Malvern

- PARTICLE SIZE
- ZETA POTENTIAL
- MOLECULAR WEIGHT
- MOLECULAR SIZE

ZETASIZER **NANO SERIES**
USER MANUAL

Zetasizer Nano User Manual

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English

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Part 1 -
Instrument guide

1

Introduction to this manual

Introduction

This manual covers the operation of the **Zetasizer Nano** particle characterisation system. The instrument measures the size of particles contained within a sample, presenting data according to the user's needs.

This user manual is a companion to the **Zetasizer Nano Basic Guide**, which gives Health and Safety, maintenance, troubleshooting and other vital information which **all users must read**.

About this manual

The manual contains the general information required by an operator; as well as supervisor information that concentrates on administration and extension of the system's functionality.

Topics covered include:

- An overview of the Zetasizer Nano hardware and software.
- A guide to making measurements and how to read the results.
- Sample preparation guidance.
- Reporting - creating and editing reports.
- System security.
- Advanced features.

Additionally an **Theory** section which describes the basic measurement theories involved with the measurements.

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.

Access to the instrument

This manual refers to the various people who will have access to the instrument, as follows.

Malvern Instruments personnel

Malvern Instruments 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 instrument covers.

Warning!
Unless advised within the content of this manual, removal of the instrument covers by unauthorised personnel, even a supervisor, will invalidate the warranty of the instrument.

Supervisor

The supervisor is the person responsible for the management and safety of the instrument and its operation. The supervisor also trains the operators and can perform user maintenance routines.

Operator

An operator is a person trained in the use of the system. The operator can perform some user maintenance routines identified.

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

Assumed information

Naming convention

Within this manual:

- The Zetasizer Nano is referred to as “the optical unit” or “the instrument”.
- The accessories or sample dispersion units are referred to as “MPT-2 Titrator”, “the vacuum degasser”, or as “the accessory” or “the dispersion unit”.
- The combination of the optical unit, one or more accessories or dispersion units and the computer is referred to as “the system”.

Menu commands

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

Where to get help

This section describes the available sources of information on the system. The primary sources of information on the system are instrument and accessory manuals and the software’s help system. The manuals give an overview of the system as a whole, while the online help system gives more detailed information on the Zetasizer software. Some features are described in both the manuals and Help system.

Manuals

The following manuals are available for the Zetasizer Nano:

Basic Guide

A concise yet essential guide that provides an introduction to the system and its usage covering **Site requirements**, **Health and Safety** and an instrument and accessory overview. Finally **a brief guide to making a measurement and viewing the results** is included.

User Guide

The primary source of information about the Zetasizer Nano hardware and its use. See **About this manual** earlier in the chapter.

Accessory and dispersion unit guides

Separate guides are supplied with the various Nano accessories and Titrator dispersion units. These provide essential information on the connection, operation and maintenance of the dispersion units.

Help System

Each key software window has a **Help** button giving information specific to it. Additional **Info Tips** are also available whenever is shown - simply move the mouse pointer over this icon to see a relevant tip.

Technical support

Malvern Instruments offers several methods of technical support, which are described in this section.

Email support

Note
This is the primary support method – only use the other methods if this is not available at your location.

When contacting your local Malvern Instruments representative - details of all local telephone numbers are available at www.malvern.com. Always quote the following information:

- **Model and serial number of the instrument**. The serial number is shown on the Zetasizer software status bar - rollover the **Nano icon** to display.
- **The software version**. To find this select **Help-About** in the software - alternatively, move the mouse over the instrument status bar icon. The **Application Version** is the main firmware version number to note.

Additional support

Contact the International Malvern Helpdesk if the local Malvern Instruments representative is not available:

Telephone: +44 (0)1684 891800 or email: helpdesk@malvern.com.

Note
This help line is primarily English speaking.

Remote support

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

2

Introducing the Zetasizer Nano

Introduction

This chapter provides information about the Zetasizer Nano hardware and software, and identifies all major features of the system.

The following topics are covered:

- A description of the purpose of the instrument, its accessories and simple explanations of the primary measurement techniques
- Detailed reference on the physical parts of the optical unit.
- An overview of the primary software features.
- Information about the parts of the cell, including how to insert and remove it. The following topics are covered:

Note
Hardware maintenance - for details on how to maintain the hardware, please refer to the Basic Guide and the Accessory manuals.

References will be made throughout the manual to the MPT-2 Titrator and other accessories. Refer to the **MPT-2 Titrator and Degasser**, and the **Zetasizer Nano accessories** user manuals where necessary.

About the Zetasizer Nano

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

These three fundamental parameters are **particle size**, **zeta potential** and **molecular weight**. Unique technology within the Zetasizer system allows these parameters to be measured over a wide range of concentrations. Depending upon

the options and accessories used, the Zetasizer system also has the ability to perform:

- **Microrheology** measurements
- **Autotitration** measurements if an MPT-2 accessory is connected,
- **Trend** measurements, including the determination of the **Aggregation point**.

The Zetasizer range features pre-aligned optics and programmable measurement position for the measurement of size and zeta potential over a wide concentration range and precise temperature control necessary for reproducible, repeatable and accurate measurements. In addition other key parameters such as conductivity and with the MPT-2 Titrator, pH can be measured.

The Zetasizer Nano range has been designed so that a minimal amount of user interaction is necessary to achieve excellent results. The use of **S**tandard **O**perating **P**rocedures (SOPs) and features such as the **Folded capillary cell** alleviate the need for constant attention.

The Zetasizer Nano range

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

Zetasizer Nano instrument	Model number	Measurement types
Nano S (Red badge)	**ZEN1600**	Particle size and Molecular weight
Nano S (Green badge)	**ZEN1500**	Particle size and Molecular weight
Nano Z (Red badge)	**ZEN2600**	Zeta potential
Nano ZS (Red badge)	**ZEN3600**	Particle size, Molecular weight and Zeta potential
Nano ZS (Green badge)	**ZEN3500**	Particle size, Molecular weight and Zeta potential
Nano S90 (Red badge)	**ZEN1690**	Particle size - 90° optics
Nano ZS90 (Red badge)	**ZEN3690**	Particle size and Zeta potential - 90° optics
Nano ZSP (Red badge)	**ZEN5600**	Particle size, Molecular weight and Zeta potential, Microrheology

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

Zetasizer Nano range options

The Zetasizer Nano series of particle analysers consists of a range of different instrument models fitted with a choice of 'red' or 'green' lasers. The models and their measurement specifications are described in the table below, with instrument options following.

Zetasizer Nano	Size range maximum (diameter)	Size range for Zeta potential (diameter)	Molecular weight range (Daltons)	Accessory option (see below)
S	0.3nm to 10µm	–	342Da to $2x10^7$Da	ⒶⒹ ⒺⒻ
Z	–	3.8nm to 100µm	–	ⒶⒷⒸ ⒹⒺⒼ
ZS	0.3nm to 10µm	3.8nm to 100µm	342Da to $2x10^7$Da	ⒶⒷⒸⒹ ⒺⒻⒼ
S90	0.3nm to 5µm	–	342Da to $2x10^7$Da	ⒶⒹ ⒺⒻ*
ZS90	0.3nm to 5µm	3.8nm to 100µm	342Da to $2x10^7$Da	ⒶⒷⒸⒹ ⒺⒻ*
Nano ZSP	0.3nm to 10µm	3.8nm to 100µm	342Da to $2x10^7$Da	ⒶⒷⒸⒹ ⒼⒽ (Ⓕincluded as standard)

ill

* Compatible but not recommended because of low sensitivity.

Ⓐ Narrow band filter	Ⓔ High temperature
Ⓑ Universal 'Dip' cell	Ⓕ Flow-mode option
Ⓒ High concentration cell	Ⓖ Microrheology
Ⓓ MPT-2 Titrator and Vacuum degasser	Ⓗ Protein mobility

For complete measurement specifications refer to the Basic Guide.

Laser type

The laser fitted is identified by the colour on the oval badge on the cover.

- Instruments with a black and **red** badge fitted to the instrument cover either have a 4mW 632.8nm 'red' laser or a 10mW 632.8nm 'red' laser (Nano ZSP only) fitted.
- Instruments with a Black and **green** badge have a 532nm 'green' laser fitted.

Generally red lasers are **least** suitable for **blue** samples, while green lasers are **least** suitable for **red** samples.

90° optics

The S90 and ZS90 instruments use optics that have a 90° scattering detector angle for size measurements.

Accessory options

A range of accessaries and options are also available for more advance measurement strategies.

These accessories are fully documented in the **Zetasizer Nano accessories guide** or **MPT-2/Vacuum degasser user manual**.

Narrow band filter	This filter improves the signal for samples that fluoresce at the wavelength of the laser fitted. If a filter is fitted, an option label will be attached to the front of the cuvette holder **ZEN9051** - Narrow band filter for 'Green' badged Zetasizer Nano S instruments **ZEN9052** - Narrow band filter for 'Green' badged Zetasizer Nano ZS instruments **ZEN9061** - Narrow band filter for 'Red' badged Zetasizer Nano S, Z and S90 instruments **ZEN9062** - Narrow band filter for 'Red' badged Zetasizer Nano ZS, ZS90 and Nano Plus instruments
High temperature	These instruments have an increased temperature range of 0° to 120°. If fitted the Zetasizer Nano instrument label will include an '**HT**' identification. An option label will also be attached to the front of the cuvette holder. (The high temperature option cannot be fitted to the Zetasizer Nano ZSP) **ZEN9063** - Extends the upper temperature range of the Nano series from 90° to 120°
MPT-2 Titrator and Vacuum degasser	The MPT-2 Titrator is used to perform pH, additive and dilution titrations. The degasser is used to remove any dissolved gases from the titrants before dispensing by the titrator. This prevents any gaseous escape during the titration, increasing the accuracy and reproducibility of the measurement.
Cells and Cuvettes	Overview of all cells available for zeta potential, size, molecular weight, flow mode and microrheology measurements.
Folded capillary cell (DTS1070)	Maintenance-free capillary cell primarily designed for zeta potential measurements, but can also be used for size measurements.

Dip cell (ZEN1002)	This is used to provide repeatable measurements of aqueous, and non-aqueous samples such as solvents. It is particularly suitable for measurements of valuable aqueous samples where minimal sample quantity is important.
High concentration cell (ZEN1010)	This is used to perform zeta potential measurements on a concentrated aqueous sample. The cell is suitable for a broad range of conductivities.
Surface zeta potential cell (ZEN1020)	The surface zeta potential cell is intended for the measurement of the zeta potential at the surface of a flat material in an aqueous environment.
Flow-mode option	Instruments fitted with the flow-mode option can be connected to the output of, for example, a chromatography column, and be used as a light scattering detector. The flow mode option is included as a standard fitment on the Zetasizer Nano Plus. **ZEN1006** - Flow-mode option for Zetasizer Nano S and Zetasizer Nano ZS.
Microrheology	Microrheology is a new measurement type available to users of the Zetasizer Nano ZS and ZSP. It allows the measurement of the viscoelastic modulus of samples within the linear viscoelastic region. Microrheology measurements require a software key to access the software features and functionality. A microrheology starter kit containing a dip cell (**ZEN1002**) and a selection of probe particles is available. **For use with the Zetasizer Nano ZS and ZSP.**
Protein mobility	Protein mobility is a measurement type available to users of the Zetasizer ZSP. The Zetasizer Nano ZSP has increased capability for the measurement of small and weakly scattering molecules and particles. This improved sensitivity improves the measurement of zeta potential, with its primary purpose being the ability to measure the zeta potential, or more appropriately, the electrophoretic mobility of **protein** samples. A dedicated measurement type is included for protein mobility and also a suite of new calculators for proteins. **For use with the Zetasizer Nano ZS and ZSP.**

Primary measurement techniques

This section describes the basics of **particle size, zeta potential** and **molecular weight** and why they are important. Greater detail on the measurement techniques is given in **Part 2 - Measurement theory**.

Particle Size?

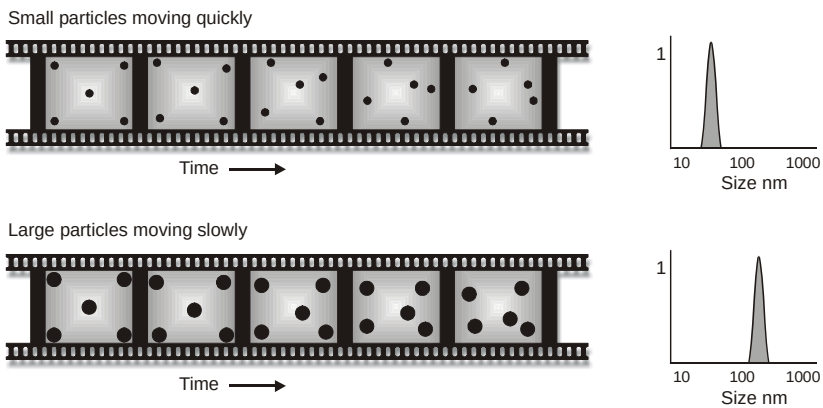
The particle size measured in a **D**ynamic **L**ight **S**cattering (**DLS**) instrument is the diameter of the sphere that diffuses at the same speed as the particle being measured.

The Zetasizer system determines the **size** by first measuring the **Brownian motion** of the particles in a sample using DLS and then interpreting a size from this using established theories.

Brownian motion is defined as:

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

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



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It is known that small particles move or diffuse more quickly in a liquid than larger particles. This movement is carrying on all the time. So if we take two 'pictures' of the sample separated by a short interval of time we can see how much the particles have moved and therefore calculate their size.

If there has been a minimal particle movement between the two 'pictures' the particles in the sample can be inferred as large; similarly if there has been a large amount of movement between the two 'pictures', then the particles in the sample can be inferred as small.

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

The above is a **very** simplistic explanation. A more detailed explanation can be found in **zeta potential theory** chapter.

Why do we use it?

A wide range of materials exist as molecules or particles that can be characterised by dynamic light scattering. These include proteins, polymers, emulsions and vesicles, as well as materials more traditionally thought of as particles, such as clays, silica, pigments and inks.

Proteins

The purity of a therapeutic protein can be investigated by the determination of the existence of aggregates in the sample. Light scattering is an excellent technique for detecting aggregates because of its sensitivity to larger particles.

Emulsions and vesicles are delicate, in that the preparation required for measurement techniques such as TEM or AFM can alter or destroy the sample. Light scattering can measure the size in the material's native environment.

Toners and Liquid inks

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

Pigments

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

Zeta potential and Electrophoresis?

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

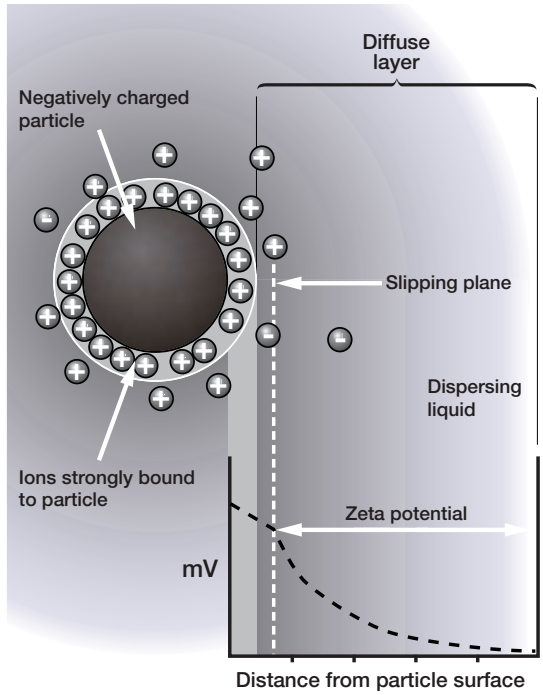
A negatively charged sample attracts positive ions from the liquid and conversely a positive charged sample attracts negative ions from the liquid.

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

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

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

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



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This technique will be further explained in Theory section of the manual.

Why do we use it?

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

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

Ceramics:

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

Waste water treatment.

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

Emulsions.

Zeta potential is used to study the chemistry involved in determining whether an emulsion will remain stable in the target environment.

Molecular weight?

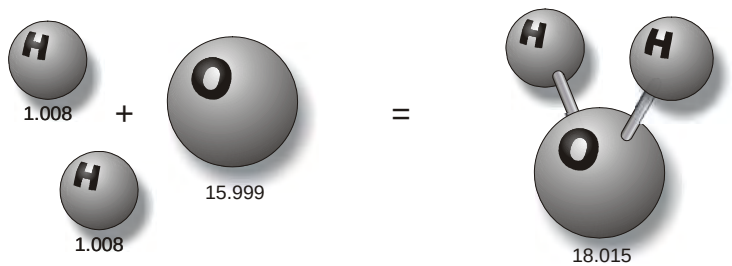
The molecular weight of a substance is the weight in **a**tomic **m**ass **u**nits (**amu**) of all the atoms in one molecule of that substance. Mathematically the **molecular weight** can be calculated from the **molecular formula** of the substance; it being the **sum** of the **atomic weights** of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

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

- In each molecule of water there are two atoms of Hydrogen (H_2) and one atom of Oxygen (O).

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

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



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

With the Zetasizer Nano series of instruments the molecular weight can now be determined by use of **S**tatic **L**ight **S**cattering (**SLS**) measurement techniques.

This technique is explained in the **Size theory** chapter.

Note
Malvern uses **Daltons** as the unit of molecular weight. Results are displayed in kiloDaltons (kDa).

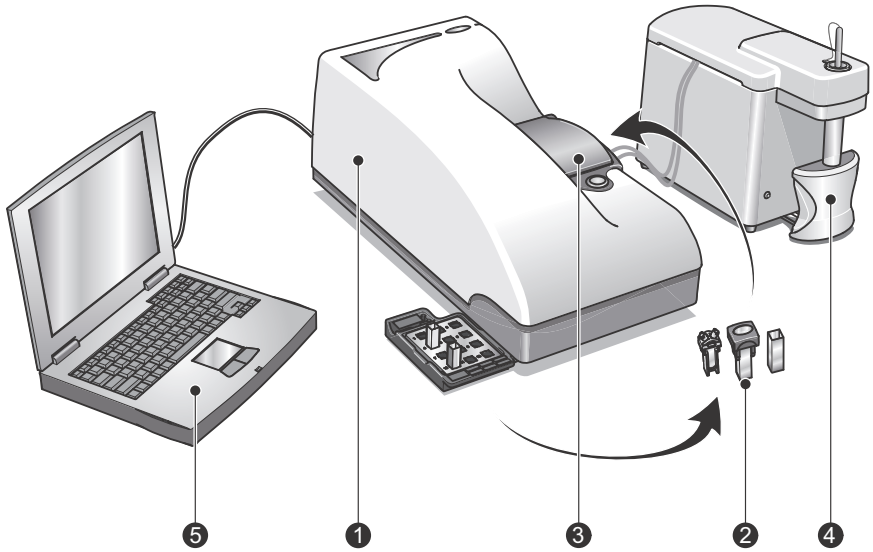
Why do we want to know it?

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

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

Hardware components

The following illustration shows a typical installation:



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① **Zetasizer Nano optical unit** (as described in the next section) – measures the sample using red and green light wavelengths.

② **Cells** – sample is presented in a selection of measurement cells so that it passes through the instrument's laser path.

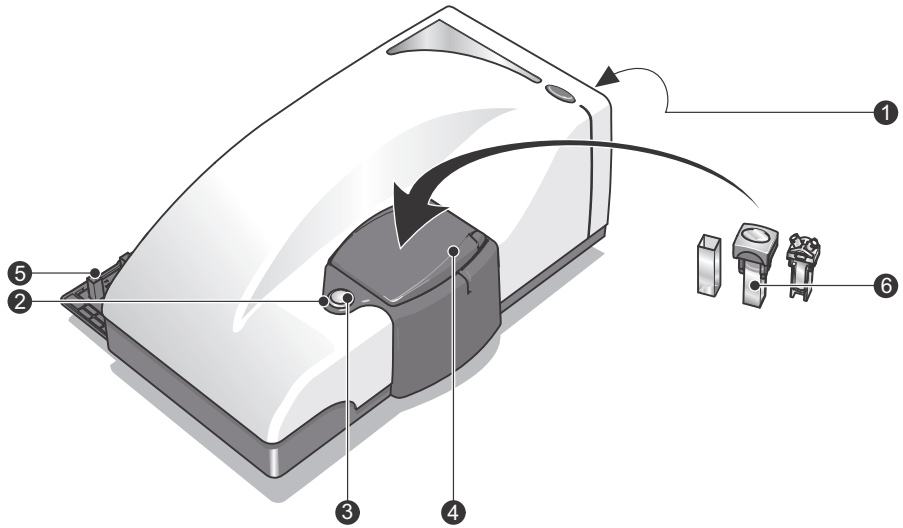
③ **Cell area** – The cell area is where all cells are inserted to undertake a measurement.

④ An **MPT-2 Titrator**, shown alongside, can be included to form part of the overall system.

⑤ **Computer running the Zetasizer software** – this controls the optical unit as well as processing and presenting the measured data to give either the size, molecular weight or zeta potential for the sample measured.

Optical unit components

This diagram shows the optical unit, the main component of the Zetasizer Nano system.



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The optical unit comprises the following:

① Rear Panel	④ Cell area
② Status indicator	⑤ Cuvette holder
③ Cell access button	⑥ Cells and Cuvettes

These components are described in the following sections.

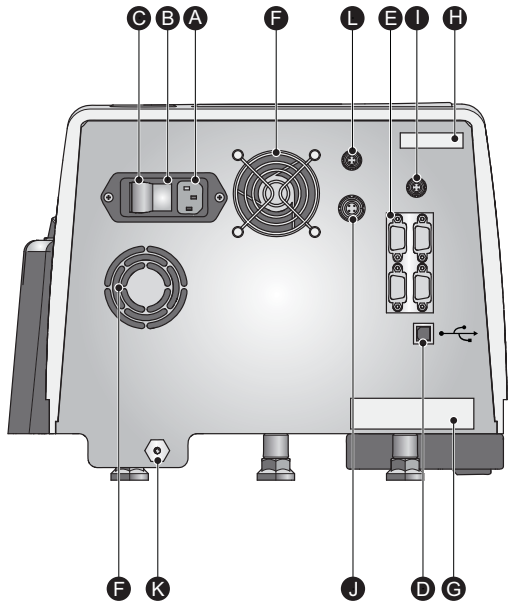
Rear Panel

The rear panel provides all the connections identified below.

Ⓐ **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. On switch on the "**status indicator**" ③ around the **Cell access button** ④ will illuminate.



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Ⓓ **Computer connection** – The **USB** cable from the computer is connected here.

Ⓔ **Accessory connections** – Two types of connection are available:

- **CAN Ports ① and ②**
  Use these ports to connect any Malvern supplied accessory that requires a CAN connection (Controller Area Network). Consult the respective accessory manual for details.
- **RS232 (I0I0I) Ports ① and ②**
  Use these ports to connect any Malvern supplied accessory that requires an RS232 connection. Again consult the respective accessory manual for details.

Control of any accessory is via the Zetasizer software.

Ⓕ **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 Nano model and its serial number. Please quote all numbers in any correspondence with Malvern Instruments.

For an explanation refer to the identification table in the I**ntroducing the Zetasizer Nano** chapter.

Ⓗ **Mod record** – Indicates what updates have been done on the instrument. This is used for reference by the Malvern service personnel.

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

Warning!
Only connect Malvern approved accessories.

Ⓙ **Green laser PSU input** – If the instrument uses a 532nm 'green' laser, connect the laser PSU to this connection.

Ⓚ **Purge connection** – If measuring samples at low temperatures there is a risk of condensation occurring on the cell; this occurs when the measurement temperature is less than the 'dew point' of the ambient air surrounding the cell. This is particularly 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 cell 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 g

For connection purposes, the purge connection uses an M5 internal thread diameter.

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 1.5ltr/min at 1 bar.
- 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 stabilise.

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.

Ⓛ **Flow-mode connection** – This connection is used when inputting a signal from an external measuring source, such as an Ultra-violet absorption detector or Refractive index detector to the Zetasizer Nano. A real-time parameter reading from the device can be directly inputted into the Nano optics unit and added to the Nano sample record.

- The Connector input voltage specification is: **-5v to +5v (analogue)**

Please refer to the **Advanced features** chapter for connection instructions and a description of the **flow-mode** capability.

Status indicator

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

Note that **Amber** is a **combination** of **red** and **green** lights.

Indicator colour and state	Function
Amber - Flashing	Shows the start-up initialisation routine is running.
Amber	Shows that the instrument is at “Standby”. The instrument is functioning correctly but is either not connected to the computer or the software has not been started.
Green	Indicates the instrument is functioning correctly and is ready to start a measurement.
Green - Flashing	Occurs when the instrument is performing a measurement.
Red	Indicates if the instrument has detected an error. The measurement will be stopped.

Cell access button

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

Cell area

Warning!
The system is capable of heating the cell to high temperatures. Care should be taken when removing the cells if a measurement has been performed at high temperatures. It is recommended that the cell area is allowed to cool before removing the cell.
A **warning triangle** is provided in the cell basin.

The cell area is where cells are inserted to undertake a measurement. The cell area is completely self enclosed and controls the sample temperature over the range 0°C to 90°C (up to 120°C for high temperature instruments).

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

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

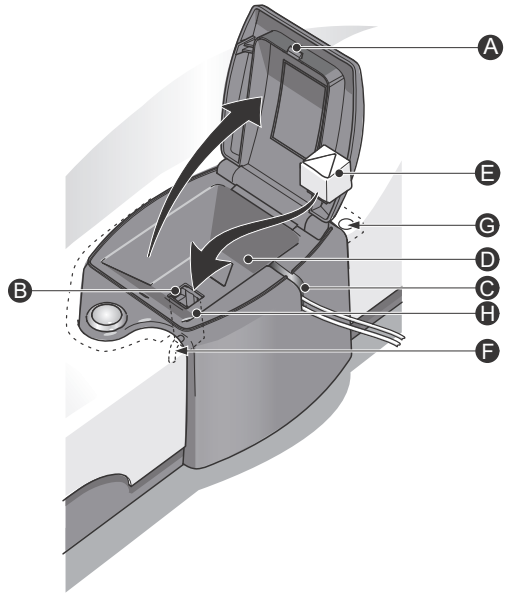
Chemical compatibility

Apart from the measurement cells, and any tubing used with the titrator, the only part of the instrument that should come into contact with any sample is the cell area, and this only if spillage occurs. The cell 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 titrant used is chemically compatible with the materials from which the cell area is manufactured.

Refer to the **Zetasizer Nano accessories guide** for a list of the cell area components that may come into contact with any spilt sample.

Features of the cell area

Other important features of the cell area are described below.



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Ⓐ **Cell area lid** – Press the **cell access button** to open the lid, the lid will slowly raise allowing access to the cell holder. On opening, two safety interlocks are activated.

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

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

Ⓑ **Electrodes** – The electrodes provide voltage for zeta potential measurements. Voltage is immediately turned off when the cell lid is opened.

Note
The maximum working voltage on the electrodes is ±150v (Measurement category I).

© **Access channel for Titrator and flow cell tubes** – A channel incorporated into the cell area allows sample tubes to be connected to the measurement cell. This facility is used with the MPT-2 Titrator accessory.

The channel includes a pinch valve to hold the tubes in place and stop sample movement during the measurement. See the **Inserting the cell** section in **Zetasizer Nano accessories guide** for precise details on fitting the tubing.

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

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

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

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

A parking position is provided in the cuvette holder to store the thermal cap. Place the cap onto the cap post as shown in the **cuvette holder** diagram. The **thermal cap** supplied with the **HT option** is gold plated. This is to give increased thermal contact for better temperature equilibration.

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

Ⓕ **Drain port** – In case of spillage within the cell area, there is a drain incorporated into the base of the cell holder.

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

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

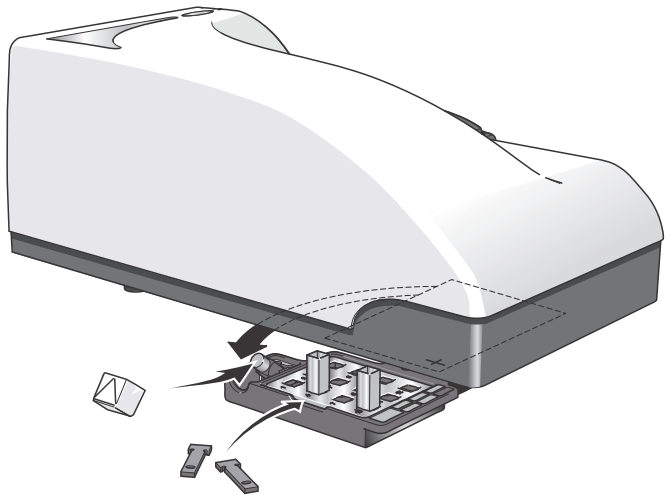
Any spillage will exit onto the bench area underneath the Zetasizer.

Ⓗ **Cell clamp** – To keep the cell in the optimum position during a measurement, a cell clamp mechanism is incorporated into the wall of the cell holder. The clamp will push against the edge of the cell when inserted.

Note
When using glass or quartz cuvettes, first insert a disposable polystyrene cuvette into the cell holder. This ensures the cell clamp moves freely, and that no spilt sample has caused the mechanism to stick.

Cuvette holder

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



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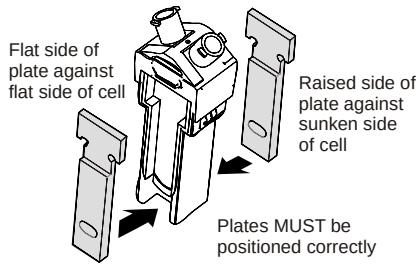
Caution!
Ensure the thermal cap is lowered and all cuvettes have been removed before swinging the holder back under the instrument base.

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

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

Similarly, storage is provided for the two cell "**thermal contact plates**" used with the folded capillary cell. These fit on either side of the cell, and work in the same manner as the **thermal cap** Ⓔ described above.

When not being used, place these in the holder to the left of the tray.



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

Cells and Cuvettes

A range of cells and cuvettes are available to use with the Zetasizer instrument. Full details are given in the **Zetasizer Nano Accessories guide**, but briefly the following cells can be used.

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

Software overview

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

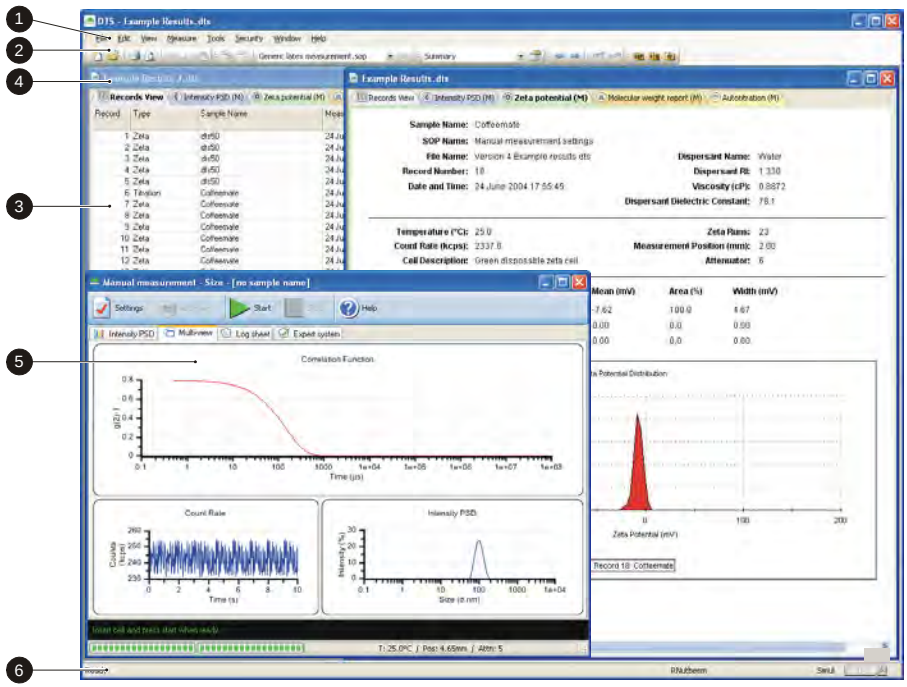
There are two software modules:

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

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

The Zetasizer Nano 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

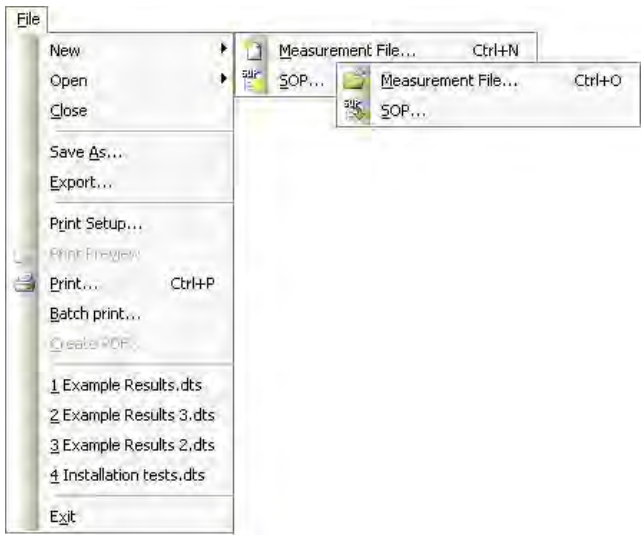
The menu bar contains the main menu headings for all software functions.

Items which end with a row of dots (**...**) will display additional windows. Similarly any items which end with an arrow (▶) will display a second menu.

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

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

File Menu



The **File** menu can be used to either create a **New** Measurement or SOP file, or **Open...** an existing Measurement or SOP file.

- The **Measurement file** is where all the measurement records (results) will be stored.
  Select **Save as…** to store a measurement file under a different file name.
- An **SOP file** contains all the measurement settings required for performing repetitive and consistent measurements.

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

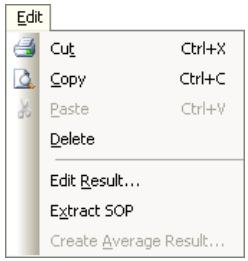
Select **Batch print...** to simultaneously print a number of measurement records.

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

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

Exit will close down the software.

Edit Menu



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

Deleted files will be stored in a **.del** file in the **Zetasizer\Measurement data** directory. This file has the same name as the original measurement file but with a **.del** extension. Refer to the **Measurement file window** chapter for more information.

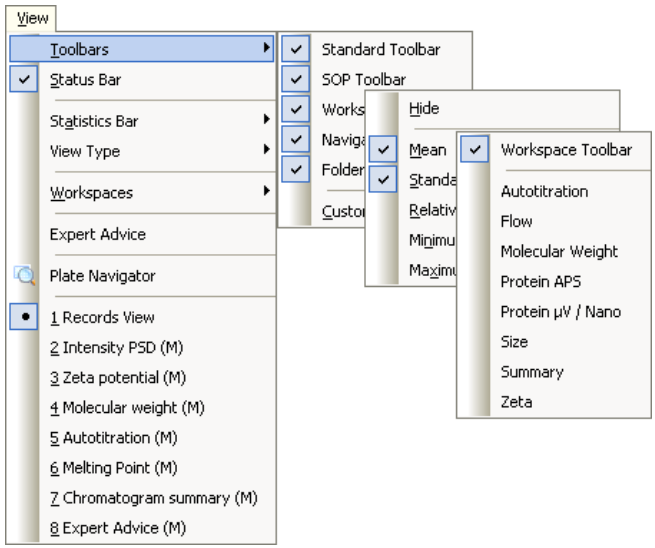
Edit result... allows an existing measurement record to be reanalysed using different dispersant and particle properties. Comments on the editing reason can be added. The edited measurement will then be added to the bottom of the Records view listing.

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

Create Average Result, enables a number of records to be selected, then recalculated to show the average result. The averaged result is added to the bottom of the record view.

Note
A combination of the **Edit** and **View** menus are displayed when the right mouse button is pressed anywhere in the measurement file window.

View Menu



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

To see the Standard deviation, the Relative standard deviation, the minimum, the maximum or the mean of a selected number of records, a **Statistics bar** can be displayed.

Additionally **View type** will display the aggregation and Titration records in either a Sorted or Outlined format.

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

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

All currently available reports are shown at the base of the menu. The icon • indicates the report that is currently displayed.

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

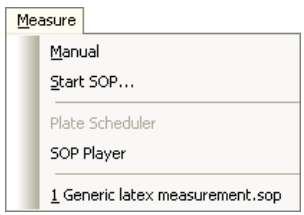
Expert Advice enables a quality check to be performed on three or more completed measurement records. This will show how good the measurements are and whether they display any unwanted attributes, such as aggregation.
The Expert advice is also available directly from the records view, and during a measurement sequence in the measurement display.

Refer to the **Expert Advice** section in **Advanced features** chapter for a description.

Plate Navigator provides a graphical view of measurements made using a **Zetasizer APS** Instrument.

Note
A combination of the **Edit** and **View** menus are displayed when the right mouse button is clicked anywhere in the measurement file window.

Measure Menu



Select this menu when ready to perform a measurement.

There is a choice of using an existing measurement SOP (**Measure-Start SOP...**) or manually setting up the measurement and sample details (**Measure-Manual**).

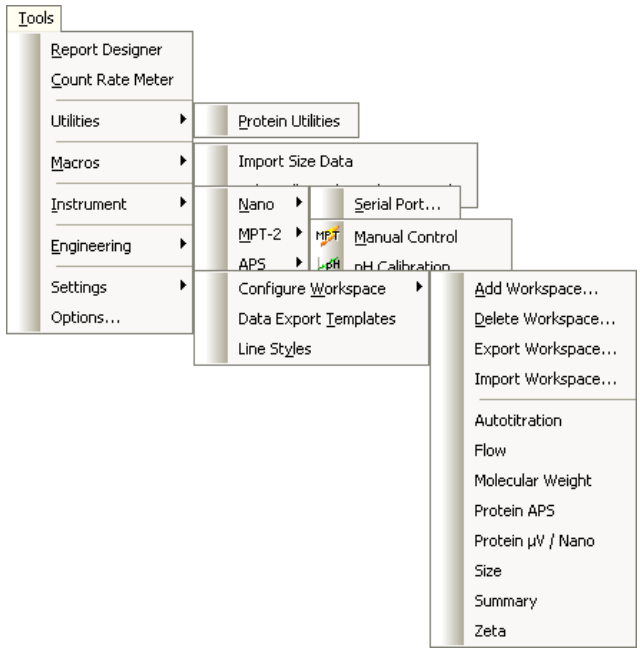
Once the measurement details have been entered or an SOP has been chosen, the **Measurement display** is displayed.

