Malvern Panalytical
a spectris company

ZETASIZER ULTRA/PRO
USER GUIDE

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CONTENTS

CHAPTER 1 INTRODUCTION

About the Zetasizer

The Zetasizer instruments provide the ability to measure particle size and zeta potential of particles or molecules in a liquid medium. The Zetasizer features unique technology for measurements over a wide concentration range, and precise temperature control to give reproducible, repeatable and accurate measurements. Depending upon the options and accessories used, the Zetasizer system also has the ability to perform:

- Autotitration measurements if an MPT-3 Multi-purpose Titrator accessory is connected.
- Trend measurements, including the determination of the Aggregation point.

Other key parameters like conductivity, particle concentration, and with the MPT-3 Multi-purpose Titrator, pH can also be measured. The Zetasizer range has been designed so that a minimal amount of user interaction is necessary to achieve precise and repeatable results.

About this manual

This manual covers the following instruments:

Table 1.1 Instrument information

Instrument	Model number	Measurement types
Zetasizer Pro	ZSU5800	Particle size and zeta potential
Zetasizer Ultra	ZSU5700	Particle size, zeta potential, Multi-Angle Dynamic Light Scattering, particle concentration

Product documentation structure

This manual provides detailed information about the operation of the Zetasizer. This manual:

- Gives an overview of the software interface
- Provides information and guidance on sample preparation

- Provides information on the measurement process and appropriate software features
- Describes the tools available for data analysis
- Explains the theory behind the operation of the Zetasizer

This manual fits into the following information structure for this product:

- Basic Guide - provides the essentials required to get started, hardware information and vital health and safety information. All users must read this manual before using the system.
- User Guide - (this manual) provides detailed information on making a measurement and using the software.
- Help - integrated with the ZS XPLORER software, provides detailed information on using the system, video tutorials, and reference on all software features.
- Accessories Guide - gives detailed information about each cell and optional accessories, including the MPT-3 Multi-purpose Titrator.

WARNING!
The instrument and the samples measured may be hazardous if misused. Users must read the Health and Safety information in the *Zetasizer Ultra/Pro Basic Guide* before operating this system.

Cover removal

Malvern Panalytical personnel (service engineers and representatives) are the only people authorized to perform any service procedures that may require the removal of the covers.

WARNING!
Removal of the main covers by unauthorized personnel will void the warranty of the instrument.

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

Assumed information

General

The Zetasizer can be used with a variety of accessories that allow it to measure a variety of samples. Some of these accessories prepare and deliver the sample to the optical unit for measurement. For more information see the *Zetasizer Ultra/Pro Accessories Guide*.

Naming convention

Within this manual:

- The Zetasizer is referred to either in full or as "the instrument".
- The accessories are referred to by name or as "the accessory".
- The combination of the instrument, one or more accessories, and the computer is referred to as "the system".

Software option selection

Software options are shown in bold and take the form **main option-sub option**. As an example, **Home-Measure size** refers to the need to select *Home* and then choose a Size measurement.

Where to get help

This section provides information on how to get help with your system.

Help desk

Direct all queries regarding the system to your local Malvern Panalytical representative, providing the following information:

- Model and serial number of the instrument. The serial number is shown when you hover over the instrument status on the bottom right of the software display, and also on the rear panel of the instrument.
- The software version. To find this, click  in the top left corner next to Home, and select About. The software version will be displayed.

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

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

If located in the United States, contact the United States Helpdesk if the local Malvern Panalytical representative is not available:

Telephone: +1 508 768 6450 or email: support.us@malvernpanalytical.com.

Note:
The help lines are primarily English speaking.

Website - www.malvernpanalytical.com

The Malvern Panalytical website offers a comprehensive range of resources for customer use 24 hours a day, 7 days a week.

CHAPTER 2 SOFTWARE OVERVIEW

The ZS XPLORER software

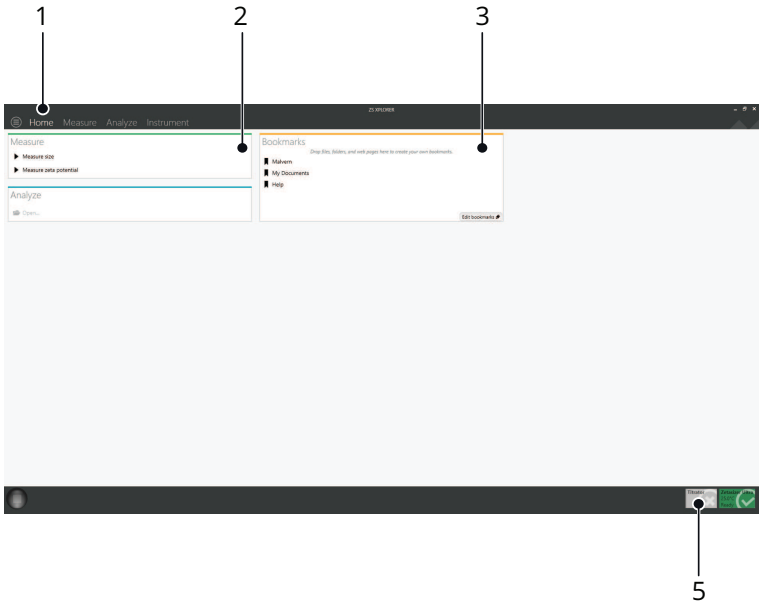
The ZS XPLORER software allows you to set up the details of the sample and parameters of your size, zeta potential, particle concentration or titration measurement. It controls the system during a measurement and then processes the measurement data to produce a result. It provides powerful analysis and export tools to help you get the best use of your data.

Starting the software

To start the software, click the ZS XPLORER icon on the desktop.

Figure 2.1 *ZS XPLORER desktop icon*

The following image shows the home page in the software. The numbered features are described in the following sections.



1. Menu bar
2. Measure
3. Instrument status icons
4. Bookmarks

Figure 2.2 ZS XPLORER software home page

Menu bar [1]

The Menu bar is the key navigation tool in the ZS XPLORER software. See Menu bar on page 15 for details.

Measure [2]

This window provides direct links to start measuring. Clicking **Measure size** or **Measure zeta potential** will set up a Method with a size or zeta potential measurement step already added. See Measurement on page 43 for full details on the measurement process.

Bookmarks [3]

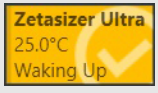
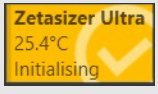
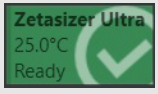
This window is a personalized bookmarks area, where you can add links to any resources you find useful. You can add links to files, folders and webpages.

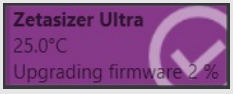
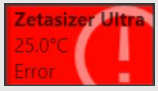
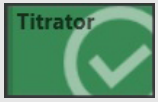
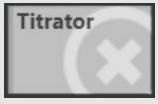
Instrument status icons [4]

The instrument status icons show the type of instrument and accessory connected, temperature, and status of the instrument.

Further information on the instruments' status can be found in the Zetasizer controls window in the Instrument menu.

Table 2.1 Connection status of instrument and MPT-3 Multi-purpose Titrator

Icon	Description
Zetasizer Ultra / Pro	
	Not detected – the instrument is disconnected or no connection can be made.
	Waking up - the instrument is connected but has not yet initialized.
	Initialising – the instrument is self configuring and is not ready for use.
	Ready – the instrument is ready for use.
	Measuring – the instrument is currently running a measurement.

Icon	Description
	Upgrading firmware – the instrument is currently upgrading firmware and is not ready for use.
	Error – the instrument is in an error state. In this state hover over the icon for further information. Contact the Malvern Panalytical helpdesk.
MPT-3 Multi-purpose Titrator	
	Connected – the accessory has been successfully connected.
	Disconnected – the accessory is disconnected or no connection can be made.

Measurement display

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

The following image shows how the software looks when a measurement is running.

1. Stop button
2. Progress bar
3. Measurement log
4. Live graph display
5. Method list

Figure 2.3 Live measurement display

Stop button [1]

Click this button to stop the current Method. You will be asked for confirmation before this is done. The aborted measurement will still be displayed under the **Complete** tab in the Method list, and you can click on it to view the measurement details.

Note:
You cannot restart a Method after aborting it. However, you can duplicate the measurement by clicking on it in the method list and clicking the **Copy Method** button.

Note:
When a measurement is aborted, any collected data is lost. Other measurements in the method that have already been completed are automatically saved.

Progress bar [2]

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

Measurement log [3]

The measurement log records data about the measurement progress. It updates in real time as the measurement is carried out. The measurement log contains data about the measurement temperature, attenuator setting, and number of runs, as well as other information.

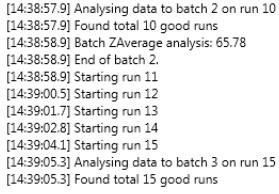


Figure 2.4 A typical measurement log

Note:
Measurement logs are saved by session, and will reset each time the software starts up. These are filed under Documents\Malvern Instruments\ZS XPLORER\logs.

Live graph display [4]

The graphs show the measurement data and are plotted in real time as the measurement progresses.

Note:
The live graph display is not configurable. The graph types shown will be automatically selected based on the type of measurement running.

Method list [5]

The Method list displays the planned measurements, measurements currently running, and completed measurements for the current software session. The Method list resets when you restart the software. More information is provided in Method list on page 61.

Menu bar

The Zetasizer menu bar is shown below.

Figure 2.5 Software menu bar

The Home window is described in The ZS XPLORER software on page 8. The other options are outlined below.

Options and About

Click in the top left corner to show the following panel.

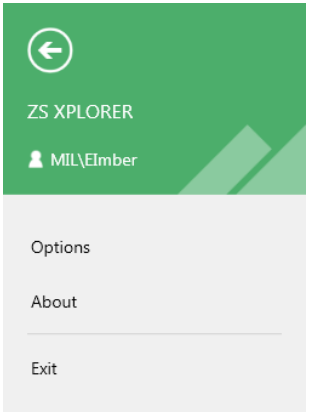


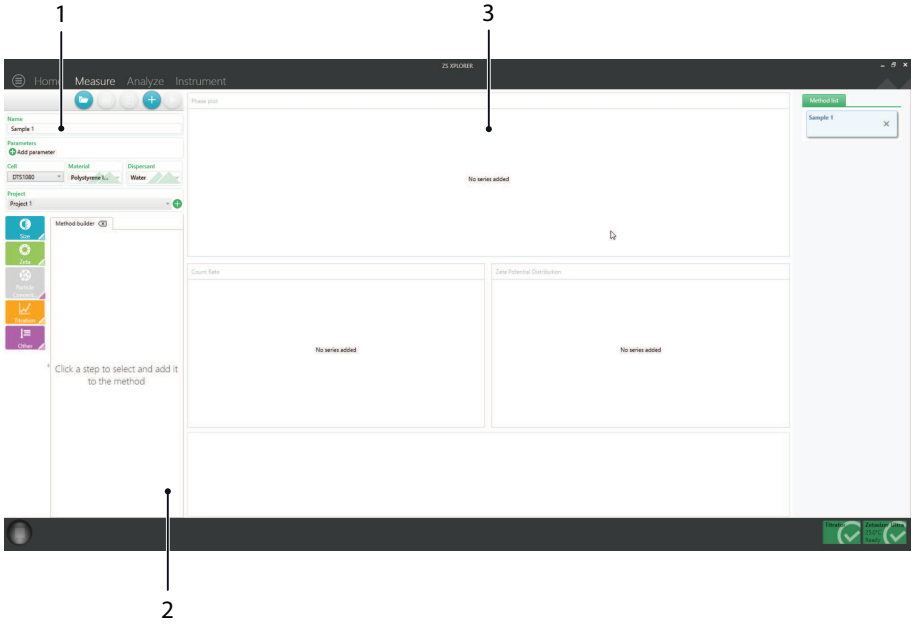
Figure 2.6 Options and About menu

From here, you can access the software **Options** and **About** sections.

- **Options** – choose which folder measurements are stored in
- **About** – view the current software version and find useful links

Measure

The **Measure** window is shown below. In this window, add Steps to a Method and specify your measurement settings to run a measurement. More detail is given in Measurement on page 43.



1. **Sample details** – see Measurement settings on page 49
2. **Method builder** – see Method Builder on page 38
3. **Measurement display area** – see Measurement display on page 12

Figure 2.7 Measure window

Analyze

The **Analyze** window is shown below. In this window, you can view, sort and edit your results. See Analysis on page 83 for further information.

Figure 2.8 Analyze window

1. **Measurement Explorer** – see Explorer on page 112
2. **Record selector** – see Record selector on page 84
3. **Workspace** – see Workspaces on page 100

Instrument

In the **Instrument** window there are **Zetasizer** and **Titrator** tabs.

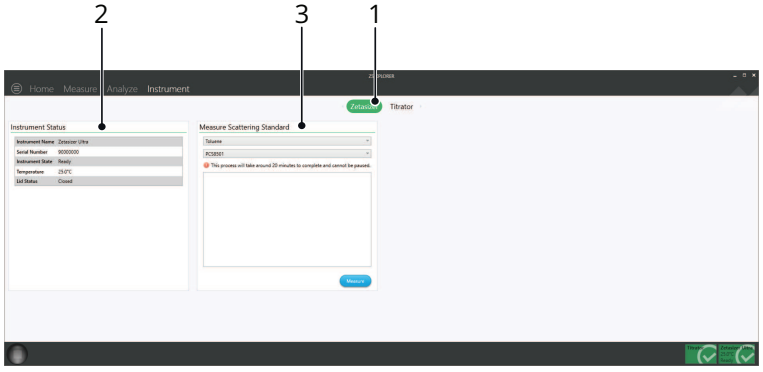


Figure 2.9 Zetasizer tab in the Instrument window

1. Zetasizer tab button
2. Instrument Status
3. Measure Scatter Standard

The **Zetasizer** tab contains the **Instrument Status** window and the **Measure Scatter Standard** window.

The **Instrument Status** window contains further information about the instruments' status including instrument name, serial number, instrument state, temperature and lid status.

The **Measure Scatter Standard** window can be used to measure a scattering standard. This is done to normalize **Particle Concentration** measurements. Measuring the scattering standard allows the ZS XPLORER software to find a mean count rate, which is then used to standardize concentration results. This makes sure that results are consistent across instruments.

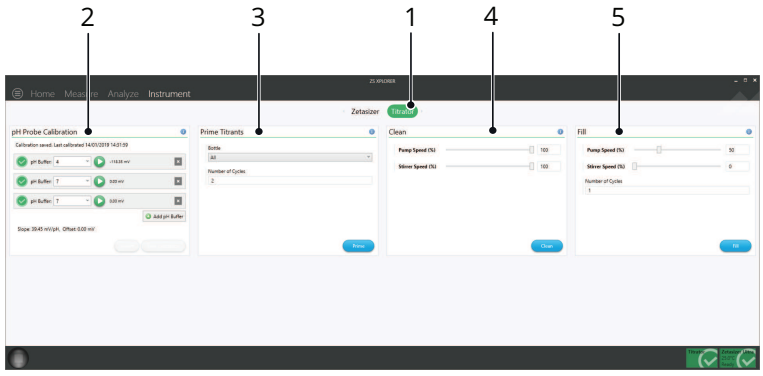


Figure 2.10 *Titrator tab in the Instrument window*

1. Titrator tab
2. pH Probe Calibration window
3. Prime Titrants window
4. Clean window
5. Fill window

The **Titrator** tab can be used to setup the MPT-3 Multi-purpose Titrator before a measurement. It contains the pH Probe Calibration, Prime titrants, Clean and Fill functions. For more information on these settings, see Other measurement types on page 68.

CHAPTER 3 SAMPLE PREPARATION

Introduction

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

Preparing the sample for different types of measurement involves specific preparation techniques. For each measurement type follow the guidelines described in this section.

Sample preparation: size

You must consider the physical properties of the sample such as its particle size and sample concentration. Particle size can impact the maximum measurement concentration of a sample. Sample concentration can have an impact on scattering levels, as described in the following section.

Sample concentration

Each sample has its own ideal concentration range 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.

Particle size is an important factor in determining the maximum concentration at which the sample can be measured.

The table below gives an approximate guide to help determine the maximum and minimum concentrations for different sizes of particles. The figures given are approximate values for samples with a density near to 1 g/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.

Table 3.1 Particle size and the appropriate sample concentrations

Particle size	Min. concentration (recommended)	Max. concentration (recommended)
< 10 nm	0.5 mg/mL	Only limited by the sample material interaction, aggregation, gelation, etc.
10 nm to 100 nm	0.1 mg/mL	5% mass
100 nm to 1 µm	0.01 mg/mL (10^{-3} % mass)	1% mass
> 1 µm	0.1 mg/mL (10^{-2} % mass)	1% mass

Whenever possible, the sample concentration should be selected such that the sample develops a slightly milky appearance - i.e. becomes 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 (e.g. particle interactions). 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.

MADLS® measurements

The factors discussed in the previous section regarding optimum sample concentration apply to all detection angles. However, the significance of each effect will vary between angles due to the differences between optical path length and detection volume within the sample.

For a reliable MADLS measurement, the concentration should be optimized in order to give good quality data in all angles.

Considerations for small particles

This section lists considerations for small particles.

Minimum concentration

For particle sizes smaller than 10 nm, 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 (10 kcps) in excess of the scattering from the dispersant. As a guide, the scattering from water should give a count rate in excess of 10 kcps, and toluene in excess of 100 kcps.

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 might not give reliable particle size results, though other result types (eg. diffusion) can be reliable and of interest.
- Particle interactions. If there are interactions between the particles then the diffusion constant of the particles usually changes, leading to less reliable size results. A concentration should be chosen to avoid particle interactions if size is being measured. Other results, such as diffusion, may still be reliable and of interest.

Considerations for large particles

This section lists 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 (e.g. 500 nm) were measured at low concentration (e.g. 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.

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/cm³.

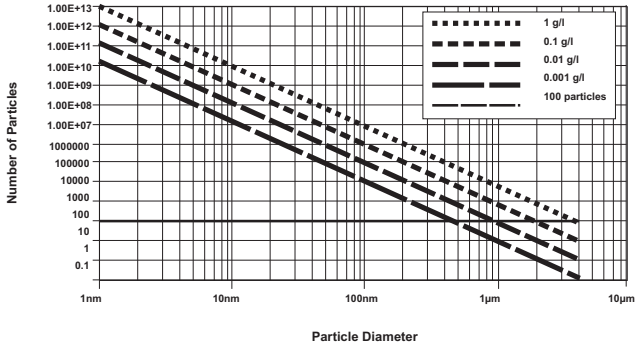


Figure 3.1 *Number of particles per scattering volume for different concentrations*

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, its effect becomes increasingly dominant, until so much multiple-scattering is generated that the results will be affected. Of course, such a high con-

centration should not be used for accurate measurements, and rough estimates are given for maximum concentrations for the different size classes in the table in Sample preparation: size on page 23.

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. Therefore, 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 10 nm, then 50 nm dust will be an important contaminant in the dispersant. Samples may be filtered down to 20 nm using an appropriate filter. Chemical compatibility of the filter material should be considered when using non-aqueous solvents.

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 break up 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 made to aggregate using ultrasound.

Emulsions and liposomes should not be ultrasonicated.

Sample preparation: zeta potential

The optical configuration for zeta potential measurements is discussed in Zeta potential theory on page 147. 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 2000 kcps. The incident laser beam passes through the center 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 starts, the intensity of the reference beam is measured and displayed in the **Measurement log** on the **Measure tab**. The intensity of the incident beam is automatically adjusted so that the intensity of the scattered light being detected is no greater than 300 kcps.

The attenuator in the Zetasizer 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 10 kcps. If the sample scattering detected is less than 10 kcps, the measurement will proceed, though the concentration should be increased if possible.

Minimum concentration

The minimum count rate of scattered light required to make a measurement is 10 kcps above the dispersant scattering level. 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 300 nm 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. The Zetasizer can measure a minimum concentration of 1 mg/ml lysozyme, but some larger proteins can be measured at lower concentrations.

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

Maximum concentration

There is no simple answer for the maximum concentration for a zeta potential measurement in the Zetasizer. 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. Therefore, the laser beam has to penetrate through the sample. If the concentration of the sample becomes too high, the laser beam will become attenuated by the particles, reducing the scattered light being detected. To 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 noting the effect on the zeta potential value.

