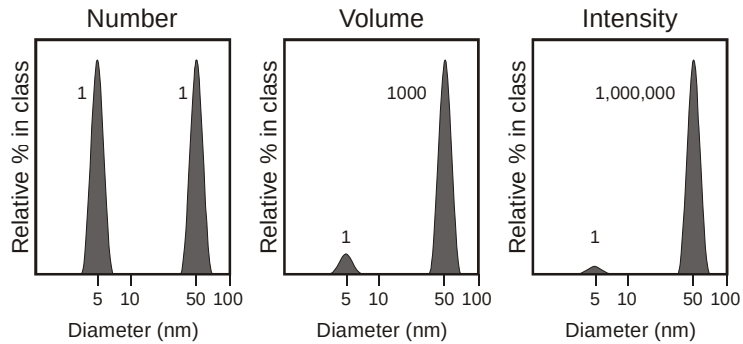
What is the difference between intensity, volume and number distributions?

A very simple way of describing the difference 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 shows the result as a 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 $4/3\pi(r)^3$).

The third graph shows the result as an intensity distribution. The area of the peak for the 50nm particles is now 1,000,000 times larger than 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).



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It is worth repeating that the basic distribution obtained from a DLS measurement is intensity - all other distributions are generated from this.

- **Z-average Size** (also known as the "cumulants mean")
  In **D**ynamic **L**ight **S**cattering (**DLS**) 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 solvent.

In any other case, the Z-average size can only be used to compare results with samples measured in the same solvent, by the same technique.

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 G_1 correlation function.

$$Ln[G_1] = a + bt + ct^2 + dt^3 + et^4 + \ldots\ldots$$

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 solvent 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).

- **PdI**

  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 documents 13321:1996 E and 22412.

- **Intercept**

  This is the amplitude of the G_1 correlation function at time 0. For a good measurement it will be 0.85 to 0.95.

  Lower values will be obtained for measurements on weaker scattering sample, or in turbid sample where background scattering is significant. Values >1 will only occur for samples with dust that makes the sample fluctuate wildly.

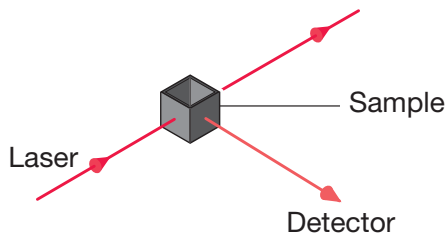
- **Peak means**

  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.

Zetasizer APS operating principles

The Zetasizer APS comprises six main components. Firstly a **laser** provides a light source to illuminate the **sample** particles within a **cell**. Most of the beam passes straight through the sample, but some is scattered by the particles within the sample. A **detector** positioned at 90° to the laser path measures the intensity of this scattered light.



To ensure the intensity of the scattered light is of a suitable level for the detector to function accurately an **attenuator** is used to reduce the laser intensity and hence 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 processor called a **correlator**. This compares the scattering intensity at successive time intervals to derive the rate at which the intensity is varying. This information is

then passed to a computer running the **Zetasizer software** which analyses the data and derives size information.

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