The most recent SOPs used are displayed at the base of the menu.

SOP Player opens the SOP Player program; this enables a sequence of SOPs and other actions to be performed sequentially. See the SOP Player section in the **Advanced features** chapter for a description.

Plate Scheduler is only available for use on the Zetasizer APS.

Tools Menu



The operation of **Report designer** is explained late in the manual.

The **Count Rate Meter** displays the number of photons detected per second. This is useful for determining both the sample quality and concentration before the start of a measurement.

The **Utilities-Protein Utilities** option is described in **Zetasizer Nano accessories guide**. With this tool, estimates of the molecular weight and particle shape can be generated.

The **Macros** submenu will show additional available programs. These are programs written by Malvern to enable the system to operate in a mode required for a specific application. These can be installed and selected using the **Options** feature.

The **Instrument** Sub-menu allows access to the manual controls of the attached instrument or each connected accessory.

- **Serial port...** allows the USB communications port to which the instrument is connected to be chosen. **MPT-2** allows access to the manual control windows of the MPT-2 Titrator. This menu item will be 'greyed' out if the MPT-2 is not connected.

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

The **Settings** sub-menu enables the user to **Configure Workspaces**, and set-up **Data export templates** and **Line styles**.

- **Configure Workspace** enables workspaces to be **added** and **deleted**, workspaces to be **imported** or **exported**, and previously created workspaces to be displayed and edited. Configure workspaces allows the user to specify what reports are viewed for each created workspace, and what parameters will be shown in that workspace's **Records view** tab.
- **Data Export Templates...** opens a window that defines the parameters and the format in which the record data will be exported. Once a template is created the measurement data can be exported to other software packages such as Excel or Wordpad, by using **File-Export data**.
- **Line styles...** allows the colour and style of lines to be changed in the report graphs.

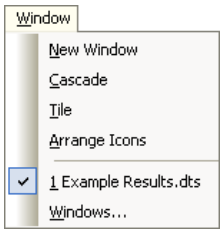
Options opens a window that allows the following options to be selected:

- **Folders** - Indicates and defines where the measurement data, SOPs and Export data are saved.
- **Macros** - Enables the selection and installation of additional programs.
- **External Inputs** - This page configures the external inputs into the Nano instrument. This is required for setting the timing between the Nano instrument and any connected accessories; and for setting any relevant calibration parameters.
- **Feature Keys** - This page allows extra features of the Zetasizer software to be enabled using a licence key.
- **Measurement** - This allows the look of the measurement display to be altered.

Security Menu

To prevent unauthorised changes, the Malvern software can be configured to limit each user's access to various functions - e.g. modifying an SOP. Users are assigned operating **permissions** that allow, or restrict, access; refer to **Security** chapter for details.

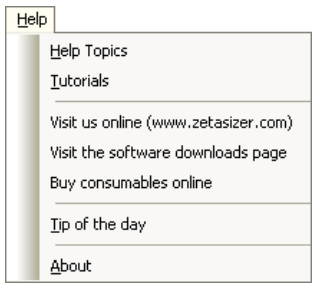
Window Menu



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

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

Help Menu



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

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

Links to the Malvern website... including downloads and consumables.

Tip of the day... will give hints on how to use the Zetasizer software; a different tip will be displayed each time this is selected. An option is given for turning off the Tip of the day window.

About... gives details on the software version installed. Quote this if contacting Malvern Instruments.

② Toolbars

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

To identify each tools function, move the cursor over a tool - a tool tip will be displayed under each tool with a short description of its action displayed in the status bar. As with the menu bar, if a tool, or accessory, is not available it will be shown "greyed 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. The plate navigator button is for viewing measurements that were made using a Zetasizer APS instrument. Greyed out for results made using a Zetasizer Nano.
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 SOPs used to enable quick selection. This list will also include any recent **SOP player** (.**sopl**) files. The eject button is greyed out and reserved for use when using a Zetasizer APS instrument.
Workspace	Allows selection and configuration of the Workspaces.
MPT-2	Opens the **Manual control** and **pH calibration** windows for the MPT-2 Titrator accessory. These functions are discussed in the MPT-2 Titrator manual.

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

③ Measurement file window

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

Refer to the **Measurement file window - workspaces** chapter.

Measurement file workspace

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

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

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

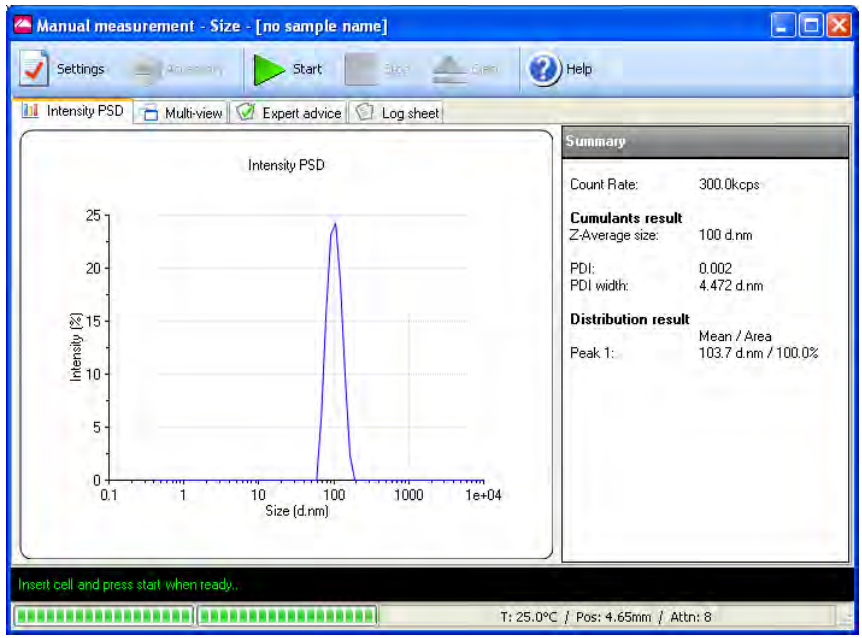
Details on interpreting the reports can be found in the **Report designer** chapter**.**

④ Title bar

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

⑤ Measurement display

When a measurement is being performed the **measurement display** is shown.



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.

⑥ 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-Customise...**. window to disable it.

On the right of the status bar, icons are shown to detail which instrument is connected and what additional features may be installed. In this case a Zetasizer Nano is connected.

Nano icon

Double-clicking the Nano icon will display the Zetasizer Nano model, serial number, software and firmware version of the instrument (only if the instrument is connected and switched on).
If the instrument is disconnected from the software the icon will be 'greyed' out:

21 CFR 11 icon

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

If unavailable the icon will be 'greyed' out:

Double-clicking the icon will display the feature key number.

Please note that the **21 CFR part 11** option is **not** described in this manual.

Research icon

If the **Research** feature key is installed, additional features will be available within the SOP windows of the software.

Double-clicking the icon will display the feature key number.

Please note that the additional **Research** features are **not** described in this manual.

Microrheology icon

If the **Microrheology** feature key is installed, additional features will be available within the SOP windows of the software.

Double-clicking the icon will display the feature key number.

The additional microrheology features are described in the accessories manual.

Advanced protein features icon

If the **Advanced protein features** feature key is installed, additional features will be available within the SOP windows of the software.

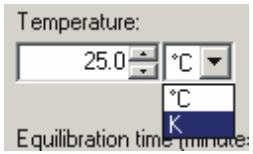
Double-clicking the icon will display the feature key number.

The additional advanced protein features are described in the accessories manual.

Malvern defined features

Within the software, various parameters, settings or reports will have either a small Malvern logo ( ) or an (M) alongside. This identifies it as Malvern defined and cannot be overwritten. However, 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, i.e. Temperature has the option to use either **ºC** (Celsius) or **K** (Kelvin).

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 windows 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 windows 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 the **Record and Reports chapter**.

3

Making measurements

Introduction

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

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

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

The Zetasizer Nano is capable of making two types of measurement:

- **Manual measurements** - all of the measurement settings are specified before the measurement and some further user input is required during the running of the measurement. The measurement process is also split into key stages, which are paused after the completion of each stage.

  Manual measurements tend to be used for one-off measurements or as part of method development (i.e. establishing the optimal settings for measuring the sample and then saving into an SOP file).

- **Standard Operating Procedure (SOP) measurements** - most of the measurement settings are stored within an SOP file which has been previously created by the user. Once an SOP has been initiated, the measurement sequence requires less user intervention than a manual measurement.

  As SOPs lock-down most of the measurement settings they improve consistency and provide greater repeatability; features that are important in quality-controlled environments.

The sections that follow, **Quick guide to making a measurement**, etc, will focus on SOP measurements. Refer to the **Using SOPs** chapter for details on creating and managing a user's SOPs.

Quick guide to making a measurement

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

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

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

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

  Follow any onscreen instructions that are displayed.

  The **Measurement display** will now be shown.
- When requested, **insert the cell** into the instrument and wait for the temperature to stabilise.
- Click **Start** . The measurement will be made and the results displayed and saved to the measurement file that was selected when the SOP was started.

Powering up the system

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

Turning on the instrument

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

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

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

Note
Important! All laser based measuring instruments should be 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 Nano software

Double click on the icon to start the software.

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

Sample preparation

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

Please refer to the **Sample preparation** chapter for sample preparation guidelines, and count rate check for the different measurement types.

Selecting and preparing the measurement cell

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

The cells available for each measurement type are fully documented in the **Zetasizer Nano Accessories manual** with some discussion on their use and application.

The accessories manual covers the following topics on choosing and using the cells.

- Choosing the correct cell

- Filling the cell
- Inserting the cell
- Configuring the SOP parameters for the cell chosen.

Making an SOP measurement

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

This process is outlined below. Refer to the **Using SOPs** chapter for information on creating new SOPs.

Opening or creating a Measurement File.

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

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

IMPORTANT: Due to the continually saving nature of the measurement files, running the software directly onto a removable USB drive can result in loss of data if the drive is removed while the software is run.

Note
The measurement record will be saved to the measurement that was active when the SOP was started.

To open an existing measurement file:

1. Select **File-Open-Measurement File** or .
2. A window is displayed allowing selection of a measurement file.
3. Select **Open**.

To create a new measurement file:

1. Select **File-New-Measurement File** or .
2. A window is displayed allowing the new measurement file to be named and specify where it will be saved.
3. Select **Save**.

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

Starting an SOP measurement

Everything should now be ready to make the actual measurement.

To start an SOP measurement, select **Measure-Start SOP**. The **Open SOP** window is displayed. Select the SOP that will be used and select **Open**. If an SOP has not been specified for the sample, refer to the **Using SOPs** chapter.

The **Documentation** window appears allowing **Sample name** and **Notes** to be added. Clicking on **Instructions** on the tree view, on the left of the window, will display the operator comments. Select the **OK** button to continue.

The **Measurement display**, discussed below, will now be displayed.

Note
It may be that the SOP was not configured to automatically show the **Sample** window. If the window is not displayed, but is required, select the **Settings** button in the measurement display.

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

The progress of the measurement can be viewed in the **measurement display**. The measurement duration depends strongly on the measurement type and SOP settings.

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

Making a manual measurement

Making a manual measurement is essentially the same as making an SOP measurement, except that, where as in an SOP measurement all the measurement options are pre-specified, here it will be necessary to set them immediately.

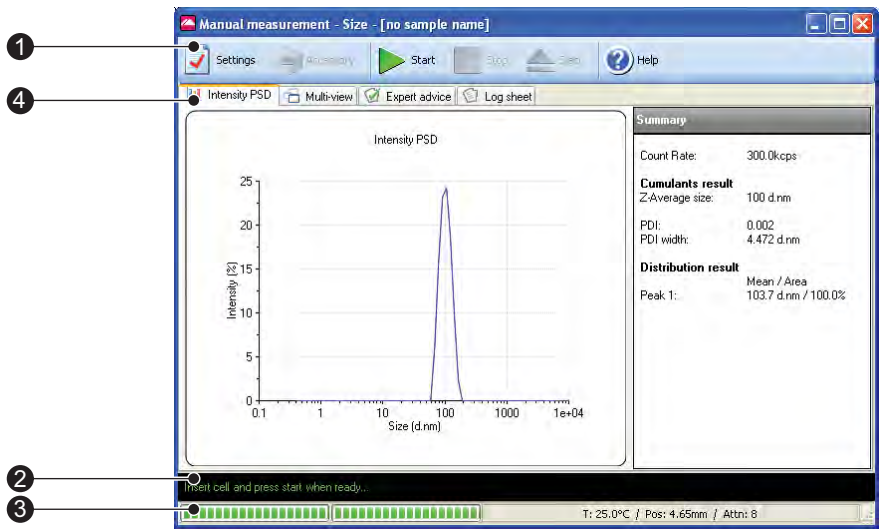
Instead of starting an SOP, select **Measure-Manual**. This will open the **Manual measurement** editor allowing any measurement types to be chosen and the settings to be configured.

The windows are virtually identical to those in the **SOP editor** when used to define a new SOP, please refer to the **Using SOPs** chapter.

Once all settings have been made, if required, select the **Save as...** icon or **File-Save as...** from the menu to store the settings. Click the **OK** button to close the **Manual measurement** editor and return to the measurement display.

Note
The manual measurement settings can be viewed and subsequently saved by selecting **Edit-Extract SOP** from each record in the **Records view**

The Measurement display



ill 8443

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

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

The features of the measurement display are:

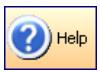
① Button bar

The button bar provides the control for the measurement operation.

The **Settings** button opens the measurement settings window. Extra comments and changes to the measurement parameters can be added prior to the measurement being started.

/

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 is displayed asking "**Are you sure you wish to abort the measurement**".



Opens the **Help** file.

The **Accessory** button will open the **MPT-2 Titrator Manual control** window when pressed.
When the **Start** button is pressed the Manual control window will be **closed**.

The eject button is greyed out and reserved for use when using a Zetasizer APS instrument.

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 three tabs windows - **Multi-view**, **Log sheet** and **Expert advice** - 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.

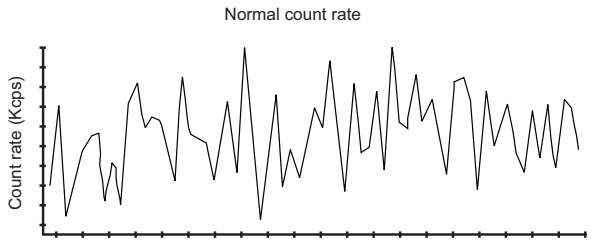
The **Result** tab is named after the result view chosen, the default result view shown is **Intensity PSD**. Different views can be selected by right-clicking on the graph and selecting from the list displayed. A graph only, or a graph with a summary table will be shown depending upon the view chosen.

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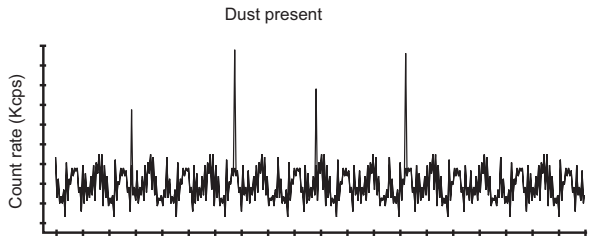
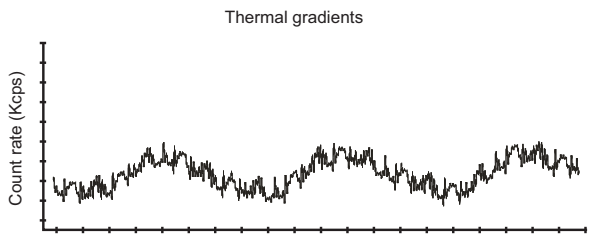
If dust is present then sharp spikes will be observed. Measurement runs with dust present will be removed from the final measurement calculation by a dust filtration algorithm.



ill 8452

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



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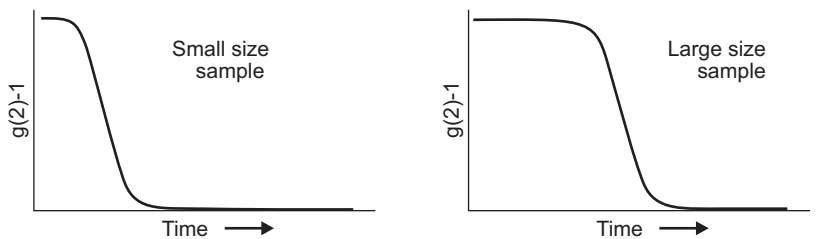
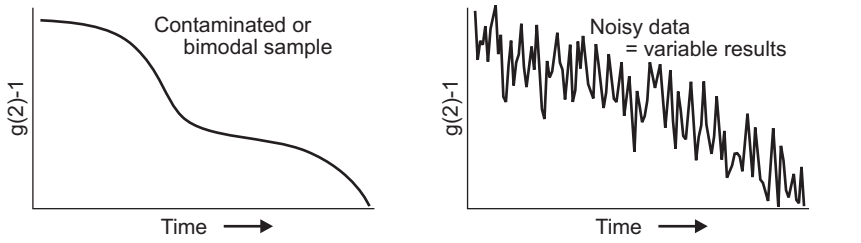
Correlation function

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



ill 8494



ill 8495

- **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.

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 displayed **Save to file...** button and saving it as a **.txt** file.

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 the **Advanced features** chapter for a description.

Molecular weight measurements

Result tab (1st tab)

Please also refer to size description above.
For molecular weight measurements the default result view shown is a **Debye** plot. The Different views that can be selected are described below: **Intensity PSD**, **Count Rate**, **Correlation Function**, **Volume PSD**, **Number PSD** and **Debye**.

- **Intensity PSD**
  The result view will be updated after each of the individual concentration measurements. The result shown will be an evolving value from the data collected so far.
  Please refer to the description in the previous section.
- **Count rate**
  Please refer to the description in the previous section.
- **Correlation function**
  Please refer to the description in the previous section.
- **Volume PSD**
  Please refer to the description in the previous section.
- **Number PSD**
  Please refer to the description in the previous section.
- **Debye**
  Displays the current result as a Debye plot. The Debye plot displayed will show an evolving plot generated from the data collected so far.

Multi-view Tab

This is the same as the description in the previous section, except for molecular weight specific view choices.

Log sheet Tab

Please refer to the description in the previous section.

Expert Advice Tab

Please refer to the description in the previous section.

zeta potential measurements

Result tab (1st tab)

Please also refer to size description above.
For zeta potential measurements the default result view shown is **Electrophoretic Mobility Distribution**. The Different views that can be selected are described below: **Zeta Potential Distribution**, **Electrophoretic Mobility Distribution**, **Count Rate**, and **Phase Plot**.

- **Zeta potential Distribution**
  This displays the zeta potential result. The view will be updated after every individual measurement run, with the result being the sum of the completed measurement runs.
- **Electrophoretic Mobility Distribution**
  This displays the Electrophoretic Mobility.

- **Count rate**
  This displays the number of photons detected in kilo-counts per second.
- **Phase Plot**
  This displays the phase shift that occurs during the zeta potential measurement. This is difference between the phase of the light scattered by the particles and the phase of the reference beam.

Multi-view Tab

As the description in the previous section, except for zeta potential specific view choices.

Log sheet Tab

Please refer to the description in the previous section.

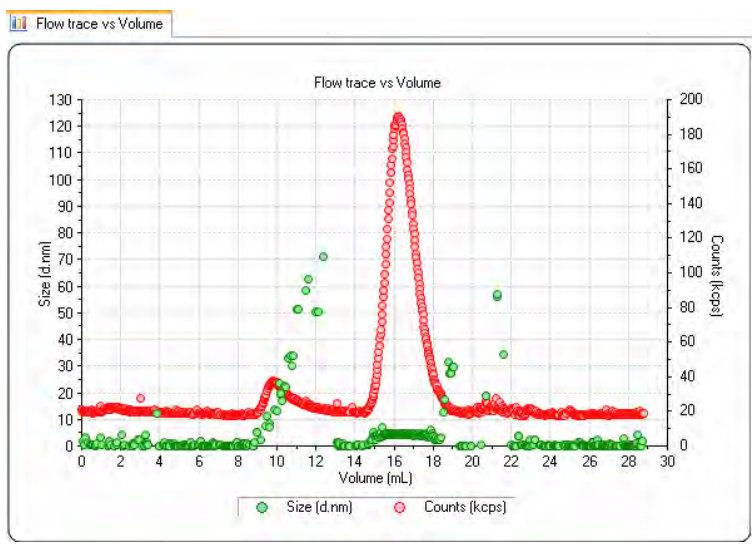
Expert Advice Tab

Please refer to the description in the previous section.

Flow-mode 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 tab is named after the result view chosen, the result view shown above is **Flow trace vs Time**. Different views can be selected by right-clicking on the graph and selecting from the list displayed.

The views available are: **Count Rate**, **Correlation Function**, **Flow trace vs Volume** and **Flow trace vs Time** and **Monitor**.

- **Monitor** enables the count rate signals to viewed before a measurement is run.

Multi-view Tab

As the description in the previous section, except for Flow-mode specific view choices.

Log sheet Tab

Please refer to the description in the previous section.

Expert Advice Tab

Please refer to the description in the previous section.

Trend measurements

The trend windows are generally the same as those used when either a size or zeta potential measurement SOP is chosen. The difference is the inclusion of an extra **Trend** graph view - this will show an evolving plot as the measurement progresses. **Aggregation** measurements can be performed using the Trend measurement type.

Titration measurements

As with trend and aggregation measurements, the windows are generally the same as those used when either a size or zeta potential measurement SOP is chosen. The difference is the inclusion of an extra **Titration/Measurement type** graph view (i.e. pH/Zeta) - this shows a plot of the titration against the measurement type selected. This is explained further in the Titrator accessory manual.

Measurement sequence

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

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

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

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

The paragraphs that follow describe the three **main measurement types** the Zetasizer Nano performs: **size**, **molecular weight** and **zeta potential**.

Size measurements

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

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

As soon as a run is completed, the result, 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.

Molecular weight measurements

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

- Press **Start** to begin the dark count measurement.
  The laser is turned off and a measurement is taken of the background light level.
- Insert the scattering standard cell (i.e. Toluene) and press **Start** when ready.
  The measurement will measure the scattering intensity of the scattering standard used.
- Once the standard has been measured, a window will be displayed to prompt insertion of the first sample concentration (i.e. the pure solvent). Insert the first sample concentration and press **Start**.
- The software displays another window where the sample concentration can be entered. Type in the concentration and press **Enter**. The measurement will then continue.
- On completion of the first sample measurement, a window is displayed - answer **Yes** to "Repeat measurement of concentration 1?" or **No** to continue with the second concentration.

- Continue as above until all sample concentrations have been measured.
- On completion of the last concentration the final result will be calculated.

The progress meter indicates the measurement progress during each stage.

Zeta potential measurements

The cell is inserted and **Start** is pressed. The status bar will indicate the instrument is now "**Performing the measurement**".

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

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

Note

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

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

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

4

Records and Reports - Viewing the Results

Introduction

Once a measurement has finished the results will need to be reviewed. This chapter describes 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

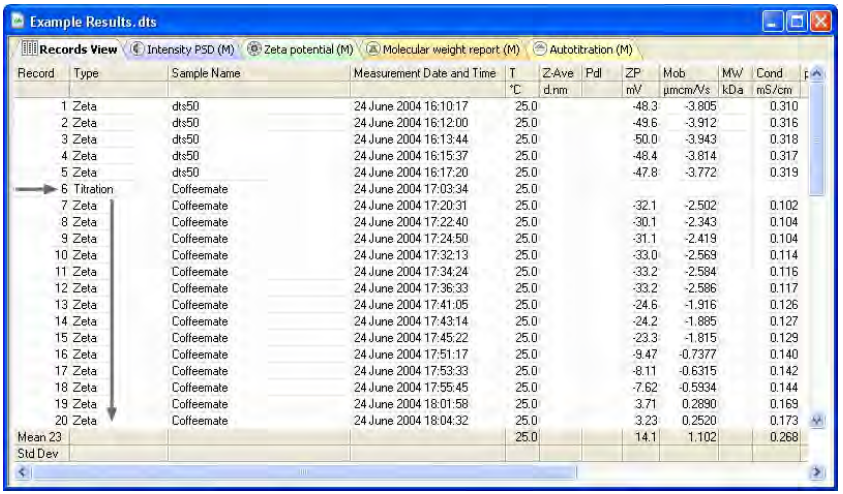
The results are displayed in two ways. A **Records view** that shows a list of the measurement records in a measurement file, and the **Report tabs** which show all measurement details of a selected measurement record, or records.

Note
The **Records view** parameters and **Report** tabs that are displayed are dependent upon the **Workspace settings** selected.

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-Workspaces** windows, the record view shown below depicts the default **Summary** workspace.



Record	Type	Sample Name	Measurement Date and Time	T °C	Z-Ave d.nm	PdI	ZP mV	Mob µmcm/Vs	MW kDa	Cond mS/cm
1	Zeta	dts50	24 June 2004 16:10:17	25.0			-48.3	-3.805		0.310
2	Zeta	dts50	24 June 2004 16:12:00	25.0			-49.6	-3.912		0.316
3	Zeta	dts50	24 June 2004 16:13:44	25.0			-50.0	-3.943		0.318
4	Zeta	dts50	24 June 2004 16:15:37	25.0			-48.4	-3.814		0.317
5	Zeta	dts50	24 June 2004 16:17:20	25.0			-47.8	-3.772		0.319
6	Titration	Coffeemate	24 June 2004 17:03:34	25.0						
7	Zeta	Coffeemate	24 June 2004 17:20:31	25.0			-32.1	-2.502		0.102
8	Zeta	Coffeemate	24 June 2004 17:22:40	25.0			-30.1	-2.343		0.104
9	Zeta	Coffeemate	24 June 2004 17:24:50	25.0			-31.1	-2.419		0.104
10	Zeta	Coffeemate	24 June 2004 17:32:13	25.0			-33.0	-2.569		0.114
11	Zeta	Coffeemate	24 June 2004 17:34:24	25.0			-33.2	-2.584		0.116
12	Zeta	Coffeemate	24 June 2004 17:36:33	25.0			-33.2	-2.586		0.117
13	Zeta	Coffeemate	24 June 2004 17:41:05	25.0			-24.6	-1.916		0.126
14	Zeta	Coffeemate	24 June 2004 17:43:14	25.0			-24.2	-1.885		0.127
15	Zeta	Coffeemate	24 June 2004 17:45:22	25.0			-23.3	-1.815		0.129
16	Zeta	Coffeemate	24 June 2004 17:51:17	25.0			-9.47	-0.7377		0.140
17	Zeta	Coffeemate	24 June 2004 17:53:33	25.0			-8.11	-0.6315		0.142
18	Zeta	Coffeemate	24 June 2004 17:55:45	25.0			-7.62	-0.5934		0.144
19	Zeta	Coffeemate	24 June 2004 18:01:58	25.0			3.71	0.2890		0.169
20	Zeta	Coffeemate	24 June 2004 18:04:32	25.0			3.23	0.2520		0.173
Mean 23				25.0			14.1	1.102		0.268
Std Dev										

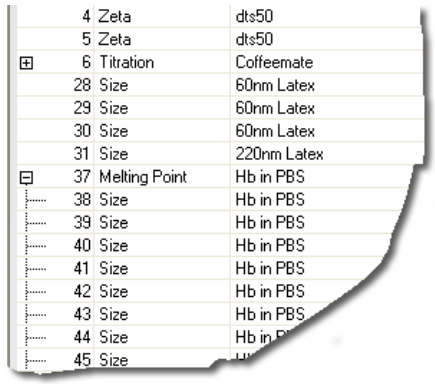
Aggregation and Titration records

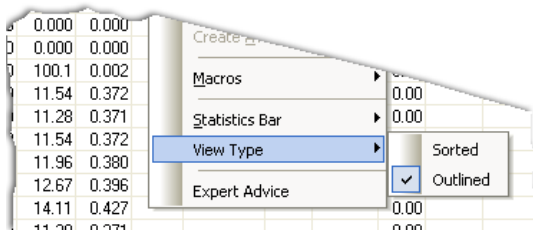
To view a sequence of Aggregation or Titration records, select the first record in the sequence; all subsequent records in that sequence will then automatically be selected.

Outliners

For both Aggregation and titration 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.

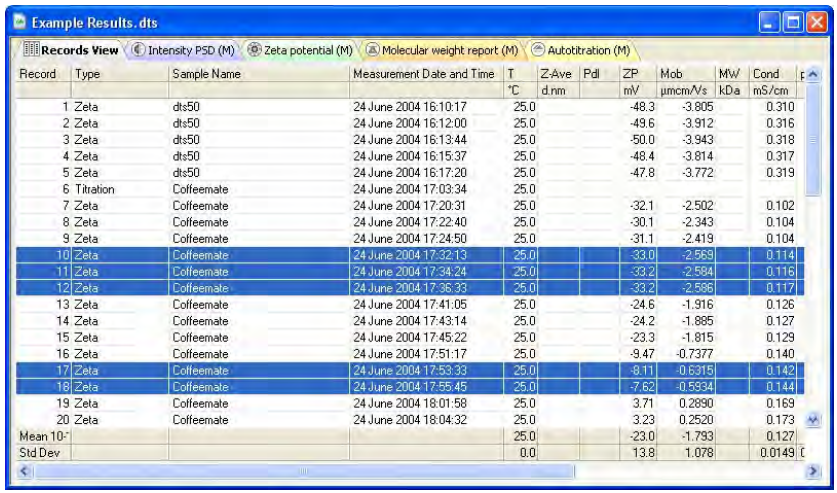




The Outlined record view can be turned off and on by selecting **View-View type-Sorted** or **Outlined** or by right-clicking the mouse on the table, as shown above.

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.



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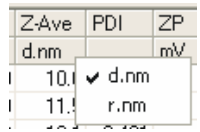
3	Zeta	dts50	24 June 2004 16:13:44	25.0			-50.0	-3.943		0.318
4	Zeta	dts50	24 June 2004 16:15:37	25.0			-48.4	-3.814		0.317
5	Zeta	dts50	24 June 2004 16:17:20	25.0			-47.8	-3.772		0.319
6	Titration	Coffeemate	24 June 2004 17:03:34	25.0						
7	Zeta	Coffeemate	24 June 2004 17:20:31	25.0			-32.1	-2.502		0.102
8	Zeta	Coffeemate	24 June 2004 17:22:40	25.0			-30.1	-2.343		0.104
9	Zeta	Coffeemate	24 June 2004 17:24:50	25.0			-31.1	-2.419		0.104
10	Zeta	Coffeemate	24 June 2004 17:32:13	25.0			-33.0	-2.569		0.114
11	Zeta	Coffeemate	24 June 2004 17:34:24	25.0			-33.2	-2.584		0.116
12	Zeta	Coffeemate	24 June 2004 17:36:33	25.0			-33.2	-2.586		0.117
13	Zeta	Coffeemate	24 June 2004 17:41:05	25.0			-24.6	-1.916		0.126
14	Zeta	Coffeemate	24 June 2004 17:43:14	25.0			-24.2	-1.885		0.127
15	Zeta	Coffeemate	24 June 2004 17:45:22	25.0			-23.3	-1.815		0.129
16	Zeta	Coffeemate	24 June 2004 17:51:17	25.0			-9.47	-0.7377		0.140
17	Zeta	Coffeemate	24 June 2004 17:53:33	25.0			-8.11	-0.6315		0.142
18	Zeta	Coffeemate	24 June 2004 17:55:45	25.0			-7.62	-0.5934		0.144
19	Zeta	Coffeemate	24 June 2004 18:01:58	25.0			3.71	0.2890		0.169
20	Zeta	Coffeemate	24 June 2004 18:04:32	25.0			3.23	0.2520		0.173
Mean 10				25.0			-23.0	-1.793		0.127
Std Dev				0.0			13.8	1.078		0.0149

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 unit.



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 or Titration 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.

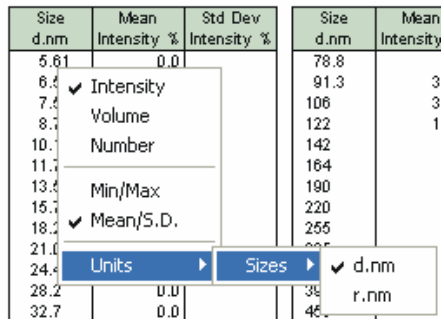
Each measurement type has a 'standard' report associated to it. The same result information is present in both the computer and printed versions, except for the diagnostic reports which contain extended information on the printed version.

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 unit.

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.

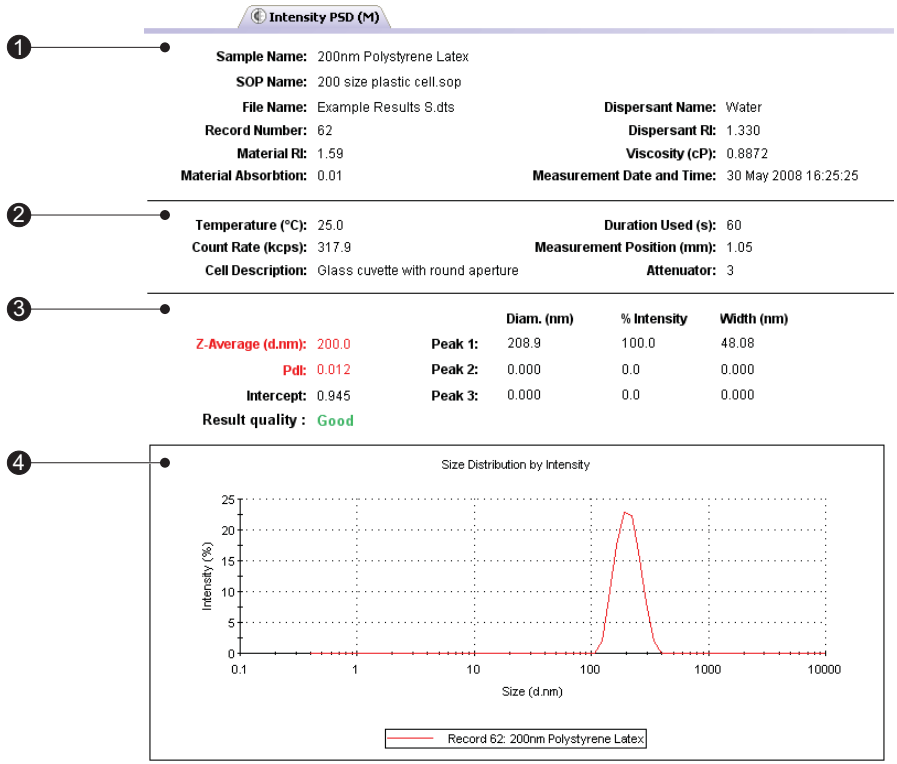


Note

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).



ill 8444

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

① Sample details

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

② System details

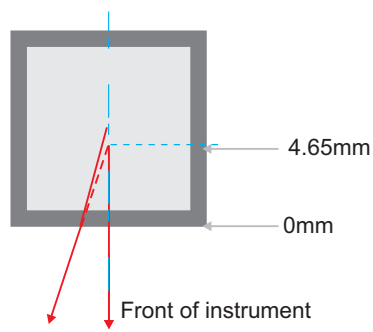
This section gives details on instrument settings for this particular measurement. Specifically, these are:

- **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.
- **Attenuator index**
  The laser power is automatically attenuated so that the count rate from the sample, especially high scattering samples, is within acceptable limits. An attenuation index of 11 denotes no attenuation (full laser power), while 0 denotes full attenuation (total laser block).
  The attenuation range is shown in the following table; the transmission value is the percentage of laser light that enters the sample cuvette.

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

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



ill 8890

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

③ Results

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

The results section gives three pieces of information:

- **Z-average size** (also known as the "cumulants mean")
  In **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 dispersant.

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

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

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

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

  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 dispersant viscosity and some instrumental constants.

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

- **Pdi**

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

  The calculations for these parameters are defined in the ISO standard document 13321:1996 E and 22412.

- **Intercept**
  This is the amplitude of the G1 correlation function at time 0. For a good measurement it will be between 0.85 and 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 count rate 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.

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** window is then displayed. This window 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.

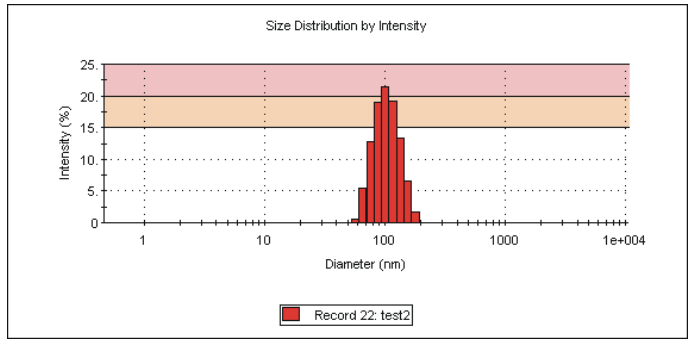
  The **Options** tab allows the graph key position to be chosen. A graph tips option can also be set, this allows the setting of pop up tips (flags showing data points on the graph) on the report itself.

- **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 window to display a size distribution by Intensity graph as a histogram, with logarithmic x-axis and linear y-axis settings. Graticule, or grid, lines have been included and upper action and warning limits have been set.



ill 6606

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 Statistics (M)

Volume PSD (M)

Volume Statistics (M)

Number PSD (M)

Number Statistics (M)

Size diagnostics report (M)

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

Size quality report (M)

This is a summary report detailing whether the result meets the Malvern Instruments quality criteria for a size measurement. The report indicates **Result meets quality criteria**, or **Result does not meet quality criteria** in which case

a list of parameters that do not meet the criteria, and probable reasons will be displayed. Some of these parameters will only be displayed if the research software has been used for the measurement.