Many samples will require dilution and this procedure is absolutely critical in determining the final value measured. For meaningful measurements the dilution medium is very 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 be put into one of two categories:

- Polar dispersants - those with a dielectric constant greater than 20 e.g. ethanol and water.
- Non-polar or low polarity dispersants - 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. Do this by filtering or centrifuging some clear liquid from 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. 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 250 mV. In such non-polar systems, equilibration of the sample after dilution is the time dependent step. Equilibration can take in excess of 24 hours.

Diffusion barrier

The diffusion barrier technique is a patented method from Malvern Panalytical that allows you to reliably measure the electrophoretic mobility of proteins or other fragile samples by minimizing the impact of the measurement process on the sample.

Note:
The license to use this patented method is granted to Zetasizer users in conjunction with Zetasizer hardware only.

Contact between the protein and the electrodes in the cell damages proteins and can cause them to denature and aggregate. Using diffusion barrier helps to minimize these effects.

Diffusion barrier can also be used with precious samples, as it allows for lower volumes of sample to be used, as low as 20 µL.

Note:
Diffusion barrier can only be used in a folded capillary cell (DTS1070 or DTS1080).

How does it work?

The diffusion barrier technique separates the sample molecules from the electrodes, reducing potential damage to the sample particles. The diffusion barrier technique does this by introducing a small plug of sample, which is separated from the electrodes by the same buffer in which the sample is dispersed. The physical distance between the sample and the electrodes protects the sample from damage caused by contact with the electrodes.

The sample is protected for as long as it takes for it to diffuse to the electrodes, which can be many hours.

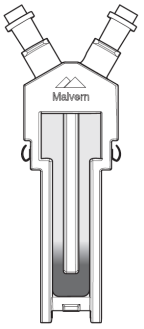


Figure 3.2 *Diffusion barrier in the folded capillary cell*

Method

1. Fill the folded capillary cell with 0.7 - 0.8 mL of the buffer that the sample is dispersed in using a Luer fitting syringe.
2. Insert the cell into the Zetasizer for 2 to 3 minutes to allow the temperature to equilibriate. This will reduce any temperature related fluid motion.
3. Remove the cell from the Zetasizer and pipette between 20 and 100 µL of the sample directly into the bottom of the cell. Use a gel loading tip to reach the bottom of the cell.
4. Carefully insert the plugs into the top of the folded capillary cell.
5. Allow the temperature to equilibriate before starting the measurement.

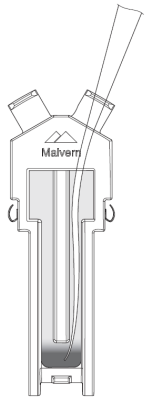


Figure 3.3 *Pipetting the slug of sample*

Note:
Minimize mixing of sample and buffer when loading sample into the cell.

Note:
Keep the cell upright and handle with care to prevent the sample from diffusing around the cell. The sample must remain at the bottom of the capillary, in the measurement area.

Software settings

When using the diffusion barrier technique, use the monomodal analysis model. In the software, you can change this in the Data processing settings window shown when setting up a Method.

Make sure to choose the correct analysis model to prevent any sample from being drawn towards the cell electrodes during measurement.

Further improvements

In principle, a mobility measurement using the diffusion barrier technique is exactly the same as when making a conventional measurement in the Zetasizer. In practice, a couple of changes can help improve the data quality of the measurements made using the diffusion barrier technique.

- In order to avoid significant Joule heating, select a voltage based on the conductivity of the buffer used. Buffers with higher ionic strength have higher conductivity, resulting in increased Joule heating at a given voltage. An appropriate voltage is selected automatically in the software, but for these measurements, improvements can be made by setting a lower voltage.

Table 3.2 Appropriate voltages for different salt concentrations

Salt concentration, mM	Voltage, V	Runs
200	20	20
100	40	20
10	150	40

- Make at least 5 measurements to make sure that the measured values are repeatable.
- A delay between measurements of at least 120 seconds, and at least 180 seconds for precious samples, ensures that the temperature is equilibrated between measurements.
- Do a size measurement both before and after the zeta potential measurements to check the sample has not changed as a result of the measurement. If the mobility measurement has affected the sample and caused it to aggregate, these aggregates should be present in the data from the size measurement following the mobility measurements. Example data is shown in the following graphs.

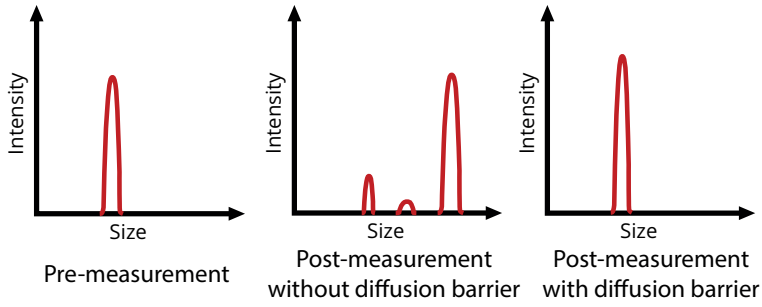


Figure 3.4 *Reduced aggregation visible after using diffusion barrier*

CHAPTER 4 METHODS

About Methods

In the ZS XPLORER software, you set up and define measurements using methods.

A method can be defined as the complete set of processes performed on a single sample during measurement. A method is composed of a number of steps compiled to create a whole measurement process. A step is a single type of measurement or other operation within a method. See Steps on the facing page for more details.

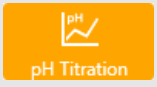
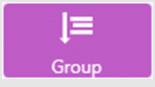
A method could be made of a single Size measurement, or of multiple Zeta, Size, Particle concentration or/and pH titration (with Size or Zeta) measurements compiled in a sequence that is repeated a number of times. Methods are created using the Method Builder. The **Method Builder** is described in more detail in Method Builder on page 38.

Methods can be saved, opened, and extracted from previous results. More details on managing Methods can be found in View Method on page 42 and Saving and opening methods on page 41.

Steps

A **Step** is a single measurement type or other operation that can be added to a method. Steps are the building blocks of methods. The following table outlines the steps available to use in the ZS XPLORER software.

Table 4.1 Available steps in the Method Builder

Step	Description
	Size measurement. Collects data from one of three measurement angles to measure the particle size of the sample. You can also measure the diffusion coefficient with this step.
	MADLS Size measurement. Collects data at three angles which is then used to produce an angle-independent particle size distribution at a higher resolution. To use this option click the **Size** icon, and then **MADLS**.
	Zeta potential measurement. Measures the Zeta potential of the particles in the sample.
	Particle concentration measurement. Conducts a MADLS measurement and uses built in algorithms to calculate the particle concentration.
	A pH Titration measurement. Conducts a titration over a set pH range and performs whichever measurement is within the titration action at each pH. For more information on the software controls see Other measurement types on page 68 or see the *Zetasizer Ultra/Pro Accessories Guide* for more information on the MPT-3 Multi-purpose Titrator.
	Groups steps together. Steps can be collected within a Group, making it easier to repeat sequences of steps. To use this option click **Other**, and then **Group**.
	Shows an instruction pop up window during a method. The instruction can remind the user of anything they need to do during a measurement. To use this option click **Other**, and then **Show Instruction**.

Pause	Pauses the experiment for a specified amount of time. The maximum pause duration of a single step is 300 seconds. To use this option click **Other**, and then **Pause**.

Note:
The steps available to use in a method depend on the cell type selected.

Method Builder

The Method Builder is shown below.

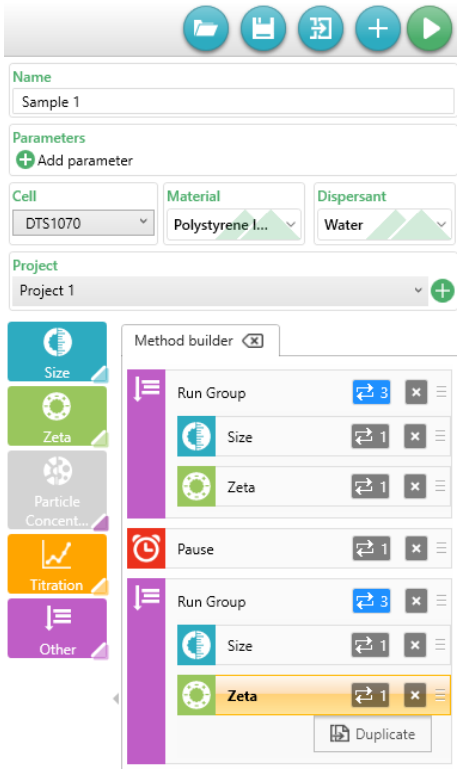
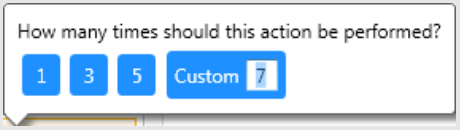
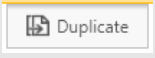


Figure 4.1 *Example method in the Method builder*

The functions of each button on the Method Builder are described in the following table.

Table 4.2 Method Builder buttons and their functions

Button	Function
	Clear the whole method and delete any steps and settings.
	By default, each step is run once. Click this button to specify the number of repeats.  Either select one of the common repeat values shown or enter your own Custom value. If you make any amendment to the repeat, this is shown in the Method in blue.
	Delete the step from the method.
	Create a copy of the step. The copy will have the same parameters and repeat settings as the original step.

- Reorder steps by clicking and dragging them up or down.

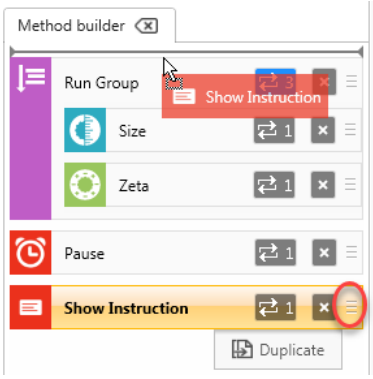


Figure 4.2 *Changing the running order of Method steps*

- To see additional steps, click on the 'parent' step (eg. *Size*) to expand the menu. You can also add a step to the Method Builder by clicking on the step in this expanded view.

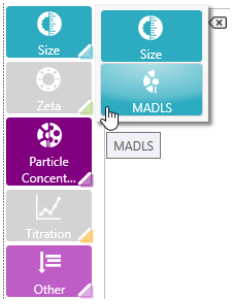


Figure 4.3 *Opening the Size step menu*

Saving and opening methods

When your method is set up, and any settings have been modified as needed (see Setting up a measurement on page 45), you can save your method. You can reopen a saved method and use it again. This helps to save time during measurement set up and improve consistency if the same methods are used frequently.

Saving a Method

1. Click the **Save** button above the Method Builder. This opens the **Save as** window.

Figure 4.4 Save button

2. Choose the location where you wish to save your method and give your method file a suitable name. Click **Save** to save your method.

Opening a method

1. Click the **Open** button above the Method Builder.

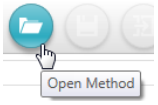


Figure 4.5 Open button

2. This opens the most recent folder a method was saved to. Select a method and click **Open**.

View Method

If you want to reuse a method, its settings can be viewed, used again, and saved. This can be useful during method development. See Saving and opening methods on the previous page.

To view a Method:

1. Click the **Analyze** tab.
2. In the Record selector, select the measurement from which you want to view the method. For information about the Record selector, see Record selector on page 84.
3. Click the **View Method** button.

Figure 4.6 View Method button

4. The selected Method will then be shown in the *Measure* tab.

The viewed method can now be used in future experiments. See Saving and opening methods on the previous page for details on opening saved methods.

CHAPTER 5 MEASUREMENT

Measurement types

The Zetasizer can measure the following:

- Particle size - including MADLS, Particle concentration and pH Titration measurements
- Zeta potential - including pH Titration measurements

Each measurement type has different cell requirements. See Measurement settings on page 50 for cell compatibility information.

This section briefly summarizes each measurement type. For detailed information on the theory behind each measurement type, see Measurement theory on page 131.

Size

The Zetasizer measures the particle size of a sample using DLS. The Zetasizer reports the hydrodynamic diameter - the diameter of a theoretical sphere which behaves in the same way as the sample. The Zetasizer software displays results in a Correlogram, Size intensity distribution and a calculated Z-average, as well as other formats. Diffusion coefficient data can also be reported.

MADLS®

Note:
A MADLS (Multi-angle DLS) measurement requires 90° measurement capability. Only a Zetasizer Ultra can perform a MADLS measurement.

MADLS is a new measurement type. It measures the size at three angles, and the data is combined to provide higher resolution results. MADLS is particularly effective at resolving populations of similar sizes at size ratios as close as 2:1

Particle concentration

Note:
A Particle concentration measurement requires 90° measurement capability. Only a Zetasizer Ultra can perform a Particle concentration measurement.

Particle concentration is a new measurement type. Algorithms are applied to a MADLS size distribution, and the Particle concentration can be derived from this using scattering intensity information.

Zeta potential

A Zeta potential measurement measures the electrophoretic mobility of a sample, and then uses this to find the Zeta potential using the Henry equation. The Zeta potential can be used as an indication of the stability of a colloidal system at a certain pH, though other factors will also affect stability.

Titration

Note:
Titration measurements require an MPT-3 Multi-purpose Titrator. For more information on the hardware setup of the MPT-3 Multi-purpose Titrator, see the *Zetasizer Ultra/Pro Accessories Guide*.

pH Titration measurements involve titrating acids and bases into a sample to change the sample pH, and then performing the desired measurement(s) at each pH point in a sequence. The measurement is done at regular intervals so that a trend can be found. The results graph will show how the sample characteristics are affected by pH.

For more information on titration measurements see Other measurement types on page 68.

Setting up a measurement

To set up a measurement you'll need to:

- Select a cell
- Select material and dispersant
- Add steps to the method
- Modify the parameters of each step if necessary

This process is outlined below.

1. Select your measurement cell from the drop down menu. The cell choice will affect which steps are available to add. For example the default cell choice, the DTS0012, will disable the Zeta measurement step as this cell is incompatible.

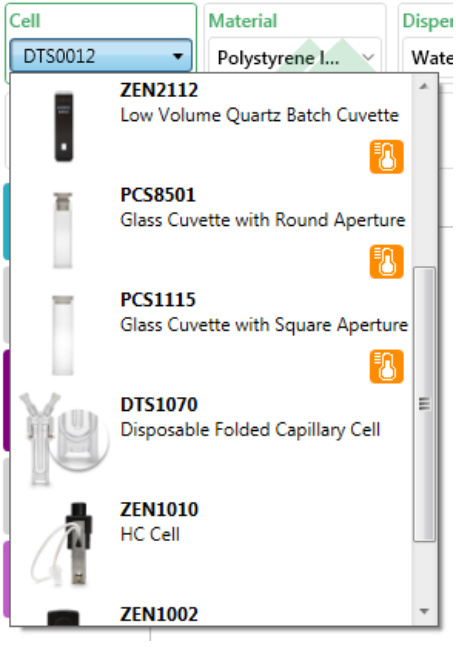


Figure 5.1 Choosing a cell

2. Select the Material and the Dispersant making up the sample from the menu. Materials and Dispersants can be edited or added to this list if necessary. See Materials and dispersants on page 62 for further details.
3. On the **Measure** tab, click or drag a step to add it to the Method builder.

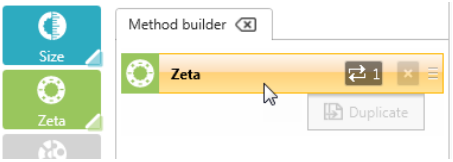


Figure 5.2 *Adding a step to the Method*

4. Fill in the remaining measurement settings as outlined in Measurement settings on page 49.

Note:
If you complete the measurement settings incorrectly, a red outline is shown around the step as shown in the following image. The Method will not run if this red line is present.

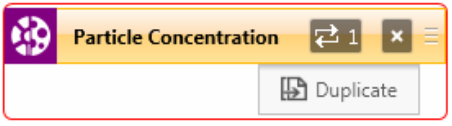


Figure 5.3 *An incorrectly set up step*

If you have an incorrectly set up step, an orange guidance panel is shown in the measurement settings telling you how to amend your setup. The incorrect setting will also be outlined in red.

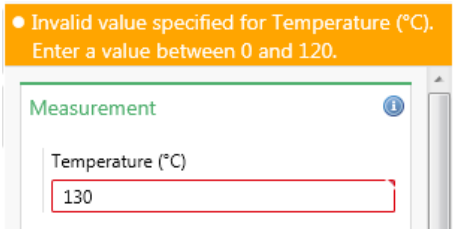


Figure 5.4 Incorrect setting in the Parameters window

Measurement settings

This section outlines the measurement settings available to complete in the ZS XPLORER software. The following sections describe the settings and how to complete them.

Basic information

Fill in the basic sample properties first.

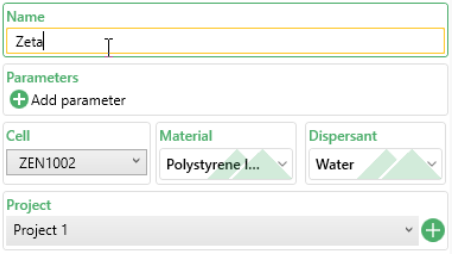


Figure 5.5 Basic sample information

Note:
Select a cell type before adding a step to the Method.

Table 5.1 Basic sample information

Setting	Description
Name	This is the name your sample will have in your results. Give the sample a meaningful name that will be easy to understand when you go back to review the data.
Parameters	In this box you can add any custom parameter to the sample, such as Batch number, which you can use to help filter results later on.
Cell	The cell type to be used for the measurement. The cell selection will affect the measurement types and settings available for the sample in the software.
Material / dispersant	The material and dispersant used in the sample should be selected from the drop down list and specified here. For more information on the materials and dispersants menu, see Materials and dispersants on page 62.

Project	Select which project to add your result to, or create a new project. For more information on projects, see Projects on page 85.

Cell selection

The following table shows which cells are usuable for which measurements. For further details on cells, their applications, and compatibility, see the *Zetasizer Ultra/Pro Accessories Guide*.

Table 5.2 Cell and measurement compatibility

Cell		Size	Zeta	MADLS	Particle concentration	pH Titration
	DTS0012 Disposable 10 x 10 mm plastic cell	✓		✓	✓	
	ZSU1002 Low volume disposable sizing cell	✓				
	ZEN0040 Low volume disposable cuvette	✓				
	ZEN2112 Low volume quartz batch cuvette	✓		✓	✓	
	PCS8501 Glass cuvette with round aperture	✓		✓	✓	
	PCS1115 Glass cuvette with square aperture	✓		✓	✓	
	DTS1070 Disposable folded capillary cell	✓	✓			✓
	DTS1080 Disposable folded capillary cell	✓	✓			✓
	ZEN1010 High concentration cell	✓	✓			✓

	ZEN1002 Dip cell	✓	✓			

Measurement properties

In this box, you can define the temperature settings for the measurement.

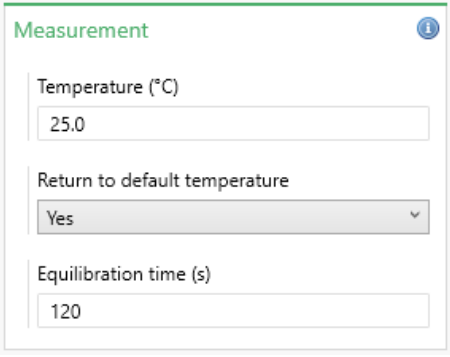


Figure 5.6 Measurement properties

Table 5.3 Measurement properties

Setting	Description
Temperature	The temperature at which to perform the measurement. The upper limit is affected by the cell type and the dispersant used.
Return to default temperature	Returns the instrument to 25 °C once the measurement is complete if **Yes** is selected.
Equilibration time	The length of time given for the sample to thermally stabilize once it has reached the specified temperature. 120 s is the default, but this can be adjusted as required.

Data processing

In this box, select the most appropriate data processing type for your sample. The data processing types for Size and Zeta potential measurements are different.

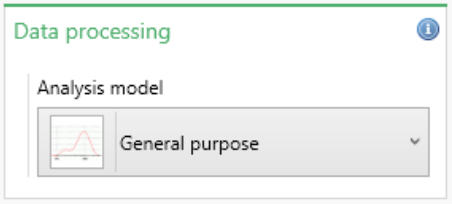


Figure 5.7 Analysis model selection

Size

Table 5.4 Size analysis models

Analysis model	Description
General purpose	The default processing type for Size measurements. **General purpose** should be suitable for most samples other than the special cases mentioned in the next two options.
Multiple narrow modes	This analysis model should be used when you know you have more than one peak, and the peaks are narrow. This analysis method gives a higher resolution than the general purpose model and will resolve the peaks more effectively.
L-curve analysis	This analysis method optimizes the distribution result to give the highest possible resolution while maintaining minimal noise. This process is suitable for low scattering samples such as proteins that may produce correlation functions that contain noise.

Zeta potential

Table 5.5 Zeta potential analysis models

Analysis model	Description
Auto-mode	In **Auto-mode**, the software automatically selects the most appropriate Analysis model to use based on the cell type chosen, dispersant properties, and measured sample conductivity. This is the default setting for Zeta potential measurements.
General purpose	Applicable for most Zeta potential measurements where a distribution plot is needed.
Monomodal	This should be used for samples in high conductivity dispersant, fast measurements, protein samples, diffusion barrier measurements, and when a distribution plot is not needed. Protein samples are small and fragile compared with other colloidal samples. This means that they may become damaged if too much energy is applied to the system, meaning that Brownian diffusion is not negligible during electrophoretic measurements. This zeta analysis uses a specific monomodal waveform that is designed to reduce the energy imparted on the sample during the measurement. It also maintains the signal to noise ratio by using an optimized signal processing method.

Advanced settings

In this box, additional measurement settings can be altered. For a quick standard measurement, you might not need to alter these settings. The Advanced settings available are different for Size and Zeta potential measurements.

Note:
Some of these settings are automatically fixed depending on the cell type selected.

Size

The default Advanced settings for a Size measurement are shown below.

Click ▲ to expand the drop-down menu. Click it again to hide the expanded view.

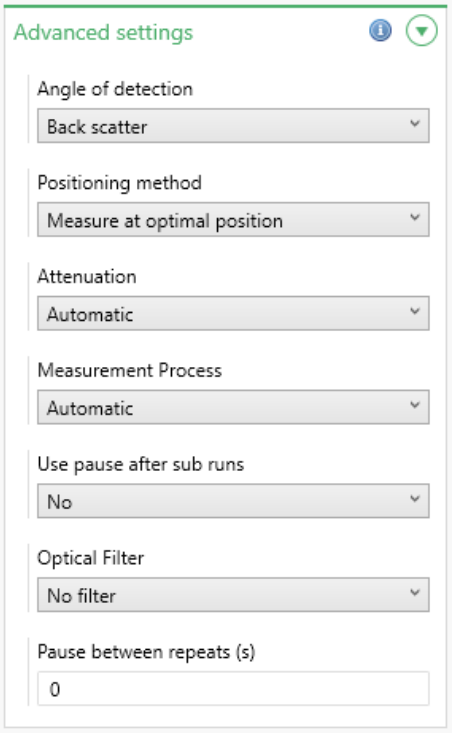


Figure 5.8 Advanced size measurement settings

Table 5.6 Advanced size settings

<table>
<tr><th>Setting</th><th>Description</th></tr>
<tr><td>Angle of detection</td><td>Select which detector to use to measure scattering. Choose from Forward scatter, Backscatter, and Side scatter. For some cells this is limited or fixed.</td></tr>
<tr><td>Positioning method</td><td>Select the measurement position to use. It is recommended to use the default setting, Measure at optimal position, during method development so that the best measurement position can be found. When the optimum measurement position is known, use Measure at fixed position to specify a fixed value. The measurement position is measured from the front of the cuvette. Smaller values are closer to the front of the cell. For some cells, the measurement position cannot be changed.</td></tr>
<tr><td>Attenuation</td><td>Select the attenuator level to use. This can be found automatically or fixed at a specific level. We recommend using the default setting, Automatic, during method development so that the optimum level can be found.

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

<table>
<tr><th>Attenuator index</th><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td></tr>
<tr><th>Transmission (% nominal)</th><td>0.0003</td><td>0.003</td><td>0.01</td><td>0.03</td><td>0.1</td><td>0.3</td><td>1</td><td>3</td><td>10</td><td>30</td><td>100</td></tr>
</table>

The optimum count rate should be between 300 and 500 kcps where possible. If the count rate is below 100 kcps when using attenuator 11 for a size measurement, a longer sub run length may improve data quality.</td></tr>
<tr><td>Measurement process</td><td>This sets the data capture method to either Automatic or Manual. If you select Manual, you can specify the duration and number of measurement runs to complete.</td></tr>
<tr><td>Use pause after sub runs</td><td>If Yes is selected, the length of the pause after each sub run can be specified.</td></tr>
<tr><td>Optical filter</td><td>Choose from Fluorescence filter, Horizontal polarization, Vertical polarization, or No filter. These options are only available in backscatter detection mode.</td></tr>
<tr><td>Pause between repeat(s)</td><td>Adds a pause between repeat measurements of a specified length. This allows the sample to re-equilibrate between measurements.</td></tr>
</table>

Zeta potential

The default Zeta potential advanced settings are shown below.

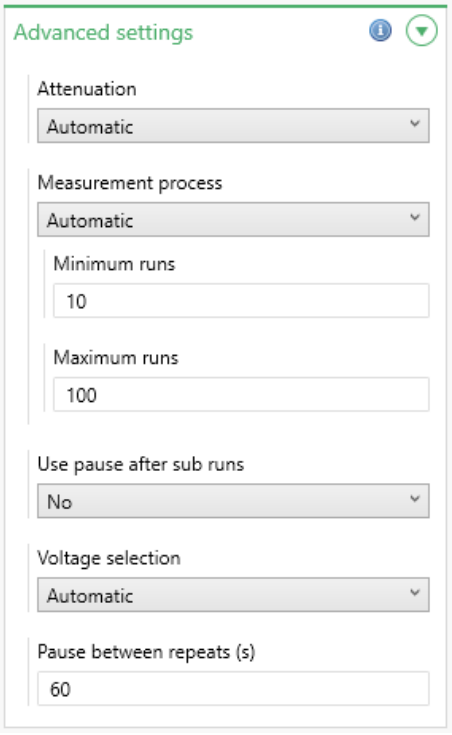


Figure 5.9 Advanced zeta potential measurement settings

Table 5.7 Advanced zeta potential settings

<table>
<tr><th>Setting</th><th>Description</th></tr>
<tr><td>Attenuation</td><td>Select the attenuator level to use. This can be found automatically or fixed at a specific level. We recommend using the default setting, Automatic, during method development so that the optimum level can be found.

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

<table>
<tr><th>Attenuator index</th><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td></tr>
<tr><th>Transmission (% nominal)</th><td>0.0003</td><td>0.003</td><td>0.01</td><td>0.03</td><td>0.1</td><td>0.3</td><td>1</td><td>3</td><td>10</td><td>30</td><td>100</td></tr>
</table>
</td></tr>
<tr><td>Measurement process</td><td>Choose between Automatic and Manual. If Automatic is selected, specify the minimum and maximum number of runs the software is allowed to carry out. If Manual is selected, specify the exact number of runs to carry out.</td></tr>
<tr><td>Use pause after sub runs</td><td>If Yes is selected, the length of the pause after each sub run can be specified.</td></tr>
<tr><td>Voltage selection</td><td>We recommend leaving this on Automatic during method development so that the optimum voltage setting can be found. Choosing Manual will enable you to specify a voltage. The voltage range is cell dependent.</td></tr>
<tr><td>Pause between repeat(s)</td><td>Adds a pause between repeat measurements of a specified length. This allows the sample to re-equilibrate between measurements.</td></tr>
</table>

Configuring the Properties window

Click ⛶ to expand the properties view. The live measurement display will be hidden in this view.

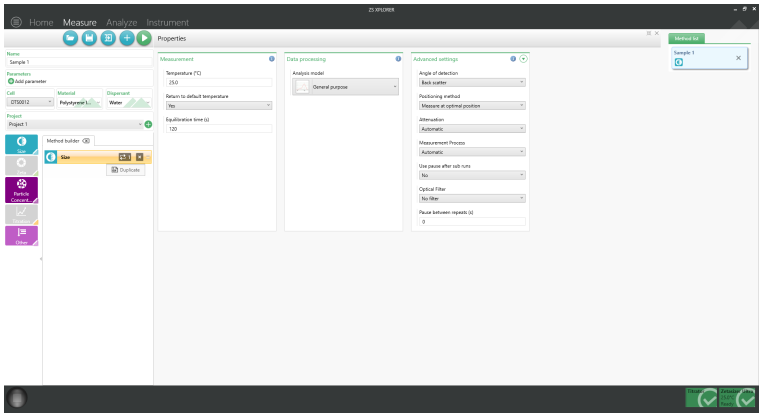


Figure 5.10 *Expanded properties view*

Click ⛶ to return to the default view.

The Properties window can be closed by clicking ✕. Click once on the step to re-open the window. Your settings will not be lost.

Running a measurement

When the Method is set up and ready to be run, click the **Start method** button.

Figure 5.11 *Starting a Method*

The method will start, and the sample will show as running in the **Method list**. See Method list on page 61.

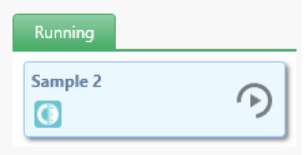


Figure 5.12 Running measurement in the Method list

Note:
The method step that is currently running is shown by the relevant icon flashing, as in *Sample 2* above, where a size measurement is in progress.

Tip:
You can carry on working in other areas of the software during the measurement - it is not necessary to observe the measurement taking place.

The display will start updating as the measurement progresses. First, the measurement log starts updating to show information about the measurement progress.

```
[11:44:34.9] Equilibrating sample for 120 seconds...
[11:46:34.9]
[11:46:34.9] ----------------------------------------------------------------
[11:46:34.9] OPTIMISATION
[11:46:34.9] ----------------------------------------------------------------
[11:46:34.9] Beginning optics optimisation.
[11:46:35.1] Translation stage set to 2.00mm.
[11:46:35.1] Cell compensation set to 7.
[11:46:39.5] REFERENCE_COUNTRATE 3117.7
[11:46:43.2] Instrument configuration Att 7, Cell Comp 7, Trans 2.00 mm.
```

Figure 5.13 Measurement log

As the method continues to run, graphs will begin to update with live data.

Figure 5.14 *Live graphs on the measurement display*

Tip:
While the measurement is running, the count rate can give an indication of your data quality. See Interpreting data on page 123.

The overall measurement progress is shown at the bottom of the window in the progress bar.

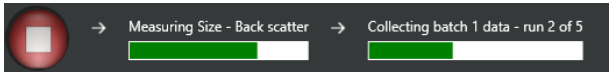


Figure 5.15 *Measurement progress bar*

Note:
During a measurement, the quality of the data is assessed after every 5 sub runs, and these sets of 5 are called 'batches'.

Tip:
You can set up another measurement while a measurement is running. Click + to set up a new method.

When the measurement is finished, the sample will show as **Complete** in the **Method list**. The measurement data will now be available to view in the **Record selector**. See Record selector on page 84.

Stop the measurement at any point by clicking one of the **Stop** buttons. These are located near the top and bottom of the left side of the window.

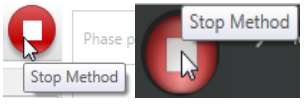


Figure 5.16 *Stop buttons*

Method list

In the **Measure** tab, the **Method list** displays all measurements from the current software session: those yet to run, measurements currently running, and those completed/aborted/failed.

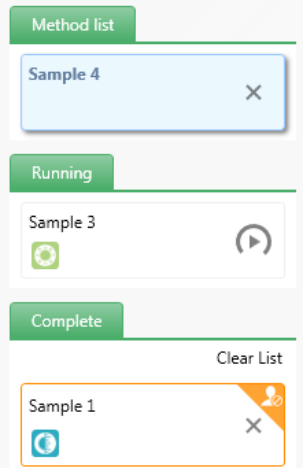


Figure 5.17 *The Method list*

The *Method list* is a useful tool for navigating the *Measure* tab when running many measurements. Click completed measurements to view their method settings. Click on a measurement that has not yet run to set up the next experiment, while another measurement is running.

To delete a measurement from the *Method list*, click the **X** next to it. Click **Clear list** to delete all complete measurements from the *Method list*.

Note:
Deleting a completed measurement from the Method list does not delete any measurement data. The results of the measurement can still be viewed in the Analyze tab.

Materials and dispersants

When setting up a method you need to select an existing material (the sample) and dispersant, or add a new one.

Each material and dispersant has unique optical properties that are factored by the software to ensure that your results are as accurate as possible.

Tip:
If you do not know the properties of the dispersant or material that is being used, choose the closest known match.

The software includes both a dispersants and a materials database that contains a number of predefined materials and dispersants, shown with the Malvern hills logo:

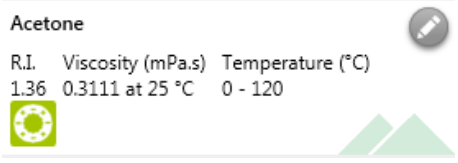


Figure 5.18 Malvern Panalytical predefined dispersant shown by the Malvern hills logo

Predefined materials and dispersants cannot be removed, but you can modify them to create your own variants.

You do not have to add an item to the database in order to use it, but if you will need to use it in the future it is a good idea to do so.

Search the database

As your list of materials and dispersants grows, it can be quicker to use the Search Database feature when selecting an item. Enter the first few characters of the item you need - the list contracts automatically to show matches:

Figure 5.19 Searching for a material

Add or edit a material or dispersant

The overall process for editing or adding a material or dispersant is the same. In both cases you need to modify an existing material or dispersant, which you can then use in your measurement as a "one off", or save for further re-use.

1. First, reveal the drop down list of dispersants or materials:

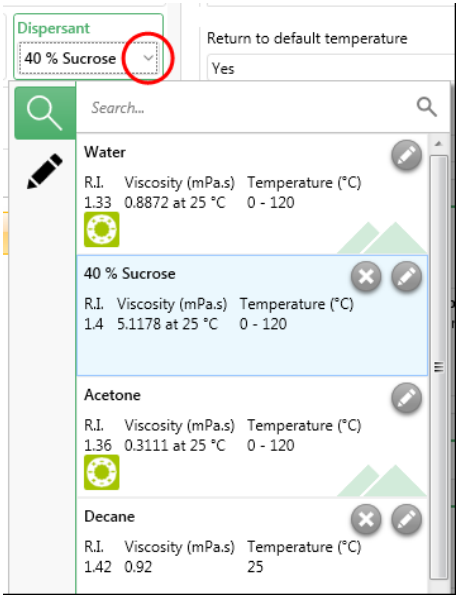


Figure 5.20 *Revealing the list of dispersants*

2. Then click the pencil icon next to an item to edit its properties:

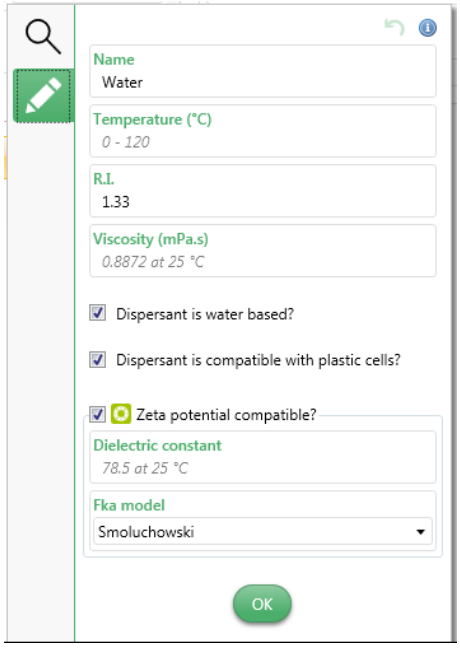


Figure 5.21 Editing a dispersant

Table 5.8 Dispersant properties

Setting	Description
Name	The name of the dispersant.
Temperature °C	Maximum temperature at which the dispersant can be used 0-120.
R.I.	Refractive Index - value of between 0 and 5. This value relates to the speed of light within the material relative to the speed of light in a vacuum, which in turn allows the degree of refraction (light bending) to be predicted when light passes from one medium to another.
Viscosity mPa.s	Value between 0 and 100. Note this is the viscosity at a defined temperature.
Dispersant is water based?	Check if the dispersant or solvent is aqueous (water based). If checked, only compatible measurement cells will be shown - incompatible cells will not be shown.

Setting	Description
Dispersant is compatible with plastic cells?	Check this box to make the dispersant available to use when a plastic cell is selected.
Zeta potential compatible?	Check this box if the dispersant capable of being used for zeta potential measurements. If you do this an icon is shown next to the item in the selection list:
Dielectric constant	Select a value in the range 0-200.
F(ka) model	Smoluchowski (F(ka) 1.5), Huckel (F(ka) 1.0) or Custom - if you select custom, enter an F(ka) value of 0-1.5.

Table 5.9 Material properties

Setting	Description
Name	The name of the material.
R.I.	Refractive Index - value of between 0 and 5. This value relates to the speed of light within the material, which in turn allows the degree of refraction to be predicted when light passes from one medium to another.
Absorption	Enter a value between 0-8.
Is sample small?	Use this field to specify whether the sample is thought to be very small or fragile - for example a protein. If selected the system modifies the zeta potential measurement settings to minimize heating and measurement duration.

Note:
RI and Absorption of the material will have no bearing on the Z-average, PdI and Intensity distribution results.

3. When you have modified all the properties as required, click **OK.** This will ensure these settings are used in the current method.
4. To re-use these settings in further methods, save them to the database - click **Add to Database** to do this. If the button is not visible, you have already added this item to the database.

Delete a material or dispersant

You can only delete materials or dispersants that you have added to the database. It is not possible to delete any of the default materials or dispersants that are identified by the Malvern hills logo.

To delete a material or dispersant, click the button circled in the following image:

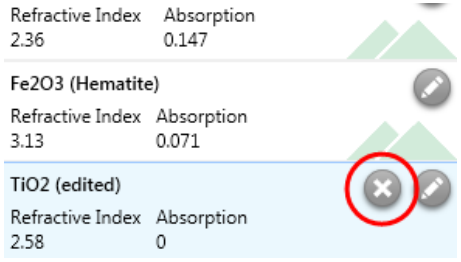


Figure 5.22 Deleting a material from the database

Other measurement types

This section provides information about the following measurement types, and gives details on the settings required:

- pH Titration measurements
- Particle concentration measurements
- MADLS measurements

Connecting the ZS XPLORER software to the MPT-3 Multi-purpose Titrator

Connect the hardware of the MPT-3 Multi-purpose Titrator to the Zetasizer. See the *Zetasizer Ultra / Pro Accessories Guide* for more information. Then, connect the ZS XPLORER, as outlined below.

1. Click the icon from the Menu bar.
2. The Options and About menu will show. Click **Options**.
3. In the Options window, click the **Titrator** tab.

Figure 5.23 Titrator tab in the Options window

4. From the COM Port drop down menu, select **USB Serial Port (COMXX)**.

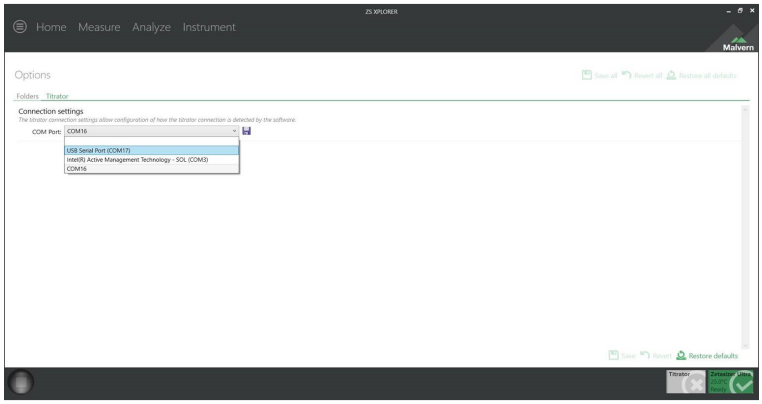


Figure 5.24 Connecting the MPT-3 Multi-purpose Titrator in the ZS XPLORER software

pH Titration measurements

Note:
For an overview of the MPT-3 Multi-purpose Titrator hardware and maintenance, see the *Zetasizer Ultra/Pro Accessories Guide*.

Note:
pH Titration measurements require the MPT-3 Multi-purpose Titrator. Not all cells will be compatible.DTS1070/1080, and ZEN1010 are the only cells you can use for this measurement.

A standard workflow for pH titration measurements is as follows:

1. Calibrate the pH probe before each titration.
2. Make sure the titrant syringe pumps and tubes are primed before each titration.

3. Prepare the sample before each titration session.
4. Fill the measurement cell before each titration session.
5. Allow the system to equilibrate for 5-10 minutes.