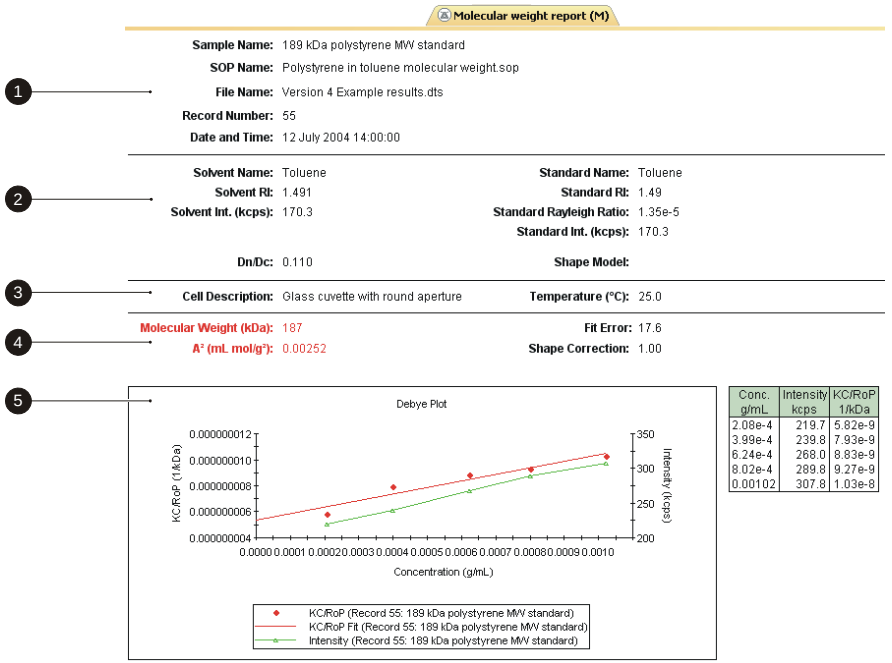
The parameters, followed by probable reasons displayed are:

- **z Average is smaller than lower size display limit**
  Wrong size limits used in display range
- **z Average is larger than upper size display limit**
  Wrong size limits used in display range
- **Polydispersity index is very high**
  Sample is very polydisperse and may not be suitable for DLS measurements
  Sample contains large particles/aggregates/dust
  Wrong measurement position selected
- **Correlation function intercept out of range**
  Sample concentration too high (multiple scattering)
  Sample concentration too low
  Sample fluorescence
  Sample absorbance (coloured samples)
  Wrong measurement position selected
- **In range figure is low**
  Presence of large or sedimenting particles
  Sample fluorescence
  Sample absorbance (coloured samples)
- **Count rate is out of range (too low)**
  Attenuator not set to automatic
  Sample concentration too low
  Sample absorbance (coloured samples)
  Sample is not stable during measurement
- **Count rate is out of range (too high)**
  Attenuator not set to automatic
  Sample contains large particles/aggregates/dust
  Sample is not stable during measurement
- **Insufficient signal collected**
  Measurement duration not set to automatic
  Filter factor in research software not set to default (50%)
- **Cumulant fit error high**
  Data quality too poor for cumulant analysis
  Sample too polydisperse for cumulant analysis
  Inappropriate cumulant analysis settings in Research Software

- **Multimodal fit error high**
  Data quality too poor for distribution analysis
  Sample too polydisperse for distribution analysis
  Inappropriate distribution analysis settings in Research Software
- **Check cumulants and distribution errors**
  Sample too polydisperse for cumulant analysis - suggest rely on distribution analysis.

Molecular weight measurements - standard report

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



ill 7795

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

①,② Sample and measurement details

These two sections show measurement details and details of parameters relating to the sample. Please see the **size** description for details.

Settings specific for molecular weight are:

- **Dn/Dc**
  This is the specific refractive index increment; the change in refractive index as a function of the change in concentration.
- **Shape model**
  Shows the shape correction model used for the measurement. With knowledge of the sample configuration it is possible to improve the result of the measurement by adding the value that most closely corresponds to the probable sample shape; i.e. sphere, coil, cylinder or no shape correction.

③ System details

The System section gives details on settings configured during the measurement process. Please see the **size** description for details about **Temperature**, and **Cell type**.

④ Results

The results section gives four pieces of information:

- **Molecular weight**
  Shows the measured weight of a molecule within the measured sample expressed in atomic mass units; indicated in kiloDaltons (kDa).
- **Shape correction**
  Displays the shape correction model used for the calculation as mentioned above.
- **Second Virial Coefficient (A_2)**
  A property describing the interaction strength between the molecule and the solvent.
- **Fit error**
  This is an indication of the quality of the measurement. The lower the fit error the better the measurement.

⑤ Graph

The results are also shown in graphical form. Please see the **size** description above for details on altering the graph.

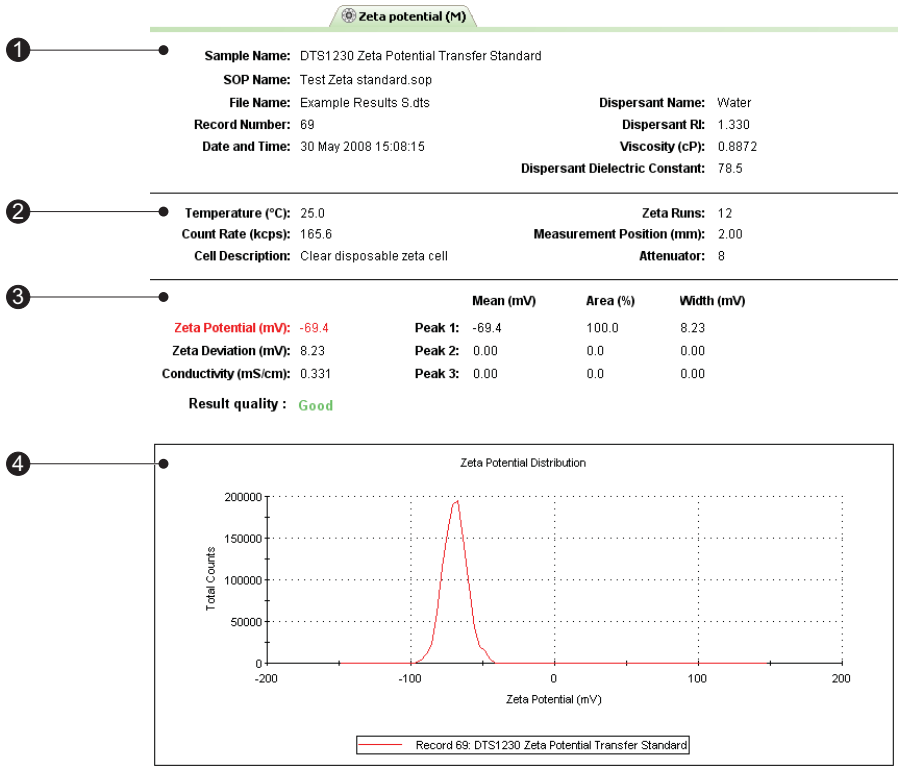
Zeta potential measurements - standard report

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

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

① Sample

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



ill 8445

② System

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

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

Settings specific for zeta potential measurements are:

- **Count rate (kcps)**
  Average count rate for the measurement.

③ Results

The results section gives four pieces of information:

- **Zeta potential**
  Displays the complete zeta potential result of the measurement in mV.
- **Zeta deviation**
  The zeta deviation displays 1 standard deviation of the zeta potential distribution around the mean result, in millivolts (mV).
- **Conductivity**
  The ability of a sample to conduct electrical current. Higher salt concentrations generally allow higher currents to pass and so have higher conductivities. Conductivity can be temperature dependent.
- **Peak means**.
  Displays the mean zeta potential for up to three peaks within the result.

④ Graph

The results are also shown in graphical form.

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

Other Zeta potential reports

Other Malvern views available for zeta potential measurements include:

Electrophoretic mobility (M)

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

Zeta potential quality report (M)

This is a summary report detailing whether the result meets the quality criteria for a zeta potential measurement. The report will indicate "Result meets quality criteria", or "Result does not meet quality criteria" in which case a list of parameters that doesn't meet the criteria, and probable reasons will be displayed.

The parameters, followed by probable reasons displayed are:

- **Phase data poor - signal to noise ratio low**
  Sample concentration too low
  Sample concentration too high (sample looks turbid)
  High conductivity causing sample/electrode degradation
  Measurement duration manually set
- **Distribution data poor**
  Sample concentration too low
  Sample concentration too high (sample looks turbid)

High conductivity causing sample/electrode degradation
Measurement duration manually set

- **Increase Zeta potential analysis range**
  Sample has a wide zeta potential distribution
  High conductivity resulting in an automatic reduction in the applied voltage
  Sample has low dielectric constant or high viscosity
  Mean zeta potential value > +50mV and wall potential >+50mV
- **Suggest use of Monomodal analysis as conductivity is >10mS/cm**
  High sample conductivity
- **Flare from cell wall - check for bubbles, increase sample concentration**
  Sample concentration too low
  Bubbles present in the path of the laser beam
  Capillary cell wall contaminated
- **Sample concentration may be inappropriate (too high or low)**
  Sample concentration too low
  Sample concentration too high (sample looks turbid)
  Bubbles present in the path of the laser beam
  Cell not inserted correctly into the instrument

A **Technical note** is available from Malvern that gives further details about this report.

Flow-mode measurements - Standard report

The Standard Flow-mode report, **Chromatogram Summary (M)**, gives the same information as seen in a standard size report, plus additional information relating to the flow duration and rate used.

The three main peaks in the measurement will also be shown, displaying the sample intensity, width, molecular weight and start and end flow volumes.

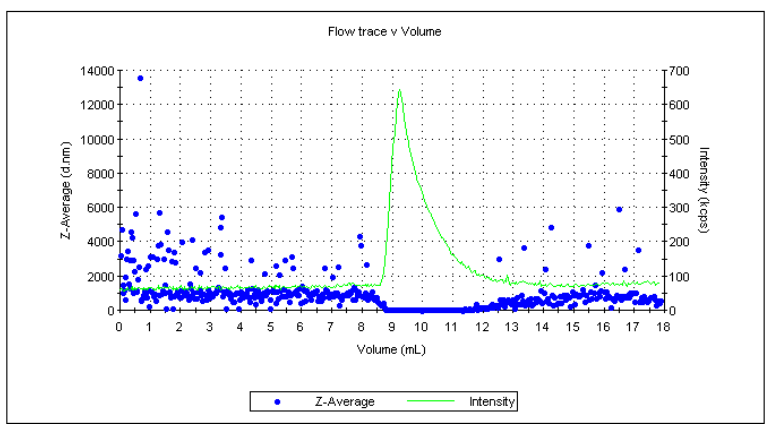
Additionally a the flow trace result graph will be displayed. This can be viewed either in **Time** or **Volume**.:

Chromatogram summary (M)

Sample Name:	Dextran 410 kDa 1 mg/mL		
SOP Name:	mansettings.dat		
File Name:	Flow Test 4-28-06.dts	**Dispersant Name:**	Water
Record Number:	5	**Dispersant RI:**	1.330
Material RI:	1.45	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.00	**Measurement Date and Time:**	28 April 2006 15:49:36

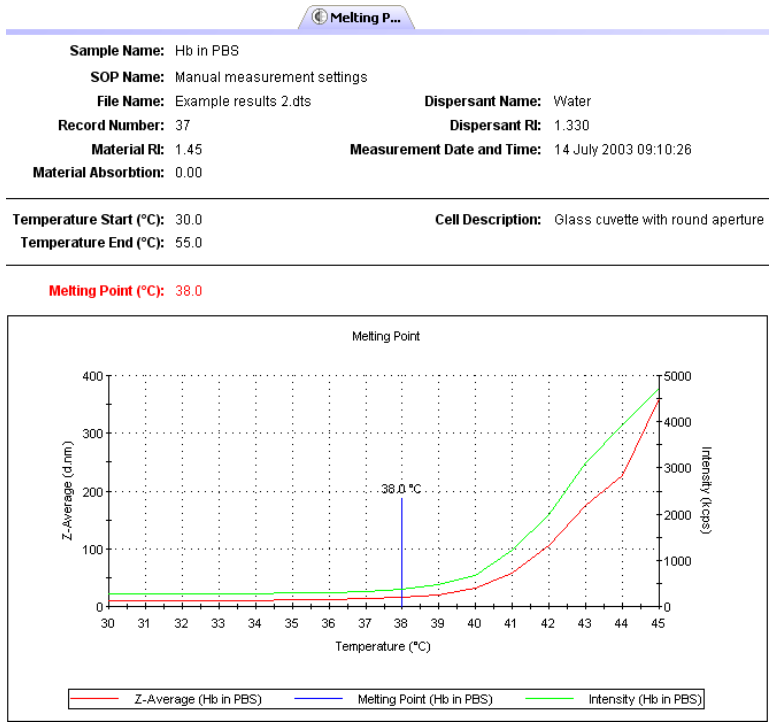
Temperature (°C):	25.0	**Flow Measurement Duration (s):**	0
Sample Time (s):	0	**Measurement Position (mm):**	4.20
Cell Description:	Quartz flow cell	**Attenuator:**	11
Flow Rate:	0.500	**Intensity Threshold:**	0.00

	Diam. (nm)	% Int.	Width (nm)	MW (kDa)	Width (kDa)	Start (mL)	End (mL)
Peak 1:	0.000	0.0	0.000	0.00	0.00	: 0.00	: 0.00
Peak 2:	0.000	0.0	0.000	0.00	0.00	: 0.00	: 0.00
Peak 3:	0.000	0.0	0.000	0.00	0.00	: 0.00	: 0.00



Trend measurements (including Aggregation)

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



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

Additionally an Aggregation 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 (°C)
The temperature at which the Aggregation is achieved.

Titration measurements

How Titration measurements are viewed is explained in the Titrator accessory manual.

Sample preparation

Introduction

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

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

Preparing the sample - Size

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

Sample concentration

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

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

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

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

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

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

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

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

Considerations for small particles

Minimum concentration

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

Maximum concentration

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

- **Gelation**. A gel is unsuitable for measurement using the Zetasizer (this is true for all instruments based on Dynamic Light Scattering).
- **Particle interactions**. If there are interactions between the particles then the diffusion constant of the particles usually changes, leading to incorrect results. A concentration should be chosen to avoid particle interactions.

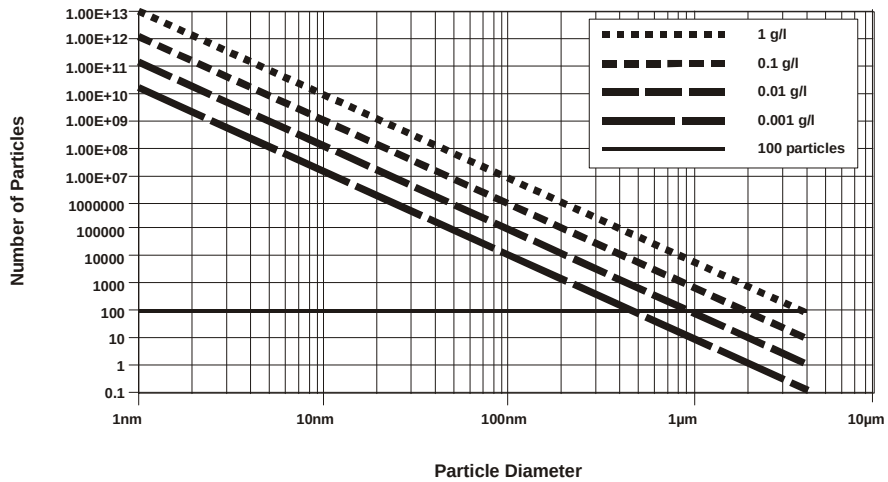
Considerations for large particles

Minimum concentration

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

As an example, if a sample of large particles (say 500nm) were measured at low concentration (say 0.001 g/l (10^{-4} %)), the amount of scattered light generated would be more than sufficient to perform a measurement. However, the number of particles in the scattering volume is so small (fewer than 10) that severe fluctuations of the momentary number of particles in the scattering volume occur.

These fluctuations are not the type assumed by the calculation method used, or will generally be misinterpreted as larger particles within the sample.



ill 6670

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

Maximum concentration

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

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

Filtration

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

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

Using ultrasonics

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

length of time ultrasonication is applied. Some materials can even be forced to aggregate using ultrasound.

Emulsions and liposomes should not be ultrasonicated.

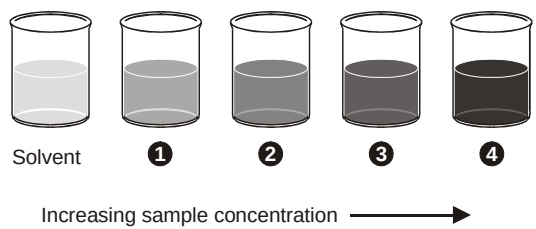
Preparing the sample - Molecular weight

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

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

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

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



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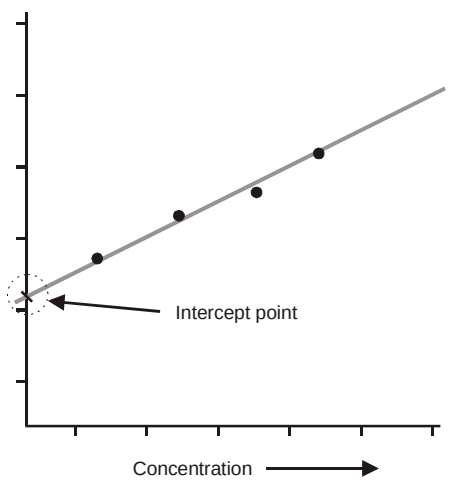
The solvent scattering is measured followed by the various concentrations of sample. From these measurements a Debye plot can be generated.

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



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Minimum concentration

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

Maximum concentration

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

Preparing the sample - zeta potential

The optical configuration on the Zetasizer Nano for zeta potential measurements is discussed in the **zeta potential theory** chapter. A laser is used as the light source and is split to provide an incident and reference beam. The intensity of the reference beam is factory set and is normally between 3500 and 2000kcps. The incident laser beam passes through the centre of the sample cell, and the scattered light is detected at a forward angle. The minimum and maximum sample concentrations that can be measured will depend on the following factors:

- Optical properties of the particles.
- Particle size.
- Polydispersity of the particle size distribution.

When a zeta potential measurement is started, the intensity of the reference beam is measured and displayed in the **Log Sheet** on the **Measurement display**. . The intensity of the incident beam is automatically adjusted so that the intensity of the scattered light being detected is no greater than 300kcps.

The attenuator in the Zetasizer Nano has 11 positions covering an attenuator range of 100% to 0.0003%.

The minimum count rate that is acceptable for a zeta potential measurement to proceed is set to 10kcps. If the sample scattering detected is less than 10 kcps, the measurement will proceed, though the **Expert advice** will suggest that the concentration should be increased if possible.

Minimum concentration needed for zeta potential measurements

The minimum count rate of scattered light required to make a measurement is 10kcps. Therefore, the minimum concentration required will depend upon the relative refractive index (the difference in refractive index properties of the particle

and the medium) and the particle size. The larger the particle size, the more scattered light it produces and hence the lower the concentration that can be measured.

For example, consider a dispersion of a ceramic powder such as titania which has a particle refractive index of around 2.5. This relative refractive index results in a very high level of scattering. Therefore, the minimum concentration of titania with a mean particle size of around 300nm that can be measured could be as low as 10^{-6}% w/v.

If the relative refractive index becomes lower, such as with proteins for example, the minimum concentration will be much higher. A minimum concentration of between 0.1 and 1% w/v may be required to obtain sufficient scattering from a protein solution in order to make a successful zeta potential measurement.

Ultimately, the minimum concentration required for successful zeta potential measurements of a particular sample has to be determined experimentally.

Maximum concentration needed for zeta potential measurements

There is no simple answer for the maximum concentration for a zeta potential measurement in the Zetasizer Nano. The factors discussed above, all have to be taken into account - i.e. the particle size and polydispersity and the optical properties of the sample.

The scattered light from a sample in a zeta potential measurement is detected at a forward angle in the Zetasizer Nano. Therefore, the laser beam has to penetrate through the sample. If the concentration of the sample becomes too high, then the laser beam will become attenuated by the particles reducing the scattered light that is being detected. To try and compensate for these effects, the attenuator position in the instrument will be adjusted to a higher index - i.e. a higher transmission.

The reduced path length of the **high concentration cell** also allows higher concentration zeta potential measurements than either the Folded capillary or Dip cells.

Ultimately, the concentration limits of any sample must be determined experimentally by measuring the sample at different concentrations and seeing the effect on the zeta potential value obtained.

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

Dilution Medium

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

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

Aqueous/Polar Systems

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

If extraction of a supernatant is not possible, then just letting a sample naturally sediment and using the fine particles left in the supernatant is a good method.

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

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

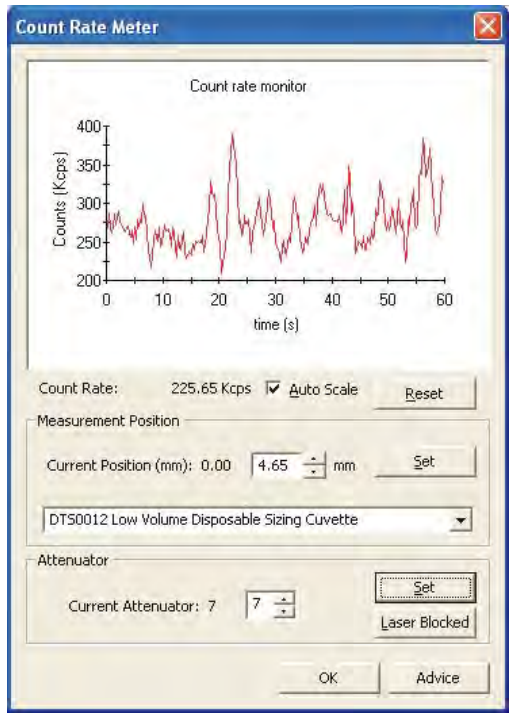
Non-Polar Systems

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

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

Count rate meter

After preparing the sample, the count rate can be checked by using the count rate meter. Selecting **Tools-Count rate meter** will display the following window.



The **Count rate** is displayed in **kcps** (kilo counts per second), and enables the sample quality and concentration to be checked without having to start a measurement.

Count rate monitor

By inspecting the count rate monitor the quality of the sample can be observed. Selecting the **Auto Scale** check box will automatically scale the graph as the count rate monitoring progresses. **Reset** will delete the current display.

- **Normal count rate**
  The monitor will show a relatively stable count rate.
- **Dust in the sample**
  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 unless all measurement runs contain spikes from contaminants.

- **Drifting count rate**
  This may indicate that thermal gradients are present in the sample, and further time is required for temperature equilibration.

- **Increasing/Decreasing count rate**
  A steadily increasing count rate will indicate an aggregating sample, while a decreasing count rate will indicate a sedimenting sample.

In addition to displaying the count rate, the window allows changes to the cell and attenuator position settings. For normal operations both will be at the default setting.

Measurement position

Measurement Position. This will change the measurement position in the cell depending on the cell selected. The measurement position is automatically determined by the software and will be appropriate for most sample measurements. By changing the cell position the effect on the count rate can be investigated.

- **To change the measurement position**, alter the position using the up/down arrows and press Set; this can be done in 0.05mm steps. The detection optics will move to the new requested position.

- **Select the cell** fitted by using the scroll down list. The measurement position will initially move to the optimum position for the chosen cell.

Attenuator

The Attenuator setting can be used to enable the count rates of two samples to be compared. A setting of 1 corresponds to maximum attenuation, and 11 to no attenuation.

- To change the attenuator position, alter the value using the **up/down** arrows and press **Set**. The attenuator holder will move to the new requested position.

- **Select laser blocked** will give 100% attenuation. No laser light will enter the measurement zone.

Measurement angle

For instruments capable of collecting scattered light at more than one angle, i.e. the ZS, ZS90 and Nano Plus, the measurement angle can be selected. Thus the level of scattered light in both the size and zeta potential optics can be measured.

Cell type

The option to select the cell type being used is also available. This allows the instrument to optimise the measurement depending on the type of cell being used at the time.

Security

Introduction

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

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

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

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

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

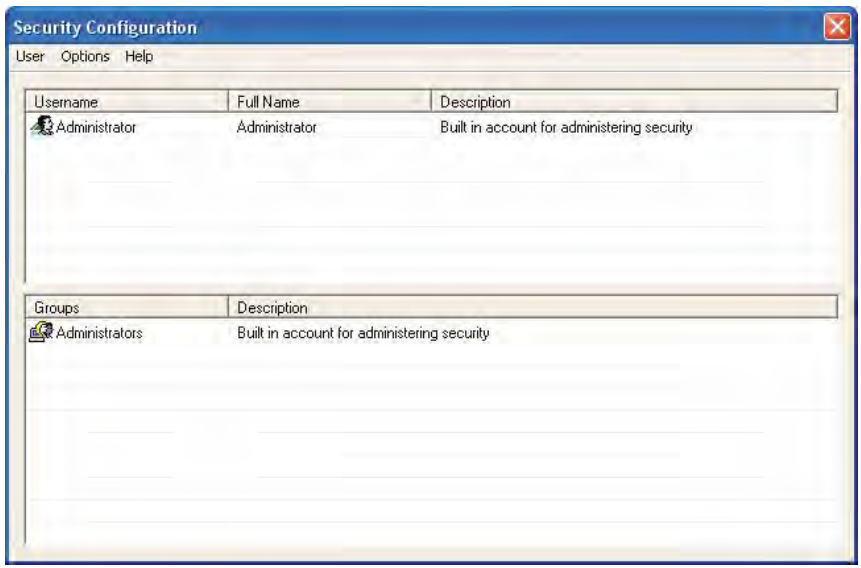
Note
The Malvern security system can be upgraded to 21 CFR part 11 compliance by installing a "Feature key". Once this is installed, 21 CFR part 11 security settings can be applied and 'Audit trails' can be viewed. If the feature key has been installed, the grey 21 CFR 11 icon on the status bar will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

- **The first task is to set up the administrator account.**

Initial start-up - set up the administrator

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

Select **Security-Configure Security**.



The first time the system is run, there will be 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 window is displayed on exiting to give a reminder on saving changes.

Enabling security

Within the **Security configuration** window 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 greyed out.

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

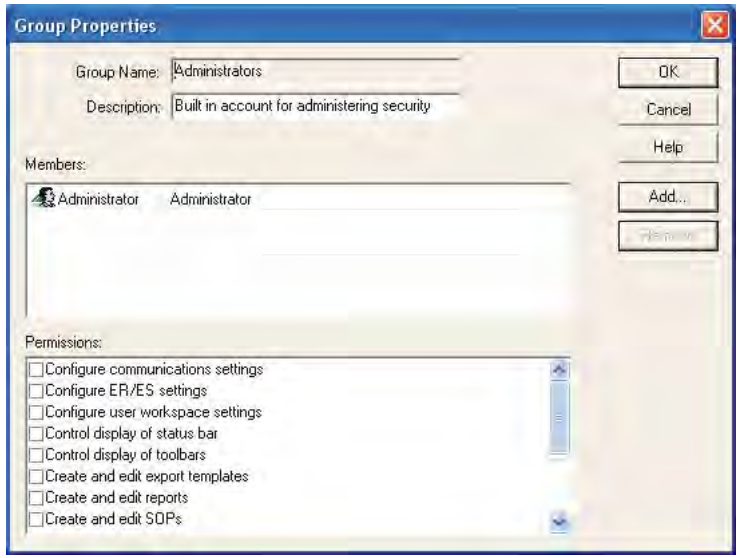
To change operators when the software is already open select **Security-Logout** and then **Security-Login** and enter the appropriate passwords.

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



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

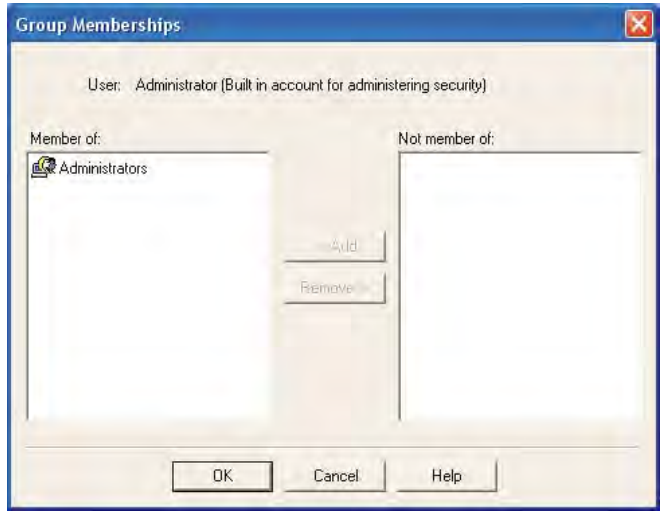
Each user must have a unique **Username**. This, along with the user **Password**, forms the unique key required to identify each individual using the system. The **Username** is commonly an abbreviated form of the individual name or a unique identifier such as an employee code.

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.

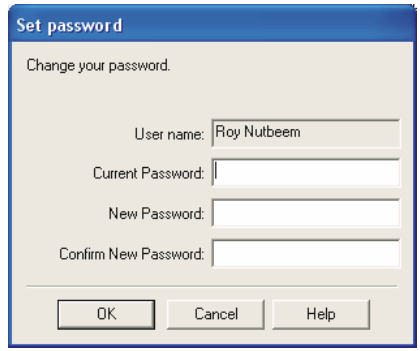
Press the **Groups** button to allocate the user to the required groups. Use the **Add** and **Remove** buttons to allocate the group(s) appropriate.



Password options

- **User must change password at next logon**
  The change password window will be shown when the user next logs in, allowing the user to change their password. This allows the security administrator and a user to set up a user account without the administrator knowing the users final password.

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



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

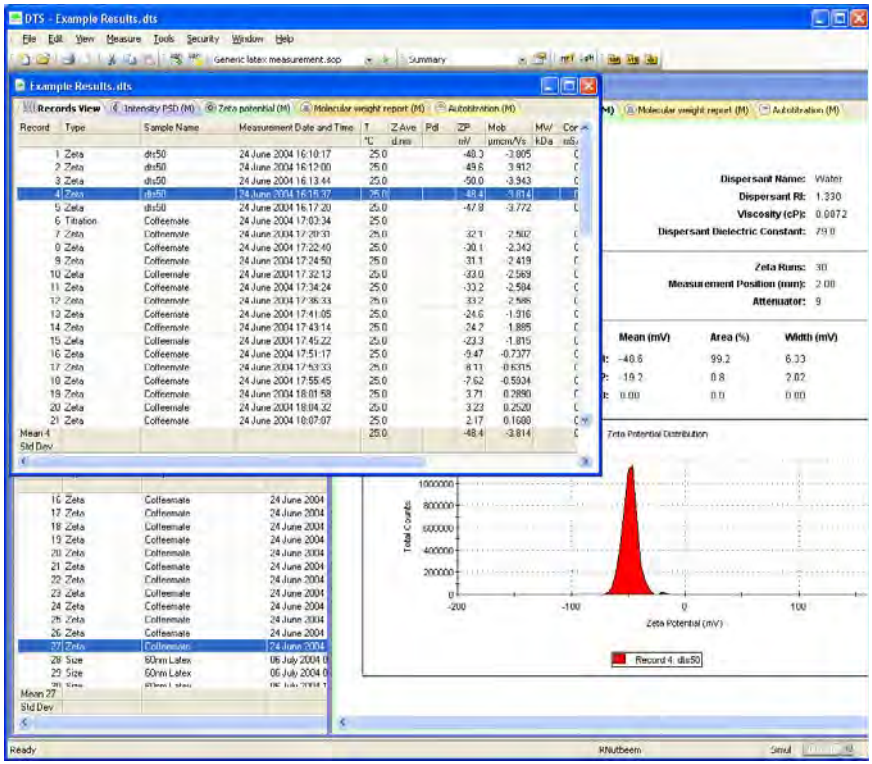
- **User cannot change password**
  This option prevents a user from changing a password once it has been initially set.

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.

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 analysed. Depending upon the **Window** options set, multiple measurement files can be viewed at the same time.



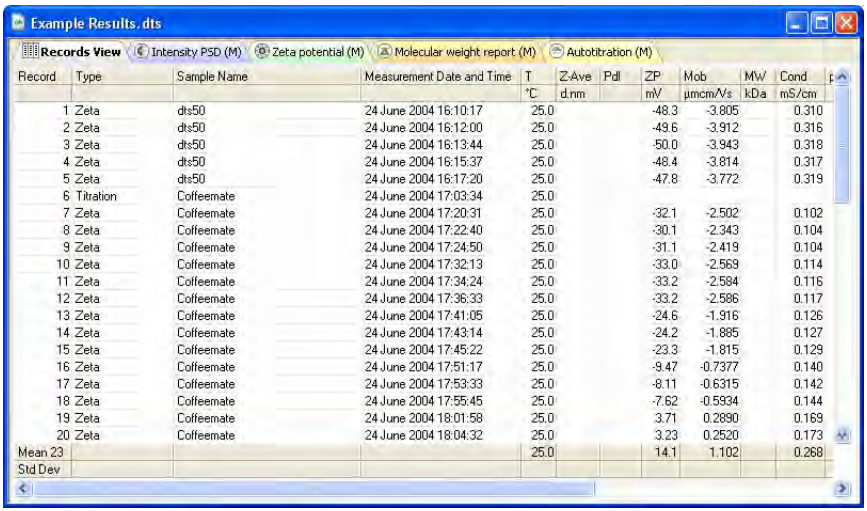
Once a result has been calculated, the data can be displayed as either a record or report - by selecting the appropriate tab.

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 and using custom reports are given in the **Report Designer** chapter.

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 is displayed.



Record	Type	Sample Name	Measurement Date and Time	T	Z-Ave	PdI	ZP	Mob	MW	Cond
				°C	d.nm		mV	µmcm/Vs	kDa	mS/cm
1	Zeta	dts50	24 June 2004 16:10:17	25.0			-48.3	-3.805		0.310
2	Zeta	dts50	24 June 2004 16:12:00	25.0			-49.6	-3.912		0.316
3	Zeta	dts50	24 June 2004 16:13:44	25.0			-50.0	-3.943		0.318
4	Zeta	dts50	24 June 2004 16:15:37	25.0			-48.4	-3.814		0.317
5	Zeta	dts50	24 June 2004 16:17:20	25.0			-47.8	-3.772		0.319
6	Titration	Coffeemate	24 June 2004 17:03:34	25.0						
7	Zeta	Coffeemate	24 June 2004 17:20:31	25.0			-32.1	-2.502		0.102
8	Zeta	Coffeemate	24 June 2004 17:22:40	25.0			-30.1	-2.343		0.104
9	Zeta	Coffeemate	24 June 2004 17:24:50	25.0			-31.1	-2.419		0.104
10	Zeta	Coffeemate	24 June 2004 17:32:13	25.0			-33.0	-2.569		0.114
11	Zeta	Coffeemate	24 June 2004 17:34:24	25.0			-33.2	-2.584		0.116
12	Zeta	Coffeemate	24 June 2004 17:36:33	25.0			-33.2	-2.586		0.117
13	Zeta	Coffeemate	24 June 2004 17:41:05	25.0			-24.6	-1.916		0.126
14	Zeta	Coffeemate	24 June 2004 17:43:14	25.0			-24.2	-1.885		0.127
15	Zeta	Coffeemate	24 June 2004 17:45:22	25.0			-23.3	-1.815		0.129
16	Zeta	Coffeemate	24 June 2004 17:51:17	25.0			-9.47	-0.7377		0.140
17	Zeta	Coffeemate	24 June 2004 17:53:33	25.0			-8.11	-0.6315		0.142
18	Zeta	Coffeemate	24 June 2004 17:55:45	25.0			-7.62	-0.5934		0.144
19	Zeta	Coffeemate	24 June 2004 18:01:58	25.0			3.71	0.2890		0.169
20	Zeta	Coffeemate	24 June 2004 18:04:32	25.0			3.23	0.2520		0.173
Mean 23				25.0			14.1	1.102		0.268
Std Dev										

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 greyed out if no measurement is selected.

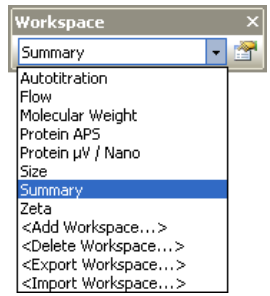
What measurement information is presented in the **Report** and **Record** tabs is controlled by the **Workspace** selection windows (below). The tabs can be configured to display either all the measurement file records and associated reports (i.e. Summary), or only those that are relevant to one measurement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

The following sections deal with the various aspects of the measurement file window. They 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 customise the window views so that, for example, only zeta potential measurements from that file are shown. Similarly a user can create a personalised workspace so that only parameters and reports relevant to 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.



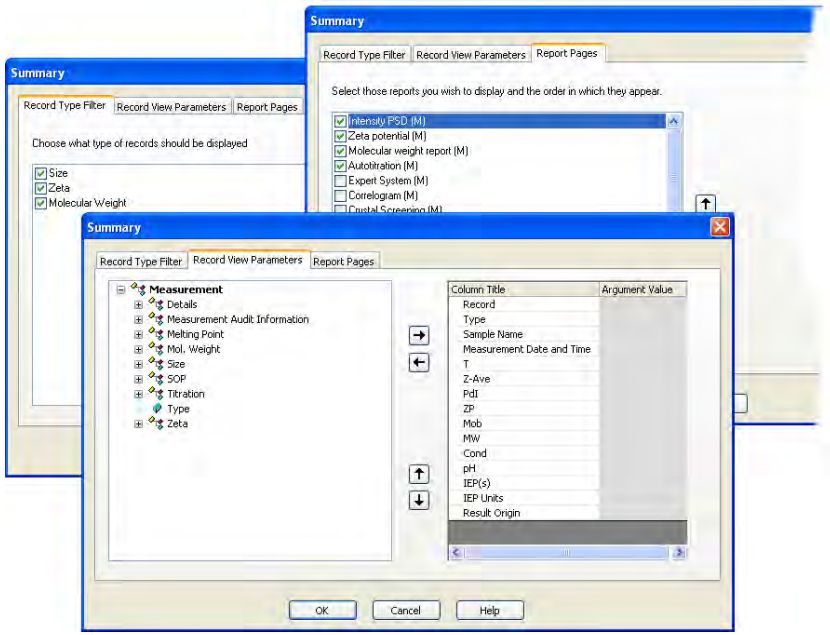
- Workspaces can be **selected** using the **View-Workspaces** menu option or the Workspace toolbar.
- Workspaces can be **managed** using the **Tools-Settings-Configure Workspace** menu option or the Workspace toolbar.
- The workspace toolbar can be turned on and off by selecting **View-Workspace-Workspace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

To Displaying 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 Workspace-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** windows.



3. Use the **Workspace settings** window to display what is required.
4. Use the **Record type filter** to display only specific measurement types in the **Record View**.

   If only the size records are required, select **only** the **size** check box, and leave the zeta potential and molecular weight ones blank.

   If all three measurement types are selected, then all size, zeta potential and molecular weight measurement records will be displayed.
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 customised 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 list and the workspace configure icon , or from the **Tools-Settings-Configure Workspace** menu.
2. This will display the **Workspace Settings** window 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 press **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 window that is displayed, 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

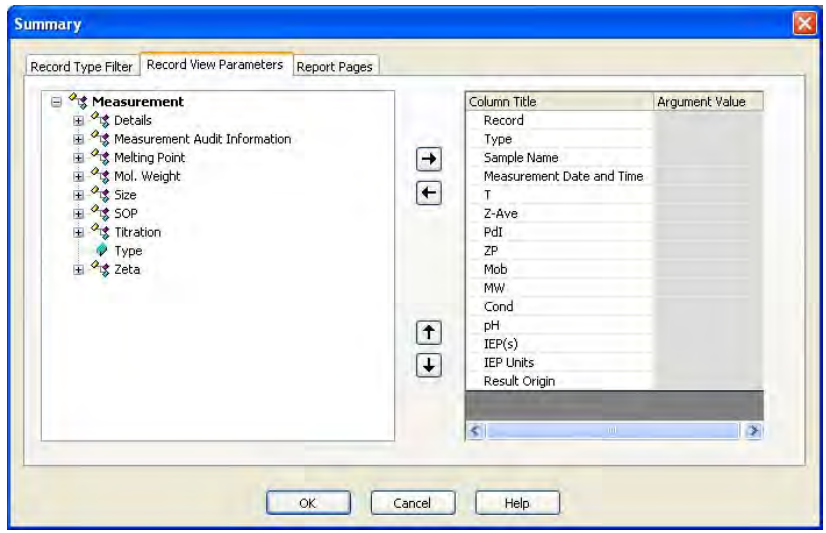
With the **Summary** workspace selected, the default parameters displayed when the software is first installed are : **Record**, **Type**, **Sample Name**, **Measurement Date and Time**, **T** (Temperature), **Z-Ave**, **PDI, ZP** (Zeta potential), **Mob** (Mobility), **MW** (Molecular weight), **Cond**,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

For each workspace the record view can be customised by adding or removing any of the parameters that are stored with the measurement record. The order in which the parameters are displayed can also be rearranged.

Adding and deleting a parameter in the record view

The **Record View Parameters** window 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 are displayed.



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.

Record Number	
Type	
Sample Name	
Measurement Date	
Temperature (°C)	
Dv(90)	
D(v) argument (0 to 1)	0.90

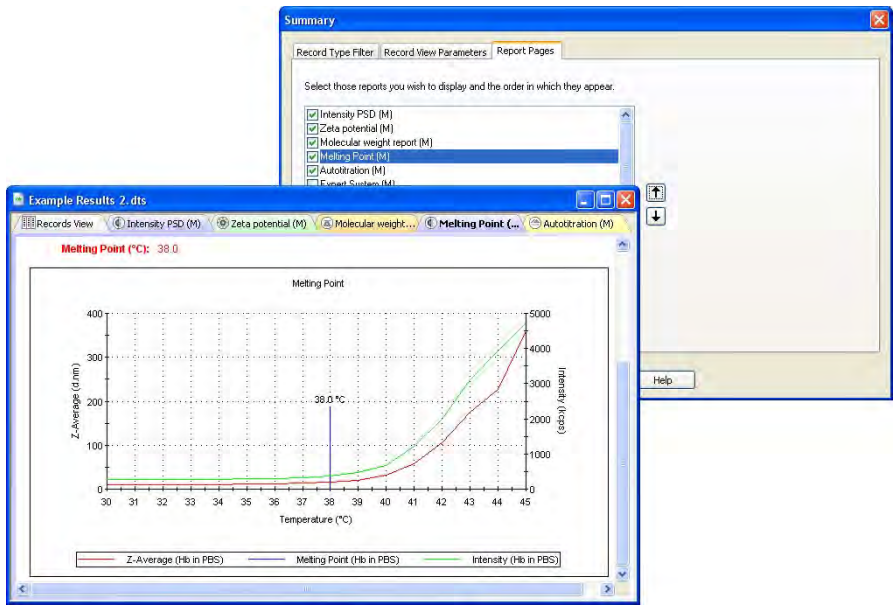
Note
If the software is subsequently updated with a new version, the changed parameter names will be preserved.

Report tabs

The report tabs show every report that has been selected for a workspace. With the **Summary** workspace selected the default reports are **Intensity PSD (M)**, **Zeta potential (M)** and **Molecular weight (M)**. These and other reports can be added or removed using the workspace windows.

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 or records, then move the cursor to within the selected records. Press the left mouse button down until a small rectangle and plus sign appears, then 'drag' the selected record(s) to the other file, and 'drop' - release the mouse button.

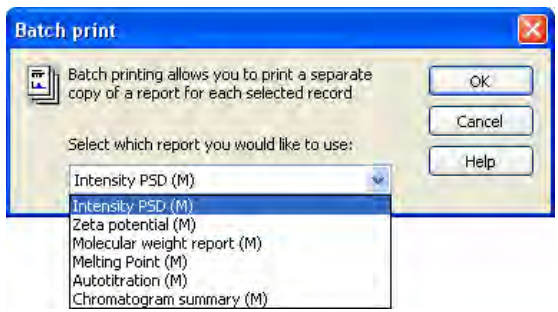
- A record can also be copied by selecting the item then selecting **Edit-Copy**. The item can then be pasted into another file (**Edit-Paste**).

- 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 **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** window is displayed, requesting which report template to print out.



8

Using SOPs

Introduction

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

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

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

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

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

A further measurement type is **Autotitration**. Details on Titration SOPs are described in the Titrator accessory manual.

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

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

SOP Player

The **SOP player** is a stand alone window that enables a sequence of **actions** - SOPs, measurements, pauses or macros - to be constructed and then played. This sequence is called a **playlist**.

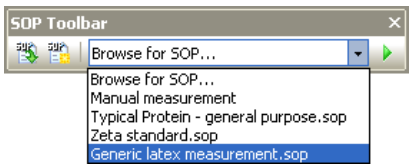
This is useful for performing different measurements types consecutively without any user interaction; i.e. performing size and zeta potential measurements in sequence with appropriate pauses, or macros.

An example application for using the SOP player is a **Temperature trend with titration measurement**:

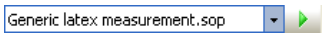
i.e. increase the temperature of a sample - measure,
add some titrant - measure,
decrease the temperature - measure,
and repeat.

SOP Toolbar

The **SOP Toolbar** looks like this:



The **SOP toolbar** allows three functions to be performed.

- Selecting the **New SOP** icon from the toolbar. This will open a new SOP.
- Selecting the **Existing SOP** icon from the toolbar. This will open an existing SOP.
- The **SOP toolbar browser** 

allows an SOP that has been created specifically for a particular type of measurement to be easily selected. This is done by selecting the SOP from the drop-down list and press-

ing the icon. The measurement will now start without first showing the SOP editor.

Alternatively if **Browse for SOP...** is selected and the icon is pressed. The SOP directory will be displayed where an SOP can be manually selected.

The title of the SOP shown is that typed into the **Sample name** entry box in the **Sample** SOP window. Ensure a suitable name is chosen that will identify the measurement has been created to perform.

The drop-down list can show a maximum of seven SOPs. This will always include **Browse for SOP**, **Protein measurement** and **Manual measurement**, plus the last four SOPs used. The last four SOPs will also be shown in the **Measure** menu.

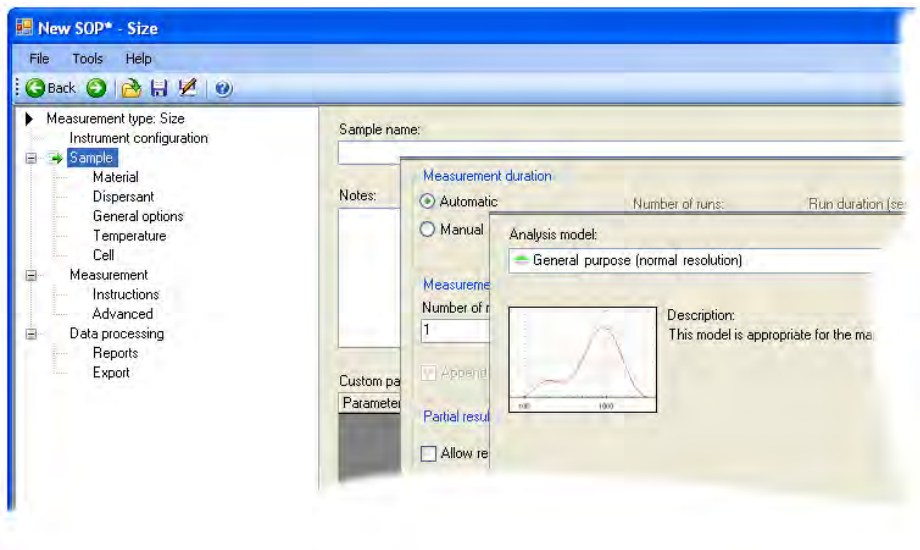
The SOP editor

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).
- Selecting **Measure-Manual** from the Menu bar. This will open the SOP editor (the title bar will show Manual Measurement) where all settings can be configured **immediately** before starting a measurement. Once configured, the settings can be saved as an SOP by using **File-Save as** or pressing the **Save as** icon **.**
- Using the **SOP search** facility in the SOP toolbar (on the main software application). See the **SOP Toolbar browser** description on previous page. Depending upon choice, this will show an SOP that is already configured for a specific measurement.

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 windows available for each measurement type will be displayed down the left hand side of the window, with the respective highlighted SOP window shown alongside. The window will alter as each SOP option is selected.



Note
Always select the Measurement type first when editing an SOP otherwise any changes will not apply.
If the Measurement type is subsequently changed while the SOP is being created a warning will appear giving the option to save any changes.

The windows can be stepped through by using the Back navigation buttons, the **up** / **down** keys on the keyboard, or selecting directly from the record list.

Most of the SOP windows are common to all measurement types; other SOP windows are **unique** to the **measurement type** selected and are discussed in individual **Size**, **Molecular weight**, **Zeta potential**, **Flow** and **Trend SOP** sections. The **Titration** SOPs are discussed in the Titrator accessory manual.

Throughout the SOP windows, some parameters will be available with an option to change the units used in their description. For example, Temperature has the option to use either **°C** (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 windows available are:

Measurement type

Allows selection of the type of measurement that the SOP will perform, i.e. **Size**, **Aggregation**, **Zeta potential**, **Molecular weight**, **Flow**, **Trend** or **Autotitration**.

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.

Instrument Configuration

This window is used to specify the **Instrument configuration** and indicate the options fitted to the instrument.

This window will not be displayed if the computer is connected to a Zetasizer that is switched on. In this case the software will automatically set up the correct configuration.

Instrument configuration - External input

This window allows the characteristics of the specific flow mode input to be configured.

This is a unique window for **Flow** SOPs.

Sample

This window allows the measurement record to be named. All measurements made using this SOP are given the sample name entered in the SOP by default, though an option is available to allow the sample name to be changed when the measurement is made. Information can be entered in the **General notes** box, such as a batch number.

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

Sample - Material

This window 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 window for **all** SOPs with unique features dependent upon the measurement type.

Sample - Dispersant / Solvent

This window allows the physical properties of the sample **dispersant** (or **solvent**) to be set. The refractive index and viscosity can be entered for the dispersant used.

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

Sample - Standard

This window allows the physical properties of the standard that will be used for molecular weight measurements to be set. The Refractive index and Rayleigh ratio can be entered for the standard used.

This is a unique window for **Molecular weight** SOPs.

Sample - General Options

This window allows additional physical properties of the sample to be set.

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

Sample - Temperature

This enables selection of the measurement temperature to be set. A equilibration delay option is also available.

This is a common window for **all** SOPs **except** Trend and Aggregation.

Sample - Cell

This window allows selection of the cell to be used.

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

Measurement

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

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

Note

The features available will alter dependent upon the cell type fitted. Where applicable consult the relevant cell user guide for measurement details.

Measurement - Instructions

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

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

Measurement - Advanced
Use the **Advanced** window to alter the cell and attenuator position settings.

Measurement - Flow settings
Enables the flow rate of the sample through the instrument and connecting tubing to be entered.

This is a unique window for **Flow** SOPs.

SZP measurement

This option enables measurements specific to surface zeta potential measurements to be input. These include the number of displacements from the sample surface and the number of individual zeta potential measurements to be made at each displacement.

This is a unique window for **surface zeta potential** SOPs.

SZP measurement - Advanced
Under this option, custom attenuation and voltage settings can be selected.

Tracer measurement
This option allows the measurement duration and the number of measurements of the tracer particles to be set. The distance from the sample surface at which this measurement is made, is also set.

This is a unique window for **surface zeta potential** SOPs.

Data Processing

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

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

Data Processing - Reports
This window 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 window 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 window 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 a common window for **Size** and **Zeta** trend measurements.

Titration - Titrants

The **Titrants** window enables the titrants used to be specified, and the pump and stirrer speed to be set.

Titration - Sequence

The **Titration sequence** window enables the titration measurement details to be selected, such as the number of points, the range of the titration and the equilibration time.

- Refer to the Titrator accessory manual for full details of the Titration SOP windows.

Microrheology SOP settings

Refer to the Zetasizer Nano accessories guide for full details of the Microrheology SOP windows.

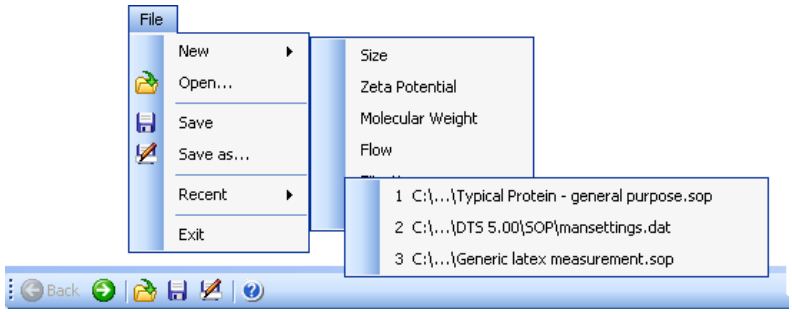
Protein mobility SOP settings

Refer to the Zetasizer Nano accessories guide for full details of the Protein mobility SOP windows.

All SOP windows are described in detail in the following sections or the respective accessory manual

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
		Use to move up and down the SOP tree menu. The SOP window will alter to match the selection

Creating an SOP

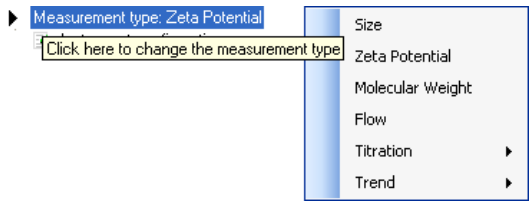
- Select **File-New-SOP**. This will open up the **SOP Editor**.
- Select the ▶ **Measurement type** required (see next section).
- Change the settings within the SOP windows. These are described in the following pages under the Measurement type selected (e.g. Size SOPs).
- When all the SOP settings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

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

  Zeta potential

  Molecular weight

  Flow

  Surface Zeta potential

  Microrheology
  Measures the mobility of tracer particles of known size in order to calculate the viscoelastic modulus of a sample in the linear viscoelastic range.
  Note that this option is a paid for accessory option in the software.

  Titration
  Titration has two sub-menus where the titration can be selected to measure pH, Dilution, Additive or Additive log (conductivity) as a function of Size, zeta potential, Size and zeta potential or Intensity.

  Trend
  Trend has two sub-menus where a temperature trend can be selected to measure against size or zeta potential or intensity.
  Aggregation measurements can be performed using the Trend measurement type.

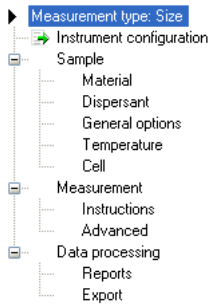
- When selected, the content of the SOP tree menu changes to match the measurement type.

Note
The following **Size SOP** section describes the SOP windows shown when a **Size Measurement type** is selected. This **includes** all sizing SOP windows **and all** windows that are common to **all** SOPs. When requested in the Molecular Weight, Zeta potential and other SOP sections refer back to the **Size** SOP section for a description of the window.

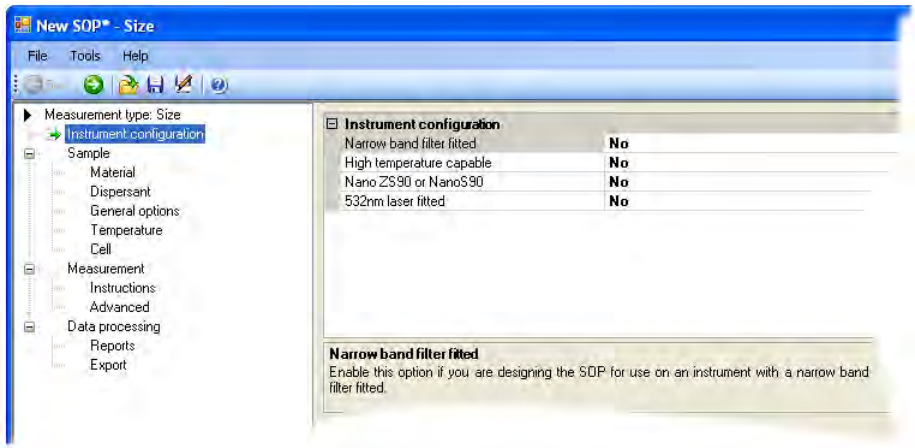
Size SOPs

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

The **Size SOP Editor** menu is:



Instrument Configuration



Note
The Instrument configuration window is **only** displayed when the software is **not** connected to an instrument. When the instrument is connected, the software automatically detects the instrument type and sets the parameters accordingly.

When creating an SOP, with **no** instrument connected, it is necessary to specify the **Instrument configuration** and indicate the options fitted to the instrument.

The options available are:

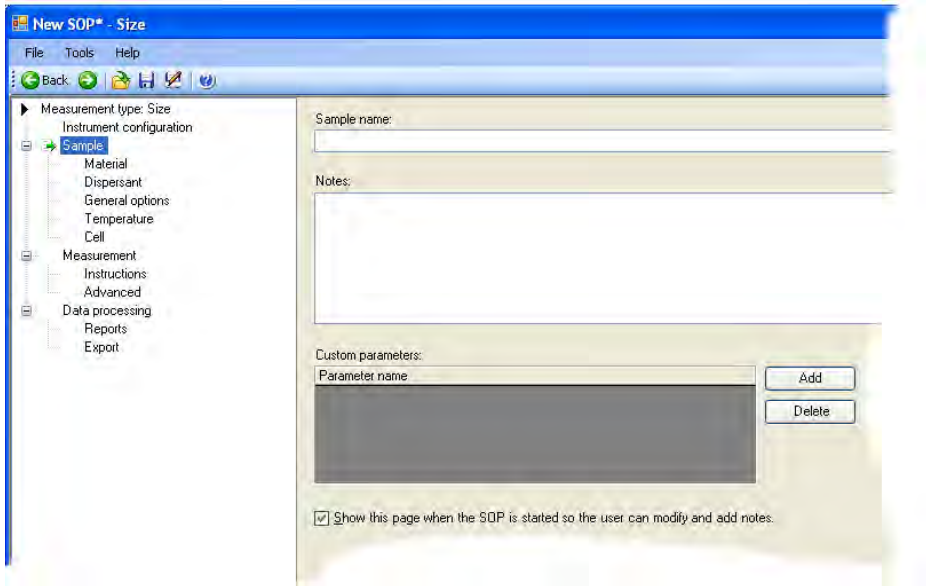
- Narrow band filter fitted
- High temperature capable
- Nano ZS90 or Nano S90
- 532nm laser fitted

To enable the options fitted, double-click on the required option to indicate **Yes** or **No** as appropriate.

After connecting a Zetasizer Nano and switching it on, the warning message (below) may be shown depending on the measurement or instrument settings selected.

- **The selected settings do not match your...**
  If the instrument settings do not match the instrument connected, a warning is displayed on the window. Ignore this message if the SOP is for another instrument or the computer is not connected.

Sample



The components of the window 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.

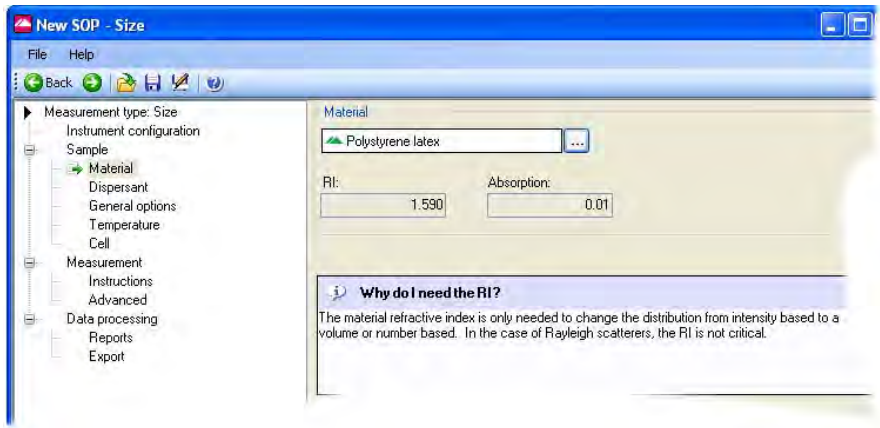
Show this page when the SOP is started…

With this check box selected the **Sample identification** window is displayed 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 is displayed immediately when the SOP is started. To add comments, press the **Settings** button on the display.

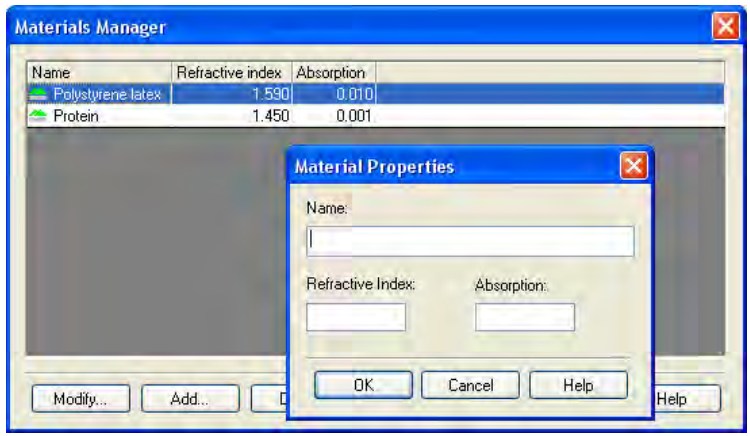
Sample - Material

The Zetasizer software requires certain information about the physical properties of the dispersant that is used in the sample that is to be measured. Selecting the **Sample - Material** window 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** window. 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.

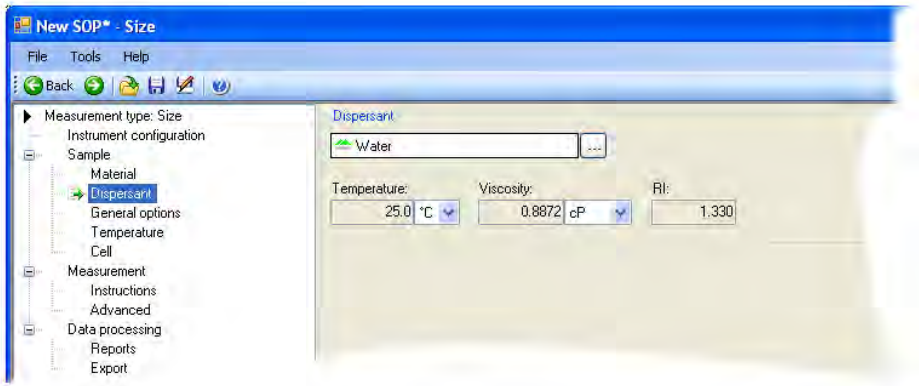
- The **Refractive index** can be obtained from a number of sources and is only required to two decimal places. Note that the refractive index is wavelength dependent.
- The **Absorption** is the quantity of light absorbed by the material when suspended in the dispersant. Generally clear samples will have a low or zero absorption while coloured or black samples will have a high value.

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

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

Sample - Dispersant

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

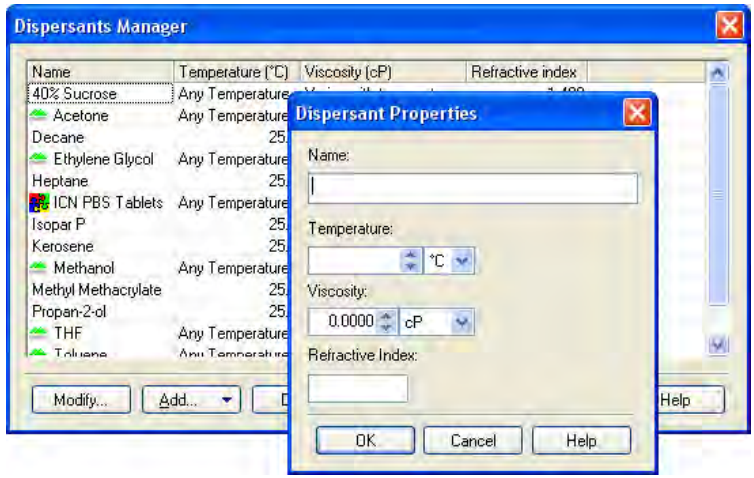


Dispersants Manager

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 **Advanced features** chapter.

On selection of a **Simple dispersant or solvent** the **Dispersant Properties** window is displayed, 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.

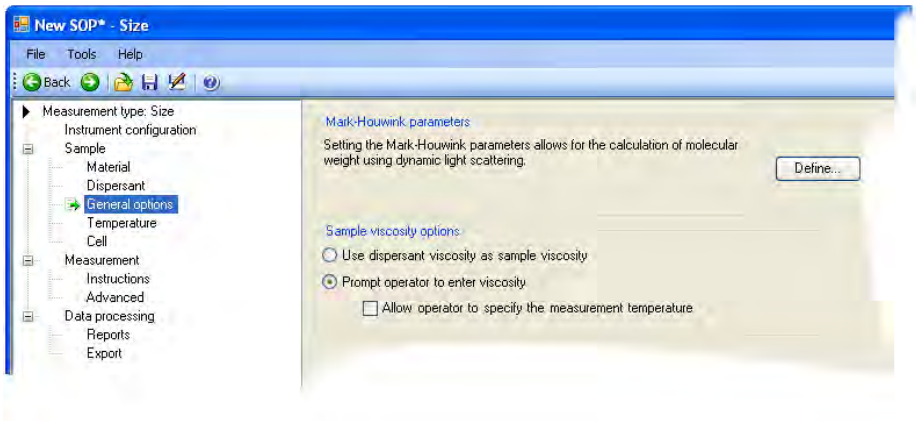
Note
Viscosity is temperature dependent. The Malvern defined dispersants include a built in viscosity calculation that determines the correct viscosity of the dispersant at any sample temperature.

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** window is displayed, 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** window.

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 the 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 viscosity options

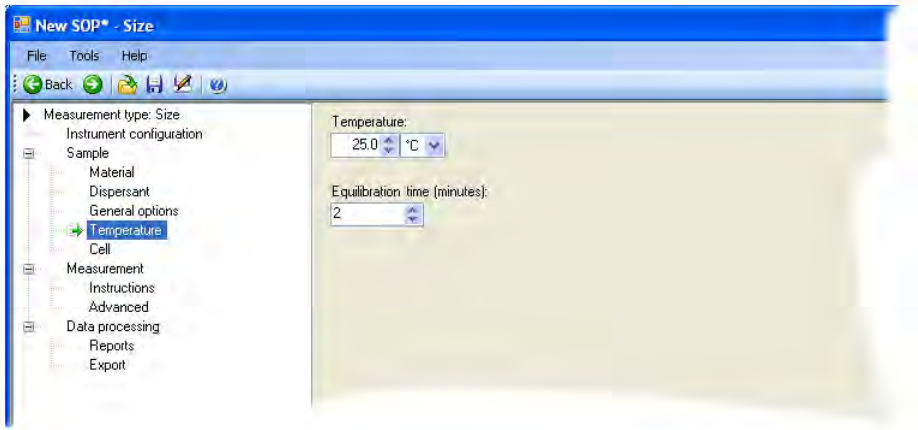
This area is useful for customising the viscosity parameters.

If working at low sample concentrations it may be useful to use the **dispersant viscosity** as **sample viscosity**. At high dilutions, the assumption that the sample

viscosity is the same as the dispersant viscosity is usually valid. If this is not the case, or at higher sample concentrations (over 0.1%) then the sample viscosity should be measured and this value used.

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

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.

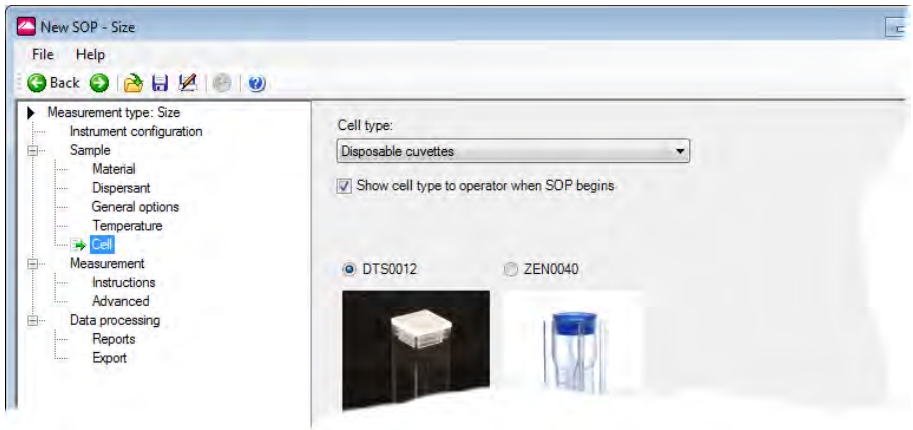
Note
To improve the stability of temperature control when using the system as a Gel Permeation Chromatography (GPC) detector, the measurement temperature should be set to be 5°C or greater, above or below the ambient temperature.

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 - Cell



Cell type

Select the appropriate cell from the list. The choices match the range of cuvettes and cells available from Malvern Instruments. Once selected a description of the cells is given.

- To choose a cell, select the **Cell type** from the drop down list, then from the displayed cell choices, select the specific cell that will be used for the measurement.

Note
Some cells look very similar so it is important that the cell selected matches the one being used. Consult any advice on the cell window page.

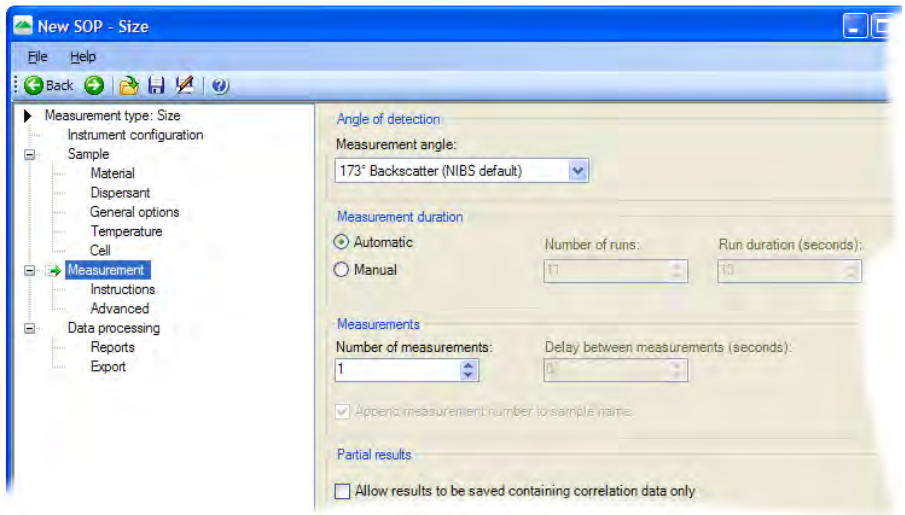
Note that only the cells appropriate for the measurement type selected will be displayed. E.g. if a **size** measurement is selected, then only the size cells will be listed. For measurements using other cell types please contact Malvern for compatibility.

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

The **Default** selection shown will be appropriate to the measurement type selected - for a size measurement, the cell shown is the **Disposable sizing cuvette**.

Measurement

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



Angle of detection

Note
This option is only available on Nano ZS, ZS90 and Nano plus instruments.

Multi-angle detection is used to ascertain the **Aggregation Index** of a sample. The Aggregation Index is used to quantify the presence of particles such as dust or aggregates within the sample. To do this an additional measurement will be performed in the 'forward' direction; this direction being more sensitive to the presence of aggregates.

The Aggregation Index parameter can be added to the **Record view** using the **Workspace** selection windows: **Size** parameters.

Choices are as indicated below:

- **175° Backscatter** performs a "standard" measurement at an angle of 175°
- **12.8° Forward-scatter** performs a measurement at an angle of 12.8°
- **Dual-angle** performs a measurement at both angles - this measurement is used for calculating the Aggregation Index.

  For each Dual measurement a "Parent" record will be entered into the Records view. On opening this record, both the forward and backward measurements can be viewed. Highlighting both the forward and backward measurements and selecting **Expert advice** will also display the Aggregation Index value.

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.

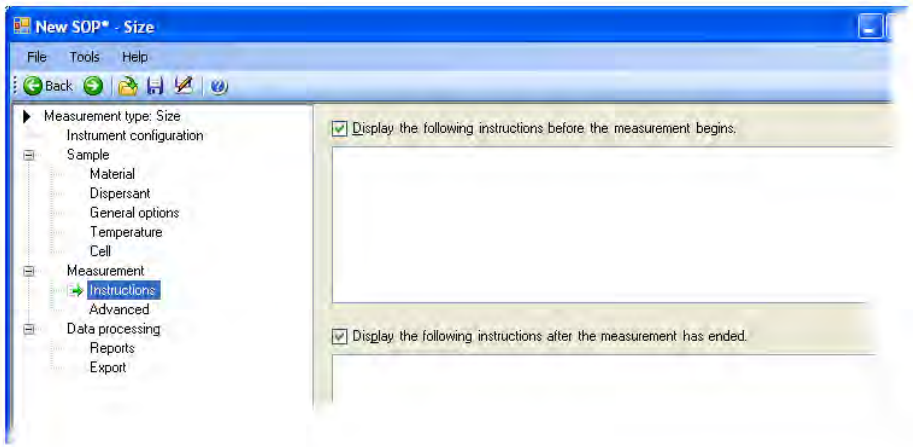
The **Append measurement number…** check box will append an incremental number, i.e. 1, 2, 3 etc, to the record name for each measurement made. The incremental number is reset back to 1 each time the SOP is started.

Partial Results

If it is likely that a measurement will not produce a correlation function that can be analysed - 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 - Instructions

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



This window will not be displayed when doing a manual measurement.

Display the following instructions…before…

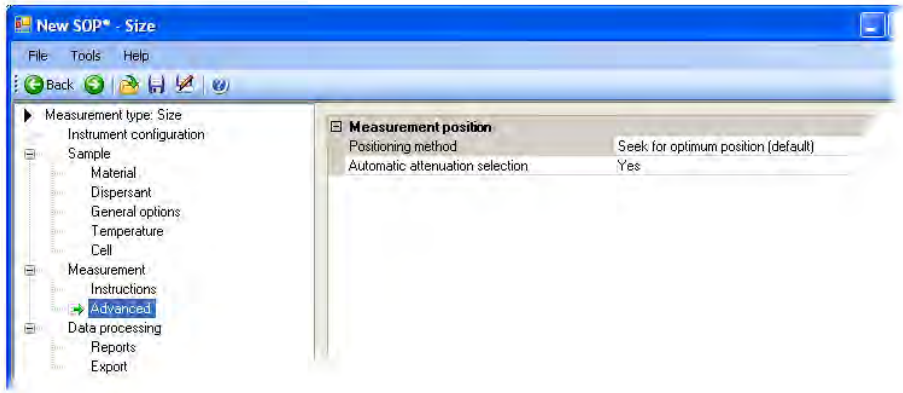
This area is useful to give details that will allow measurements to be made correctly and consistently; e.g:

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

Display the following instructions…after…

With this check box selected post-measurement instructions can be displayed.

Measurement - Advanced



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

Measurement position

Positioning method

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

- Choices are: The **Centre of the cell**, **Seek for optimum position** (**default** setting) or **Fixed Position** where a appropriate measurement position can be used for the particular sample.