Further information on the software settings for these steps are listed below.

To open the titrator settings, select the **Titrator** tab from the **Instrument** window:

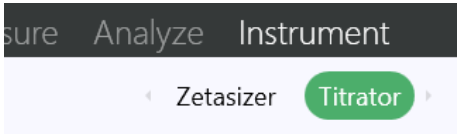
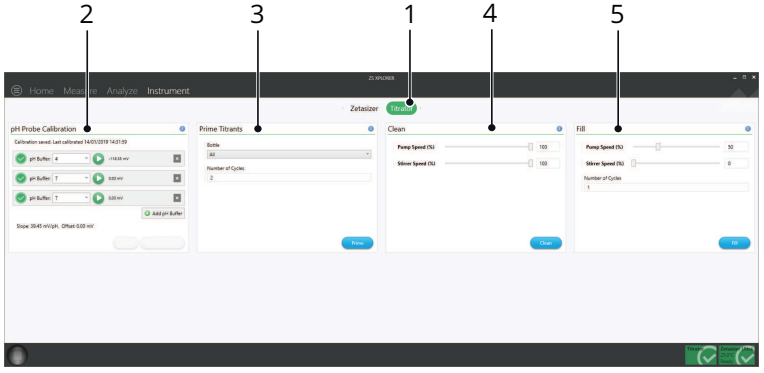


Figure 5.25 *Titrator tab (button)*

The **Titrator** tab contains the following settings panels:



1. Titrator tab
2. pH Probe Calibration — calibrate the pH probe with two or more pH buffers.
3. Prime Titrants — prime titrants.
4. Clean — clean the titrant lines and cell.
5. Fill — fill the sample container.

Figure 5.26 *The Titrator controls tab*

Calibrating the pH probe

If the pH probe has not been calibrated the pH values will be incorrect. Calibrate the pH probe before each titration.

Before calibrating the pH probe, you will need:

- **At least two pH buffers**. The default pH standards are pH 4 and pH 9 (supplied with the probe). See Other measurement types on page 73 for advice on selecting pH standards. You can use a maximum of five buffers for the calibration.
- **A container of clean deionised water to rinse the probe between measurements**. Ideally calibrate the pH probe while fitted to the system and using the standard sample containers.

Note:
Recalibrate the pH probe if the room temperature changes by more than 5 °C.

Calibration process

1. Open the **Titrator** tab from the **Instrument** window and the **Calibration** settings are shown.

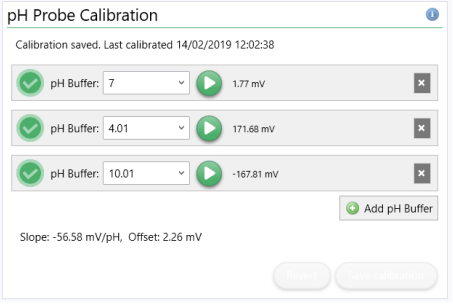


Figure 5.27 pH Probe Calibration tab

2. Select the matching pH buffer from the drop down menu to the known pH buffer.
3. Rinse the electrode in deionised water and dab dry with a lint-free tissue.
4. Place the pH probe into the known pH buffer.
5. Click the icon. If you need to stop the calibration, click the icon.
6. The software checks that the pH reading is stable before continuing. When the calibration of the first pH point is successfully completed, this is indicated by the icon.
7. Remove the probe from the first pH buffer, rinse with deionised water and dab dry.
8. Repeat the above for all required calibration points, e.g. pH 4, 7 and 9. Default pH points are pH 4 and pH 9 as these are the buffers originally supplied with the pH probe. The highest and lowest pH need to be a minimun of 3 pH units apart.
9. Once the measurements have been complete you can save the calibration by clicking Save Calibration. If further changes are made after this, you can click Revert to revert to the last saved calibration.

The icons shown during a calibration indicate the following:

Table 5.10 Calibration steps

Icon	Description
	A pH point has been chosen but not yet calibrated
	A buffer is being measured
	A buffer has been successfully measured
	An issue has occured with the calibration as the wrong buffer has been used
	A calibration measurement has been canceled or has failed. Hover over the icon to see further information.

Click the icon to delete pH buffers from the calibration, if required.

Choosing the buffers when calibrating

The default calibration buffers are pH 4 and pH 9. Other values may be used but ensure that the lowest and highest values are at least 3 pH values apart. For example, if your titration measurements tend to be in the lower end of the pH table, use pH 2 and pH 5 buffers to calibrate the probe.

Adding a pH buffer point

To use a different pH value, click Add pH Buffer.

1. Select a defined pH point from the Calibration pH list.
2. If your pH buffer is not in the list, type the value manually.

For best accuracy over a wide pH range, use 3 or 4 buffers.

The pH probe is supplied with pH 4 and pH 9 buffers. Follow the instructions supplied with the buffer sachets. Contact Malvern Panalytical for advice on sourcing further buffers.

Note:
The MPT-3 Multi-purpose Titrator sample cell is not temperature controlled and the pH probe does not compensate for temperature. We recommend that pH buffers are at room temperature during calibration. The pH value used for any standard reflects the true pH value at the temperature measured.

Priming

Priming makes sure that the titrant delivery tubes are free of air and full of liquid. If you do not prime, the titrant may evaporate and the correct volume will not be delivered when first used.

CAUTION!
DO NOT prime the Auto Degasser membranes by pushing titrant through the degassing systems. This can generate high pressure which might rupture the Teflon AF® membrane. The maximum recommended pressure on the membrane is 0.7 MPa (100 psig, 7 Bar).

How many prime cycles?

- One prime cycle - every morning, or at the beginning of a new session.
- Three prime cycles - when a titrant container is changed, to remove traces of the original titrant.

Prime titrant tubes either individually or simultaneously. The colors mentioned in the software match the LED's colors next to the titrant container and also the titrant tubing connectors.

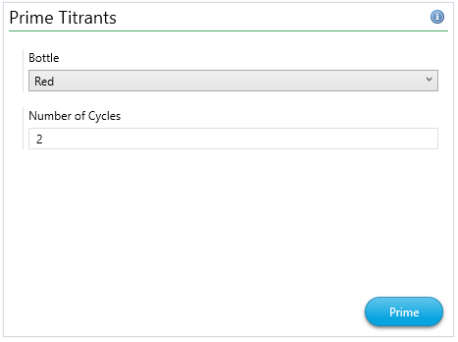


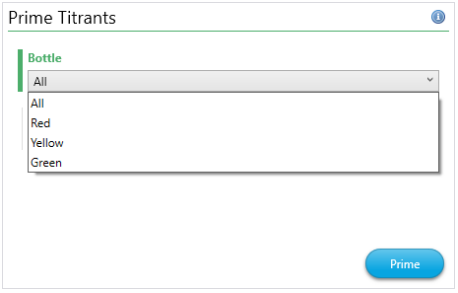
Figure 5.28 Priming Titrants tab

Priming the titrant tubes:

1. Place the filled titrant containers under the container cover, making sure that the ends of the titrant tubes are immersed in the titrants.

Note:
Water in a sample container can be attached instead of using a waste beaker. This is not essential, but will eliminate the effect of surface tension at the end of the titrant tube.

2. Open the *Titrator controls* window by clicking the **Instrument** menu, and then click the **Titrator** tab.
3. In the *Prime* area, select the titrant line that is to be primed, or select **All** to prime all three titrant delivery tubes at once.



4. Select the cycles required and click the Prime button to begin the operation. Each cycle will pump the volume of the titrant tubes from the container.

Note:
Click **Cancel** to stop the operation.

5. Priming will stop once the number of cycles has been completed.
6. When the prime operation has finished, titrant should be visible in the bottom of the beaker. If none is dispensed, click the **Prime** button again to repeat the process.

Note:
The titrant tubes can also be cleaned using the same tab. Simply insert containers of cleaning fluid in place of the titrant containers.

Setting up a pH titration measurement

In a pH Titration measurement, actions can be placed within a Titration step. This means that the titrator will perform each action at every pH point in the sequence set up in the pH titration properties.

- When setting up a measurement, choose a cell that is compatible with the titrator, DTS1070/1080 or ZEN1010, or the titration action will be unavailable.
- The pH titration action can be found under **Titration** actions in the **Measure** tab, and can only be run with another action within it.
- Click **Titration** and then **pH Titration**. Drag any other actions, in this case a Size measurement, within a pH Titration action step.

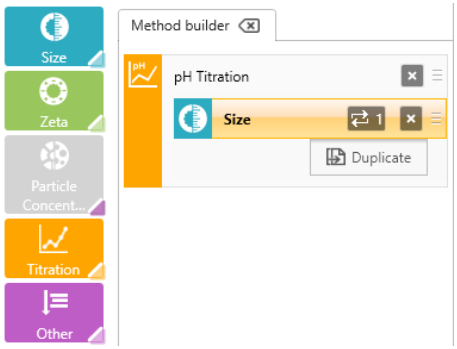


Figure 5.29 A size measurement within a pH titration action

- Each action still has its own independent settings which can be altered by clicking onto one of the actions and amending the properties.
- In the **Analyze** tab, there are three available graph types that can be used for titrator measurements - **Titration Zeta Potential Trend**, **Titration Z-Average Trend** and **pH Trend** graph. Titration Zeta Potential Trend and Titration Z-Average Trend plot titration measurement(s), display any corresponding zeta and size measurements, and display any isoelectric point (s). The ph Trend graph plots individual pH points generated during a titration.

Note:
The MPT-3 Multi-purpose Titrator sample cell is not temperature controlled and the pH probe does not compensate for temperature. We recommend that pH buffers are at room temperature during measurements.

Titration steps have their own specific measurement settings.

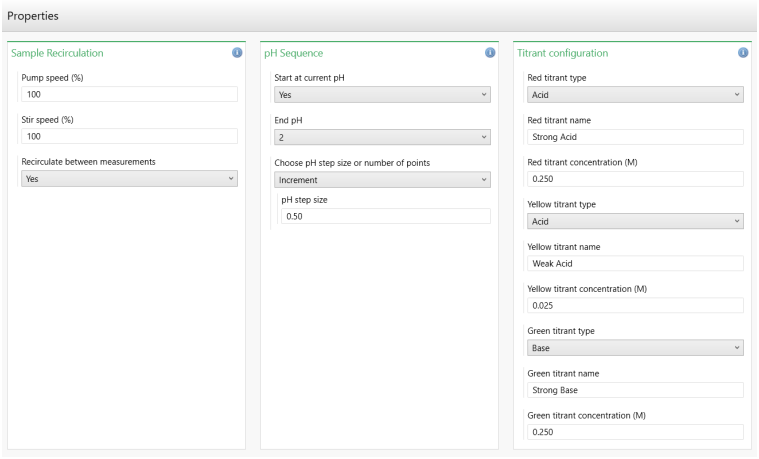


Figure 5.30 pH Titration properties

Table 5.11 pH Titration settings

Setting	Description
Pump speed (%)	The percentage speed of the pump, where 30% is the minimum speed and 100% is the maximum speed.
Stir speed (%)	The percentage speed of the magnetic stirrer, where 100% is the maximum speed.
Recirculate between measurement	If **Yes** is selected, the sample will be recirculated between repeat measurements at every pH point in the titration sequence.
Start at current pH	If **Yes** is selected, the measurement pH sequence will start at the sample pH. If **No** is selected, you can specify a starting pH value.
End pH	The target pH value the titrator will finish titrating at.
Choose pH step size or number of points	If **Increment** is selected, you can specify the amount by which you'd like the pH value to change between measurements. Smaller increments mean more pH values in the pH sequence will be measured. If **Points** are selected, specify the number of data points you'd like to measure. For example, if Increment was selected and a titrator's Start pH was 6, the End pH was 7, and the pH step size was set to 0.5, a total of three measurements would be made.
Red / Yellow / Green titrant type	Acid or Base titrant to be dispensed from the red / yellow / green inlet.

Setting	Description
Red / Yellow / Green titrant name	The name of the titrant dispensing from the red / yellow / green inlet.
Red/ Yellow / Green con-centration (M)	The concentration (in M) of the titrant to be dispensed from the red / yellow / green inlet.

Particle Concentration measurements

Note:
Particle concentration measurements require size measurement at three angles, and can only be performed on a Zetasizer Ultra. Not all size cells will be compatible. DTS0012, ZEN2112, PCS8501, and PCS1115 are the only cells you can use for this measurement.

Before performing a Particle Concentration measurement, you must measure a scattering standard to normalize the instrument sensitivity. A Particle Concentration measurement can only run if a scattering standard measurement has been made.

This will be done as part of the instrument OQ and service. Check your service records to see when the last measurement was made.

You can also do this yourself in the Zetasizer tab within the Instrument window. This only needs to be done once as the instrument will store the results.

Note:
You don't need to measure a scattering standard unless you would like to make sure the instrument characterization is up to date, or you would like to measure your own standard.

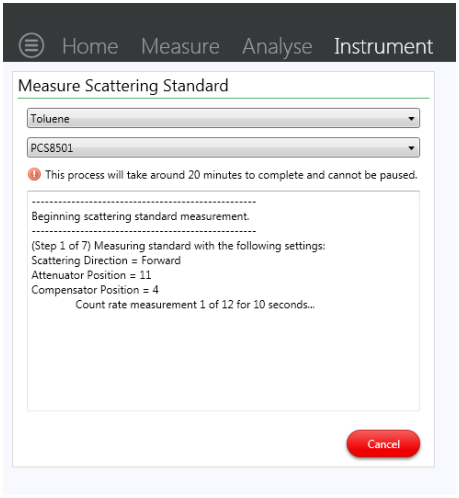


Figure 5.31 *Measuring a scattering standard*

When setting up the measurement, you will also need to enter a Buffer scattering mean count rate (kcps). This can be recorded by performing a Size measurement on your dispersant only.

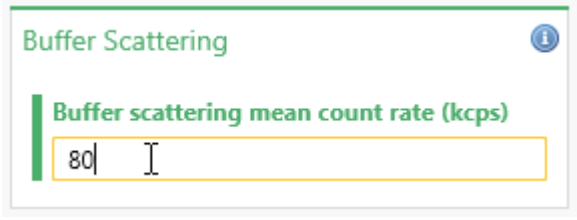


Figure 5.32 *Entering buffer count rate*

In this box you need to enter the mean scattering count rate of your buffer. This is so that any background scatter can be subtracted from the size measurement data.

Note:
The particle optical properties (RI and Absorption) are required for particle concentration measurements. All of the other measurement settings remain the same as a size measurement.

MADLS measurements

Note:
MADLS measurements require size measurement at three angles, and can only be performed on a Zetasizer Ultra. Not all size cells will be compatible. DTS0012, ZEN2112, PCS8501, and PCS1115 are the only cells you can use for this measurement.

Most of the settings remain the same as for a standard Size measurement, except the Advanced settings. Positioning method and angle of detection are unalterable, as measurements are taken at all three angles, and the position must stay fixed. Optical filters are also not available for MADLS measurements, as these are only compatible with backscatter measurements.

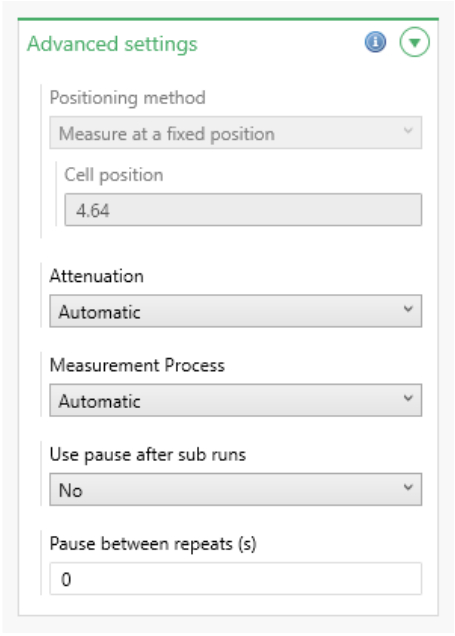


Figure 5.33 *MADLS advanced settings*

CHAPTER 6 ANALYSIS

Introduction

In the **Analyze** window you can:

- View results in tabular and graphical form
- Group and sort results by different parameters
- Customize your results workspace
- Create, view and edit Projects
- Use the Measurement Explorer

This section explains how to use these features.

Record selector

The Record selector on the left of the **Analyze** window allows you to view, filter and edit your measurement data.

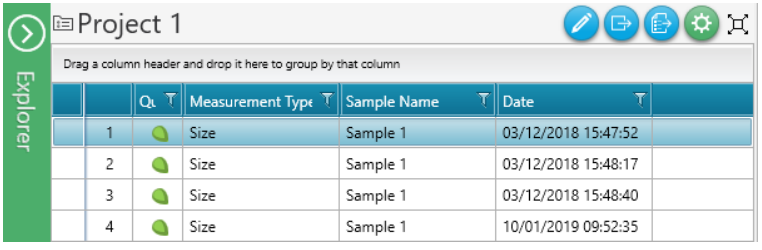


Figure 6.1 The Record selector

Projects

About projects

By default, each measurement you make is added to a project by the ZS XPLORER software. Projects can help you to find and organize your measurement results, and to also group results made from different sets of measurements. This can be particularly useful when doing retrospective analyses of results that were made over a broad time-frame.

Selecting a project

The data shown in the Record selector depends on the project you have selected. Click the Explorer arrow icon to show the Project Explorer.

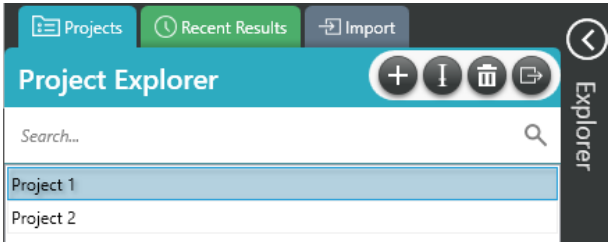


Figure 6.2 Switching between projects

Click on the required project to display it in the *Record selector*.

Note:
You choose which project to place a result into when setting up the measurement. Make sure you choose the right Project during measurement set up - see Measurement settings on page 49. You can also move results into other projects later on - see Explorer on page 112.

Add a new project from Method editor

To create a new project during measurement set up, click the button:

Figure 6.3 *Adding a new project in measurement setup*

Enter a name for your new project, and click the ✓ button to save your project.

When you run the measurement the results will be added to the new project.

Add a new project in Project Explorer

To add a new project from the Project Explorer, click the ⊕ button.

Enter the name of the project:

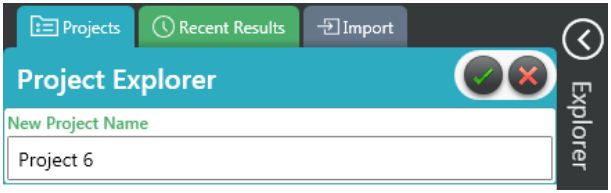


Figure 6.4 *Adding a new project in the Project Explorer*

Click the ✓ button to add the new project, or click ✕ to cancel.

Rename a project

To rename a project in the Project Explorer, select the project and click I:

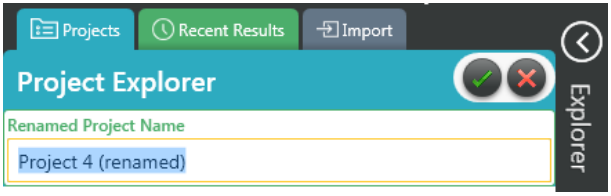


Figure 6.5 *Renaming a project*

Click the button to confirm the change, or click to cancel.

Delete a project

To delete a project, select it in the Project Explorer and click .

The system asks you to confirm this:

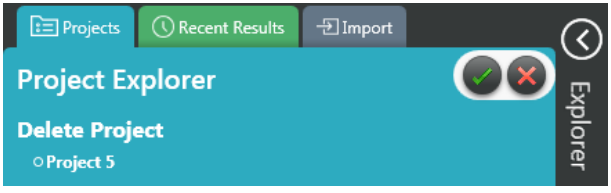


Figure 6.6 *Deleting a project*

Click the button to delete the project, or click to cancel.

Move results between projects

You can move results between projects in the Project Explorer.

Select the results in a project that you would like to move, then drag and drop the results into another project.

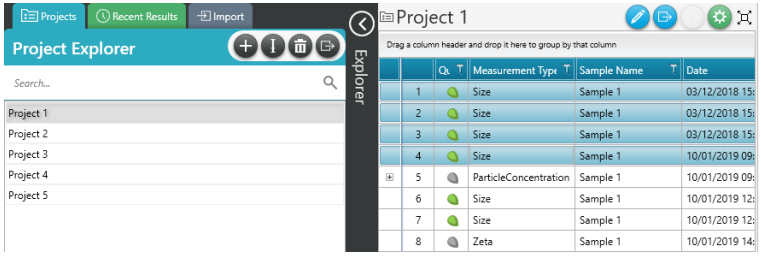


Figure 6.7 *Moving results between projects*

The results will now only show in the project they were moved into.

Sorting results

You can sort results by clicking on the column headers to sort by that parameter.

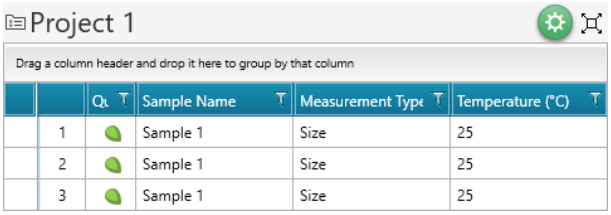


Figure 6.8 *Sorting results by date*

The displayed parameters can be changed by clicking . This opens the parameter selection window. You can change the order in which parameters are displayed by using the up and down arrows.

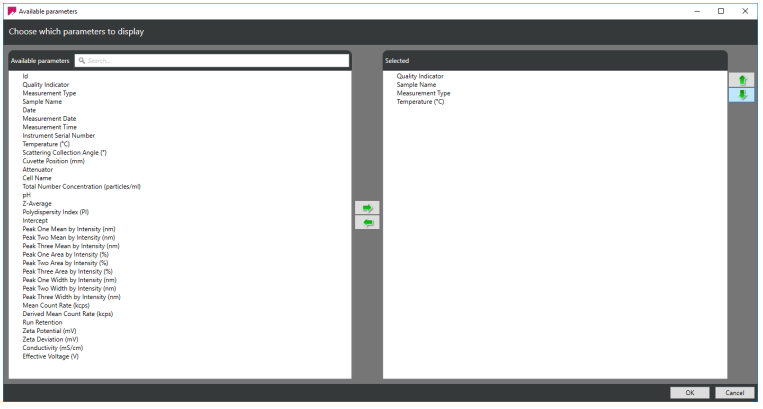


Figure 6.9 *Changing the parameters in the table*

You can change the order of the columns by clicking the row headers and dragging to rearrange.

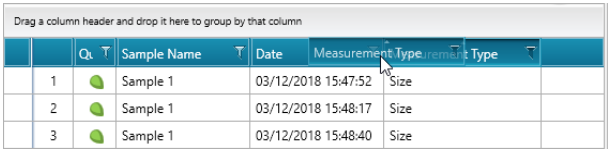


Figure 6.10 Rearranging columns in the Record selector

The available parameters are described in the following section.

Parameters

The parameters available for display in the Record selector are as follows:

Table 6.1 Parameters available in the Record selector

Parameter	Description
ID	A unique identifier of each measurement.
Quality Indicator	A symbolic indicator of the quality of a given measurement, currently available for size measurements only.
Measurement Type	The type of measurement - i.e. size, zeta potential, MADLS, particle concentration or pH titration.
Sample Name	The name given to the sample when setting up the experiment. The default name begins at Sample 1, with the number increasing every time a new measurement is set up.
Date	The date and time at which the measurement was taken.
Measurement Date	The date at which the measurement was taken.
Measurement Time	The time at which the measurement was taken.
Instrument Serial Number	The serial number of the instrument the measurement was made on.
Temperature (°C)	The temperature at which the experiment ran in °C.
Scattering Collection Angle (°)	The angle at which scattering was detected from the sample, either 174.7, 90 or 12.78 degrees - e.g. back, side and forward scatter.
Cuvette Position (mm)	The position in the cell where the measurement was taken. This is determined at set up.
Attenuator	The attenuator used when performing the measurement. The attenuator controls the intensity of light incident upon the sample.
Cell Name	The type of cell used in the experiment.

Total Number Concentration (particles/ml)	The total number of particles detected in a sample, calculated for a Particle concentration measurement.
pH	The pH value input as a parameter when setting up a measurement.
Z-Average	Average hydrodynamic diameter of the sample in nm.
Polydispersity Index (PI)	An indicator of the spread of particle sizes measured for the sample, ranging between 0 and 1.
Intercept	The intercept at zero lag time of the measured autocorrelation function. This should theoretically be between zero and 1 and is an indicator of signal to noise for a DLS measurement. An intercept of greater than 1 may indicate instability of the sample.
Peak One Mean by Intensity (nm)	Hydrodynamic diameter of the most prominent particle size population in the intensity weighted distribution analysis.
Peak Two Mean by Intensity (nm)	Hydrodynamic diameter of the second most prominent particle size population in the intensity weighted distribution analysis.
Peak Three Mean by Intensity (nm)	Hydrodynamic diameter of the third most prominent particle size population in the intensity weighted distribution analysis.
Peak One Area by Intensity (%)	Area of the most prominent peak, signifies proportion of particles of this size.
Peak Two Area by Intensity (%)	Area of the second most prominent peak, signifies proportion of particles of this size.
Peak Three Area by Intensity (%)	Area of the third most prominent peak, signifies proportion of particles of this size.
Peak One Width by Intensity (nm)	Width of the most prominent peak - indicates the range of sizes in a population.
Peak Two Width by Intensity (nm)	Width of the second most prominent peak - indicates the range of sizes in a population.
Peak Three Width by Intensity (nm)	Width of the third most prominent peak - indicates the range of sizes in a population.
Mean Count Rate (kcps)	Average amount of light detected during the measurement.
Derived Count Rate (kcps)	Average amount of light detected from the sample, corrected for by the attenuator.
Run Retention	The percentage of sub runs that were used in the steady state result of the size analysis. A percentage of < 100 indicates that some data has been identified as being transient.
Zeta Potential (mV)	Zeta potential of the sample in mV.
Zeta Deviation (mV)	Width of the distribution of measured zeta potential (mV). Only available when General Purpose analysis has been used.
Conductivity (mS/cm)	The conductivity of the sample measured during a zeta potential measurement in mS/cm.
Effective Voltage (V)	Voltage applied to the sample during a zeta potential measurement

Grouping results

In the Record selector, you can group results by any parameter. This feature is useful to get an overview of result distribution. For example, you can organize measurements by type, allowing you to quickly assess the number of Size measurements in a Project, or compare results with similar measurement parameters more easily, e.g. scattering angle.

Click and drag a column header into the blue bar.

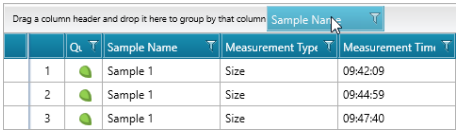


Figure 6.11 *Dragging a column header*

The results will now be shown in a grouped view.

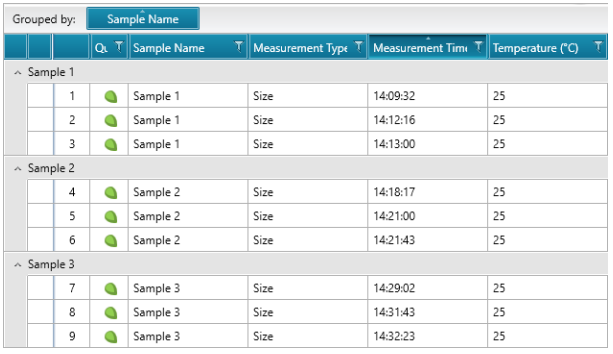


Figure 6.12 *Grouped view*

To return the Record selector to an ungrouped view, hover over the parameter in the blue bar until an **X** is shown. Click the **X** to remove the grouping.

Figure 6.13 *Removing the groups*

Click the parameter at the top of a column to change the order in which the groups are displayed. For example, you can make the Date display from least to most recent instead of most to least recent, as shown in the example below. The arrow shown at the top of the cell indicates the order in which results are being sorted.

Figure 6.14 *Sorting results by a parameter*

Tip:

You can add more parameters to nest groups. In the following example, the results are first grouped by measurement type, then by measurement time, and then sample name. You can make your grouping structure as complicated or as simple as you like. However, if you have too many nested groups, results may become hard to read.

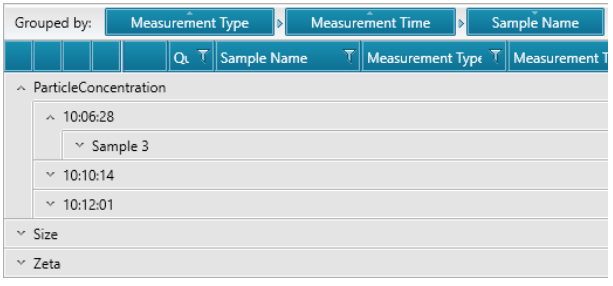


Figure 6.15 *Three level grouping tree*

Tip:
You can modify this view by using the drop down arrows on the left to collapse and expand the groups of data.

Filtering results

In the Record selector, you can filter results within their projects based on certain parameter values. Click the icon on the parameter and the filter settings are shown.

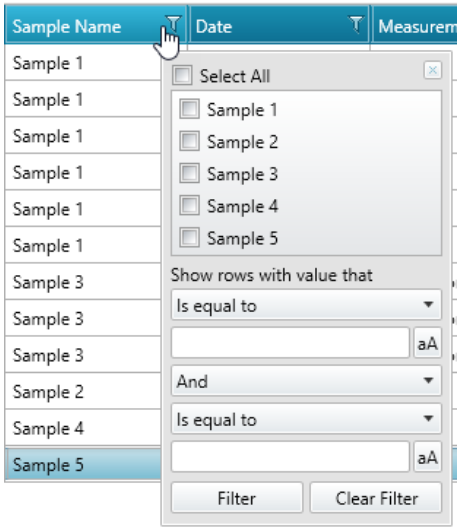


Figure 6.16 Filtering results by sample name

Select the name of the parameter, or choose values to filter the results. Click **Filter** to filter the results.

Click **Clear Filter** to remove the filter.

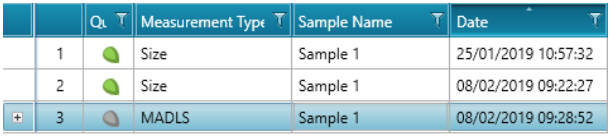
Compound results

Some measurement types will necessarily result in multiple measurements, which together form a single compound result. The following measurement types do this:

- MADLS - three scattering angles per measurement
- Particle Concentration - three scattering angles per measurement
- Titration - one record for every measurement at each pH point

The ZS XPLORER software groups these measurements in the Record selector for easier analysis. This section shows how each of the different compound measurement types are presented.

In the example below, a MADLS measurement has been made:

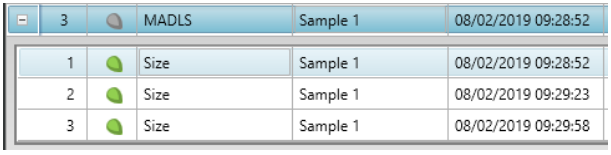


		Qu	Measurement Type	Sample Name	Date
	1		Size	Sample 1	25/01/2019 10:57:32
	2		Size	Sample 1	08/02/2019 09:22:27
+	3		MADLS	Sample 1	08/02/2019 09:28:52

Figure 6.17 *MADLS compound measurement - contracted*

The + symbol in the leftmost column indicates that the measurement can be expanded to show sub-data. The top level (or parent) record is the combined result - the result that the ZS XPLORER software has calculated as the aggregate of the sub (or child) data.

Click the **+** symbol to expand the result, showing child results nested below:



−	3		MADLS	Sample 1	08/02/2019 09:28:52
	1		Size	Sample 1	08/02/2019 09:28:52
	2		Size	Sample 1	08/02/2019 09:29:23
	3		Size	Sample 1	08/02/2019 09:29:58

Figure 6.18 *MADLS compound measurement - expanded*

You can select the individual child components of a compound measurement and also the parent record. This displays the plots for the selected records within the workspace.

Analyzing titration results

The ZS XPLORER software contains powerful features that enable you to quickly assess your titration data. This section provides information on how to view and select titration data for tabular or graphical analysis.

Titration plots and tabular data

Within the data grid, titration results are presented as compound measurements - see: Compound results on the previous page. The data is grouped by parent records that contain child sub-data. This is an example of a titration measurement in the Record selector:

		Qt	Measurement Type	Date
	1		Size	25/01/2019 10:57:32
⊟	2		Titration	30/01/2019 13:07:39
	1		Zeta	30/01/2019 13:07:39
	2		Zeta	30/01/2019 13:08:42
	3		Zeta	30/01/2019 13:09:45
	4		Zeta	30/01/2019 13:23:18

Figure 6.19 Titration result parent and child data

The workspace also provides information that can be used to analyze titration results, such as the parameter table and specific titration trend plots. The following titration plots are available:

- pH Trend - plot of the Z-Average (nm) and zeta potential (mV) as a function of pH. Only child records and an iso-electric point (IEP), if present, are shown.
- Titration Z-Average Trend - the Z-Average for all measurements that produce them within the titration.
- Titration Zeta Potential Trend - all zeta potential results produced during the titration. An IEP, if present, is shown.

Note:
To show data on the Titration Z-Average and Titration Zeta Potential Trend plots you must select the titration result in the Record selector - do not select individual sub-records.

The following example shows a Zeta potential titration result in the *Titration Zeta Potential Trend* graph. The vertical line shows the Isoelectric Point (IEP) value of pH 4.696 - move the mouse over the IEP line to reveal the IEP (pH) value. The IEP line is only shown if the plot intersects 0 mV. As with other data shown in plots, move the mouse pointer over individual data points to show measured pH and Zeta Potential (mV) values.

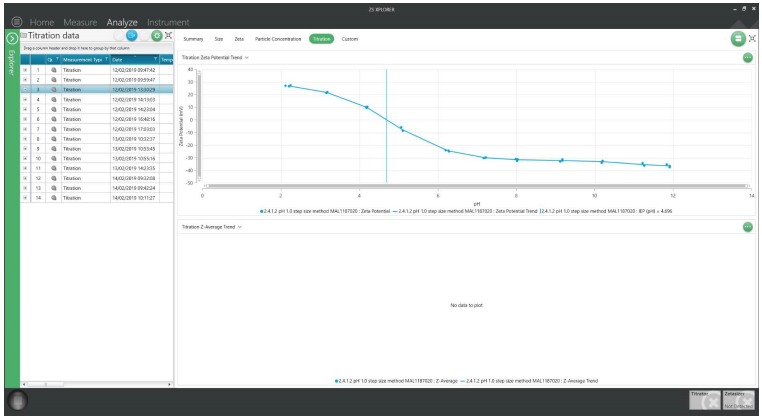


Figure 6.20 Titration Zeta Potential Trend plot

Multiple titrations

To overlay multiple titrations:

1. Make sure that you have selected an appropriate titration plot in the workspace, for example the Titration Zeta Potential Trend plot.
2. Hold **CTRL** and click to select multiple parent titration records from the Record selector.

The plot updates to show the information:

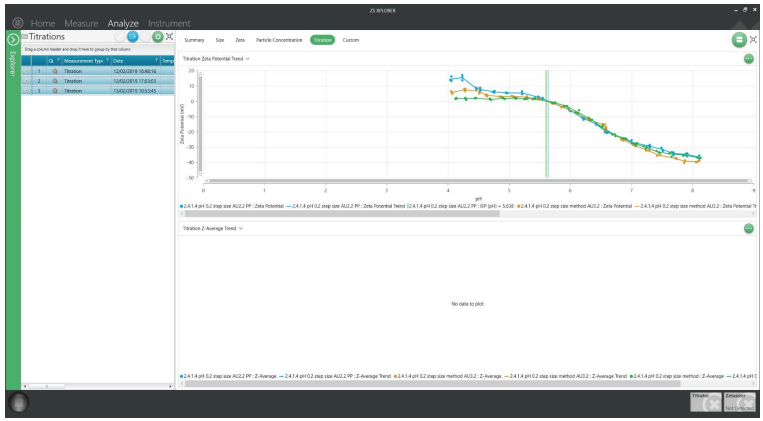


Figure 6.21 *Titration zeta potential trend plot*

The titration plot automatically colors the trend lines and data points in the same color to allow easy differentiation. Regardless of the order in which the records are selected, the legend shows the oldest in the leftmost position through to newest, rightmost.

Repeated steps within a titration

Sometimes your titration measurement will contain repeated steps. The example below shows a titration that has been set up in this manner. In this example, the initial zeta potential measurement will be repeated three times, before running a separate size titration measurement once.

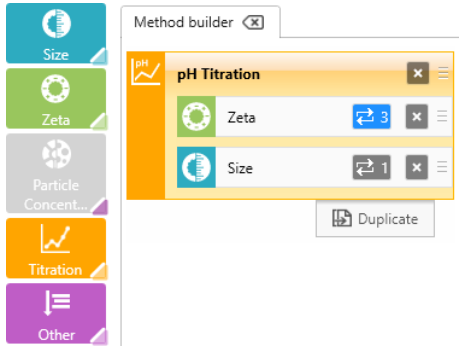
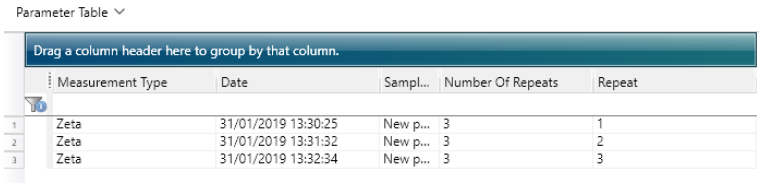


Figure 6.22 *Repeated steps or separate steps in a titration*

After running a measurement like this, the Record selector shows a compound result. However, repeat information is not shown in the Record selector. To view this you may wish to select both the **Repeat** and **Number of Repeats** parameters in the workspace's Parameter Table.

The following example shows the first three measurements in this data series in an adapted Parameter Table. The *Repeat* number increments with each measurement.


Parameter Table

Drag a column header here to group by that column.

	Measurement Type	Date	Sampl...	Number Of Repeats	Repeat
1	Zeta	31/01/2019 13:30:25	New p...	3	1
2	Zeta	31/01/2019 13:31:32	New p...	3	2
3	Zeta	31/01/2019 13:32:34	New p...	3	3

Figure 6.23 *Number of Repeats/Repeat*

Result editor

You can right-click a result or multiple results in the Record selector and click **Edit result** to access the Result editor. Alternatively, you can also select a result or multiple results and click the edit button .

Note:
Only multiple results of the same type can be edited together and pH titration records cannot be edited.

Note:
To select multiple results hold **Ctrl** and click the results you would like to select, or hold **Shift** and select the range of records you would like. You can hold **Ctrl** and **A** to select all of the records in a project.

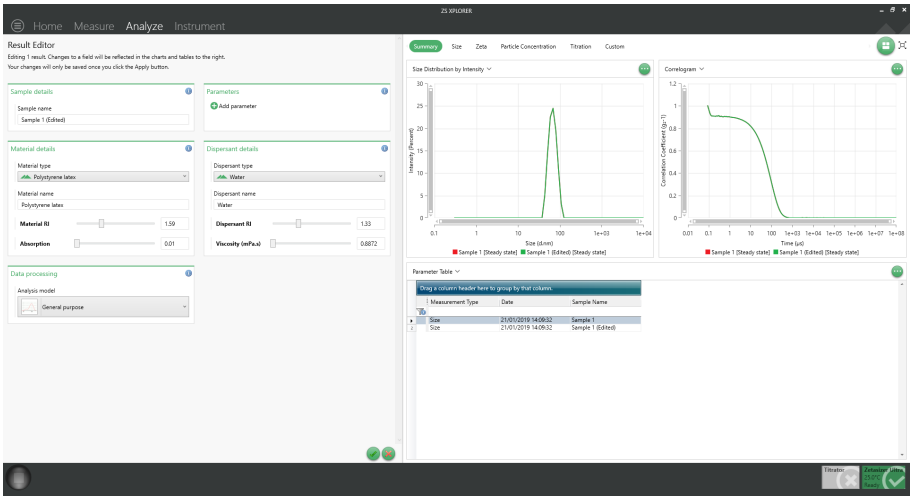


Figure 6.24 *The Result editor*

In the Result editor you can:

- Change the Sample name
- Add or remove parameters
- Change basic material and dispersant properties
- Change the analysis model used for data processing

The graphs will update as changes are made to show how the edited result(s) will appear in the workspace.

Save your changes by clicking . Click to discard your changes.