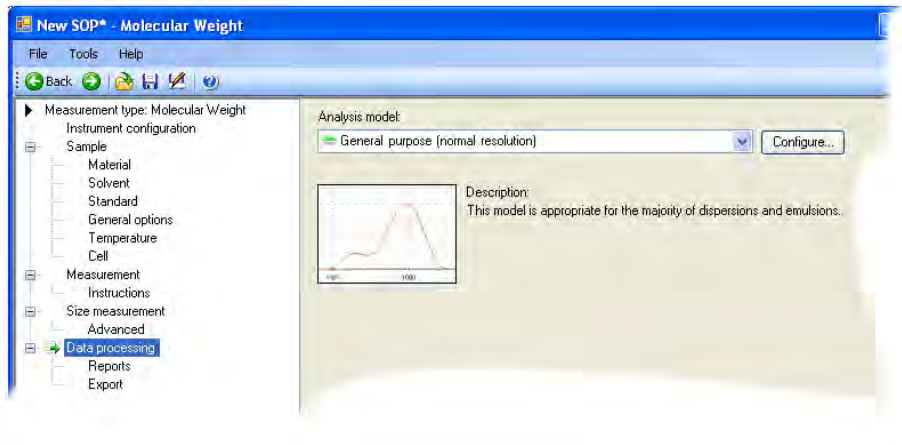
By changing the cuvette position the effect of performing a measurement at different positions within the cell can be investigated.

Automatic attenuation selection

This can be used to enable the count rates of two samples to be compared, or to speed up the optimization stage prior to a measurement being performed.

If **No** is selected, a parameter appears that allows setting of the attenuator position.

Data Processing



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

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

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

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

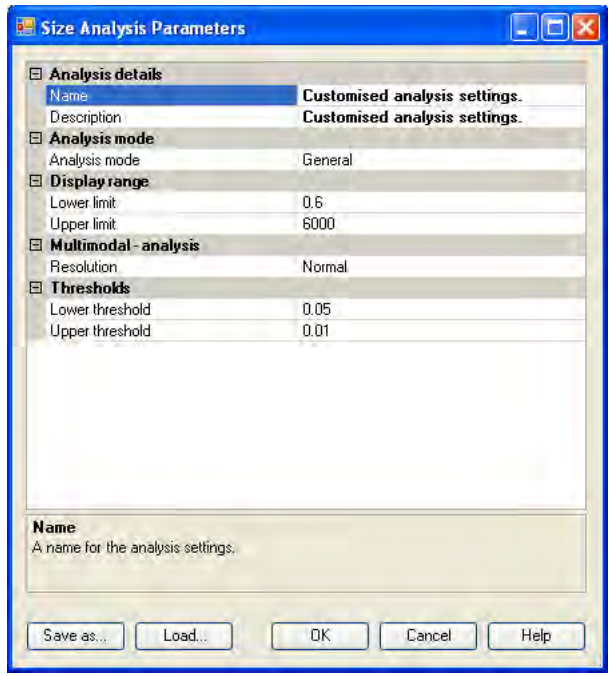
Analysis Models

- **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.
- **Protein analysis**
  The Protein Analysis method is suited to protein applications where samples typically consist of narrow peaks at small sizes plus aggregates.
- **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 window 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 window and use the analysis model; the new analysis model name will now be displayed in the **Analysis model** entry box on the **Data Processing** window.

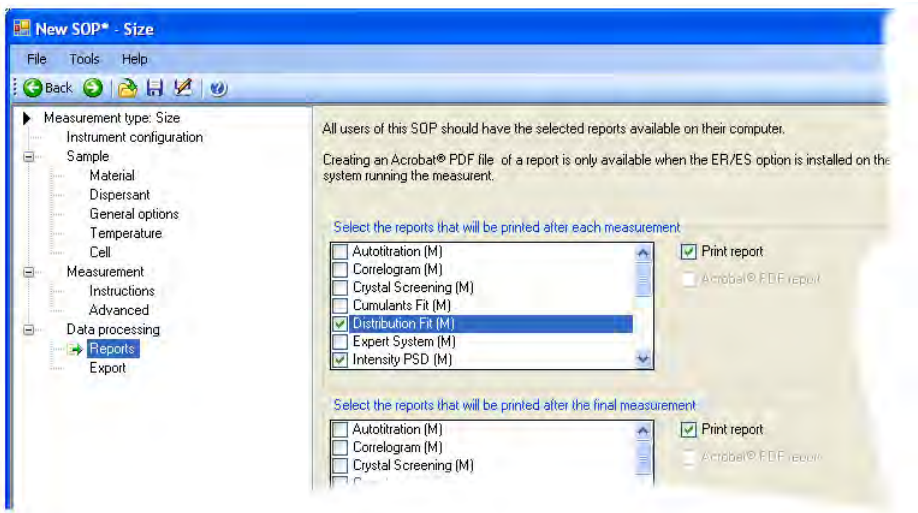
Note
If no name has been given, the analysis model will be displayed as **Customised analysis settings** in the **Analysis model** entry box.

Previously saved analysis models can be used, viewed and altered by pressing the **Load** button. Pressing **OK** will exit the window 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** window 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

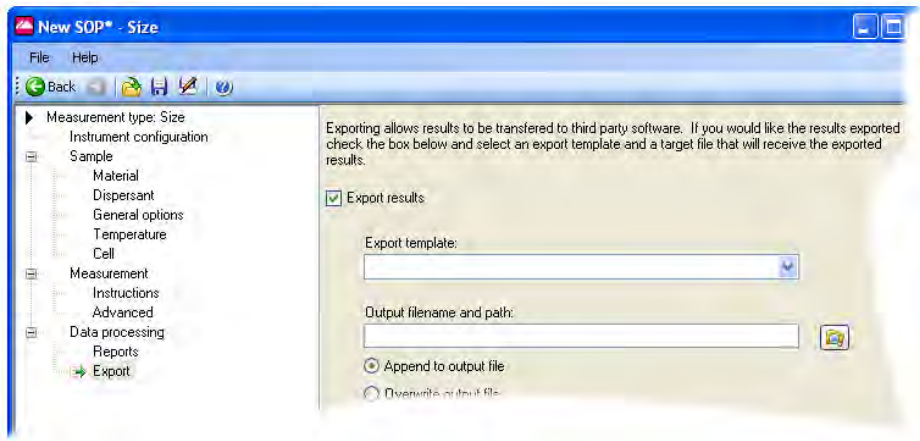
and then the reports required. Any reports created using **Report Designer** will automatically be added to this list.

If an **Autotitration** measurement is being performed, the print reports after each measurement option will be 'greyed' out and unavailable. This is to prevent a report being printed after each 'individual' titration measurement. A report will be printed only after the **final** titration has been completed.

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** window 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 windows described in **Advanced features** chapter. Refer to this chapter for full details on creating the export templates.

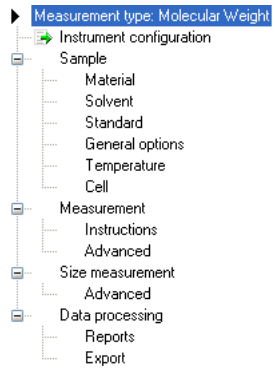
The parameters will be exported as a text file to the specified **output filename…** and saved.

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.

Molecular weight SOPs

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

The **Molecular weight SOP editor** menu is:

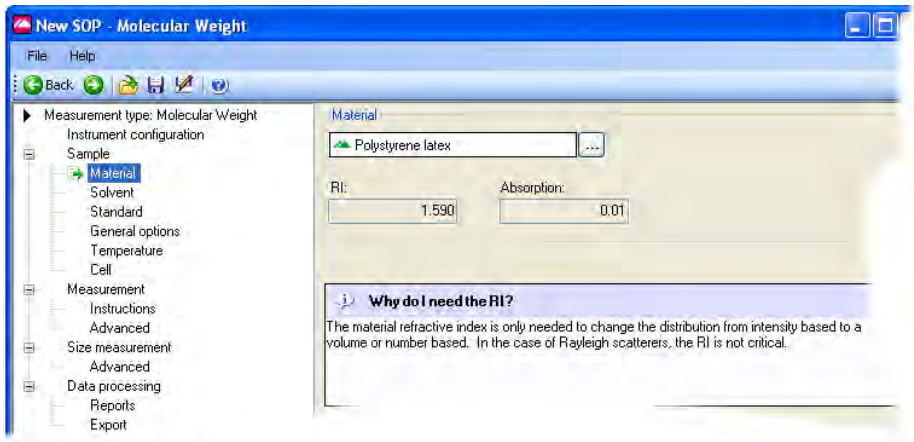


Sample

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

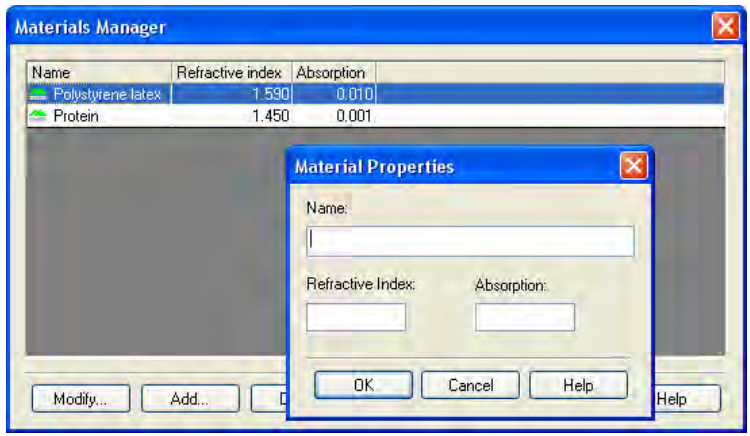
Sample - Material

The Zetasizer software requires certain information about the physical properties of the dispersant used. By selecting the **Sample - Material** window and pressing the ... button, the **Materials properties manager** is displayed where these properties can be defined.



Materials Manager

Selecting this window allows new material to be added, modified and deleted.



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

Please refer to the **Sample - Material** description in the **Size SOP** section for details.

Sample - Solvent

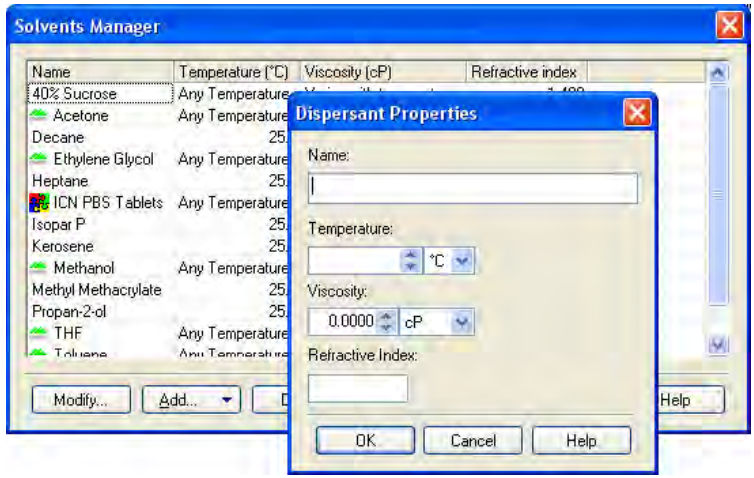
The Zetasizer software requires certain information about the physical properties of the dispersant that is used in the sample that is to be measured. By selecting the **Sample - Solvent** window and pressing the ... button, the **Solvents properties manager** is displayed where these properties can be defined.

Solvents Manager

Selecting this window enables solvents to be added, modified and deleted.

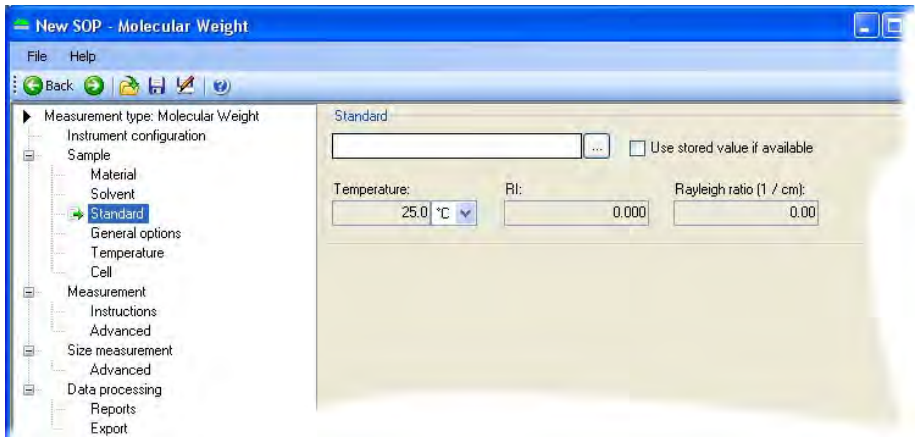
To define a new Solvent, press the **Add...** button and choose from defining either a **Simple dispersant or solvent** or a **Complex solvent**.

Please refer to the **Sample - Dispersant** description in the **Size SOP** section for details.



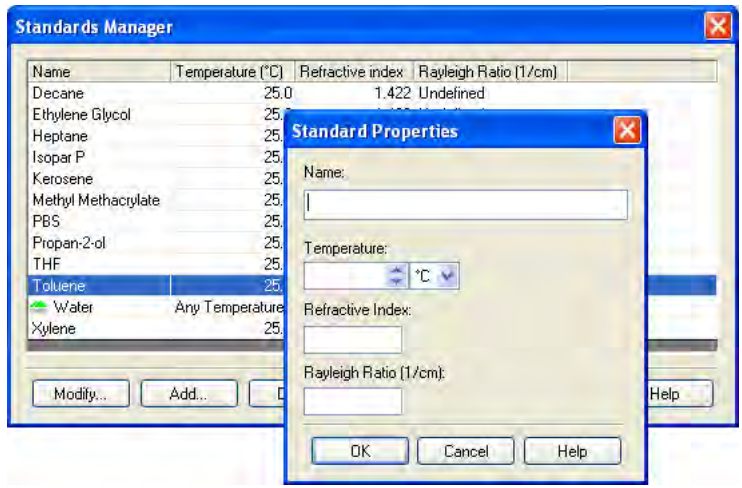
Sample - Standard

The Zetasizer software requires certain information about the "Standard" used for laser intensity calibration. By selecting the **Sample - Standard** window and pressing the [...] button, the **Standards properties manager** is displayed where these properties can be defined.



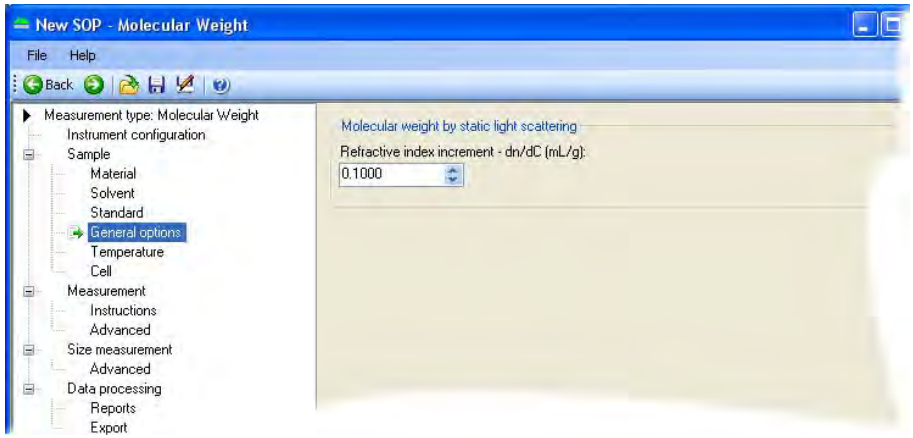
Standards Manager

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



The standard properties can be added, modified and deleted in the same way as detailed for the **Solvent Manager** above.

Sample - General Options



Molecular weight by Static Light Scattering

Input here the differential refractive index increment (dn/dc) of the solvent. This is the change in refractive index as a function of the change in concentration. For

many sample/solvent combinations this may be available in literature; while for novel combinations the dn/dc can be measured by use of a sensitive differential refractometer.

Note
This will require a refractometer capable of measuring to an accuracy of six decimal places.

Sample - Temperature

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

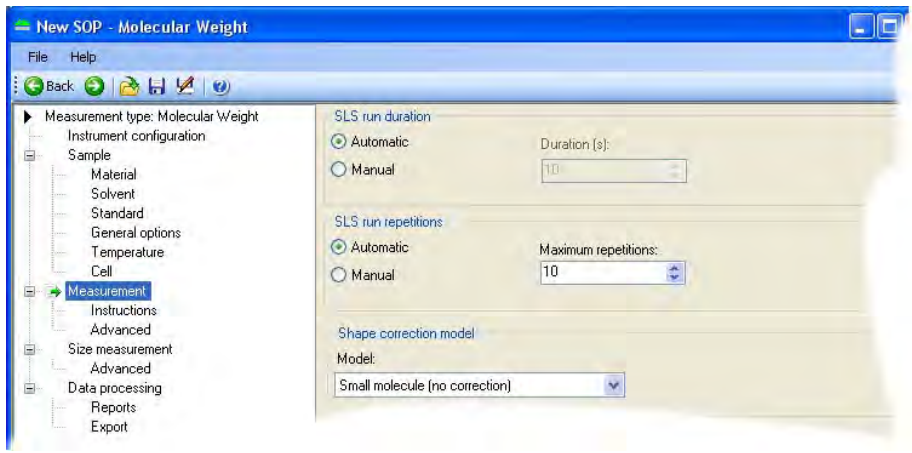
Sample - Cell

Please refer to the **Sample - Cell** description in the **Size SOP** section.

The **default** selection is a **Low volume glass cuvette**.

Measurement

The **Measurement** window allows the duration of the measurement to be set, and multiple measurements of the same sample to be made.
Also refer to the **Measurement-Advanced** window below



SLS run duration

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

With **Automatic** selected, the software will automatically determine the measurement duration.
With **Manual** selected the measurement will use the user defined settings

Shape correction model

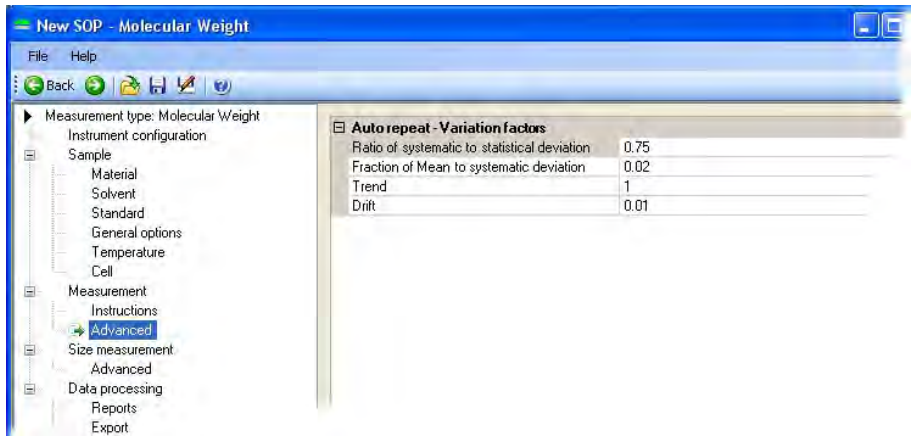
This option allows selection of the shape correction model used for the measurement. With knowledge of the sample structure it is possible to improve the result of the measurement by adding the value that most closely corresponds to the probable sample shape; i.e. sphere, coil, cylinder or no shape correction.

Measurement - Instructions

Please refer to the **Measurement - Instructions** description in the **Size SOP** section.

Measurement - Advanced

The purpose of this window is to allow users the option to adjust the (**variation**) **factors** that are used in determining the length of an '**automatic duration**' measurement; as detailed in the **Measurement** window above.



The measurement process takes a sequence of count rate measurements, one every 20 milliseconds, for the run length specified.
This automatic default will normally be 10 seconds though this may increase for lower scattering samples to help increase the accuracy. The **Run duration** range should typically be within the range 10 to 50 seconds.

Performing only one run may not always be sufficient to achieve a good measurement of the intensity. This is because the sample scattering will normally show some systematic variations, as well as an expected random variation about a mean value.

These systematic variations can occur for a few reasons; possibly thermal drifting of the sample, poorly mixed sample, or instrument and laser stabilisation after initial switch on. (Wait 30 minutes after switch on for the laser to stabilise).

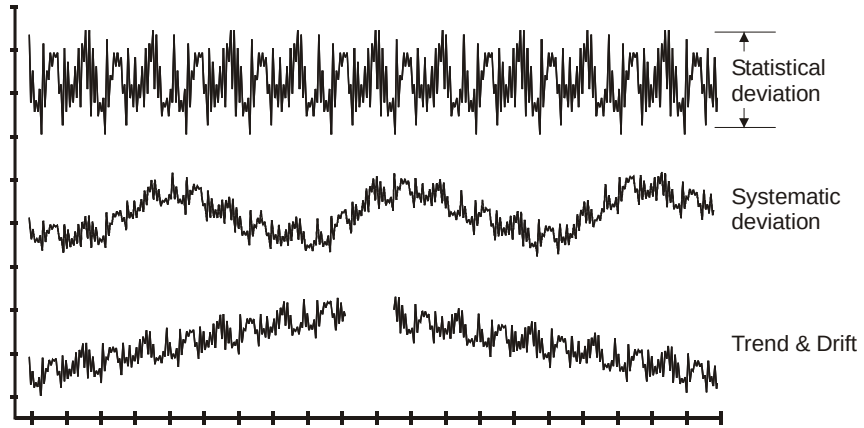
If any variations are detected that are greater than the set values, then the measurement will be repeated until a result below the thresholds is achieved.

Adjusting the **Variation factors** will enable a percentage of these systematic variations to be ignored during the measurement process, therefore allowing a quicker measurement to be performed.

The **default** variation factors are:

- **Ratio of systematic to statistical deviation**
  The systematic deviation should be < **0.75** *x* the statistical noise
- **Fraction of Mean to systematic deviation**
  The systematic deviation should be < **0.02** *x* the mean
- **Trend**
  The trend < statistical deviation (*x* **1**) - the default factor
- **Drift**
  The trend should be < **0.01** *x* the mean

During a measurement the size of these components is compared to the random noise and the mean value. The run will be accepted if they are sufficiently small.



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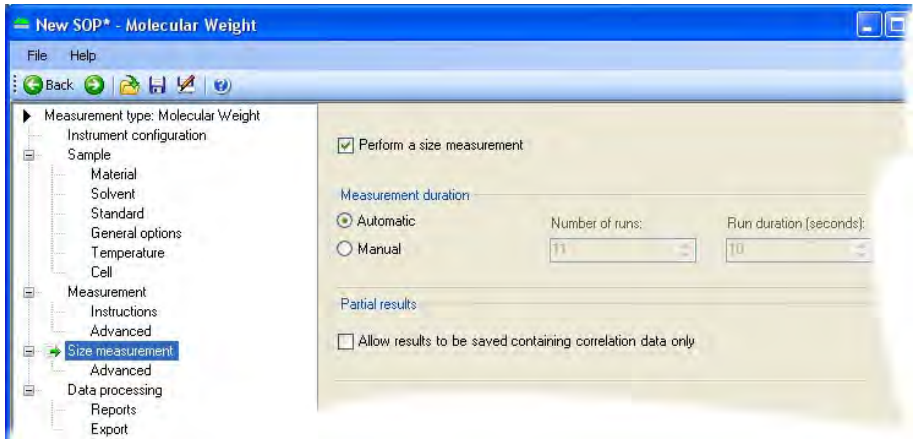
Size Measurement



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

Measurement Duration

The **Measurement Duration** setting can affect the accuracy and repeatability of the size results.

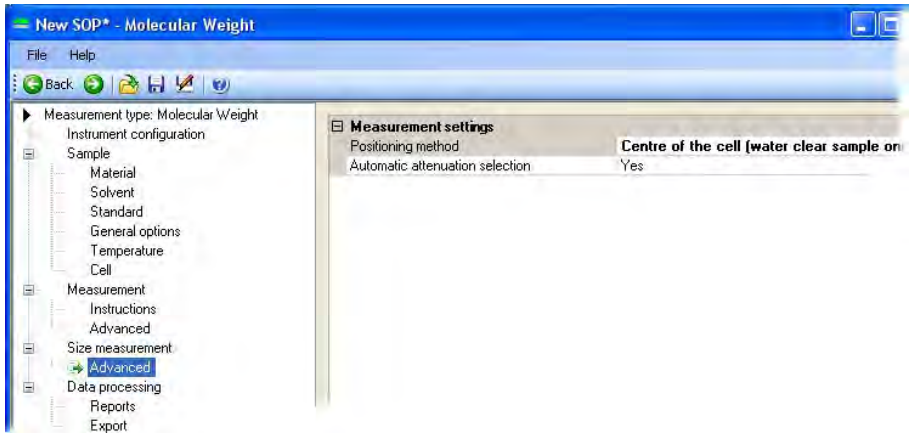
Please refer to the **Measurement** description in the **Size SOP** sections, for details of the **Measurement duration** settings.

Partial Results

If it is likely that a measurement will not produce a correlation function that can be analysed, 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. This means that the measurement will continue, with no error warning, to the next measurement.

Please refer to the **Measurement** description in the **Size SOP** section, for details of the **Partial Results** settings.

Size Measurement - Advanced



Measurement settings

Please refer to the **Measurements - Advanced** description in the **Size SOP** section for a description of the **Measurement Position** and **attenuator** functions.

Data Processing

If the characteristics of the sample to be measured are known, this window will allow an appropriate analysis model to be applied and so optimise the measurement calculation.

Please refer to the **Data Processing** description in the **Size SOP** section.

Data Processing - Reports

Please refer to the **Data Processing - Reports** description in the **Size SOP** section.

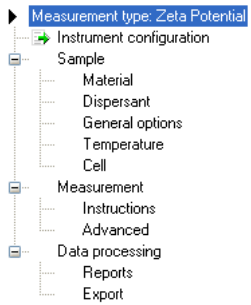
Data Processing - Export

Please refer to the **Data Processing - Export** description in the **Size SOP** section.

Zeta potential SOPs

Though the majority of the **Sample** and **Measurement** windows described below are **unique** for **zeta potential** measurements, when indicated please refer to the description in the **Size SOP** section.

The **zeta potential SOP editor** menu is:

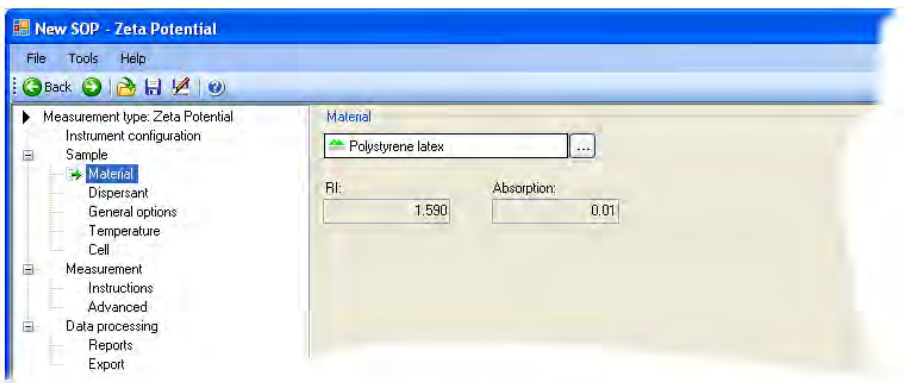


Sample

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

Sample - Material

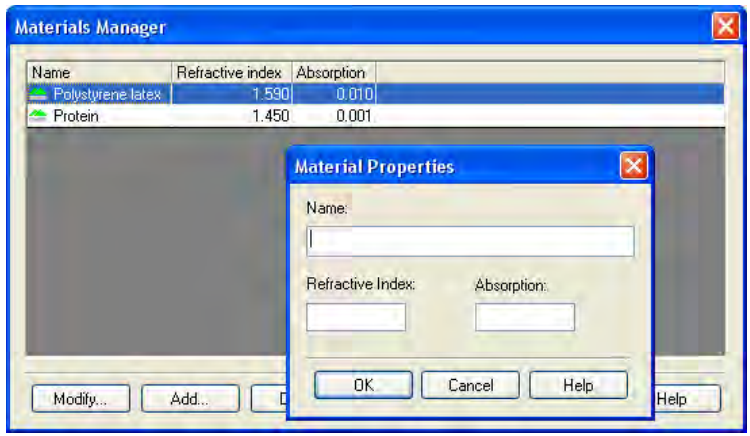
The Zetasizer software requires certain information about the physical properties of the dispersant to be used in the sample that is to be measured.



By selecting the **Sample - Material** window and pressing the ... button, the **Material properties manager** is displayed where these properties can be defined.

Materials Manager

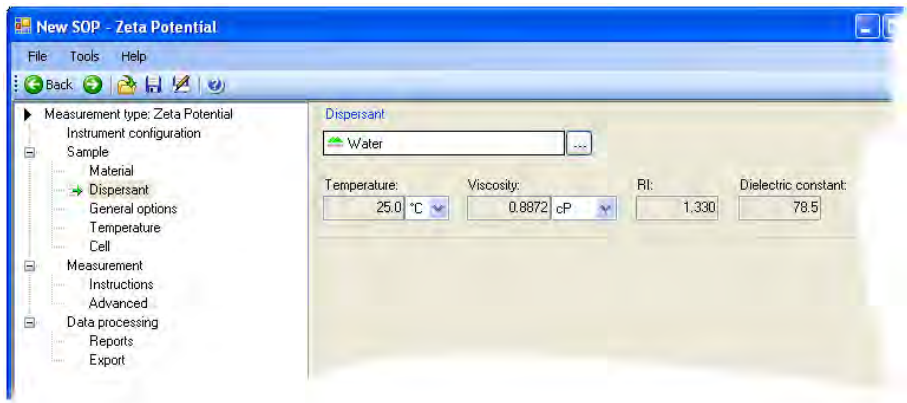
Selecting this window allows new material to be added, modified and deleted.



To define a new material, press the **Add...** button to display the **Material Properties** window. Here, the material **name**, **refractive index** and **absorption** can all be specified. Please refer to the **Sample - Material** description in the **Size SOP** section for details.

Sample - Dispersant

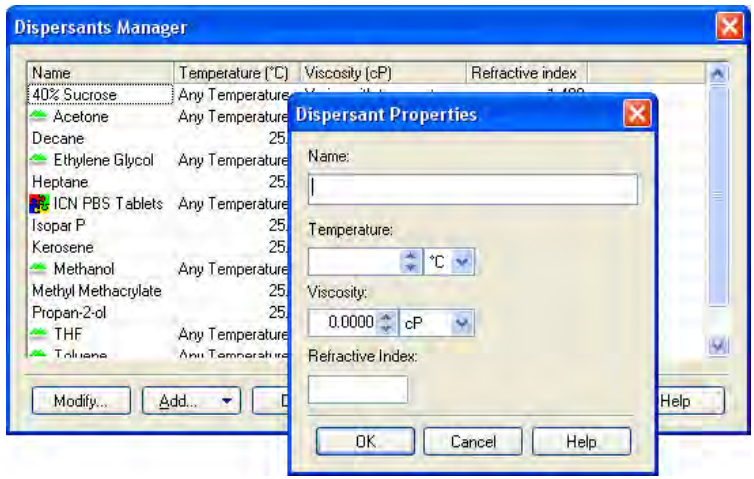
The Zetasizer software requires certain information about the physical properties of the dispersant that is used in the sample that is to be measured.



By selecting the **Sample - Dispersant** window and pressing the ... button, the **Dispersants manager** is displayed where these properties can be defined.

Dispersants Manager

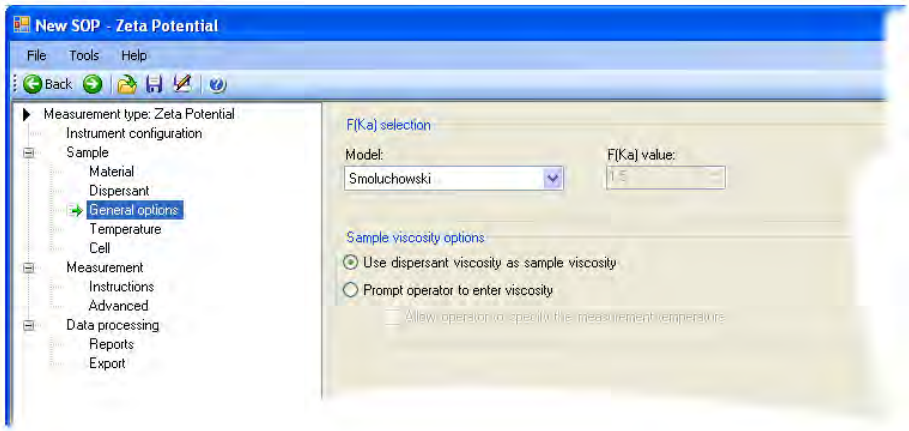
Selecting this window enables new dispersants to be added, modified and deleted.



To define a new dispersant, press the **Add...** button and choose either a **Simple dispersant or solvent** or a **Complex solvent**.

Please refer to the **Sample - Dispersant** description in the **Size SOP** section for details.

Sample - General options



F(ka) selection

Input the F(ka) model to be used in the Henry's equation.

- For most particles in **aqueous** media select **Smoluchowski**: **1.5**.
- For **non-aqueous** measurements, or for very small particles in low conductivity media measurements, the **Huckel** approximation should be used: **1.0**.

Please refer to **Zeta theory** chapter for more information on the F(ka) model.

Sample viscosity options

This area is useful for customising the viscosity parameters.

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

Sample - Temperature

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

Sample - Cell

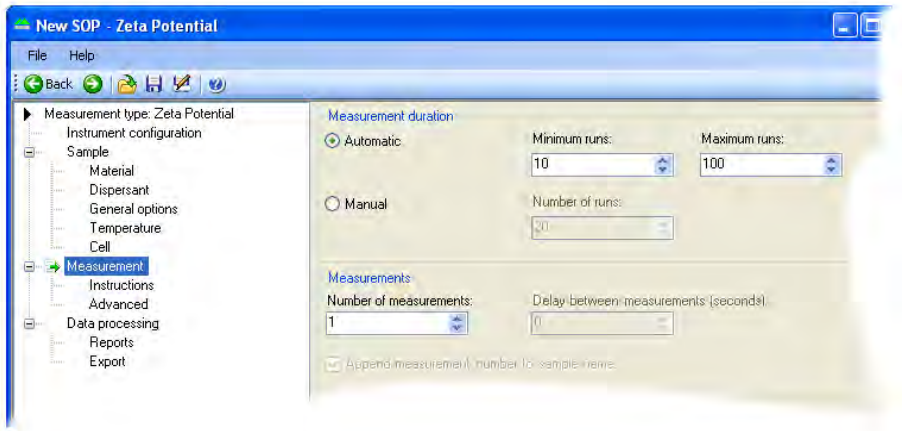
Please refer to the **Sample - Cell** description in the **Size SOP** section.

The default selection is the **Folded capillary cell**.

Measurement

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

Note
The features available will alter dependent upon the cell type fitted. Where applicable consult the relevant cell user guide for measurement details.



Measurement Duration

The measurement duration setting may affect the accuracy and repeatability of the results.

With **Automatic** selected, the software will automatically determine the measurement duration. This will be suitable for the majority of samples and can simply be left as the **default**. The **Minimum run** and **Maximum run** entry boxes can be used to define the run duration limits of the automatic measurement.

The default number of runs in 'auto' measurement duration is 30, but for a stable sample as few as 10 runs may be required. The measurement will then complete even though the displayed run total has not been achieved.

With **Manual** selected the measurement will use the user defined **Number of runs**: setting. The time may be reduced for the measurement of a latex standard, or increased to improve the repeatability of the measurement of particularly polydisperse samples. Manual measurements can be up to 10,800 runs. All the individual runs are accumulated and then averaged to give a final zeta potential result. Therefore the more runs performed the better the repeatability. Naturally the more runs selected the longer the duration of the complete measurement. However if the sample has a high conductivity, measuring for too long can affect the sample and the electrodes.

Measurements

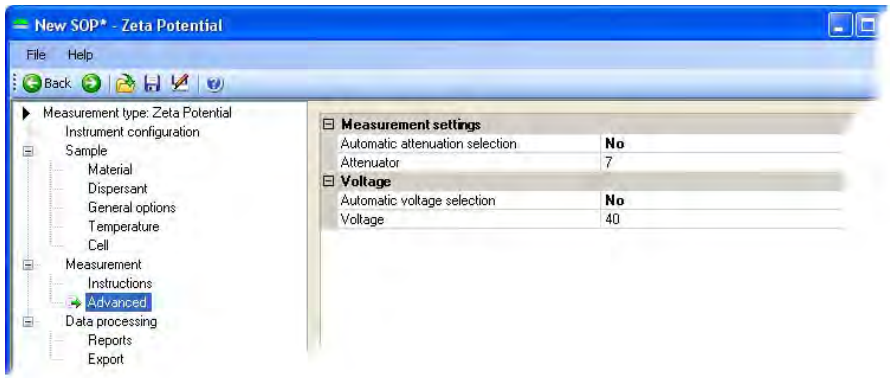
This option allows a sample to be measured more than once.

Please refer to the **Measurements** description in the size SOP section.

Measurement - Instructions

Please refer to the **Measurement - Instructions** description in the **Size SOP** section.

Measurement - Advanced



In the **Measurement - Advanced** window there are two functions: **Measurement settings** and **voltage**.
For normal operations both functions will be at the **default** settings.

Measurement settings

Fixing the attenuator setting can be used to enable the count rates of two samples to be compared, or to speed up the optimization stage prior to a measurement being performed. Either set the **Automatic attenuation selection** parameter to **Yes** to use automatic settings, or **No** to display an extra parameter line that will allow setting of the attenuator position.

Voltage

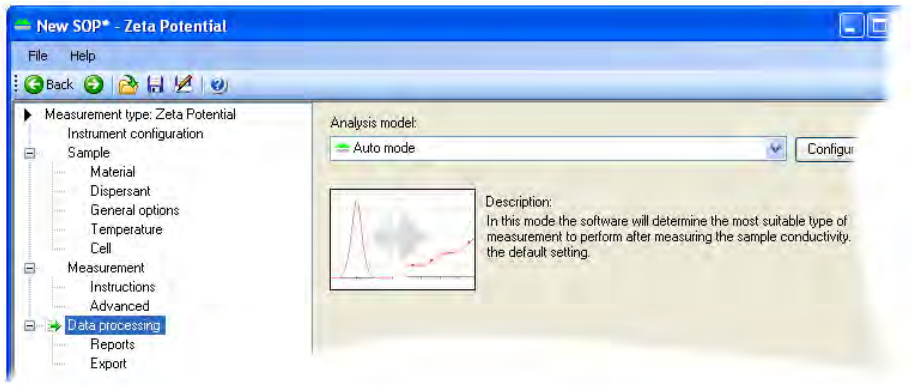
The cell voltage is the voltage applied to the cell electrodes during a measurement. The voltage applied will **automatically** be set by the software. The actual voltage applied is dependent upon the SOP settings, i.e. the cell selected and the F(ka) selection, as well as the conductivity measured during the measurement.

Sample type	Cell used	Conductivity	Voltage applied
Aqueous	Folded capillary cell	< 5mS/cm	150V
		> 5mS/cm	50V
		> 30mS/cm	10V

Aqueous	Universal 'Dip' cell	< 10mS/cm	5V
		> 10mS/cm	3V
Aqueous	High concentration cell	< 5mS/cm	40V
		> 5mS/cm	10V
		> 30mS/cm	2.5V
Non-aqueous	Universal 'Dip' cell	Negligible	40V

If the **Automatic voltage selection** is not appropriate for the sample being measured, double-click on the function (to change the settings from **Yes** to **No**). An appropriate voltage can then be manually typed in.

Data Processing



If the characteristics of the sample to be measured are known, this window will allow an appropriate analysis model to be applied and so optimise the measurement calculation.

Size ranges and measurement thresholds can be applied to the analysis to filter spurious peaks prior to the analysis being performed.

As default, **Auto mode** analysis is used. This should be used for most measurements, especially if the sample to be measured is unknown; otherwise two additional **analysis models** are available - **General Purpose** and **Monomodal**; or 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

Auto mode

This is the default setting. In this mode the software will determine the most suitable type of measurement scheme to perform after measuring the sample conductivity; this is useful if the sample to be measured is unknown.

In **Auto mode** the result will reveal details about the number of modes and the shape of the zeta potential distribution. If the conductivity exceeds 5ms/cm **Auto mode** will automatically select either a **General purpose** of **Monomodal** analysis - see analysis models below.

To obtain a detailed measurement a minimum of 10 runs should be performed. If the sample is thought to consist of a multi-modal distribution then 20 to 30 measurement runs should be performed.

General Purpose

Use this analysis model to obtain a distribution regardless of conductivity

Monomodal

The monomodal model only gives the mean zeta potential value required. In this case only five to ten runs maybe required to obtained an accurate measurement.

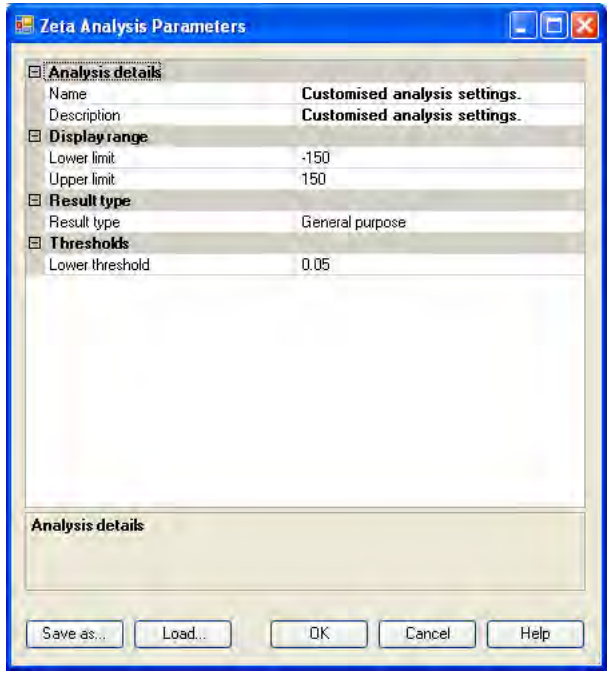
This mode is recommended for time, pH and temperature trend measurements as well for samples known to have a conductivity greater than 5milliSiemens/cm.

Load Analysis settings...

This enables a previously saved analysis model to be loaded and used for the data processing. These are saved as **.dasz** files.

Configure button

Pressing the **Configure** button will display the window below. This window enables various attributes of the analysis model to be altered. These include the **Name**, the measured **Zeta Display range**, the **Analysis model** and the measurement **thresholds**. The altered analysis model can then be saved. Analysis models are saved as **.dasz** files. (The last **z** indicates the analysis setting has been created for a **zeta potential measurement**).



If it is known that all particles within the sample will fall within a certain zeta potential range, then the Zeta **Display range** can be set to improve the repeatability of the measurement result; similarly a lower **threshold** can be applied to eliminate undesired particles that are below a defined percentage of the particle population.

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

Note
If any of the settings have been altered, the analysis model will be automatically saved under the name given in the **Name** entry box, or as **Customised analysis settings** if no name has been given.

To keep the new analysis settings, press the **Save as...** button

Previously saved analysis models can be used, viewed and altered by pressing the **Load** button.

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

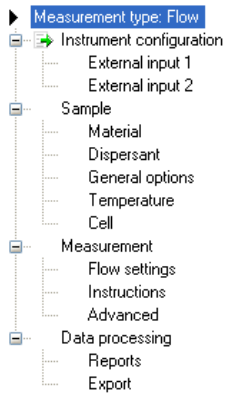
Please refer to the **Data Processing - Reports** description in the **Size SOP** section.

Data Processing - Export

Please refer to the **Data Processing - Export** description in the **Size SOP** section.

Flow-mode SOPs

The **Flow SOP editor** menu is:



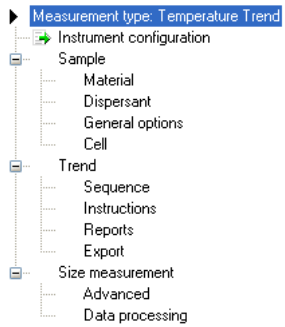
The **Flow**-mode SOP enables measurements to be performed on a flowing sample stream. The Scattered light intensity and Hydrodynamic diameter can be plotted as a trend, and, with the addition of optional external input windows, information from external measuring sources, such as a refractive index detector, can be monitored and displayed.

In general, flow-mode SOPs are the same as the standard Size SOPs, the only differences being the inclusion of **External Input** SOP windows. The Flow mode SOP windows are described in the **Zetasizer Nano Accessories manual**.

For all other SOP windows, refer to the Size SOP section.

Trend SOPs

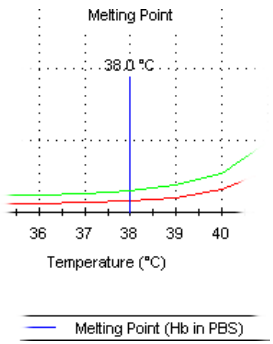
The **Trend SOP Editor** menu is:



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

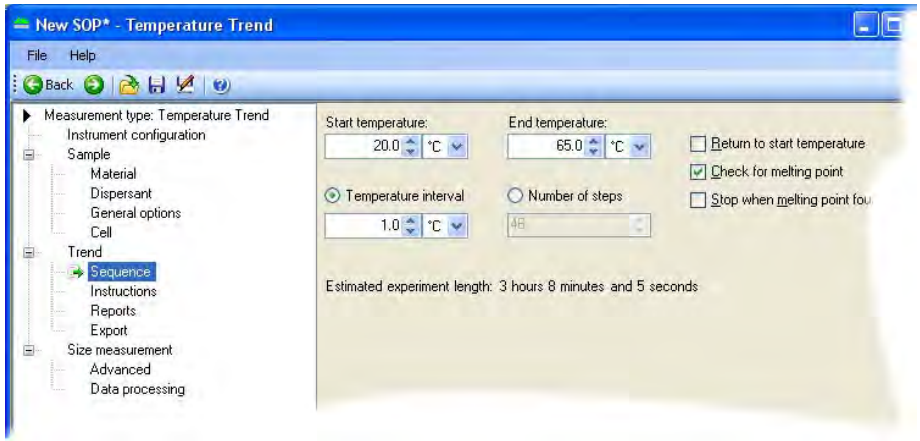
Trend SOPs are similar to the standard size and zeta potential SOPs, the differences being the inclusion of a trend and changes in the measurement windows.

Aggregation SOPs



Aggregation measurements can be performed by selecting a check box on the **Trend - Sequence** window. 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** window 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.

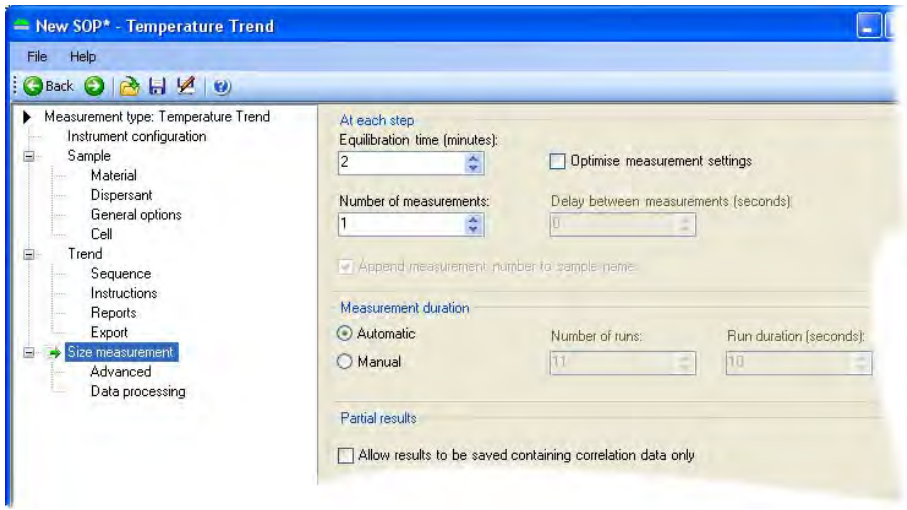
Temperature Interval / Number of steps
The measurement can be configured to change either in specific measurement intervals (e.g. every 5°C), or in a specific number of steps (e.g. 10 steps). Select the button to identify which is required.

Aggregation measurements only

Check for Aggregation
If the protein **denaturation** point is required, select the Aggregation point check box.

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

Size Measurement



The Trend and Aggregation measurement windows are generally the same as those used with either a size or zeta potential measurement SOP. The additional features are:

- **At Each step** gives the option to control the **Equilibration time** and **Number of measurements** performed.
- **Optimise Measurement settings** enables the cell position to be checked and verified before each individual measurement is performed. This option is **not** available with the Aggregation window.

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

Titration SOPs

Refer to the Titrator accessory manual for details of the SOP functions.

Extracting an SOP

The SOP (or manual settings) used when performing a measurement can be viewed by right-clicking on the measurement record and selecting **Extract SOP**. This will display the SOP windows.

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 windows will be displayed, allowing any settings to be changed.

Alter the SOP settings as required, 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 are designed to allow the measurement of the same type of sample in a consistent way - if monitoring batches of sample in a quality control environment, the results will be meaningless if different measurement parameters have been chosen for each batch.

Consider an application where a manufacturer is producing the same type of sample in different factories. It will be important that the measurement protocol is consistent between factories.

An SOP can be created and copied into the SOP directory on another Zetasizer Nano instrument so that measurements are consistent. Simply copy the .**sop** file to all computers running a Zetasizer Nano system.

If the SOP has a non-standard dispersant defined then this will not exist on the target computer. When the SOP is run on the target computer, the software will detect this and give the following choices:

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

9

Advanced features

Introduction

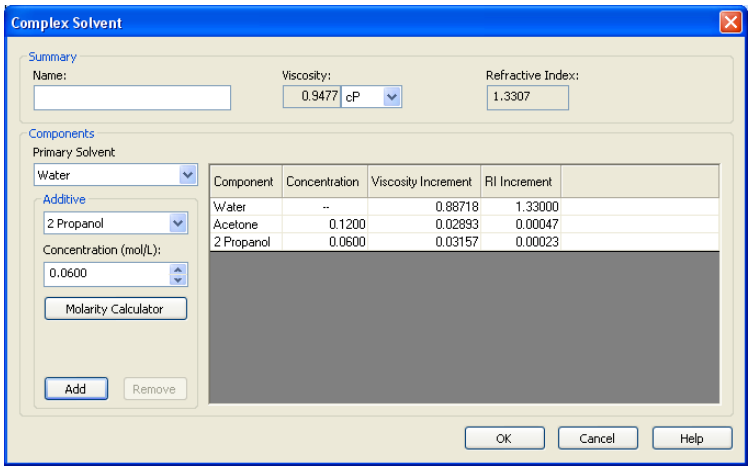
This chapter contains the following **advanced** features and capabilities of the Zetasizer nano:

- Solvent builder
- SOP player
- Averaging results
- Editing the result
- Exporting the results
- Options window
- 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 window below is displayed when **Complex solvent** is selected when adding a dispersant or solvent in the **Sample** SOP windows.



The Solvent builder is used for:

- **Size or Molecular weight SOPs -** calculating the viscosity and refractive index for non-standard solvents.
- **Zeta potential SOP -** calculating the dielectric constant and viscosity.

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.

The window above shows the Solvent builder for a Complex solvent when selected for either a size dispersant or molecular weight solvent. For a zeta potential dispersant, a **Dielectric increment** is added to the window, with Dielectric constant replacing Refractive index in the summary area.

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 window.

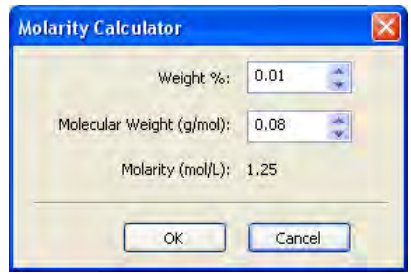
The **Summary** area details the chosen name for the new solvent, plus its calculated viscosity and refractive index; or the dielectric constant for zeta potential SOPs. Do the following:

- 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 window (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 window without updating.



- For zeta potential SOPs, enter the **Dielectric increment** for the additive.
- 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** window is displayed, allowing the parameters to be changed. The builder will only be displayed if the solvent was originally created using the builder and not the standard **Dispersant** properties window

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

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.

SOP player

The SOP player is a separate feature that enables a sequence of **actions** - SOPs, measurements, pauses or macros - to be constructed and then played. This sequence is called a **playlist**.

This is useful for performing different measurement types consecutively without any user interaction; a **playlist** can be created, the **Start** button pressed, and the measurement will continue, performing size and zeta potential measurements in sequence with appropriate pauses, or macros.

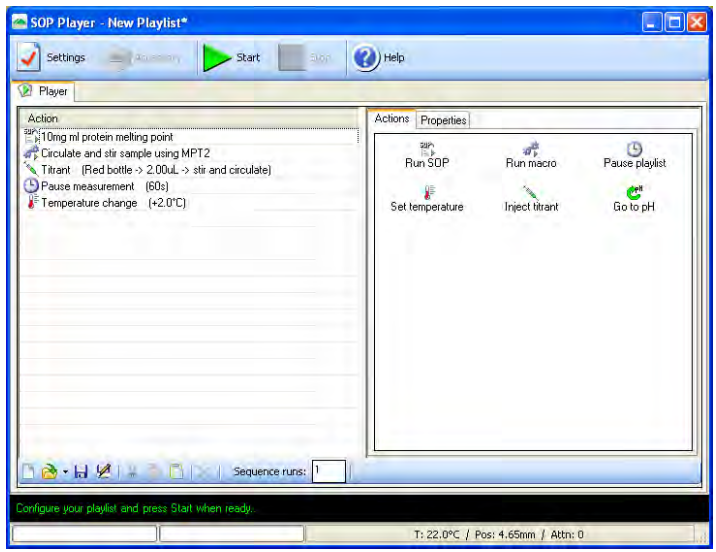
An example application for using the SOP player is a **Temperature trend with titration measurement**:

i.e. increase the temperature of a sample - measure,
add some titrant - measure,
decrease the temperature - measure,
and repeat.

Note
A playlist **must** be saved before playing

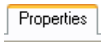
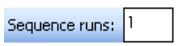
SOP player window

To open the player select **Measure-SOP Player**. The window below is displayed.



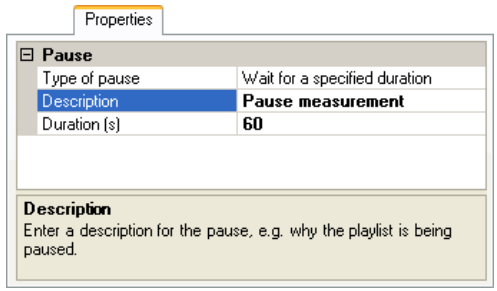
Note, that when first opened, the table on the left will normally be blank. The actions shown are only for reference.

The SOP player controls are:

Button	Description
	Select this tab to add new items (or actions) to a playlist. Choices are SOP, Macro, Temperature, pH, Inject titrant and Pause. These are described below.
	Input the properties for the selected action.
	Creates a new 'blank' playlist page. This will overwrite any playlist currently displayed. A prompt to save the current playlist will be shown.
	Loads a previously stored playlist. By default playlists are stored in the SOP directory under a **.sopl** filename.
	Saves a newly created or modified playlist. A playlist must be saved before being played.
	Saves a modified playlist under a different file name.
	Cuts the selected action(s) out of the playlist. It can then be pasted to the end of the playlist.
	Copies the selected action(s) in the playlist. It can then be pasted to the end of the playlist.
	Pastes the copied or cut action(s) to the end of the playlist
	Use this button to remove actions from a playlist. Either the selected item can be removed, or the complete playlist cleared.
	Enter how many times the playlist is required to be run.
	Opens the documentation window. Sample name and instructions can be inputted. Refer to the **Sample** description in the **Size SOP** section.
	Plays (starts) the currently viewed playlist measurement. The measurement display will then open. An extra tab will be present showing the measurement playlist.
	Stops the current playlist measurement.
	The eject button is greyed out and reserved for use when using a Zetasizer APS instrument.

Construct the playlist by selecting and configuring the **Actions** in the right pane; These are described in the following sections. The list can be arranged in order as required by dragging and dropping an action at the desired point.

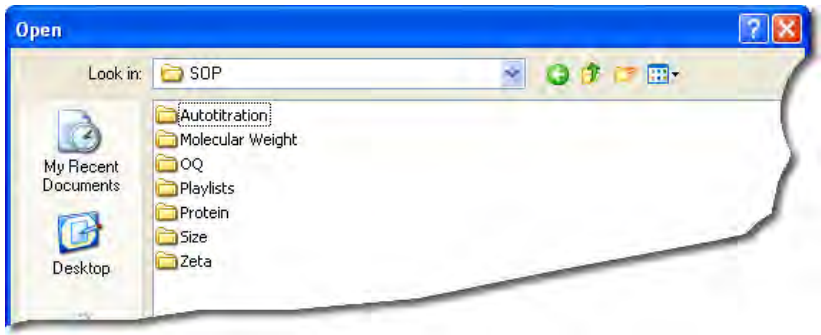
For each action, enter the action properties as required. An instruction for the parameter is displayed at the bottom of the Properties tab.



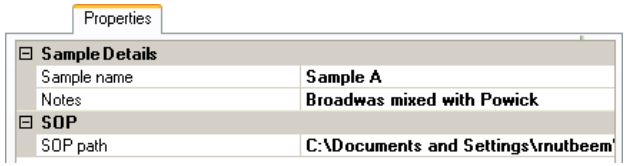
When the playlist is played using the button, the **Measurement display** is displayed; the actions being performed can be viewed by selecting the **Log sheet** tab, whilst the playlist can be viewed by selecting the **Player** tab. All other functions are of the Measurement display are as previously described in the manual.

Run SOP...

To add an SOP, select the **Run-SOP** icon; The window below is displayed.



Select the required SOP from the list and press **Open**. The SOP will be added into the playlist. The SOP will be identified in the playlist by its SOP filename.



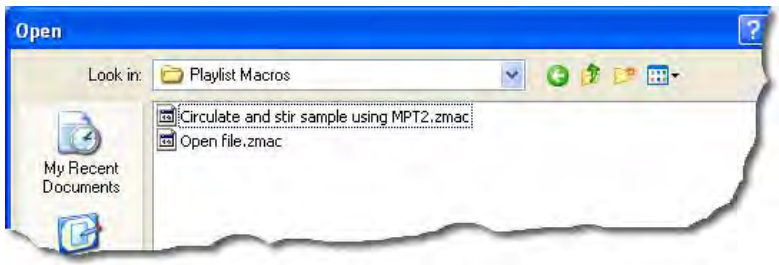
Selecting the SOP in the Playlist will display both the SOP properties and the sample details. Select each parameter to edit as required. The SOP used can be changed by selecting ...; this will open the window above.

To alter an SOP, use the **SOP Editor**.

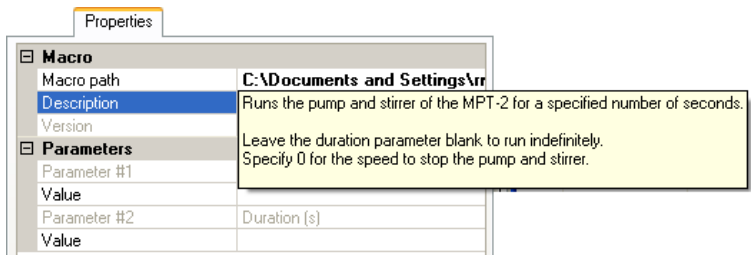
Note
It is important to ensure that the measurement cells selected in an **action** are compatible for all other actions in the playlist. This includes all SOPs and Macros selected.

Run Macro...

To add a Macro program, select the **Run Macro** icon. The window below is displayed.



Select the required Macro from the list and press **Open**. The Macro file path will be added into the entry box. The Macro will be identified in the playlist by the its filename.



Complete the relevant **Parameters** as required. Follow any instructions indicated in the **Description** field.

The **Parameters** section allows additional options to be passed to the macro. The description or details of the selected Macro will explain what should be entered here. Generally this will be the type of function or task the Macro will perform. The values entered will be shown alongside the Macro filename in the playlist.

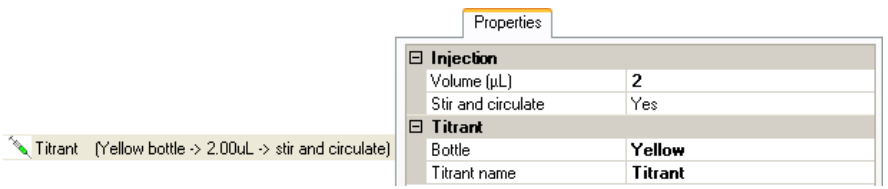
The Macro used can be changed by selecting ...; this will open the window above.

Note
The Macros available will be supplied by Malvern.

Inject Titrant

To add a titrant, select the **Inject titrant** icon. This will add a Titrant action into the playlist, with the properties displayed alongside.

Alter the properties as required. Enter the **Titrant name**; This will identify the titrant in the playlist. Select the **bottle** the titrant will be delivered from, and the **injection volume** that will be administered into the sample. The details entered will be shown alongside the Titrant icon in the playlist.

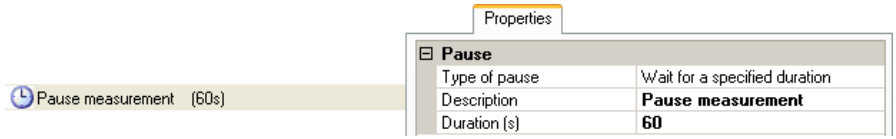


Note
The MPT-2 Titrator must be fully set-up and primed before use.

Pause Playlist

To add a pause (or time-delay) between each playlist action, select the **Pause playlist** icon. This will add a **pause** action into the playlist, with the properties displayed alongside.

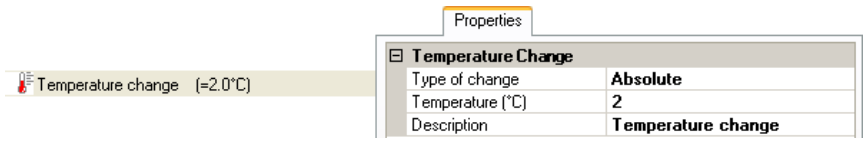
Alter the properties as required. Enter the pause **Description**, this will identify the pause in the playlist, and enter the pause **Duration**. The details entered will be shown alongside the Pause action in the playlist.



A pause may be required to allow for thermal equilibration, or to allow a titrant to be delivered and dispersed into the sample before proceeding.

Set temperature

To add a temperature change or setting between each playlist action, select the **Set temperature** icon. This will add a **Temperature** action into the playlist, with the properties displayed alongside.



Alter the properties as required. Enter the temperature **Description**, this will identify the temperature change in the playlist. Also enter the **Type of** temperature **change** and the **temperature** value to be acheived; note that this is dependent on the **Type of** temperature **change** option selected:

- **Type of change : Relative**
  Enter the temperature "change" required from the current temperature.

  If the current temperature is 25°C and the new temperature required is 23°C. then enter **-2** as the value; the instrument will cool until that 23°C is achieved.

- **Type of change : absolute**
  The temperature will go to the temperature requested.

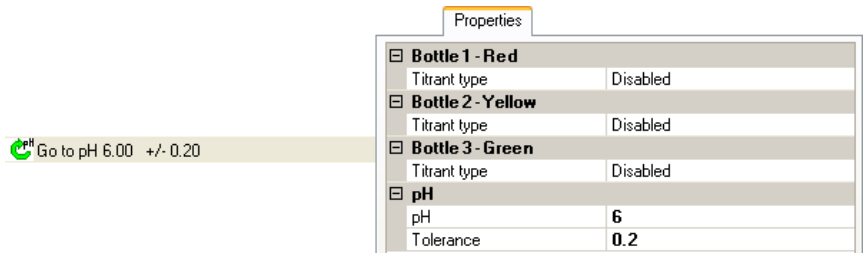
  If 10°C is entered, the instrument will cool, or heat, until that temperature is achieved. The temperature range available is that of the instrument: 0°C to 90°C, or 0°C to 120°C if the HT option is fitted.

The details entered will be shown alongside the temperature action in the playlist.

Note
Any temperature requested in the **Set temperature** action will override the temperature setting requested in any previous SOP action within the playlist.
It is recommended to amend the **Set temperature** value **back** to ambient at the end of the playlist; otherwise, when the playlist finishes, the temperature will stay at the last value requested

Go to pH

To adjust the pH value between two playlist actions, select the **Go to pH** icon. This will add a **Go to pH** action into the playlist, with the properties displayed alongside.



Alter the **Properties** as required. Enter the **Titrant type** used for each bottle - either acid, base or disabled. Also enter the **pH** value to be obtained together with its **Tolerance**. The properties entered will be shown alongside the **Go to pH** action in the playlist.

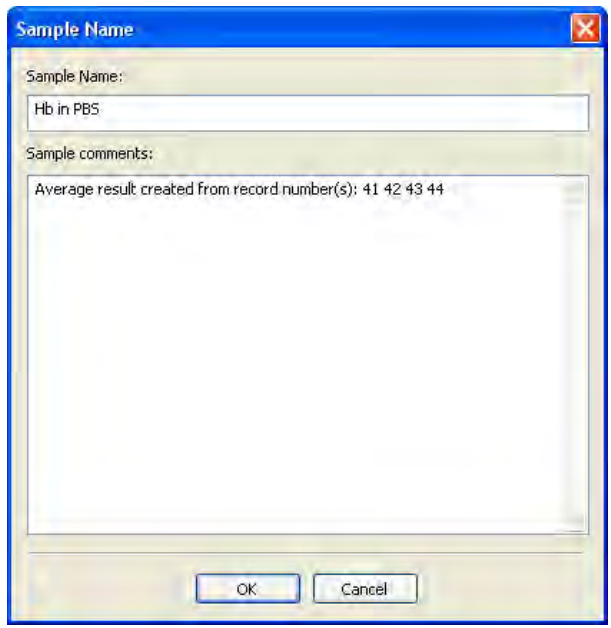
More information on pH measurements can be found in the MPT-2 Titrator user manual.

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 window is displayed so comments can be added and the sample name changed, if required.



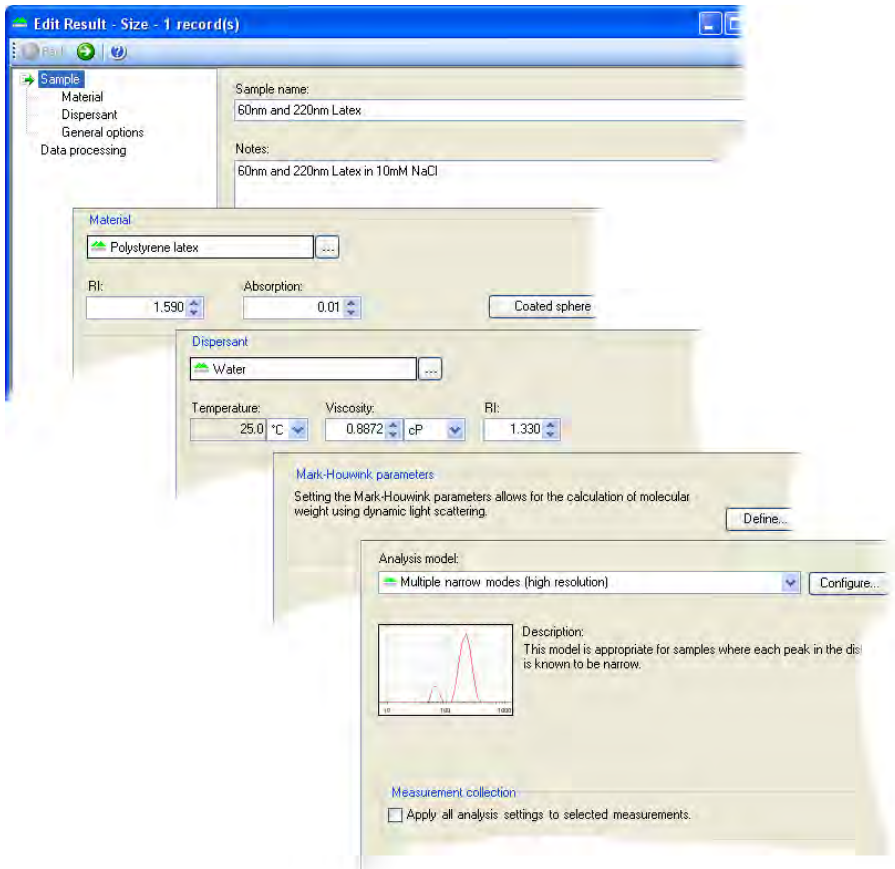
Note
When averaging, the measurement type must be the same, e.g size or zeta potential.
A previously averaged record cannot be averaged again.

Editing the result

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

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

Select the menu item **Edit-Edit result** or right-click on a measurement record and select **Edit Result**; The **Edit Result** windows are displayed - these will be similar windows to those within the **SOP Editor**. As with the **SOP Editor**, the windows shown will be different dependent upon the measurement type selected. The example below shows the **Edit result windows** available when a **size measurement** is selected.



Note
Each edit result window 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 window.

To Edit a result:

- **Right-click** on the required measurement record(s) and select **Edit Result**.
- Select the appropriate windows 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 reanalysed and the result added to the **Record view**.

Editing a Flow-mode result

When a flow-mode result is selected for editing, a flow-mode plot window will be displayed. This will allow the flow-mode result to be edited and reanalysed, and the flow-mode plot to be viewed and the peaks inspected.

Refer to the **Editing and inspecting the Flow-mode result** in the F**low-mode** section later in this chapter.

21 CFR Part 11

If the 21 CFR part 11 feature is installed, a **Reason for change** window is displayed 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** window (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 Wordpad. There are several ways to export the required information:

- One or more records can be selected in the **Records view** tab and moved via "drag and drop" directly into another 'visible' application (provided that the application supports tab or comma separated formats e.g. Excel).
- A **table** or **graph** can be dragged and dropped into another application by holding the **Ctrl** key, selecting the item and dragging into the application.
- A **table** or **graph** can be copied by selecting the item then selecting **Edit-Copy**. The item can then be pasted into another application. 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** window 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 or records, then move the cursor to within the selected records. Press the left mouse button down until a small rectangle and plus sign appears, then 'drag' the selected record(s) to the other application, and 'drop' - release the mouse button

The parameters exported will be those shown in the **Records view**.

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

To drag and drop tables and graphs, hold down the **Ctrl** key, place the cursor within the graph or table then drag the object to the target application.
The same function can be performed by using the menu item **Edit-Copy graph** or **Copy table**, then **Pasted** in to the target application. The selections in the menu will depend on the measurement type selected.

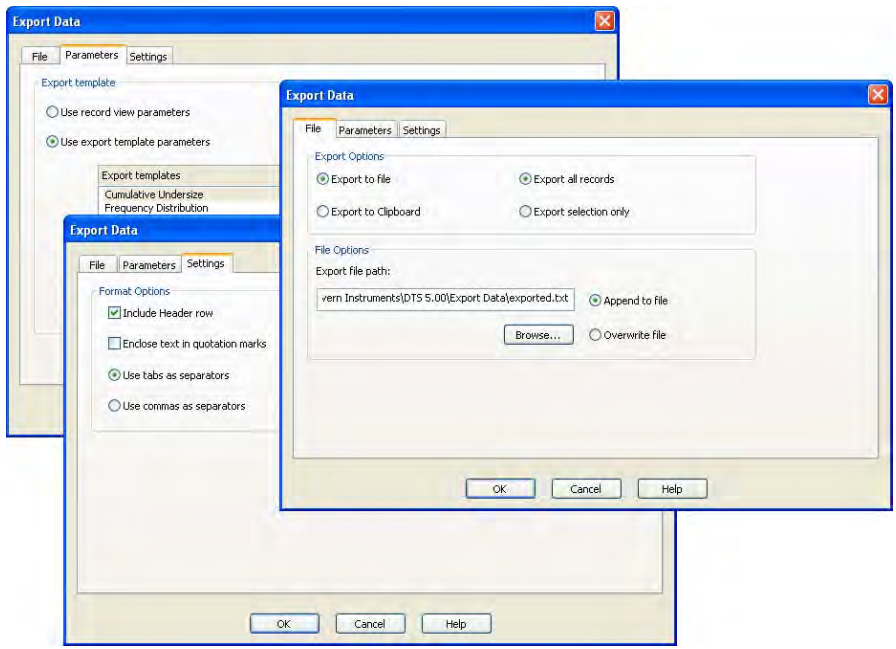
Exporting using Menu controls

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 greyed out when this option is chosen.
- **Export all records** will export the complete measurement file, whereas **Export selection only** will export only the records that have been highlighted.



Parameters tab

This enables exporting of selected parameters. Selecting **Use records view parameters** export parameters currently displayed in the Record view. User defined parameters can be exported 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**.

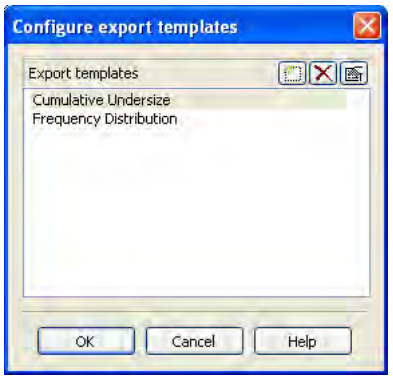
Settings tab

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 used as numerical separators in a 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 window, shown here.

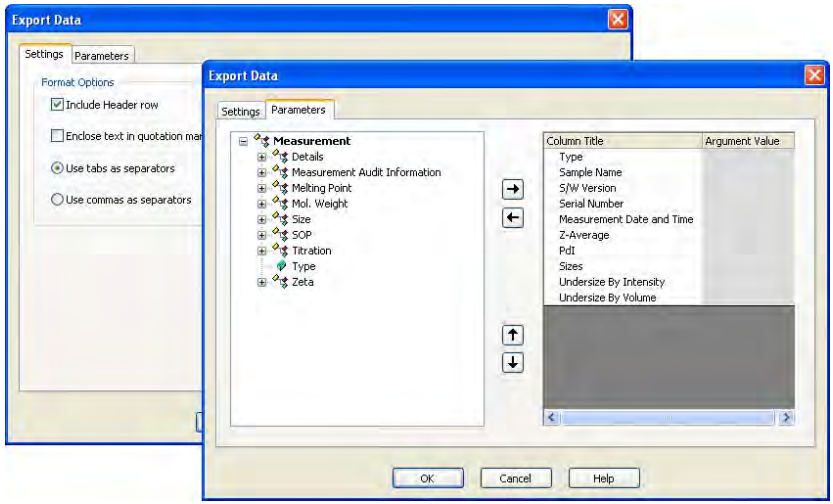


Two export templates are supplied as standard: **Cumulative Undersize** and **Frequency Distribution**.

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** window below is displayed: 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.

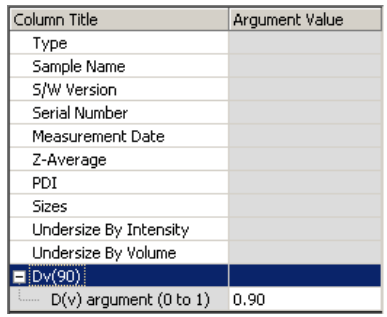
Parameters can be added or removed from this list by using the **Add** and **Remove** keys.

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** window. Press **OK** again to save the template.