When you save your changes, the original result(s) will not be overwritten. Instead, the edited result(s) will be saved as a separately in the Record selector.

Workspaces

There are six workspaces to choose from in the Analyze window: Summary, Size, Zeta, Particle Concentration, Titration and Custom.

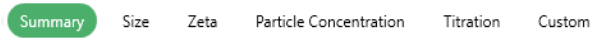


Figure 6.25 Available workspaces

Each workspace has its own default configuration containing graphs and tables. These are listed below.

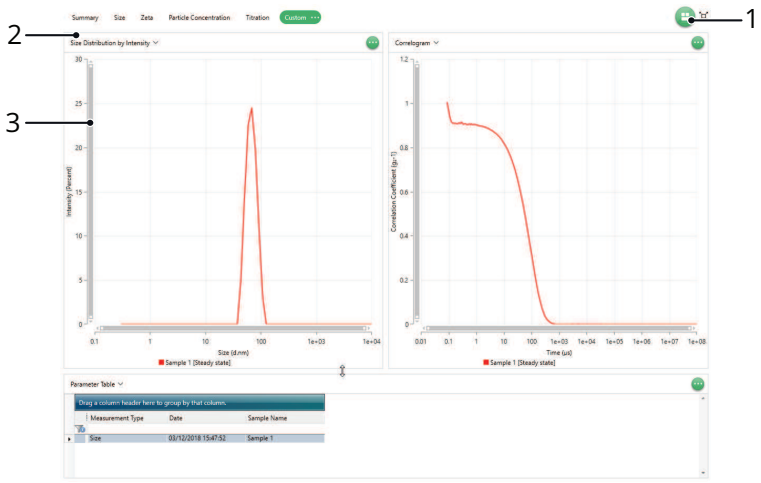
- Summary - Data Quality Guidance, Size Distribution by Intensity and Zeta Potential Distribution.
- Size - Size Distribution by Intensity, Correlogram and Statistics Table.
- Zeta - Phase Plot, Zeta Potential Distribution, Statistics Table and Zeta Potential Voltage and Current.
- Particle Concentration - Size Distribution by Volume, Distributed Particle Concentration, Size Distribution by Intensity and Cumulative Particle Concentration.
- Titration - Titration Zeta Potential Trend and Titration Z-Average Trend graphs.
- Custom - Size Distribution by Intensity, Correlogram and Parameter Table.

See Information types on page 105 for more information on each available figure type.

Customizing your workspace

Note:
ONLY the custom workspace is saved - the default workspaces return to their default state once a session is complete.

The layout and features included in a workspace can be changed to suit your requirements.



1. **Workspace layout selector** - switch between different layouts of the workspace windows
2. **Graph selector bar** - click on the bar to open a drop down workspace feature selection menu
3. **Graph axis control bar** - resize and slide this to zoom in and out of the graph, and to move the view

Figure 6.26 Workspace

Clicking on the Graph selector bar [2] opens the following window.

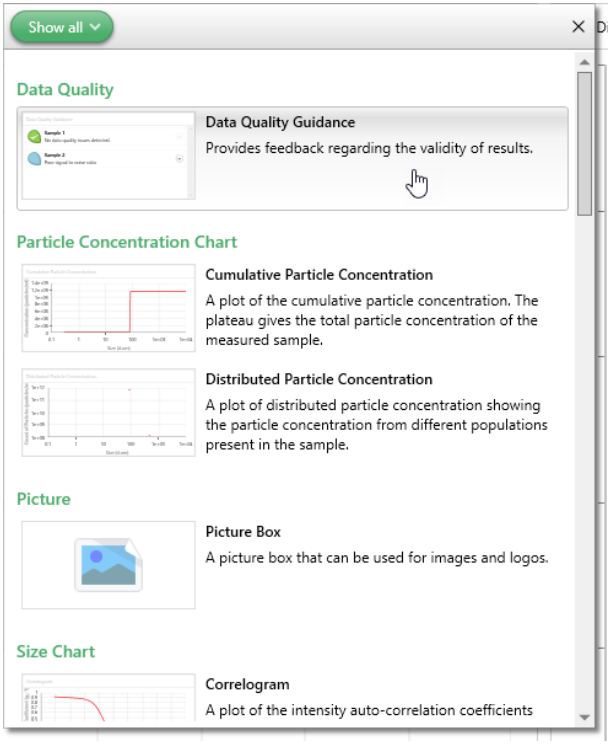


Figure 6.27 *Workspace feature selection window*

In this window, you can choose a graph or other feature to display in the workspace.

Tip:
You can overlay graphs of results from several experiments by selecting multiple records in the Record selector. See Interacting with the workspace on page 110.

Changing workspace layout

Click in the top right corner. The following drop down menu is shown:

Figure 6.28 Workspace window arrangement options

Select one of these buttons to change the window layout to that shown on the button. For example, clicking will change the workspace window layout as shown below.

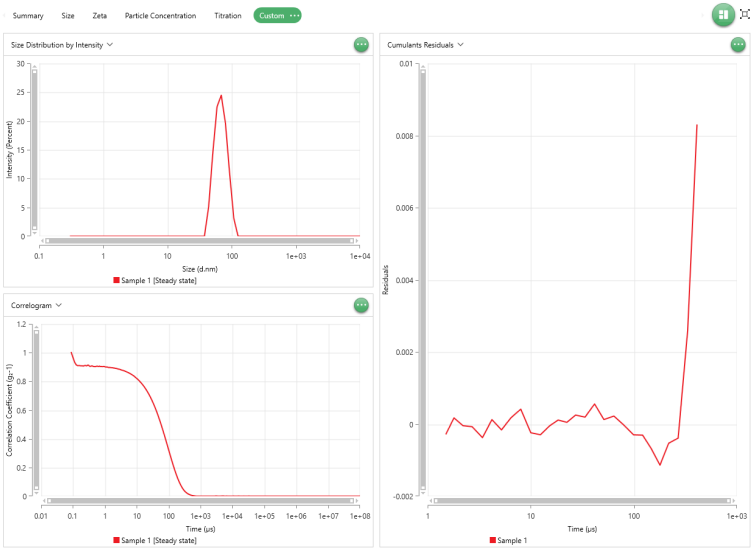


Figure 6.29 Changed workspace layout

Changing window size

Move the mouse pointer over the window border. The cursor will change to an arrow ( ). Then, click and drag until the window is the required size.

This is a useful tool if one of the windows seems too big (for example if there isn't much data in your table) or if you want to enlarge one graph.

Figure 6.30 Changing window size

Full screen view

Click ⌗ in the top right corner to make the workspace full screen.

Note:
Making the workspace full screen will hide the Record selector from view.

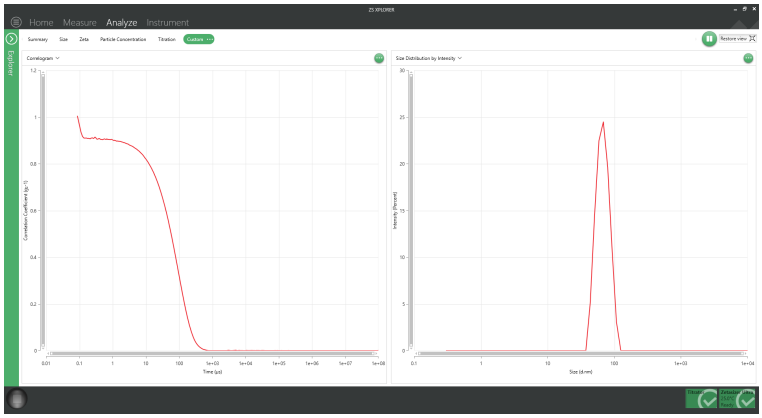


Figure 6.31 *Full screen workspace view*

Click Restore view to return to the original view.

Information types

You can show a variety of of information types on a workspace ranging from plots and data grids through to images and text boxes. The following table lists all information types that are available.

Table 6.2 *Types of figures available in the workspace*

Name	Description
Data Quality	
Data Quality Guidance	Provides feedback regarding the validity of single angled size results.
Particle Concentration Chart	
Cumulative Particle Concentration	A plot of the cumulative particle concentration. The plateau gives the total particle concentration of the measured sample.
Distributed Particle Concentration	A plot of distributed particle concentration showing the particle concentration from the different populations present in the sample.
Picture	
Picture Box	A picture box that can be used for images or logos.

Size Chart	
Correlogram	Displays the correlation function, which is the output of the contents of each of the correlation channels.
Cumulants Fit	Plots 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.
Diffusion Distribution	Distribution of diffusion coefficients that are measured for the sample.
Distribution Fit	Plots the distrubution analysis fit (green line) of the g_1 correlation coefficients (red dots) as a function of correlation time.
Distribution Residuals	Plots the intensity based distribution of relaxation times.
Relaxation Times Distribution	Distribution of relaxation times that are fitted to the correlation function.
Size Distribution by Intensity	Particle size distribution that is weighted according to the light intensity that is detected for each size component. This result does not use the material properties but may disproportionately present different size components due to the relationship between scattering intensity and particle size.
Size Distribution by Number	A particle size distribution that uses the material properties to convert the intensity distribution to a proportional result based on the number of particles in each size range.
Size Distribution by Volume	A particle size distribution that uses the material properties to convert the intensity distribution to a proportional result based on the volume of particles in each size range.
Table	
Parameter Table	Displays selected parameters for the selected data.
Statistics Table	Displays statistics on specific parameters for the selected data.
Text	
Text Box	A text box for adding notes and annotations to reports.
Titration Chart	
pH Trend	Plots the z-average (nm) and zeta potential (mV) as a function of pH. An iso-electric point, if present, is calculated from the selected data.
Titration Z-Average Trend	A trend plot of the z-average as a function of pH.
Titration Zeta Potential Trend	A trend plot of zeta potential (mV) as a function of pH. The iso-electric point (s), if present, are also displayed.

Trend Chart	
Trend	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 other numerical parameters for the y axis.
Z-Average Trend	This chart shows the Z Average as a function of record number when multiple records are selected.
Zeta Chart	
Frequency Shift	Fourier transform of the optical signal measured during a zeta potential measurement. The shape and height of the peak can be used to interpret the signal quality of the measurement.
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.
Zeta Potential Distribution	This displays the distribution of the zeta potential results, showing the peak of the measured values.
Zeta Potential Voltage and Current	Applied voltage and measured current applied across the sample as a function of time during a zeta potential measurement.

Interacting with the workspace

This section contains a number of tips to help you navigate the workspace effectively.

Selecting series data

On a Correlogram, click to open up a submenu.

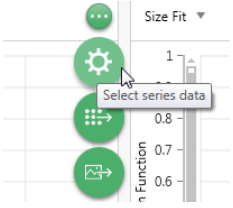


Figure 6.32 Selecting series data

From here, click the **Select series data** button. This opens up a menu where you can choose to include **Transient** and **Unfiltered** data in the graph, as well as hide your **Steady state** data (the data displayed by default).

This can be useful to view any data that was filtered out of your results by the adaptive correlation algorithms built into the software.

For more information on Adaptive Correlation see Viewing transient and unfiltered data on page 128.

Data points

Move the mouse pointer over the plot and move the cursor to show an individual data point. Data points are indicated by circles that appear when you hover over them. The data point displays the specific data values at that point.

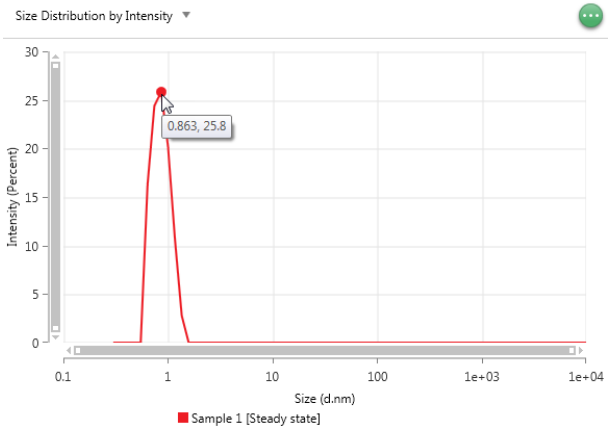


Figure 6.33 Viewing a data point

When working with the *Titration Zeta Potential Trend* graph, move the mouse pointer over the vertical IEP line (if shown) to reveal the IEP (pH) value. See: Analyzing titration results on page 95.

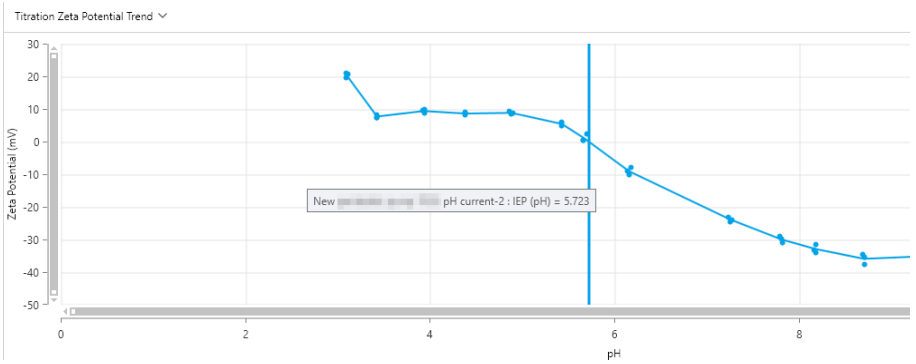


Figure 6.34 *Displaying the IEP value*

Zooming

A quick way to zoom in on a region of interest within a graph is to click and drag a selection area over the section of interest. The software will zoom in to show that part of the graph.

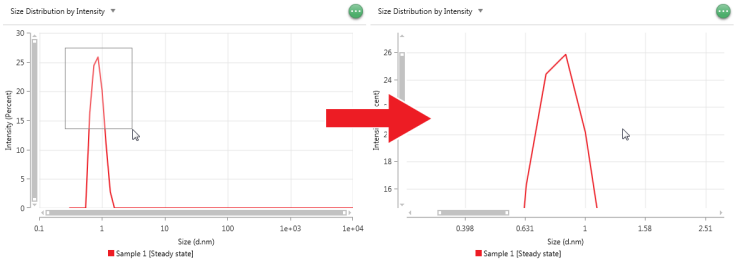


Figure 6.35 *Automatically zooming in on a graph*

You can manually zoom in or out by using your mouse wheel while the mouse pointer is positioned over the graph, or by adjusting the length of the axes scroll bars.

You can also use the scroll bars on the graph axes to move around the zoomed-in graph. Double click on the scroll bars or graph to zoom all the way back out.

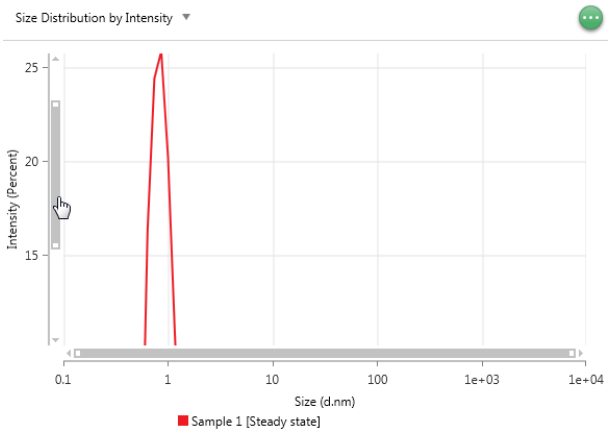


Figure 6.36 *Navigating a graph using the scroller bars*

Overlaying data

To overlay different samples on the same graph, select multiple results in the Record selector. To do this, hold **Ctrl** and click the records you would like to overlay, or hold **Shift** and select the range of records you would like. You can hold **Ctrl** and **A** to select all of the records in a project.

		Qu	Sample Name	Date	Measurement Type	Temperature (°C)	
	1		Sample 1	03/12/2018 15:47:52	Size	25	
	2		Sample 1	03/12/2018 15:48:17	Size	25	
	3		Sample 1	03/12/2018 15:48:40	Size	25	
	4		Sample 1	10/01/2019 09:52:35	Size	25	
	5		Sample 1	10/01/2019 12:18:31	Size	25	
	6		Sample 1	10/01/2019 12:24:56	Size	25	

Figure 6.37 *Selecting multiple records*

The graphs now overlay in the workspace view, as shown:

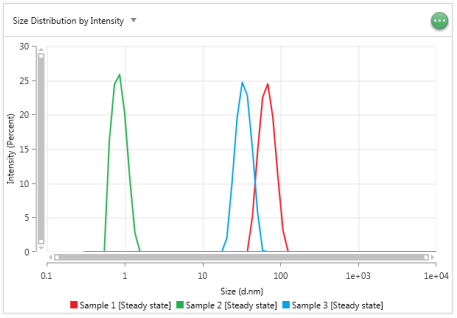


Figure 6.38 *Overlaid graphs*

Note:

If the data does not appear to overlay, check your workspace view is correct. For example, make sure the Zeta workspace is selected if you are overlaying Zeta potential measurements, as nothing will be shown in the Size workspace.

Explorer

The Explorer provides flexible features that allow you to:

- Find your measurements by date
- Organize your results by copying them between projects

Find and organize measurements

Find results by date

Select one of the pre-configured date ranges from the drop down menu as follows:

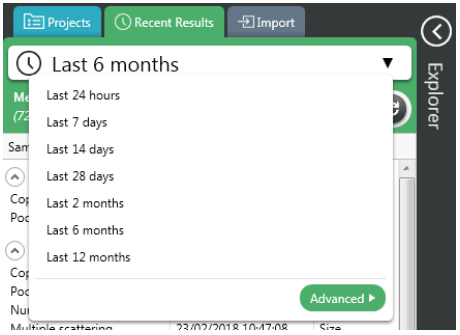


Figure 6.39 *Recent results*

Alternatively, click **Advanced** to specify a custom date range.

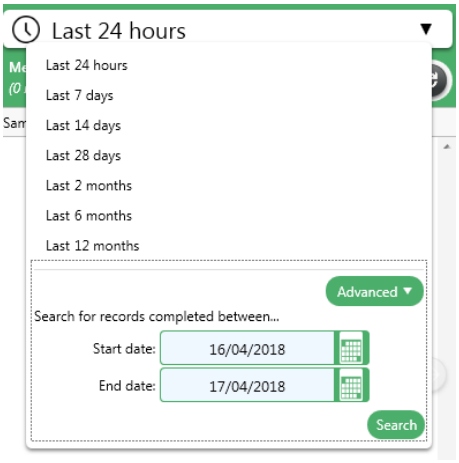


Figure 6.40 *Explorer's Advanced panel - date range*

Click **Search** to show the records.

Once you have found the results you are looking for - either using the search or a date range - the results are shown like this:

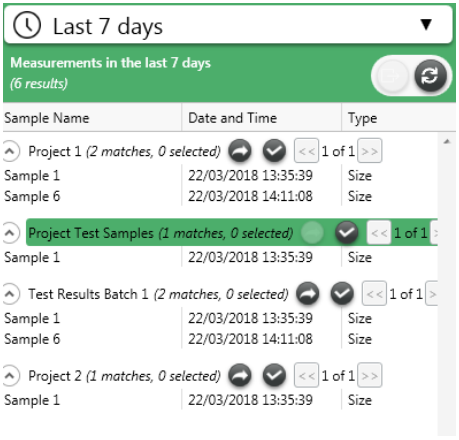


Figure 6.41 *Explorer - last 7 days' results*

The results are grouped by project. A maximum of ten results are shown within each project. To see more results, click the next page icon.

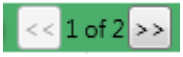


Figure 6.42 *Paging through results in the Explorer*

Click the Select all button to select all the visible records in a project.

Exporting measurement data

This section provides information on how to export measurement data. Data can be exported from the Explorer or the Workspace.