Parameters with arguments

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



Column Title	Argument Value
Type	
Sample Name	
S/W Version	
Serial Number	
Measurement Date	
Z-Average	
PDI	
Sizes	
Undersize By Intensity	
Undersize By Volume	
Dv(90)	
D(v) argument (0 to 1)	0.90

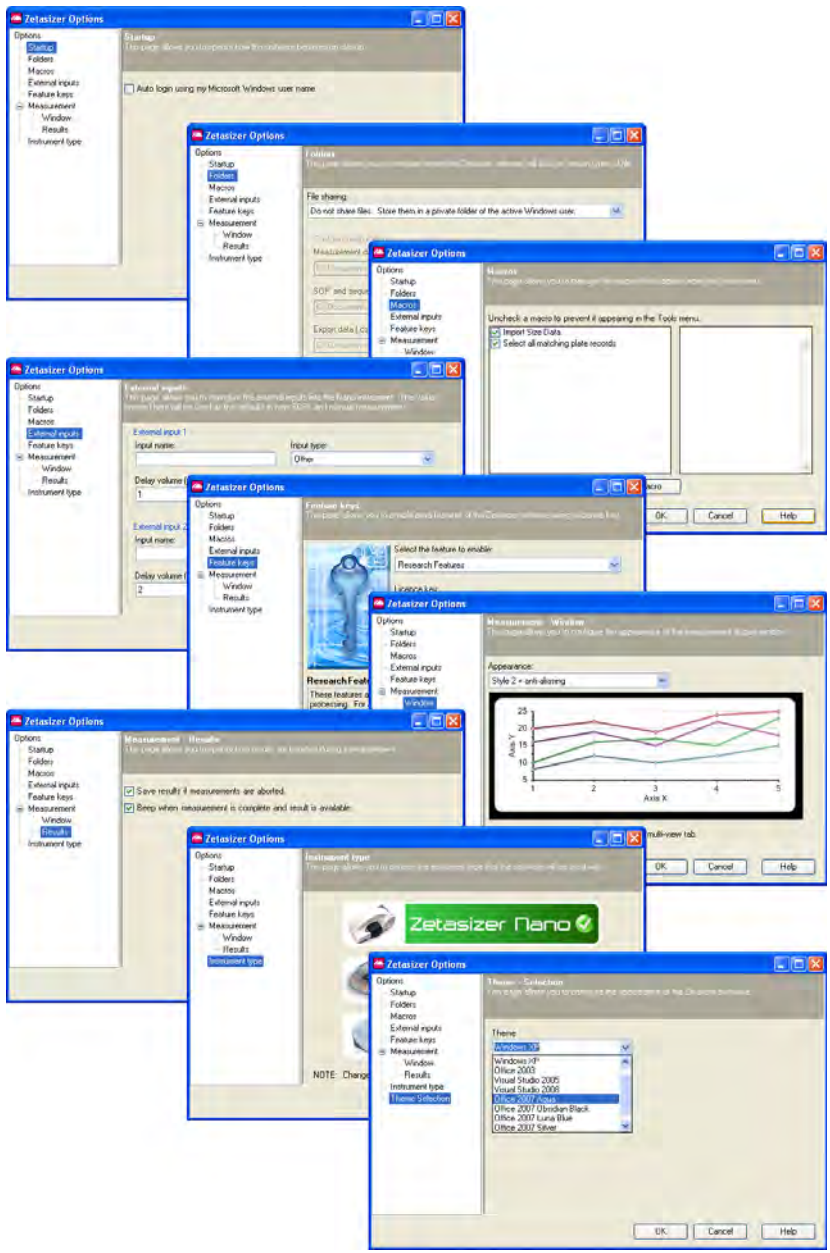
A Dv(90) percentile can be viewed by adding **Measurement-Size-D(v)** and then typing 0.90 as the argument value.

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** window.

Options window

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



The **Options** window 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 window, 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** window 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.

Select Custom from the drop-down to allow configuration.

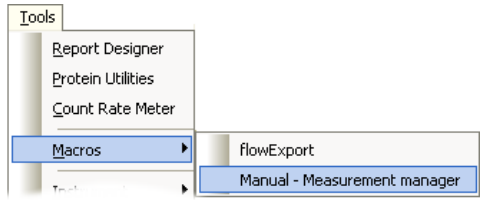
	Allows another location to be selected. This will open a **Browse for Folder** window; select the new location directory and press **OK**. Button is highlighted in Blue once selected.
	Default folder that allows each Windows user to have their own files. Button is highlighted in Blue once selected.
	Default folder that allows all Windows users to access the same files. Button is highlighted in Blue once selected.
	Reverts the file-path to the default location. Button is greyed out when default location is used.

Macros

Additional and customised software programs can be added and installed by using the Macros option. This window allows new macro 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, USB memory stick, 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 is displayed to indicate the macro will be deleted: **"Are you sure you want to delete the selected macro?"**.

External Inputs

This page allows configuration of the external inputs into the Nano instrument. These settings are required for setting the timing between the Nano instrument and any connected accessories when using the **Flow-mode** capability.

- Use **Input Name** to name the type of input. Use a name that represents the signal or reading to be measured.
- The **Delay volume** is the volume of liquid flowing between the external device and the Zetasizer Nano. This value will be obtained by experiment.
- The **Offset** and **Gain** are mathematical parameters (values) that are needed to convert the incoming signal (in volts from the external measurement device) to the measurement parameter required.
  Consult the external device documentation to ascertain the **offset** and **gain** values required and input these into their respective entry boxes.
- **Input type** (the kind of detector the instrument is connected to) and **Path length (mm)** are options that **only** require selection when using the **Research features** (see below).

Note that the values entered here will be used as the defaults in new SOPs and manual measurements. When the Flow SOP **External inputs** window is viewed, these values will be shown in the respective entry boxes.

As default settings can be configured for each external input, this will allow each input to be dedicated to a specific external accessory.

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.

Microrheology

Enables a Nano ZS or ZSP to perform Microrheology measurements. For a 30-day trial enter the licence key number indicated on the feature key window.

Advanced protein features

The advanced protein feature key enables the advance protein features in the application software. Once enabled protein mobility measurements can be performed, together with an expanded calculators utility.

For a 30-day trial enter the licence key number indicated on the feature key window.

Research Features

The Research features allow greater control over many aspects of data acquisition and processing. For a 30-day trial enter the licence key number indicated on the feature key window.

ER/ES Features (21 CFR Part 11)

The Malvern security system can be upgraded to 21 CFR part 11 compliance by installing the 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 21 CFR 11 icon on the status bar is displayed.

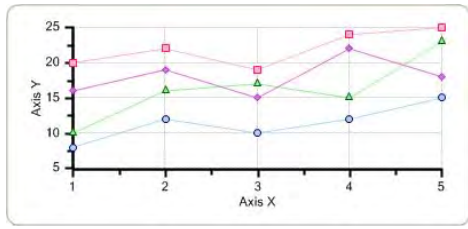
For a 30-day trial enter the licence key number indicated on the feature key window.

Measurement

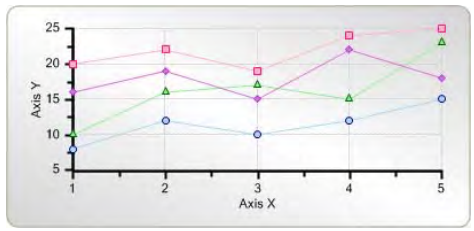
Window

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.

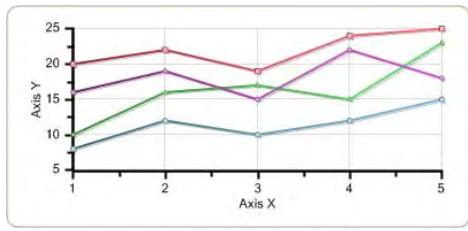
Standard



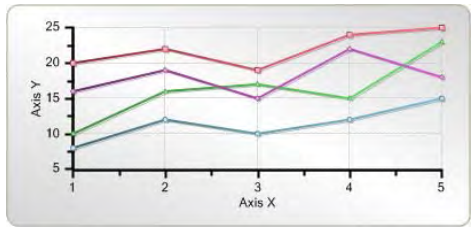
Style 1



Style 2



Style 3



Results

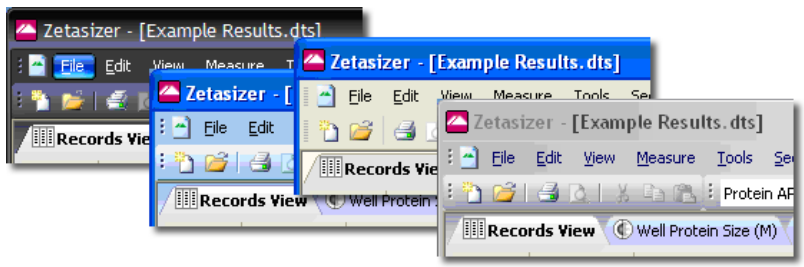
This window contains a check box that will allow measurements results to be saved even if the measurement is aborted before the measurement has finished collecting and analysing the data.

Instrument type

This window 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.

Theme Selection

This option allows the appearance of the software to be changed. The appearance is based on various Windows colour schemes such as Windows XP, Office 2003, Office 2007, etc.

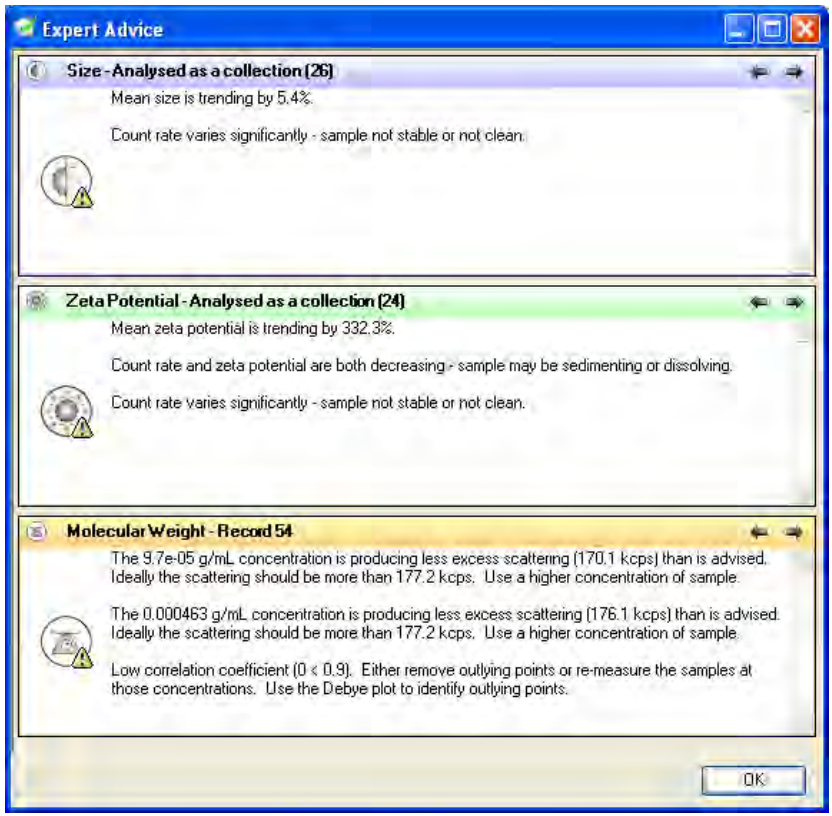


Expert advice

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** window indicating the quality of the measurement. Advice may be given about how to rectify a poor measurement.

Note
An **Expert Advice** tab is also available in the **Measurement display**. This enables the quality of the measurement to be inspected as it progresses.



Explanation

In the example above size, zeta potential and molecular weight measurements have been selected. The quantity of records selected is indicated in the measurement header line (i.e. **Size [3]**).

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 - **Analysed as a collection** - indicates that the records selected for the expert system to inspect have been analysed as one. To see each measurement records advice individually, use the ⇦ and ⇨ arrows to step through each record in turn. The record being inspected will then be indicated in the measurement type title line; e.g. **Record 39**.

Measurement type	Good Measurements (green circle)	Poor or unacceptable Measurements (Amber triangle)
Zeta Potential		
Sizing		
Molecular weight		

Note
If the Microrheology feature key has been installed, a similar symbol will be displayed to indicate microrheology measurement advice.

Note
A Technical note is available from Malvern Instruments that further describes the Expert advice.

Report designer

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 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 - print and screen reports

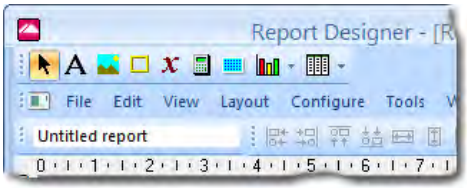
As the aspect ratio of a computer screen tends to be landscape, and a printed report tends to be portrait, each report has two versions - a **print** version and a **screen** version. Both versions are held in the same report page (.**pag**) file.

The print and screen versions do **not** have to have the same contents. For example, you may wish to have your company logo on the top of the printed report, but not on the screen version.

Toggle between screen and page layout by clicking the corresponding buttons on the toolbar:

- **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 Report names (not report filenames) entered in the **report name** entry box, should be **unique**. If no name is entered the report name will default to **Untitled report**.



- To change the report title, edit the report name field in the report designer. The report name will be displayed in the **report pages** selection window.

Note
If a user tries to select more than one report with the same title then a duplicate report warning will be displayed: A report with the same name already exists.

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 customise 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.

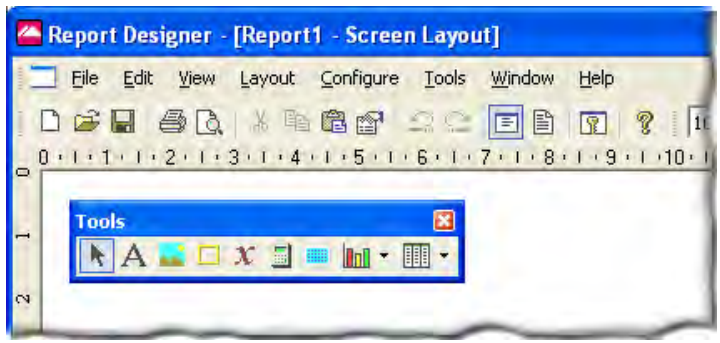
These are added using a **Control Palette** toolbar.

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 small **Control palette** has been dragged from the toolbar to make it easier to use. This tool can be used in either position, depending on the user's preference.



These **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:

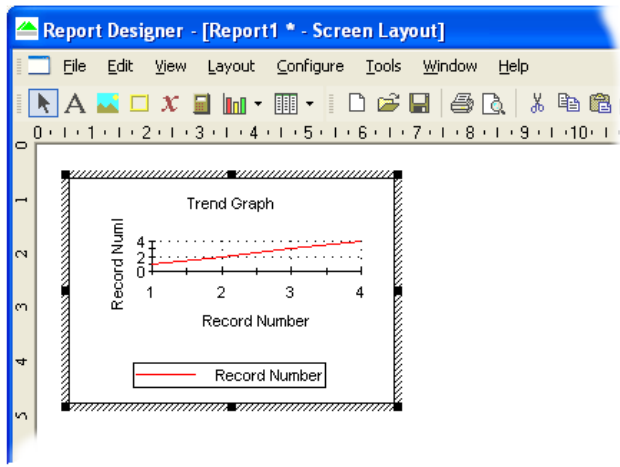
The palette can be moved anywhere on the screen. It can be switched on and off using the **View-Control Palette** command on the **Report Designer** menu.

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

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

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** window box. The on-line help in these windows describes how to use each one. The sections below show the properties each type of element has.

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

Text

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

With the element selected, use its **Properties** window 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.

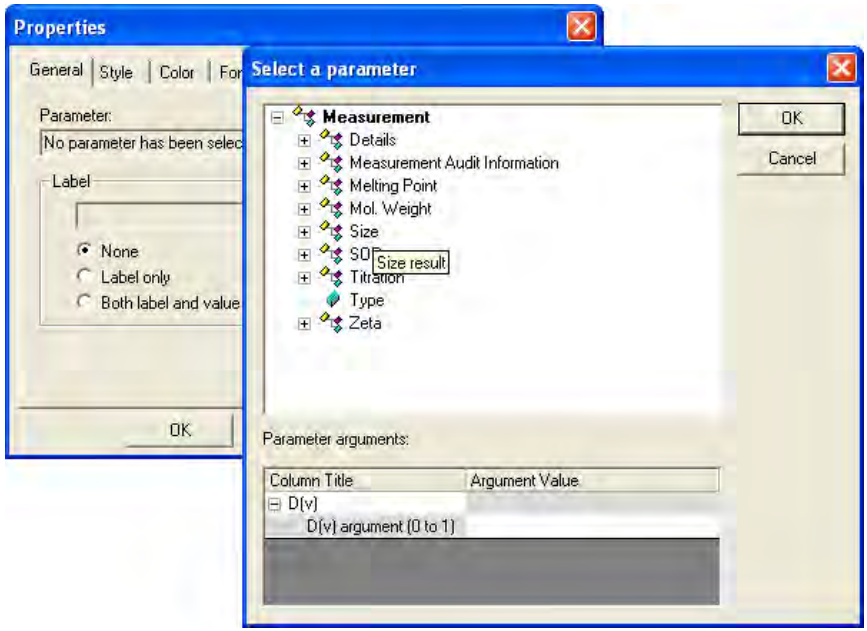
Picture

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** window 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 window, plus a **General** tab to specify what the parameter is, as shown above:

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

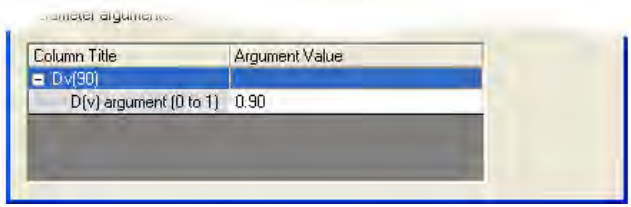
Use the **Label** selection area to add a parameter label alongside the parameter value. The style, colour and font of the label and its value can also be defined here.

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.

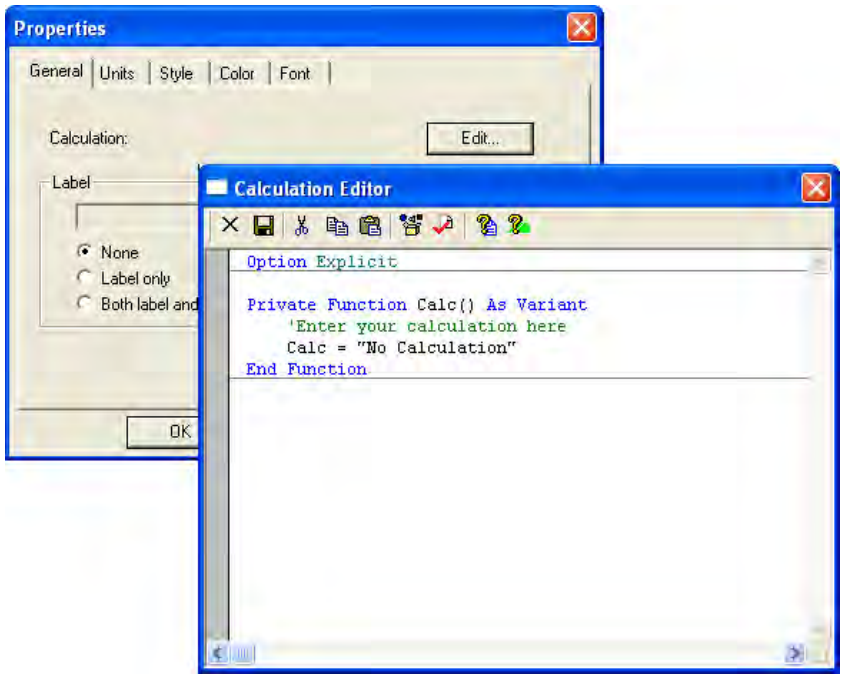
Frame

Use the **Frame** tool to split the report into relevant sections.
With the element selected, use its **Properties** window 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** window to configure the calculation.



The **Properties** window 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**.

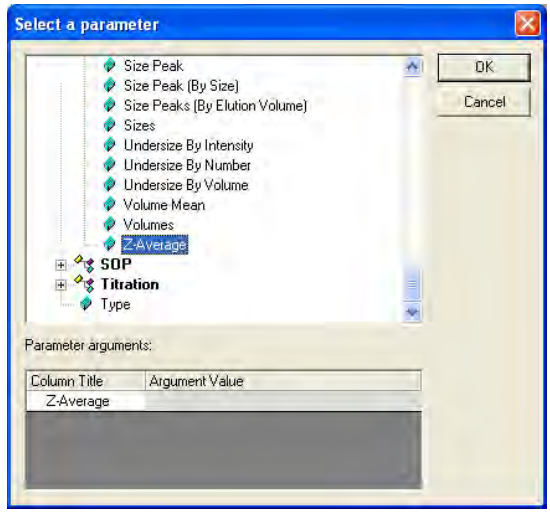
Well-plate

The **Well-plate** report 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) once added to the report despite showing a 384 well-plate image in Report Designer. Double click on the image of the plate to show the **Properties** window.

Setting Parameters

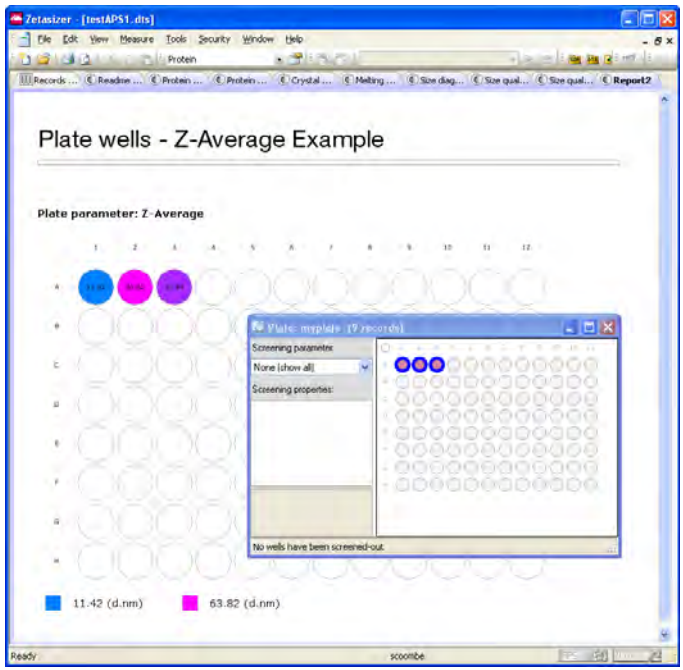
1. Select the **Parameter** tab and click **Select...**
2. Choose the required parameter, for example Z-Average from the **Size** group, and then click **OK**.
3. Ensure that **Show values in wells** is selected if numeric data is required on the wells.



Setting Colours

Choose the **Color** tab to set the high and low value colours - these are shown automatically on the 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. 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 through 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 well-plate. Then set screening parameters and range limits as required in the Plate Navigator (refer to **Zetasizer APS manual** 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.

For more information on the Plate Navigator and well-plate, please consult the **Zetasizer APS manual**.

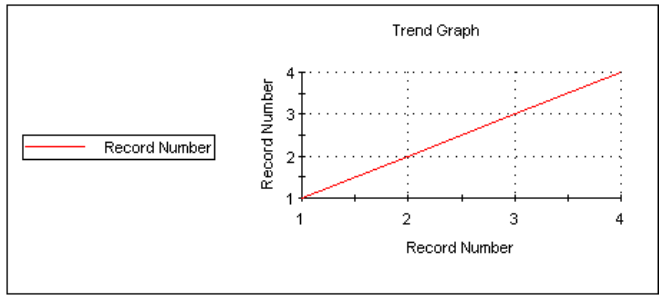
Graphs

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**, **Molecular weight** and **Zeta potential** 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** window.

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
Displays the temperature at which Aggregation 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.

Zeta potential graphs

The following zeta potential graphs can be inserted.

Zeta Potential
This shows the zeta potential distribution generated by the measurement. It can be displayed in different ways using its **Properties** box.

Statistics
The statistics graph will show the mean, standard deviation, maximum and minimum of a group of selected measurement records. The statistics shown on the table can be altered using the **Properties** window.

Titration graphs

The following Titration graphs can be inserted.

Titration
These graphs show a plot of the titration parameter against the selected measurement type, i.e. zeta potential versus pH. The graph, when used in the main Zetasizer application software, will automatically configure to the titration/ measurement type result selected from the records list.

Graph properties window - general

This window allows the attributes of the graph to be altered.

- **Display** or **Option** – Depending on the graph type inserted, **Display** or **Option** is displayed in the **Properties** window.

  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 position of the key to be chosen. A graph tips option enables pop up tips (flags showing data points on the graph to be shown on the report (not on the screen shown in the Report Designer).

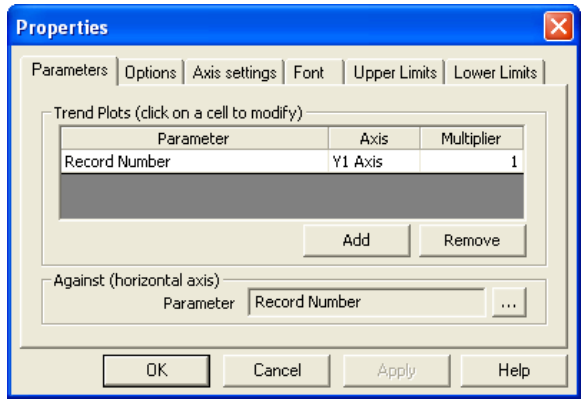
- **Axis settings** – Allows both the x and y-axis settings to be defined. Whether **logarithmic** or **linear** axis are required and the axis scales - defined or autoscaling. **Graticule** (or grid) lines can also be shown on the graphs.

- **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** window 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** window.

Use the **Against** area to select the x-axis parameter.

Line styles

Select **Configure-Line styles** to display a line styles window. 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, Molecular weight** and **Zeta potential** with accompanying table choices.

Trend tables

Trend tables allow the measurement data from multiple records to be compared to investigate any trends in the information. Any two numerical parameters can be compared.

Titration tables

This will insert a table of the titration parameter against the selected measurement type, i.e. zeta potential versus pH. The table, when used in the main Zetasizer application software, will automatically configure to the titration/measurement type result selected from the records list.

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** window.

Molecular weight tables

The following molecular weight table can inserted.

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

Zeta potential tables

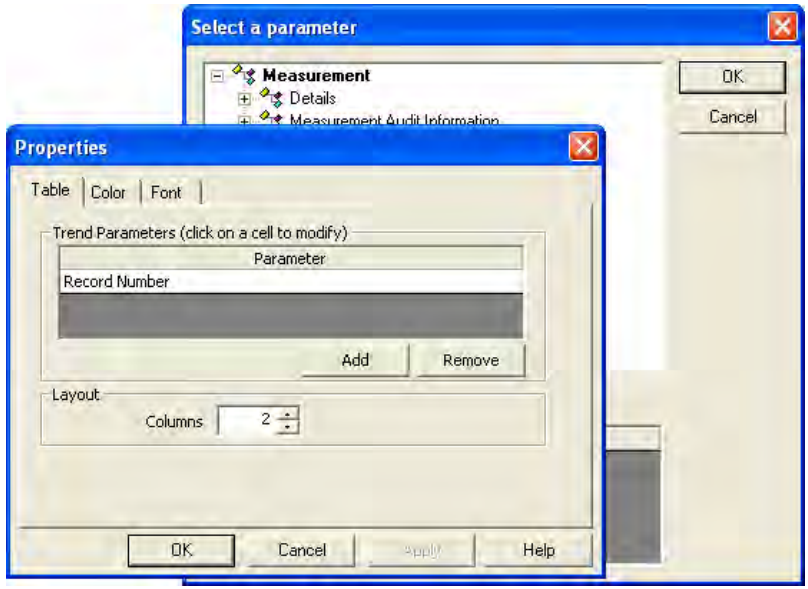
The following zeta potential tables can be inserted.

Zeta Potential
This shows the result of the measurement. It can be displayed as either zeta potential or mobility using its properties box.

Statistics
The statistics graph will show the mean, standard deviation, maximum and minimum of a group of selected measurement records. The statistics shown on the table can be altered using the **Properties** window.

Table properties window

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

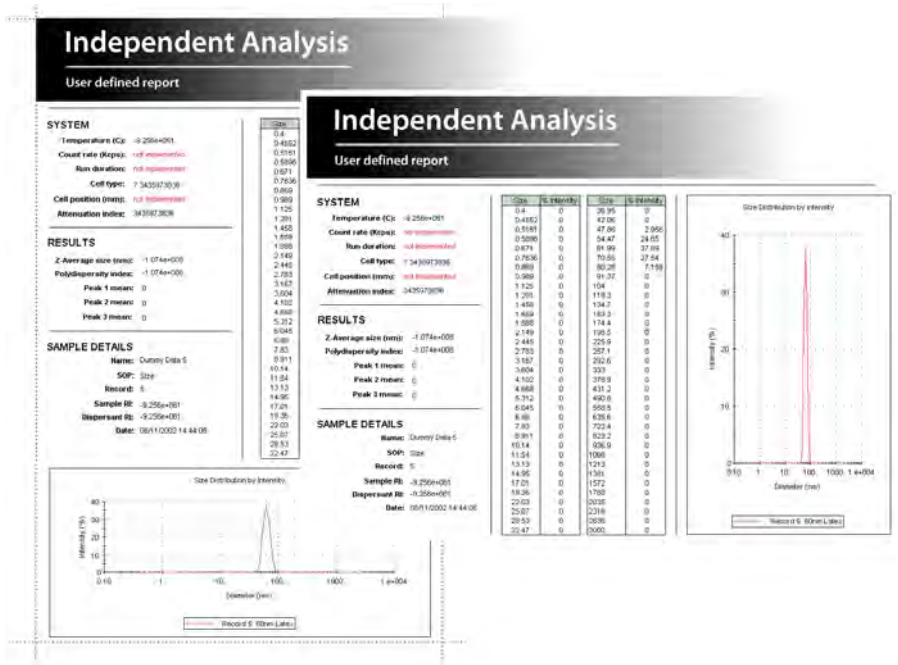
Note
Right-clicking on a selected element also brings up the layout options.

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 is displayed, 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. Some rearranging and formatting to line up all the elements may then be required.

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.

Note

New reports must be saved to the **My Documents\Malvern instruments\Zetasizer\Reports\en-US** (or **ja-JP** for Japanese) directory to be viewed in Records view.

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 Nano window:

1. Select **Tools-Settings-Workspace** and select the workspace to associate the new report with.
2. Select the **Report Pages** tab.
   The report name will be displayed in the report pages selection window. If there are any reports with duplicate titles visible to the software then they will be listed in the Report Pages tab with their file location.
3. Select the check box for the newly created report. Click **OK**.
   If a user selects more than one report with the same title then a duplicate report warning will be displayed: **A report with the same name already exists**.
4. 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 window is displayed 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**.

Part 2 - Measurement theory

Size theory

Introduction

The aim of this chapter is to describe the basic **size** principles behind the Zetasizer Nano series. This will help in understanding the meaning of the results achieved.

The chapter is divided into two major sections. **What is Dynamic light scattering?** and **Operation of the Zetasizer Nano - Size measurements**. The first section describes the theory, while the second describes the physical operation of how a size measurement is performed.

What is Dynamic light scattering?

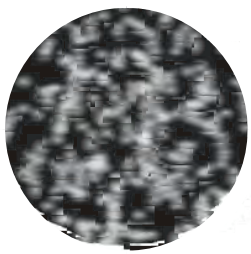
The Zetasizer Nano series performs size measurements using a process called **D**ynamic **L**ight **S**cattering (**DLS**)

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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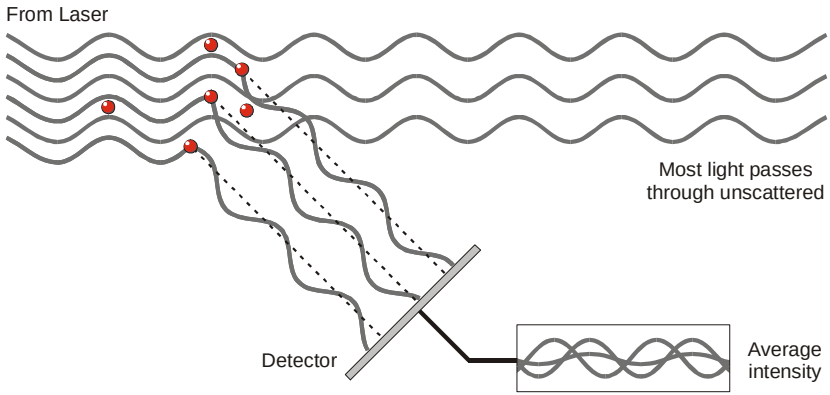
The speckle pattern will consist of bright and dark areas.

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 were stationary. 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 at any particular point appears to fluctuate. The Zetasizer Nano system measures the rate of the intensity fluctuation and then uses this to calculate the size of the particles.

Interpreting scattering intensity fluctuation data

We know that the Zetasizer measures the fluctuation in scattering intensity and uses this to calculate the size of particles within the sample - but how does it do this?

Within the instrument is a component called a digital correlator. A correlator basically measures the degree of similarity between two signals over a period of time.

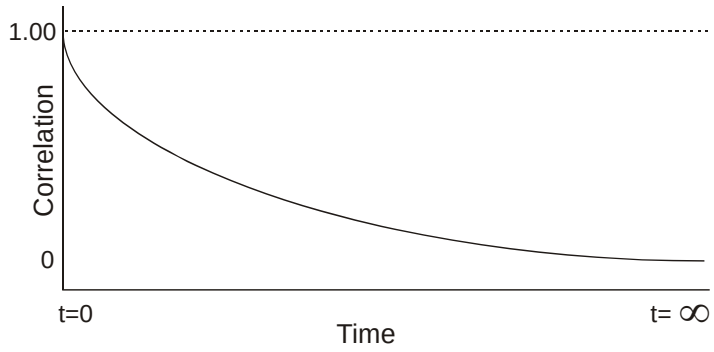
If we compared the intensity signal of a particular part of the speckle pattern at one point in time (say time = t) to the intensity signal a very short time later (t+δt) we would see that the two signals are very similar - or strongly correlated. If we then compared the original signal a little further ahead in time (t+2δt), there would still be a relatively good comparison between the two signals, but it will not be as good as at t+ δt. 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+3δt), (t+4δt), (t+5δt), (t+6δt), etc, the correlation will eventually reach zero. A typical correlation function against time is shown below.



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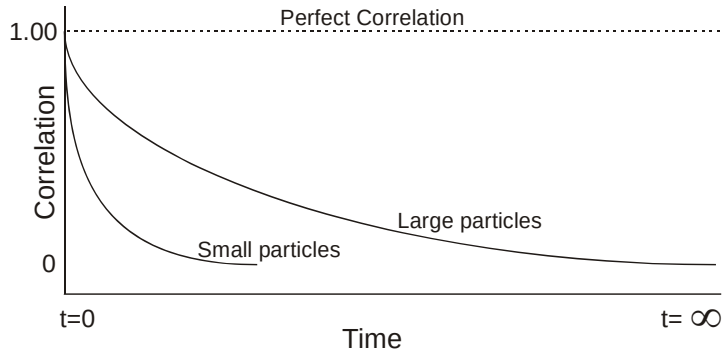
Using the correlation function

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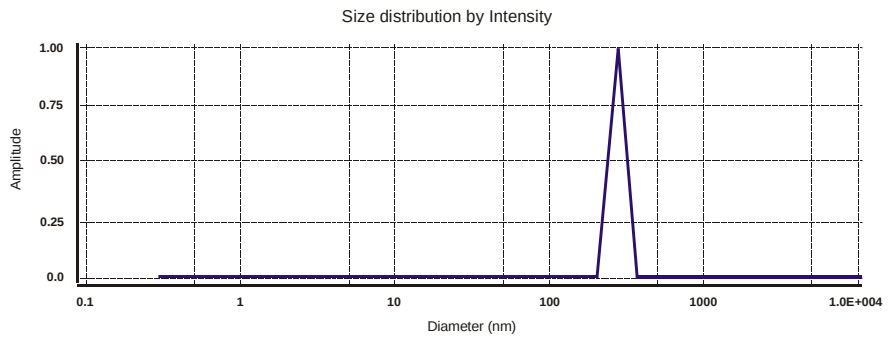
After the correlation function has been measured this information can then be used to calculate the size distribution. The Zetasizer software uses algorithms to extract the decay rates for a number of size classes to produce a size distribution.

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

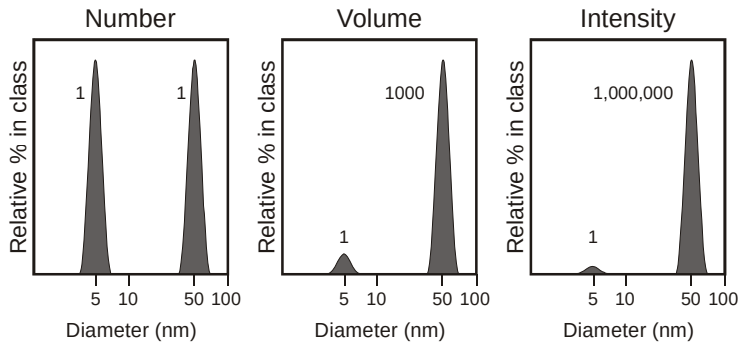
What is the difference between intensity, volume and number distributions?

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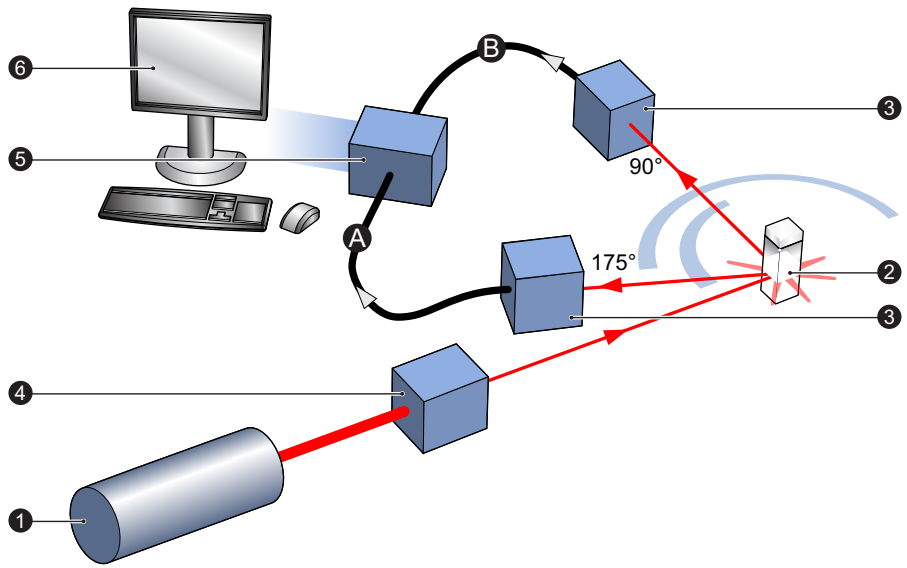
It is worth repeating that the basic distribution obtained from a DLS measurement is intensity - all other distributions are generated from this.

Operation of the Zetasizer Nano - Size measurements

A typical DLS system comprises of six main components. First of all a **laser** ① is used to provide a light source to illuminate the sample particles within a **cell** ②. Most of the laser beam passes straight through the sample, but some is scattered by the particles within the sample. A **detector** ③ is used to measure the intensity of the scattered light. As a particle scatters light in all directions, it is (in theory), possible to place the detector in any position and it will still detect the scattering.



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With the Zetasizer Nano series, depending upon the particular model, the detector position will be at either 175° or 90°.

Zetasizer	Optical Arrangement	Detection path (above)
Nano S / ZS	175°	Ⓐ
Nano S90 / ZS90	90°	Ⓑ

The intensity of the scattered light must be within a specific range for the detector to successfully measure it. If too much light is detected then the detector will become overloaded. To overcome this an "**attenuator**" ④ is used to reduce the intensity of the laser and hence reduce the intensity of the scattering.

- For samples that do not scatter much light, such as very small particles or samples of low concentration, the amount of scattered light must be increased. In this situation, the attenuator will allow more laser light through to the sample.
- For samples that scatter more light, such as large particles or samples of higher concentration, the amount of scattered light must be decreased. This is achieved by using the attenuator to reduce the amount of laser light that passes through to the sample.

The appropriate attenuator position is automatically determined by the Zetasizer during the measurement sequence.

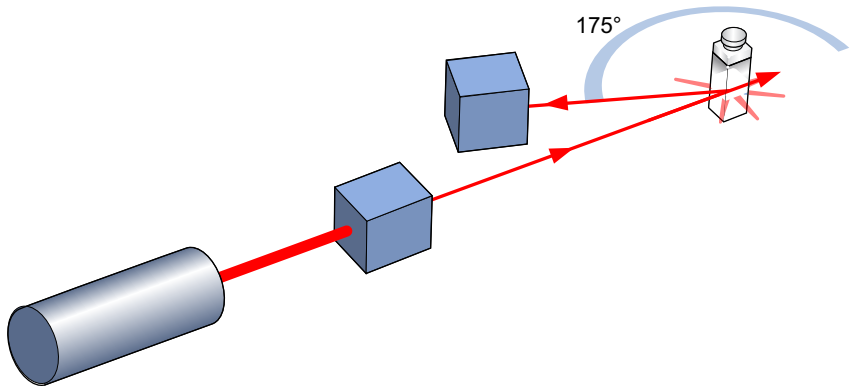
The scattering intensity signal for the detector is passed to a digital signal processing board called a **correlator** ⑤. The correlator compares the scattering intensity at successive time intervals to derive the rate at which the intensity is varying.

This correlator information is then passed to a **computer** ⑥, where the specialist **Zetasizer software** will analyse the data and derive size information.

175° detection optics - Backscatter detection

The Zetasizer Nano Z, S and ZS measure the scattering information at close to 180°. This is known as **backscatter detection**.

The application of the Backscatter detection is by a patented technology called **NIBS** (**N**on-**I**nvasive **B**ack-**S**catter).



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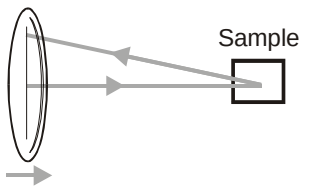
Why measure backscatter? There are several advantages to doing this:

- Because the backscatter is being measured, the incident beam does not have to travel through the entire sample. As the light passes through a shorter path length of the sample, then higher concentrations of sample can be measured.
- This reduces an effect known as multiple scattering, where the scattered light from one particle is itself scattered by other particles.

- Contaminants such as dust particles within the dispersant are typically large compared to the sample size. Large particles mainly scatter in the forward direction. Therefore, by measuring the backscatter, the effect of dust is greatly reduced.
- The effect of multiple scattering is at a minimum at 180° - again, this allows higher concentrations to be measured.

Moveable lens

A movable lens allows the focus position within the cell to be changed. This allows a much larger range of sample concentrations to be measured.



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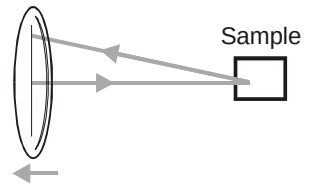
For small particles, or samples of low concentration, it will be beneficial to maximise the amount of scattering from the sample.

As the laser passes through the wall of the cell and into the dispersant, the cell wall will cause “flare”. This flare may swamp the scattering signal. Moving the measurement point away from the cell wall, towards the centre of the cell will remove this effect.



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Large particles or samples of high concentration, scatter much more light. In this situation, measuring closer to the cell wall will reduce the effect of multiple scattering.

In this instance the flare from the cell wall will have less impact. Any flare will be proportionally reduced compared to the scattering signal.

The measurement position is automatically determined by the Zetasizer software.

90° detection optics - Classical arrangement

The 90° models, Zetasizer Nano S90 and ZS90, have been included in the Zetasizer Nano instrument range to provide continuity with other systems that have 90° detection optics.

These models do not utilise a movable optical arrangement but use the ‘classical’ fixed detection arrangement of 90° to the laser and the centre of the cell area.

This arrangement reduces the detectable size range on these models.

Molecular weight theory

Introduction

The aim of this chapter is to describe the basic **molecular weight** principles behind the Zetasizer Nano. This will help in understanding the meaning of the results achieved.

The chapter is divided into two major sections. **What is Static light scattering?** and **The Debye plot**. The first section describes the molecular weight theory, while the second shows how a molecular weight measurement is displayed.

What is Static light scattering?

The Zetasizer Nano series performs molecular weight measurements using a process called **S**tatic **L**ight **S**cattering (**SLS**). This is a non-invasive technique used to characterise the molecules in a solution.

In a similar way to Dynamic Light Scattering - the size theory - the particles in a sample are illuminated by a light source such as a laser, with the particles scattering the light in all directions. But, instead of measuring the time-dependent fluctuations in the scattering intensity, Static light scattering makes use of the time-averaged intensity of scattered light instead.

The intensity of light scattered over a period of time, say 10 to 30 seconds, is accumulated for a number of concentrations of the sample. This time averaging removes the inherent fluctuations in the signal, hence the term 'Static Light Scattering'.

From this we can determine the **Molecular weight (M_W)** and the **2nd Virial Coefficient (A_2)**.

The **2nd Virial Coefficient (A_2)** is a property describing the interaction strength between the particles and the solvent or appropriate dispersant medium.

- For samples where $\mathbf{A_2>0}$, the particle 'likes' the solvent more than itself, and will tend to stay as a stable solution.
- When $\mathbf{A_2<0}$, the particle 'likes' itself more than the solvent, and therefore may aggregate.
- When $\mathbf{A_2=0}$, the particle-solvent interaction strength is equivalent to the molecule-molecule interaction strength – the solvent can then be described as being a **theta** solvent.

Static light scattering - theory

The molecular weight is determined by measuring the sample at different concentrations and applying the **Rayleigh equation**. The Rayleigh equation describes the intensity of light scattered from a particle in solution.

The **Rayleigh equation** is

$$\frac{KC}{R_\theta} = \left(\frac{1}{M_W} + 2A_2C\right)P(\theta)$$

- R_θ**:** The Rayleigh ratio - the ratio of scattered light to incident light of the sample.
- M_W : Sample molecular weight.
- A_2 : 2_{nd} Virial Coefficient.
- C : Concentration.
- P_θ : Angular dependence of the sample scattering intensity. Please refer to the Rayleigh scattering section.
- K : Optical constant as defined below.

$$K = \frac{4\pi^2}{\lambda_o^4 N_A}\left(n_o\frac{dn}{dc}\right)^2$$

N_A : Avogadro's constant.
λ_o : Laser wavelength.
n_o : Solvent refractive index.
dn/dc : The differential refractive index increment. This is the change in refractive index as a function of the change in concentration. For many sample/solvent combinations this may be available in literature; while for novel combinations the dn/dc can be measured by use of a differential refractometer.

The standard approach for molecular weight measurements is to first measure the scattering intensity of the analyte used relative to that of a well described 'standard' pure liquid with a known Rayleigh ratio.

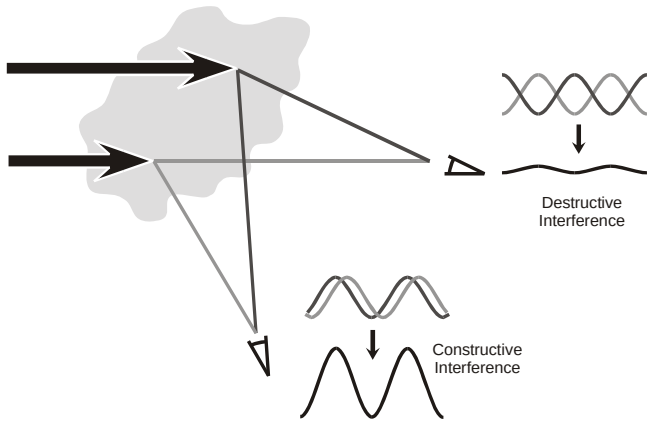
A **common standard** used in Static light scattering is **Toluene**, for the simple reason that the Rayleigh ratios of toluene are suitably high for precise measurements, are known over a range of wavelengths and temperatures and, maybe more importantly, toluene is relatively easy to obtain in a pure form. The Rayleigh ratio of toluene can be found in many reference books, but for reference purposes the expression used to calculate the sample Rayleigh ratio from a toluene standard is given below.

$$R_\theta = \frac{I_A n_o^2}{I_T n_T^2} R_T$$

- I_A : Residual scattering intensity of the analyte (i.e. the sample intensity – solvent intensity).
- I_T : Toluene scattering intensity.
- n_o : Solvent refractive index.
- n_T : Toluene refractive index.
- R_T : Rayleigh ratio of toluene.

Rayleigh scattering

The P_θ term in the Rayleigh equation embodies the angular dependence of the sample scattering intensity. The angular dependence arises from constructive and destructive interference of light scattered from different positions on the same particle, as shown below.



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This phenomenon is known as **Mie scattering**, and it occurs when the particle size is of the same order as the wavelength.

However when the particles in solution are much smaller than the wavelength of the incident light, multiple photon scattering will be avoided. Under these conditions, P_θ will reduce to **1** and the angular dependence of the scattering intensity is lost. This type of scattering is known as **Rayleigh scattering**.

The Rayleigh equation will now be:

$$\frac{KC}{R_\theta} = \left(\frac{1}{M} + 2A_2C\right)$$

We can therefore stipulate that if the particle is small, Rayleigh scattering can be assumed and the Rayleigh approximation used.

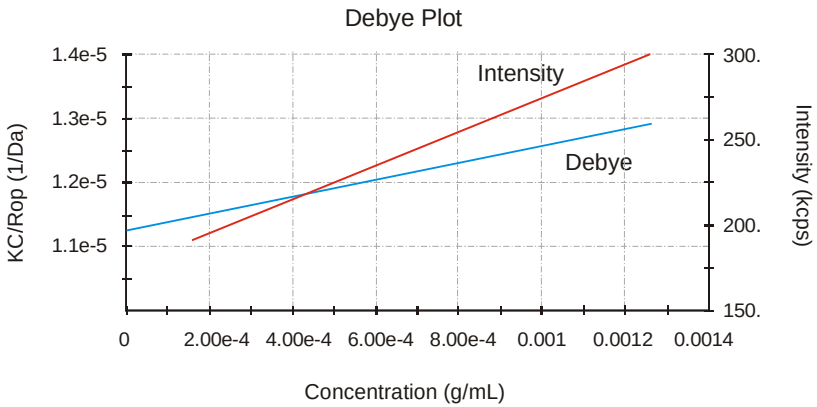
With the Zetasizer Nano series the applicable molecular measurement weight range is from a few hundred g/mol to 500,000 for linear polymers, and over 20,000,000 for near spherical polymers and proteins.

The Debye plot

The intensity of scattered light that a particle produces is proportional to the product of the weight-average molecular weight and the concentration of the particle.

The Zetasizer Nano S and ZS measure the **Intensity of scattered light** (**KC/R$_\theta$**) of various **concentrations** (**C**) of sample at **one** angle; this is compared with the scattering produced from a standard (i.e. Toluene).

The graphical representation of this is called a Debye plot and allows for the determination of both the Absolute molecular weight and 2nd Virial Coefficient.



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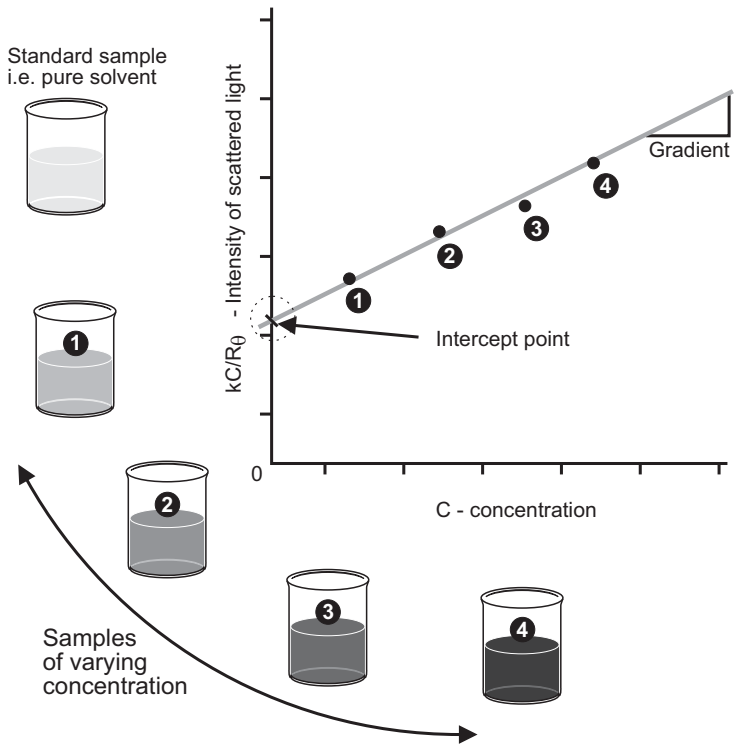
The weight-averaged molecular weight (**Mw**) is determined from the intercept at zero concentration i.e. $\mathbf{KC/R_\theta = 1/Mw}$ (for c--> 0) where the Mw is expressed in Daltons (or g/mol).

The **2nd Virial Coefficient** ($\mathbf{A_2}$) is determined from the gradient of the Debye plot.

Each plot and molecular weight measurement is performed by doing several individual measurements; from the solvent used (a zero concentration measurement), through sample preparations at various concentrations.

The diagram below shows how the molecular weight and 2nd Virial Coefficient are derived from the Debye plot.



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As only one measurement angle is used in this case, a plot of $\mathbf{KC/R_\theta}$ versus $\frac{\mathbf{C}}{}$ should give a straight line whose intercept at zero concentration will be **1/Mw** and whose **gradient** is proportional to $\mathbf{A_2}$.

Zeta potential theory

Introduction

The aim of this chapter is to describe the basic **Zeta potential** measurement principles behind the Zetasizer Nano. This will help in understanding the meaning of the results achieved.

The chapter is divided into two major sections. **What is Zeta Potential?** and **Operation of the Zetasizer Nano - Zeta potential measurements**. The first section describes the zeta potential theory, while the second describes the physical operation of how a zeta potential measurement is performed.

What is Zeta potential?

The Zetasizer Nano series calculates the zeta potential by determining the **Electrophoretic Mobility** and then applying the **Henry equation**. The electrophoretic mobility is obtained by performing an electrophoresis experiment on the sample and measuring the velocity of the particles using **Laser Doppler Velocimetry** (**LDV**).

These techniques are described in the following sections.

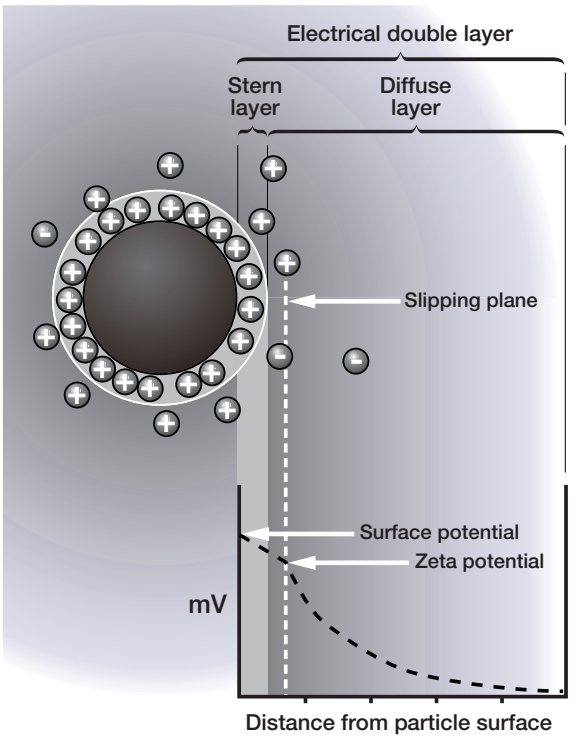
Zeta potential and the Electrical double layer

The development of a net charge at the particle surface affects the distribution of ions in the surrounding interfacial region, resulting in an increased concentration of counter ions (ions of opposite charge to that of the particle) close to the surface.

The liquid layer surrounding the particle exists as two parts; an **inner** region, called the **Stern layer,** where the ions are strongly bound and an **outer**, diffuse, region where they are less firmly attached; thus an **electrical double layer** exists around each particle. Within the diffuse layer there is a notional boundary inside which the ions and particles form a stable entity. When a particle moves (e.g. due to gravity),

ions **within** the boundary move with it, but any ions beyond the boundary **do not** travel with the particle. This boundary is called the surface of hydrodynamic shear or **slipping plane**.

The potential that exists at this boundary is known as the **Zeta potential**.



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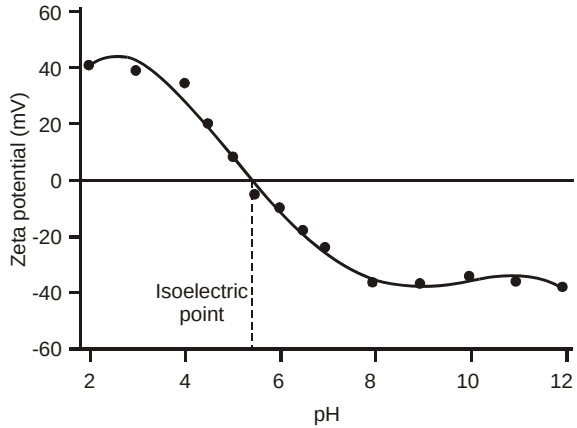
The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. A colloidal system is when one of the three states of matter: gas, liquid and solid, are finely dispersed in one of the others. For this technique we are interested in the two states of: a solid dispersed in a liquid, and a liquid dispersed in a liquid, i.e. an emulsion.

If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there is no tendency to flocculate. However, if the particles have low zeta potential values then there is no force to prevent the particles coming together and flocculating. The general dividing line between stable and unstable suspensions is generally taken at either +30mV or -30mV. Particles with zeta potentials more positive than +30mV or more negative than -30mV are normally considered stable.

The most important factor that affects zeta potential is **pH**. A zeta potential value on its own without a quoted pH is a virtually meaningless number.

Imagine a particle in suspension with a negative zeta potential. If more alkali is added to this suspension then the particles will tend to acquire a more negative charge. If acid is then added to this suspension a point will be reached where the negative charge is neutralised. Any further addition of acid can cause a build up of positive charge. Therefore a zeta potential versus pH curve will be positive at low pH and lower or negative at high pH.

The point where the plot passes through zero zeta potential is called the **Isoelectric point** and is very important in prectical terms. It is normally the point where the colloidal system is least stable. A typical plot of zeta potential versus pH is shown below.



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Electrokinetic effects

An important consequence of the existence of electrical charges on the surface of particles is that they will exhibit certain effects under the influence of an applied electric field. These effects are collectively defined as **electrokinetic effects**. There are four distinct effects depending on the way in which the motion is induced. These are:

- **Electrophoresis** :
  The movement of a charged particle relative to the liquid it is suspended in under the influence of an applied electric field.
- **Electroosmosis** :
  The movement of a liquid relative to a stationary charged surface under the influence of an electric field.
- **Streaming potential** :
  The electric field generated when a liquid is forced to flow past a stationary charged surface.

- **Sedimentation potential** :
  The electric field generated when charged particles move relative to a stationary liquid.

Electrophoresis

When an electric field is applied across an electrolyte, charged particles suspended in the electrolyte are attracted towards the electrode of opposite charge. Viscous forces acting on the particles tend to oppose this movement. When equilibrium is reached between these two opposing forces, the particles move with constant **velocity**.

The **velocity** of the particle is dependent on the following factors:

- Strength of electric field or voltage gradient.
- The dielectric constant of the medium.
- The viscosity of the medium.
- The zeta potential.

The **velocity** of a particle in an electric field is commonly referred to as its **Electrophoretic mobility**.

With this knowledge we can obtain the zeta potential of the particle by application of the **Henry equation**.

The **Henry equation** is:

$$U_E = \frac{2\varepsilon z f(\kappa a)}{3\eta}$$

where:

- z : Zeta potential.
- U_E : Electrophoretic mobility.
- ε : Dielectric constant.
- η : Viscosity.
- *f(Ka)* : **Henry's** function.
  Two values are generally used as approximations for the *f(Ka)* determination - either **1.5** or **1.0**.

  Electrophoretic determinations of zeta potential are most commonly made in aqueous media and moderate electrolyte concentration. *f(Ka)* in this case is **1.5**, and is referred to as the **Smoluchowski** approximation. Therefore calculation of zeta potential from the mobility is straightforward for systems that fit the Smoluchowski model, i.e. particles larger than about 0.2 microns dispersed in

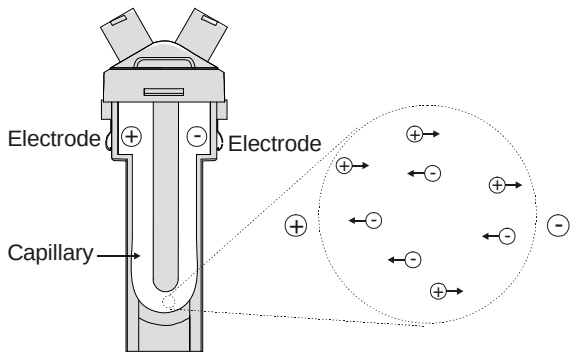
electrolytes containing more than 10^{-3} molar salt.
The **Smoluchowski** approximation is used for the folded capillary cell and the universal dip cell when used with aqueous samples.

For small particles in low dielectric constant media *f(Ka)* becomes **1.0** and allows an equally simple calculation. This is referred to as the **Huckel** approximation. **Non-aqueous** measurements generally use this.

Measuring Electrophoretic Mobility

It is the electrophoretic mobility that we measure directly with the conversion to zeta potential being inferred from theoretical considerations. How is electrophoretic mobility measured?

The essence of a classical micro-electrophoresis system is a cell with electrodes at either end to which a potential is applied. Particles move towards the electrode of opposite charge, their velocity is measured and expressed in unit field strength as their mobility.



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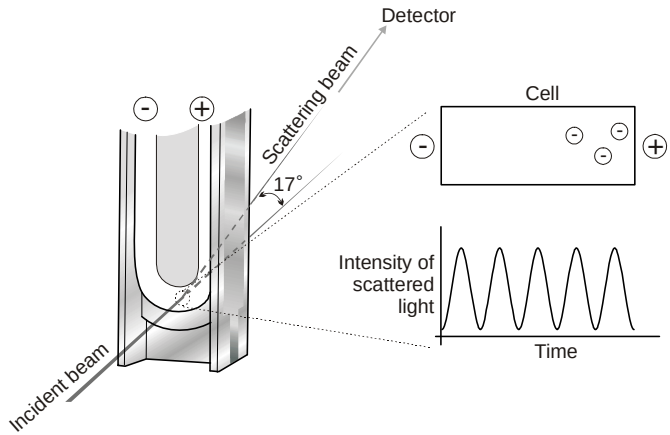
The technique used to measure this velocity is **Laser Doppler Velocimetry**.

Laser doppler velocimetry

Laser doppler velocimetry (**LDV**) is a well established technique in engineering for the study of fluid flow in a wide variety of situations, from the supersonic flows around turbine blades in jet engines to the velocity of sap rising in a plant stem.

In both these examples, it is actually the velocity of tiny particles within the fluid streams moving at the velocity of the fluid that we are measuring. Therefore, LDV is well placed to measure the velocity of particles moving through a fluid in an electrophoresis experiment.

The receiving optics are focused so as to relay the scattering of particles in the cell.



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The light scattered at an angle of 17° is combined with the reference beam. This produces a fluctuating intensity signal where the rate of fluctuation is proportional to the speed of the particles. A digital signal processor is used to extract the characteristic frequencies in the scattered light.

Optical Modulator

A refinement of the system involves modulating one of the laser beams with an oscillating mirror. This gives an unequivocal measure of the **sign** of the **zeta potential**.

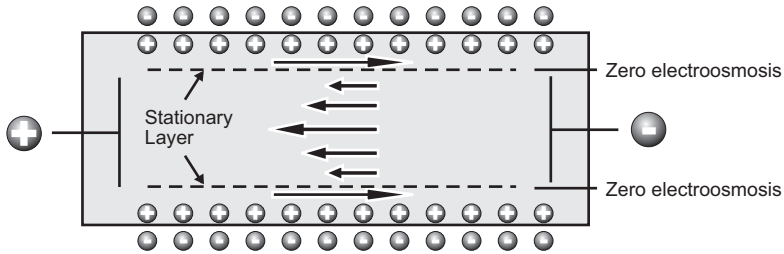
A second benefit of the modulator is that low or zero mobility particles give an equally good signal, so measurement is as accurate as for particles with a high mobility.

This technique ensures an accurate result in a matter of seconds, with possibly millions of particles observed.

The Electroosmosis effect

The walls of the capillary cell carry a surface charge so the application of the electric field needed to observe electrophoresis causes the liquid adjacent to the walls to undergo electroosmotic flow. Colloidal particles will be subject to this flow superimposed on their electrophoretic mobility. However, in a closed system the flow along the walls must be compensated for by a reverse flow down the centre of the capillary.

There is a point in the cell at which the electroosmotic flow is zero - where the two fluid flows cancel. If the measurement is then performed at this point, the particle velocity measured will be the **true electrophoretic velocity**. This point is called the **stationary layer**. It is where the two laser beams cross; the zeta potential measured is therefore free of electroosmotic errors.



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Avoiding Electroosmosis

The stationary layer technique described above has been in use for many years. Because of the effect of electroosmosis the measurement can only be performed at specific point within the cell. If it was possible to remove electroosmosis altogether then it would be possible to perform the measurement on the particles at **any point in the cell** and obtain the true mobility.

This is now possible. With a combination of Laser Doppler Velocimetry and **P**hase **A**nalysis **L**ight **S**cattering (**PALS**) this can now be achieved in Malvern's patented **M3-PALS** technique.

Implementation of M3-PALS enables even samples of very low mobility to be analysed and their mobility distributions calculated.

The M3-PALS technique

To perform measurements at any point within a cell and obtain the electrophoretic mobility, Malvern has developed its patented **M3-PALS** technique. This is a combination of Malvern's improved laser doppler velocimetry method - the **M3** measurement technique, and the application of **PALS** (**P**hase **A**nalysis **L**ight **S**cattering).

The M3 technique

As discussed earlier traditional electrophoretic measurements are performed by measurement of particles at the stationary layer, a precise position near the cell walls. With M3 the measurement can be performed anywhere in the cell, though with the Zetasizer Nano series it is performed in the centre of the cell.

M3 consists of both **S**low **F**ield **R**eversal (**SFR**) and **F**ast **F**ield **R**eversal (**FFR**) measurements, hence the name '**Mixed Mode Measurement**'.

Measurement position in the cell

The M3 method performs the measurement in the middle of the cell, rather than at the stationary layer. In principle the M3 measurement could be done at any

position in the cell, however there are a number of reasons for choosing to work at the **centre**.

- The measurement zone is further from the cell wall, so reduces the chance of flare from the nearby surface.
- The alignment of the cell is less critical.
- The charge on the cell wall can be calculated.

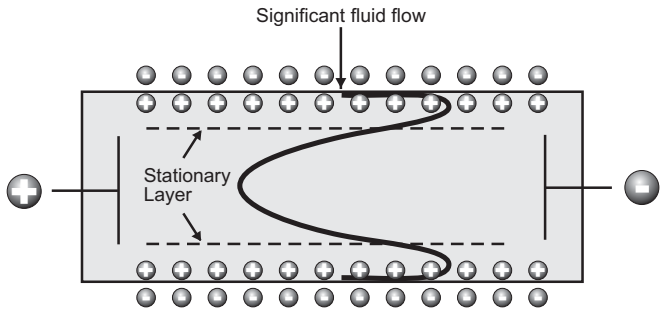
Reversal of the applied field

All systems that measure mobilities using LDV (Laser Doppler Velocimetry) reverse the applied field periodically during the measurement. This is normally just the slow field reversal mentioned below.

However, M3 consists of two measurements for each zeta potential measurement, one with the applied field being reversed slowly - the SFR measurement; and a second with a rapidly reversing applied field - the FFR measurement stage.

Slow Field Reversal (SFR)

This reversal is applied to reduce the polarisation of the electrodes that is inevitable in a conductive solution. The field is usually reversed about every one second to allow the fluid flow to stabilise.



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Fast Field Reversal (FFR)

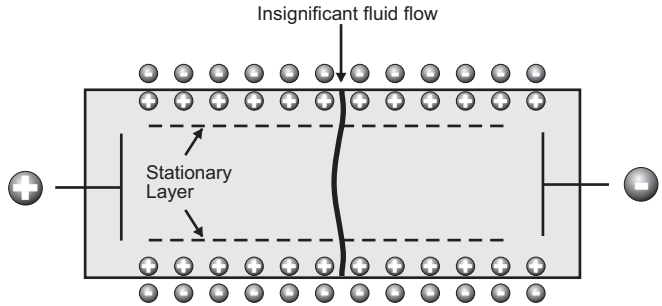
If the field is reversed much more rapidly, it is possible to show that the particles reach terminal velocity, while the fluid flow due to electroosmosis is insignificant. (The residual flow in the diagram below is exaggerated).

This means that the mobility measured during this period is due to the electrophoresis of the particles only, and is not affected by electroosmosis.

The mean zeta potential that is calculated by this technique is therefore very robust, as the measurement position in the cell is not critical.

However, as the velocity of the particles is sampled for such a short period of time, information about the distribution is degraded. This is what is addressed by M3,

the PALS technique is used to determine the particle mobility in this part of the sequence.



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M3 measurement sequence

An M3 measurement is performed in the following manner:

- A **Fast field** reversal measurement is performed at the cell centre. This gives an accurate determination of the mean.
- A **Slow field** reversal measurement is made. This gives better resolution, but mobility values are shifted by the effect of electroosmosis.
- The mean zeta potentials calculated from the FFR and SFR measurements are subtracted to determine the electroosmotic flow. This value is used to normalise the slow field reversal distribution.
- The value for electroosmosis is used to calculate the zeta potential of the cell wall.

Benefits of M3

Using M3 the entire zeta potential measurement is simplified. It is no longer necessary for the operator to select any system parameters for the measurement, as the appropriate settings are calculated as part of the M3 sequence. With a reduction in the number of measurement variables, both measurement repeatability and accuracy is improved. Additionally, alignment is no longer an issue as there are no concerns about the location of the stationary layer.

M3 is now combined with the PALS measurement.

Adding PALS and what is it?

PALS (**P**hase **A**nalysis **L**ight **S**cattering) is a further improvement on traditional Laser Doppler Velocimetry and the M3 implementation described above.

Overall, the application of PALS improves the accuracy of the measurement of low particle mobilities. This can give an increase in performance of greater than 100 times that associated with standard measurement techniques.

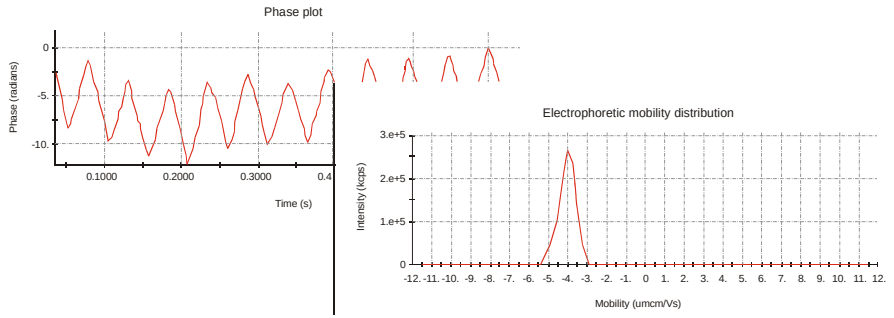
This allows the measurement of high conductivity samples, plus the ability to accurately measure samples that have low mobilities. Low applied voltages can now be used to avoid any risk of sample effects due to joule heating.

How PALS works

Rather than use the Doppler frequency shift caused by moving particles to measure their velocity, Phase Analysis Light Scattering, as the name suggests, uses the phase shift. The phase is preserved in the light scattered by moving particles, but is shifted in phase in proportion to their velocity. This phase shift is measured by comparing the phase of the light scattered by the particles with the phase of a reference beam. A beam splitter is used to extract a small proportion of the original laser beam to use as the reference.

The phase analysis of the signal can be determined accurately even in the presence of other effects that are not due to electrophoresis, for example thermal drifts due to joule heating. This is because the form of the phase change due to the application of the field is known so the different effects can be separated.

As electroosmosis is insignificant due to the implementation of M3 then the difference between the two phases will be constant, so if there is any particle movement then this phase relationship will alter. Detection of a phase change is more sensitive to changes in mobility, than the traditional detection of a frequency shift.



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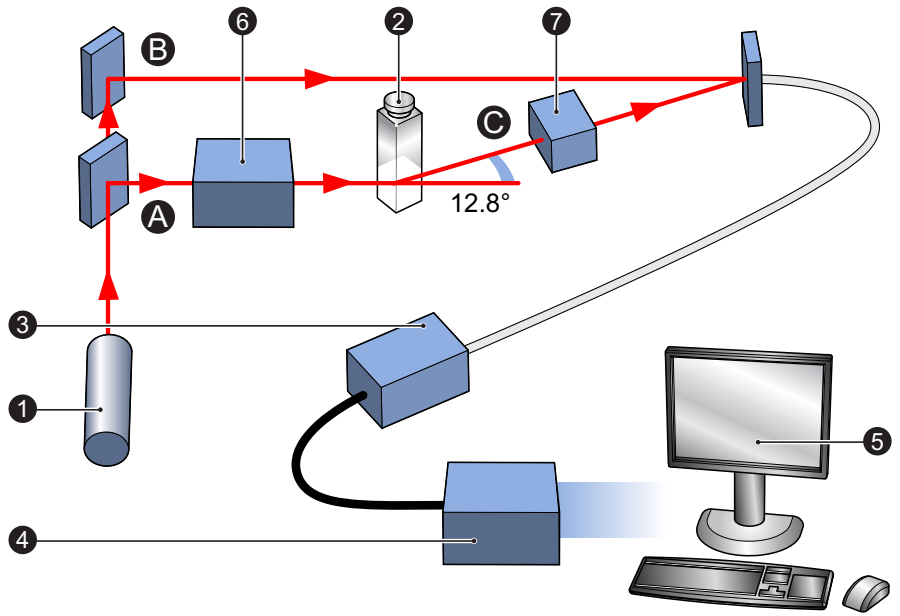
Electrophoretic mobility and consequently the **zeta potential** is then determined by summing the phase shifts measured during the FFR part of the measurement.

Operation of the Zetasizer Nano - Zeta potential measurements

In a similar way to the typical DLS system described in the size theory chapter, a zeta potential measurement system comprises six main components. First of all a **laser** ① is used to provide a light source to illuminate the particles within the sample; for zeta potential measurements this light source is split to provide an **Incident** Ⓐ and **Reference beam** Ⓑ. The reference beam is also 'modulated' to provide the Doppler effect necessary.

The laser beam passes through the centre of the sample **cell** ②, and the **scattering beam** Ⓒ at an angle of 12.8° is detected.



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When an electric field is applied to the cell, any particles moving through the measurement volume will cause the intensity of light detected to fluctuate with a frequency proportional to the particle speed.

A **detector** ③ sends this information to a **digital signal processor** ④. This information is then passed to a **computer** ⑤, where the **Zetasizer software** produces a frequency spectrum from which the electrophoretic mobility and hence the zeta potential information is calculated.

The intensity of the scattered light within the cell must be within a specific range for the detector to successfully measure it. If too much light is detected then the

detector will become overloaded. To overcome this an "**attenuator**" ⑥ is used to reduce the intensity of the laser and hence reduce the intensity of the scattering.

For samples that do not scatter much light, such as very small particles or samples of low concentration, the amount of scattered light must be increased. The attenuator will automatically allow more light through to the sample.

For samples that scatter more light, such as large particles or samples of higher concentration, the amount of scattered light must be decreased. The attenuator will automatically reduce the amount of light that passes through to the sample.

To correct for any differences in the cell wall thickness and dispersant refraction, **compensation optics** ⑦ are installed within the scattering beam path to maintain alignment of the scattering beams.

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