Exporting a whole project as .zmes

1. Select the project you wish to export in the Project Explorer.

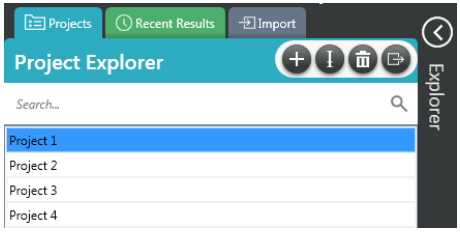


Figure 6.43 Selected project for export

2. Click . This opens the *Save As* window.

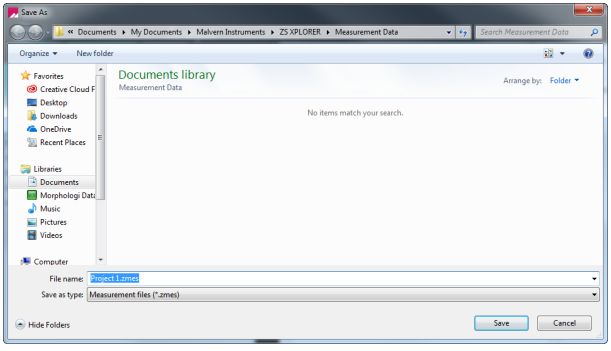


Figure 6.44 Save As window

3. Choose a save location and enter a name for the exported file, then click **Save**.

Exporting selected results as .zmes

To export specific records as a .zmes file from the Project Explorer:

1. Select the result(s) to export from the Project Explorer.
2. Click to export the selected results.

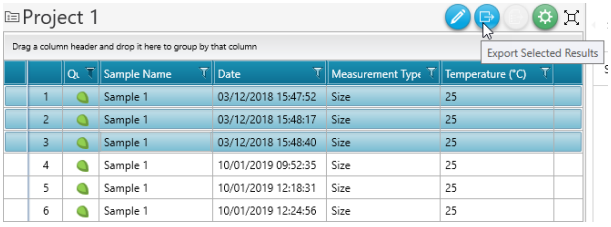


Figure 6.45 Export button

3. This opens the *Save As* window. Choose a save location and enter a file name. The default file name will be the name of the Project that the results are being exported from. Click **Save**.

Note:
The .zmes file needs to be saved in *Documents/Malvern Instruments/ZS XPLORER/Measurement Data* if you would like to import the data later on.

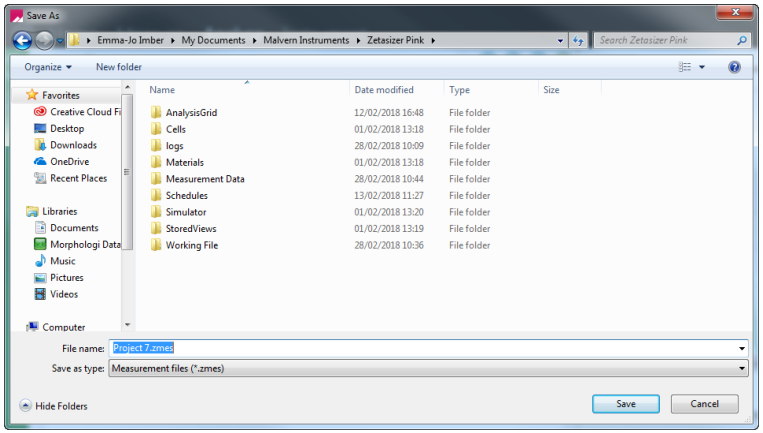


Figure 6.46 Export destination and file name

Tip:
Once exported, data can be shared and opened in the ZS XPLORER on other computers.

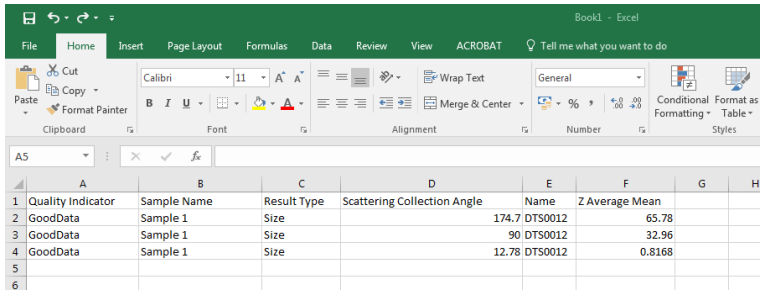
Copy and paste records into Microsoft Excel®

Records can be copied and pasted from the Record selector, parameters table or statistics table. Workspace tables allow you to create bespoke tables to suit your requirements with additional parameters that are not available in the Record selector. To copy and paste records into Microsoft Excel®:

1. Select the record(s) required and then press CTRL+C. You can select multiple records by holding down SHIFT or CTRL and clicking results.
2. In an Excel worksheet, press CTRL+V to paste the records - a header row is automatically added.

Note:
The columns pasted into the Mircosoft Excel® spreadsheet will be the same as the current columns shown in the Record selector, parameter table, or statistics table.

The results will be shown in the same format as in the following image.



	A	B	C	D	E	F	G	H
1	Quality Indicator	Sample Name	Result Type	Scattering Collection Angle	Name	Z Average Mean		
2	GoodData	Sample 1	Size	174.7	DTS0012	65.78		
3	GoodData	Sample 1	Size	90	DTS0012	32.96		
4	GoodData	Sample 1	Size	12.78	DTS0012	0.8168		
5								
6								

Figure 6.47 *Results pasted into Microsoft Excel®*

Copying graphs

Graphs in the workspace can be copied as images or data. This is particularly useful when writing a report. To copy a graph:

1. In the workspace, click one of the buttons circled in the image below from the graph drop down menu. This copies the graph as either data or an image to the clipboard, depending on the button clicked.

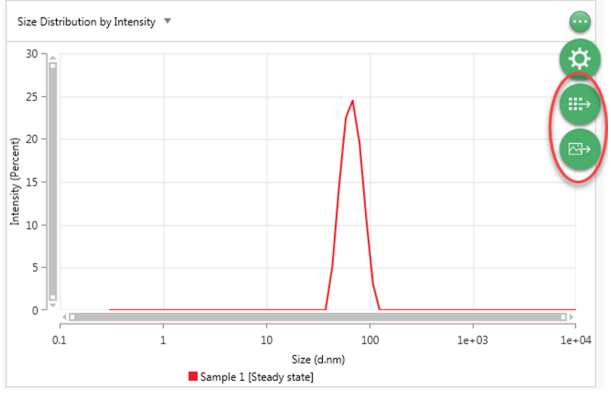


Figure 6.48 *Data and image graph copy buttons*

2. Press CTRL and V to paste the image or data into software of your choice. The image below shows an exported graph image pasted into Microsoft Word®.

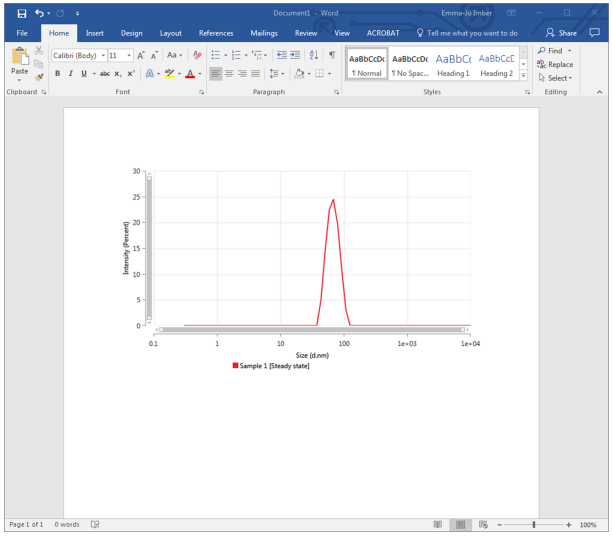


Figure 6.49 Copied graph as an image in Microsoft Word®

Importing results into a project

1. First, you must have already exported the results you now want to import. See Exporting measurement data on page 115.
2. Open the Explorer window, and select the project into which you want to import results from the Project Explorer tab.
3. Click the Import tab.
4. Select the results file that you wish to import into the currently selected project and then click .

The results are now imported into your project – the time taken to do this depends on the number of results you are importing.

CHAPTER 7 INTERPRETING RESULTS

Introduction

There are a number of tools you can use to interpret the results you obtain with the Zetasizer. This chapter outlines some of these, including:

- Interpreting graphs of data
- The Data Quality Guidance tool found in the Analyze software window
- Viewing transient data

Interpreting data

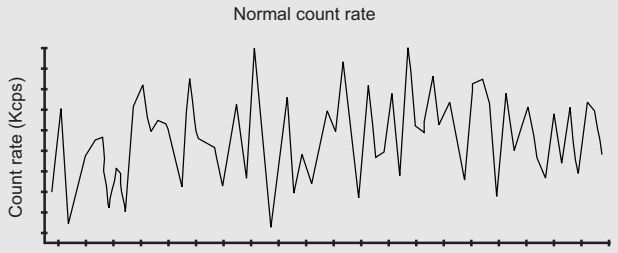
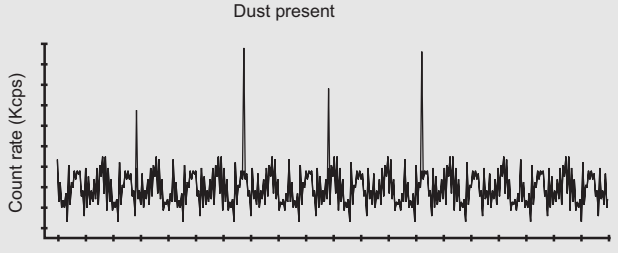
The Data quality guidance indicator gives a quick assessment of data quality and helps you to troubleshoot results. See Data quality guidance on the facing page. However, this section gives some information on irregularities you can look out for in your data and what they may indicate.

Count rate

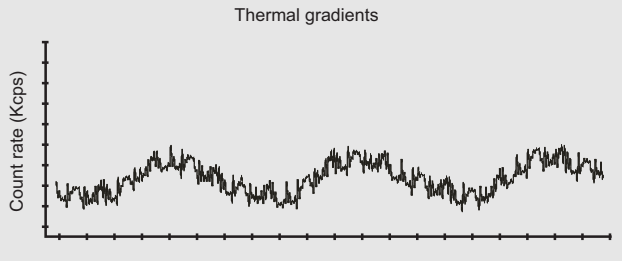
The count rate is the number of photons detected per second. Count rate is visible in the live measurement display.

The images below show some of the irregularities you may observe, and their causes.

Table 7.1 *Count rate graphs*

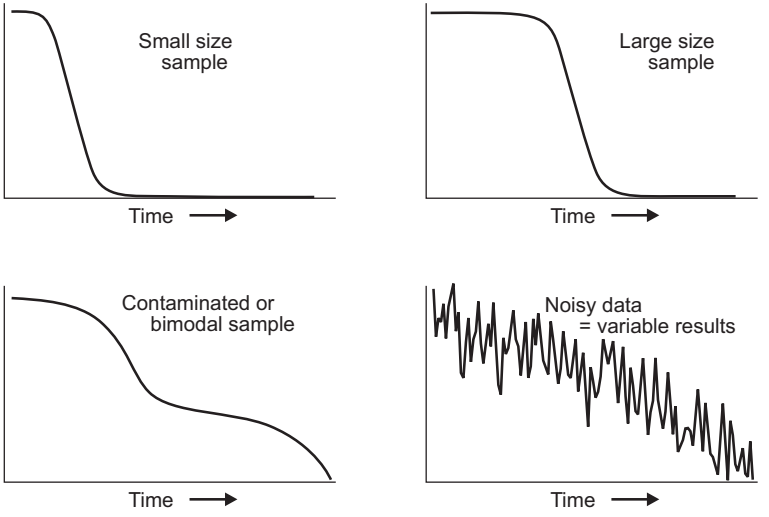
Description	Example graph
Normal count rate display.	
If dust, larger particles, or aggregates are present then sharp spikes will be observed. Measurement runs with dust present may be filtered out of the steady state results by adaptive correlation, depending upon the number of larger particles measured.	

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



Correlogram

The shape of the Correlogram can also help you to interpret your sample. The images below show how certain conditions can affect the Correlogram shape.



Data quality guidance

The ZS XPLORER has a built in tool to help you assess the quality of your data. Data quality guidance is given by a neural network trained to assess your data quality.

The data quality can be viewed in two ways:

1. In the Record selector
2. In the Workspace

Viewing the data quality in either environment is explained in detail in the following sections.

[1] The Record selector

The Quality Indicator parameter in the Record selector indicates the quality of a result. For more information about using parameters in the Record selector, see Parameters on page 89.

If the icon is green, the data is of good quality. If the icon is not green, view the Data Quality Guidance in the Workspace for further information.

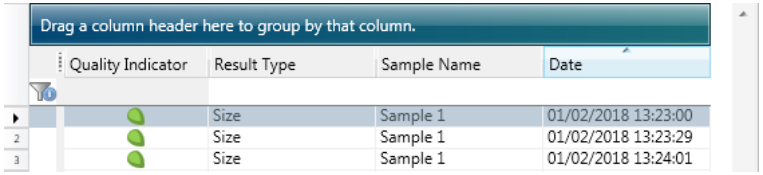


Figure 7.1 Data quality shown in the Record selector

[2] In the workspace

The Data Quality Guidance window can be shown in the Workspace display. An entry will be shown in the table for every selected result.

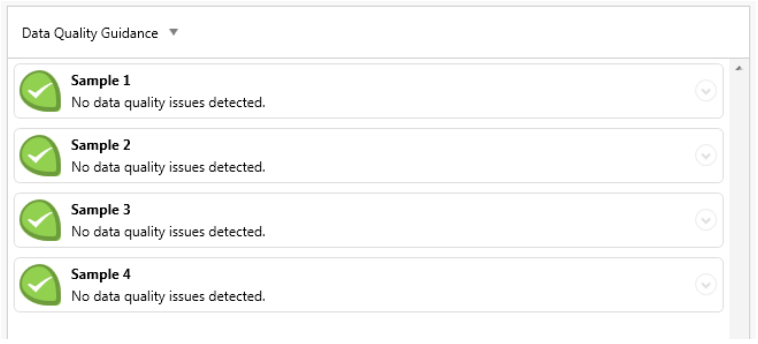


Figure 7.2 Data Quality Guidance in the workspace

See Customizing your workspace on page 100 for details about changing the workspace display.

The following table shows the meaning of each indicator color, and the actions you should take.

Table 7.2 Indicator colors and their meaning

Color	Meaning
	No Data quality information available. The measurement type may not currently be supported.
	There are no problems detected in the data. The data is reliable.
	The data is reliable, but should be interpreted carefully following the advice given in the software.
	There are problems detected in the data, and the data might not be reliable. Check your experiment set up and results thoroughly.

Examples

Some examples of the Data Quality Guidance window in the workspace are shown below.

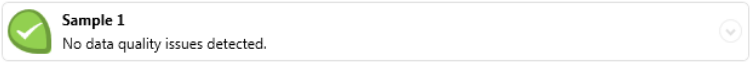


Figure 7.3 No issues detected

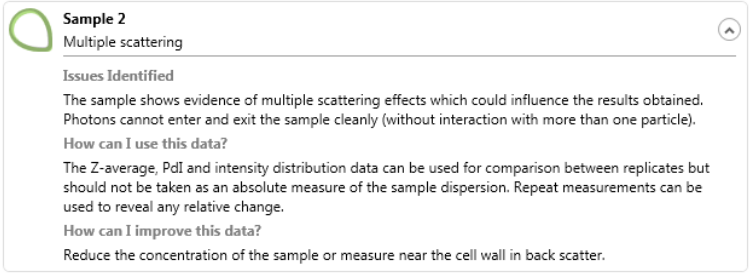


Figure 7.4 Multiple scattering detected

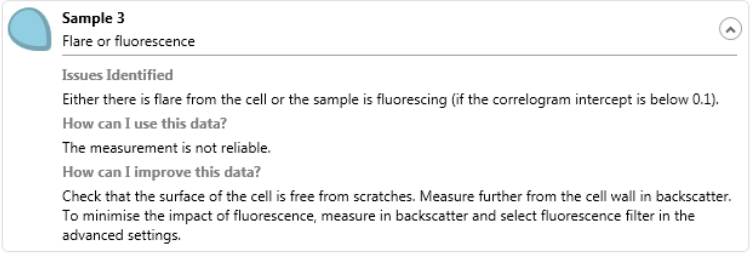


Figure 7.5 Flare or fluorescence detected

Viewing transient and unfiltered data

In the Analyze tab, you can view any transient data which the software has removed from the steady state results displayed by default.

To view transient data:

1. Open your result(s) in the workspace, and then open the drop down menu in the corner of a workspace window.

Note:
Not all workspace windows have the functionality to view transient and unfiltered data. Only the correlogram, size distribution by intensity, size distribution by number and size distribution by volume windows support this feature.

2. Click the cog button, '**Select series data**'.

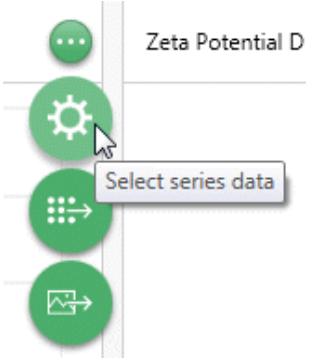


Figure 7.6 Select series data button

3. This opens the menu shown below. Select the data you would like to view.

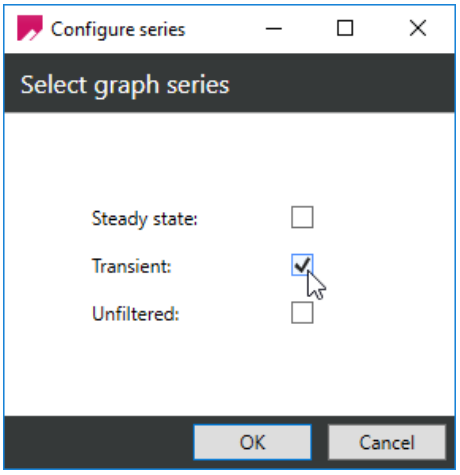


Figure 7.7 Selecting transient data

4. Click **OK** to confirm your changes.

Transient particles may not be present in your results, but if they are, viewing the transient data can give you information to help reduce the concentration of these particles.

Filtering the sample can help to remove impurities which may cause transient data. Viewing the measured size of the transient data in the workspace will give a clear indication of what filter size to try.

Tip:
The Data quality guidance tool in the software will inform you if transient data is affecting the quality of your results.

The following image shows steady state and transient data overlaid on a correlogram and on a size distribution graph.

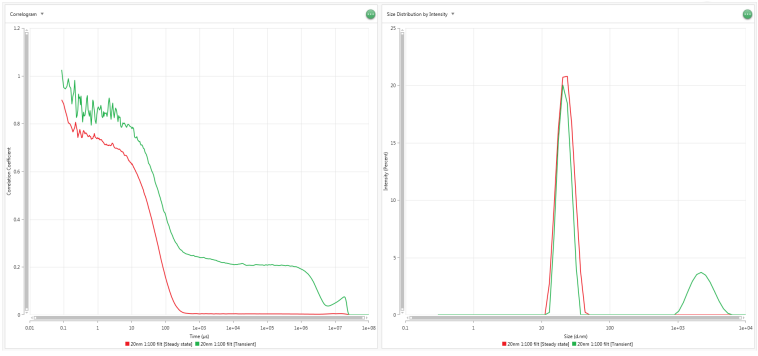


Figure 7.8 *Overlaid transient (green) and steady state (red) data*

CHAPTER 8 MEASUREMENT THEORY

Measurement theory overview

This section describes the basics of how the Zetasizer measures particle size and zeta potential and why they are important.

Particle size - Dynamic Light Scattering (DLS)

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

The Zetasizer system determines the particle 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. These are described in detail in Size theory on page 134.

Why use DLS?

A wide range of materials exist as molecules or particles in dispersion that can be characterized by DLS. These include proteins, polymers, emulsions and vesicles. Additionally, materials more traditionally thought of as particles, such as clays, silica, pigments and inks are also good candidates for DLS. The benefits of using DLS for some of these applications are described below.

- 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: emulsions and vesicles are delicate, in that the preparation required for measurement techniques such as TEM (Transmission Electron Microscopy) or AFM (Atomic Force Microscopy) 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 color and hue are highly related to particle size, this has applications in determining a pigment's properties.

Zeta potential - Laser Doppler Electrophoresis

The liquid layer surrounding a particle exists in two parts. The inner layer, called the Stern layer, consists of ions that are strongly bound to the particle. The outer layer, called the diffuse layer, consists of ions held less strongly.

There is a stable boundary in the diffuse layer called the slipping plane. When the particle moves, surrounding ions within this boundary will travel with the particle. Ions outside of this boundary will be left behind. The electric potential at the slipping plane is called the zeta potential.

For a more detailed explanation of the technique, see Zeta potential theory on page 147.

Why measure zeta potential?

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

Size theory

This section provides more in-depth information on the size theory used within the Zetasizer.

Dynamic Light Scattering (DLS)

The Zetasizer performs size measurements using DLS - a non-invasive technique used to measure the size of particles and molecules in suspension. DLS works by illuminating particles with a laser and analyzing the intensity fluctuations in the scattered light.

When a laser shines on a particle, the particle will scatter the light. When a laser illuminates many particles in a solution, the scattered light beams will interfere with each other to produce a so-called speckle pattern. The resulting pattern of light will have light and dark areas. The bright areas are where the scattered light 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.

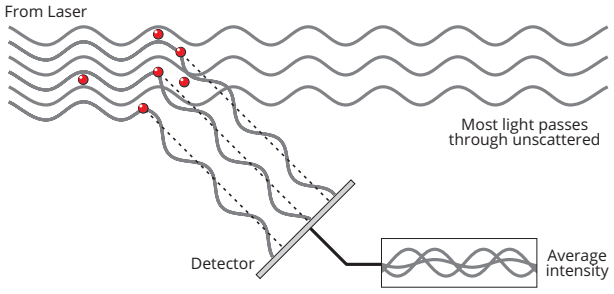


Figure 8.1 *Scattered light falling on the detector*

Because these particles are moving due to Brownian motion, the intensity of the light and dark areas will change over time. At any one point, the intensity of light will appear to fluctuate. Smaller particles move faster, and larger particles move more slowly.

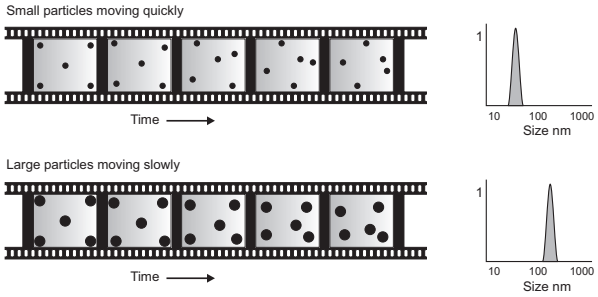


Figure 8.2 Particles in motion

By measuring this intensity fluctuation, the particle diffusion coefficient can be calculated. Using the Stokes-Einstein equation, the particle size can then be calculated. This equation relates the size of a particle to its velocity as a result of Brownian motion.

$$d(H) = \frac{k_B T}{3\pi\eta D}$$

Figure 8.3 The Stokes-Einstein equation

- $d(H)$ = hydrodynamic diameter
- D = translational diffusion coefficient
- k_B = Boltzmann constant
- T = absolute temperature
- η = viscosity

What is intensity correlation?

A digital correlator within the Zetasizer measures the degree of similarity between a signal at two different points in time. A short amount of time after starting a measurement, the particles haven't moved much. The measured intensity signal will be similar to the intensity at the

beginning of the measurement. Therefore, the correlation coefficient will be close to 1.

When more time has passed, the particles will have moved further from their original positions. So, the measured light intensity will have changed more, and will be less similar to the original signal. After enough time has passed, the correlation coefficient will reach 0 when it no longer bears any resemblance to the original signal.

If large particles are being measured, their intensity changes slowly due to their slower speed. If small particles are being measured, their intensity fluctuates quickly because the particles themselves are moving more quickly. The graph below illustrates this.

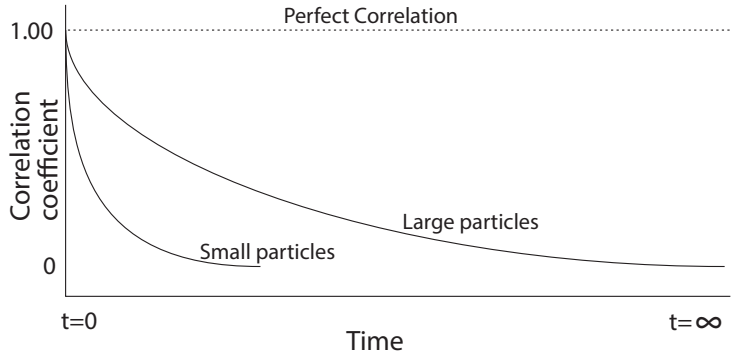


Figure 8.4 Decay rate of correlation for large and small particles

The rate of decay of the correlation coefficient provides information about the speed of the Brownian motion of the particles in the sample. The Zetasizer software then uses algorithms to produce a size distribution from the decay rate.

Although the fundamental size distribution generated by DLS is an intensity distribution, this can be converted to a volume or number distribution in the software.

Adaptive correlation

Dust particles in the sample can reduce the accuracy of your results. As the detection volume is much smaller than the total sample volume, it is possible for rare dust particles to diffuse in and out of the detection volume. By performing a series of sub runs, the Zetasizer can capture

sub runs when dust particles are, and are not present.

During a measurement, the Zetasizer software uses Adaptive correlation to identify and classify data suspected of being dust, due to its erroneous particle size. To do this the system looks at the Polydispersity index, a reliable indicator of changes in particle size distribution.

Adaptive correlation performs Cumulants analysis on each sub run, and the Polydispersity index for each is found, compared, and a hypothesis test is used to check for statistical outliers. This hypothesis test assumes a Normal distribution, where the an outlier is a value that falls outside of 3 standard deviations of the mean.

These outliers are not classified as steady state data, and are likely to be dust particles. Steady state data is then made into a final measured correlogram. Data which is not classified as steady state will not be shown by default in the results, but you will still be able to view it. See Viewing transient and unfiltered data on page 128.

The following images illustrate how much Adaptive correlation can improve the quality of results. The first graph shows results from a measurement made using a legacy algorithm, the second graph shows the same sample measured using Adaptive correlation.

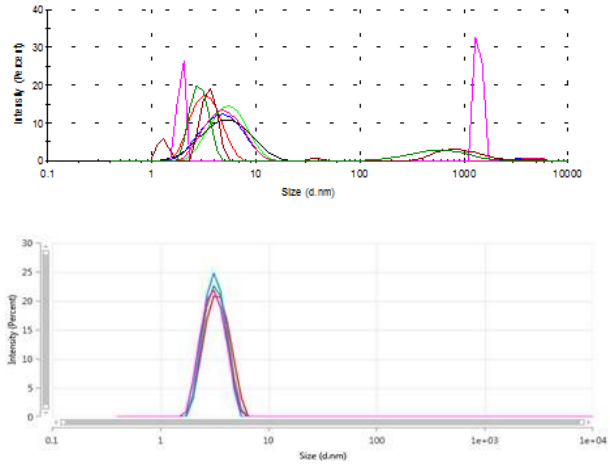


Figure 8.5 *Adaptive correlation in action*

The time taken to carry out a measurement is also reduced in Adaptive correlation. This is because the system will only take measurements for as long as it needs to, rather than collecting surplus data. Data is only collected until a stable Z-average value converges. Averaging from at least 15 sub runs is recommended for good quality results.

Intensity, volume and number distributions

This section uses an example to illustrate the difference between intensity, volume and number distributions. The example sample contains equal numbers of 5 nm and 50 nm particles.

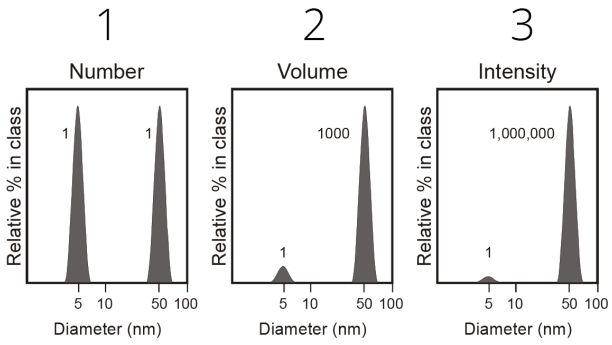


Figure 8.6 *Number, volume and intensity graphs for the same measurement*

Note:
Intensity is the base measurement obtained from a DLS measurement. Other quantities are derived from this.

[1] - 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.

[2] - Shows the result as a volume distribution. The area of the peak for the 50 nm particles is 1000 times larger the peak for the 5 nm (1:1000 ratio). This is because the volume of a 50 nm particle is 1000 times larger than the 5 nm particle (volume of a sphere is equal to $4/3\pi(r^3)$).

[3] - Shows the result as an intensity distribution. The area of the peak for the 50 nm particles is now 1,000,000 times larger than the peak for the 5 nm (1:1000000 ratio). This is because large particles scatter much more light than small particles. The scattering intensity is proportional to the sixth power of the particle's diameter. This is based on Rayleigh's approximation.

Multi-angle Dynamic Light Scattering (MADLS®)

MADLS is a fully automated process that measures the correlation function in three scattering directions - backscatter, side scatter and forward scatter - and uses that data from three angles to create an angle independent result. This gives a particle size distribution with a higher resolution, beyond that attainable with a single-angle DLS experiment alone.

Note:
A MADLS measurement can only be performed using a Zetasizer Ultra, due to the requirement for side-scatter measurement.

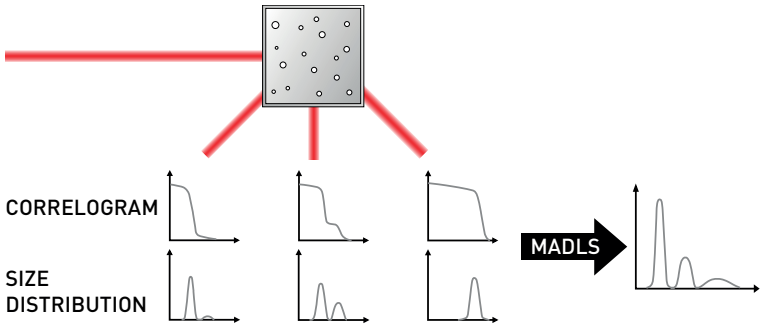


Figure 8.7 How MADLS works

A MADLS measurement works in the following way:

- The sample is illuminated by laser light and scattered radiation is detected sequentially in backscatter, side scatter and forward scatter
- Different sized particles scatter more or less light at different angles due to the interaction between illumination wavelength and the particle size
- The instrument correlator produces an auto-correlation function at each scattering angle
- The correlation functions are combined and the MADLS analysis produces the particle size distribution
- Mie theory is used to calculate the angular scattering properties of the particles and reconcile the observed auto-correlation functions with the particle size distribution

This removes angular dependence in the size result, and increases its robustness. This technique allows particles with a size ratio of 2:1 to be resolved, whereas a single angle measurement may only resolve particles with a size difference of 3:1.

Particle concentration

Measuring particle concentration using DLS requires three quantities:

- The particle size distribution
- The time averaged intensity scattered by a molecular scatterer
- The time averaged intensity scattered by the sample

The particle size measurement is performed using multi-angle dynamic light scattering (MADLS). A MADLS measurement carried out in the Zetasizer Ultra involves measurement of the auto-correlation function at three angles: backscatter, side scatter and forward scatter. From this, we obtain a particle size distribution.

The time averaged intensity scattered by a molecular scatterer is found by using the scattering measurement in the Instrument tab (see Menu bar on page 18). By measuring a molecular scatterer with a known volume scattering function, such as toluene, the instrument sensitivity can be normalised.

In order to calculate the intensity of scattering attributed to the particles in the sample, background scattering from the dispersant needs to be quantified. This intensity, or count rate, can be measured for a sample containing no particles, and can then be entered into the software measurement settings.

The Zetasizer Ultra performs this measurement automatically to determine the particle concentration as a function of size, using a specially derived equation.

Size result types

Size measurement results are given in the Statistics table in the workspace as the following values.

Z-average size

In Dynamic Light Scattering (DLS) this is the most important and stable number produced by the technique. This value is often required for quality control purposes.

The Z-average 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 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 overresolving 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 2 c/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 1.

Size optical configuration

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 in the plane perpendicular to the laser polarization, it is possible to place the detector at any angle and still detect scattered light.

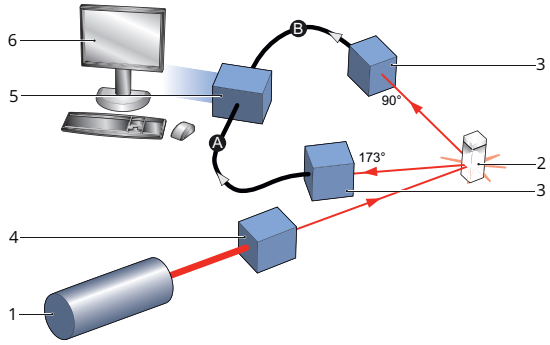


Figure 8.8 *173°/90° measurement optics*

Note:
The side-scatter detector is in the Zetasizer Ultra only.

Note:
MADLS and Particle size measurements also use the 17° forward scatter - see Zeta potential optical configuration on page 154 for illustration of this angle. Zetasizer Ultra only.

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 response will become non-linear. To overcome this an attenuator **[4]** reduces the laser intensity and hence the intensity of the scattering.

- For samples that do not scatter much light, such as very small particles or samples of low concentration, the amount of scattered light must be increased. In this situation, the attenuator will allow more laser light through to the sample.
- For samples that scatter more light, such as large particles or samples of higher concentration, the amount of scattered light must be decreased. This is achieved by using an 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 ZS XPLORER software will analyze the data and derive size information.

173° backscatter detection

The Zetasizer measures 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 (Non-Invasive Back-Scatter).

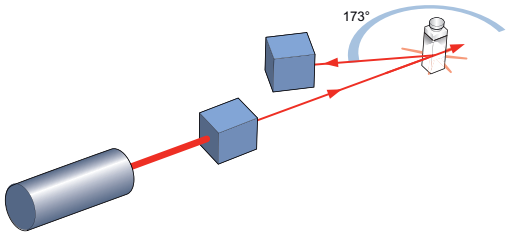


Figure 8.9 NIBS set up

There are several advantages to measuring backscatter:

- The incident beam does not have to travel through the entire sample, so higher concentrations of sample can be measured.
- Multiple scattering, where the scattered light from one particle is itself scattered by other particles, is reduced.
- Contaminants, such as dust particles, are typically large compared to the sample size, mainly scattering in the forward direction. Therefore, measuring backscatter, reduces the effect of dust.
- The effect of multiple scattering is at a minimum at 180° - again, this allows higher concentrations to be measured.

Moveable lens

The Zetasizer's movable lens allows the focus position within the cell to be changed, which means that a much larger range of sample concentrations can be measured.

For small particles, or samples of low concentration, it is beneficial to maximize the amount of scattering from the sample. When the incident light interacts with any change in refractive index, scattering occurs. The wall of the sample cell may therefore scatter some light, which we call "flare". This may swamp the sample scattering signal so moving the measurement point away from the cell wall, towards the center of the cell can remove this effect.

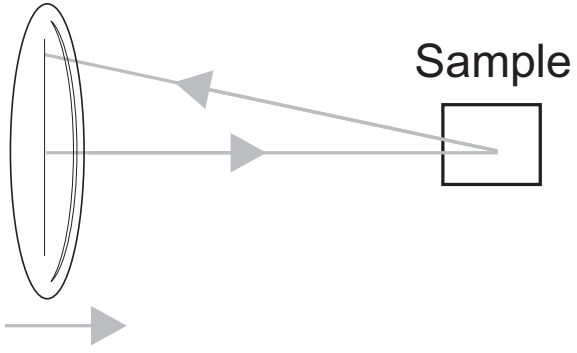


Figure 8.10 Moving the lens towards the cuvette

Large particles, or samples of high concentration, scatter much more light. Here, measuring closer to the cell wall reduces the effect of multiple scattering. In this instance the flare from the cell wall will have less impact. Any flare will be less significant compared to the scattering signal.

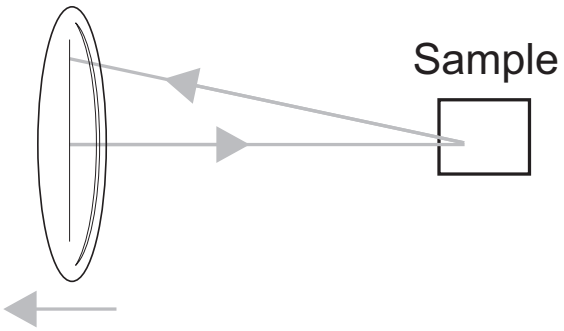


Figure 8.11 Moving the lens away from the cuvette

The measurement position is automatically determined by the Zetasizer software.

Zeta potential theory

What is zeta potential?

Most liquids contain ions. These are negatively and positively charged atoms and molecules 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 positively charged sample attracts negative ions from the liquid.

Ions close to the surface of the particle are strongly bound. Ions that are further away will be loosely bound, forming what is called a diffuse layer. Within the diffuse layer is a notional boundary called the slipping plane. Ions within this boundary will move with the particle as it moves within the liquid. Any ions outside this boundary will be left behind.

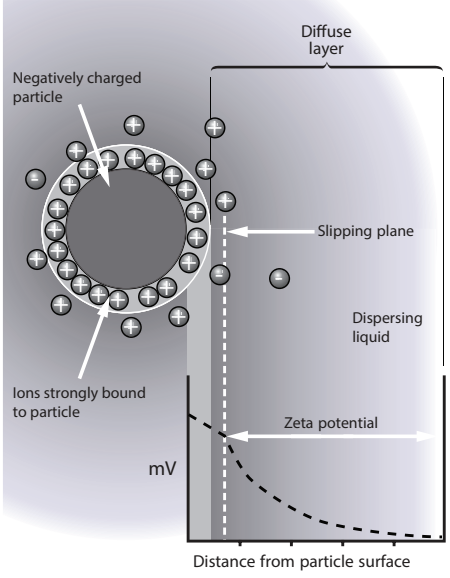


Figure 8.12 Explaining zeta potential theory

An electric potential exists between the particle surface and the dispersing liquid. This varies with distance from the particle surface. This potential at the Slipping plane is called the Zeta potential.

Zeta potential is measured using a combination of Electrophoresis and Laser Doppler Velocimetry; sometimes called Laser Doppler Electrophoresis. This technique measures the speed of a particle in a liquid when an electric field is applied. Once the velocity of the particle and the electrical field applied are known, by using two other known constants of the sample - viscosity and dielectric constant - the zeta potential can be calculated.

Significance of zeta potential

The magnitude of the zeta potential of particles in a colloidal system indicates system stability. Particles with a large zeta potential (positive or negative) repel each other. This makes flocculation less likely, so the system is more stable. When the particles have small zeta potential values, they do not repel and are more likely to flocculate. Particles with zeta potentials higher than +30 mV, or less than -30 mV, are normally considered stable, though particle size can also affect the stability.

Zeta potential is influenced by pH. Therefore, by titrating a sample over a range of pH values, the zeta potential can change. Adding alkali to a particle with a negative zeta potential makes it more negative. When acid is added instead, the particle gains positive charge and becomes neutral. The pH at which the zeta potential is zero is called the isoelectric point and is the pH value at which the sample is least stable.

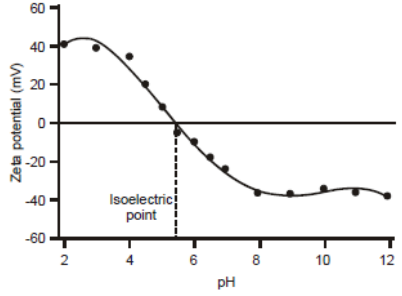


Figure 8.13 pH and Zeta potential

Electrophoretic mobility and the Henry equation

When an electric field is applied across a sample, charged particles 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 a particle in an electric field is commonly referred to as its Electrophoretic mobility. The zeta potential of a particle influences the Electrophoretic mobility.

Electrophoretic mobility and zeta potential are related by the Henry equation:

$$U_E = \frac{2\varepsilon\zeta f(\kappa a)}{3\eta}$$

Where:

- ζ = Zeta potential
- U_E = Electrophoretic mobility
- ε = Dielectric constant
- η = Viscosity
- *f(Ka)* = Henry's function

Two values are generally used as approximations for f(Ka) - either 1 or 1.5.

Zeta potential is usually measured in aqueous media and moderate electrolyte concentration. The f(Ka) value in this case is 1.5, and is referred to as the Smoluchowski approximation. The Smoluchowski approximation is used for the folded capillary cell, high concentration cell, and the universal dip cell when used with aqueous samples.

For small particles in media with a low dielectric constant, f(Ka) becomes 1.0. This is referred to as the Hückel approximation, and is used for non-aqueous measurements.

To calculate the zeta potential, the electrophoretic mobility is measured. The Zetasizer uses Laser Doppler Velocimetry to measure the electrophoretic mobility.

Laser Doppler Velocimetry

Laser Doppler Velocimetry is a technique used to study the velocity of fluid flows. A laser is split into two beams, a measurement beam and a reference beam. The measurement beam passes through the sample and is scattered by the particles.

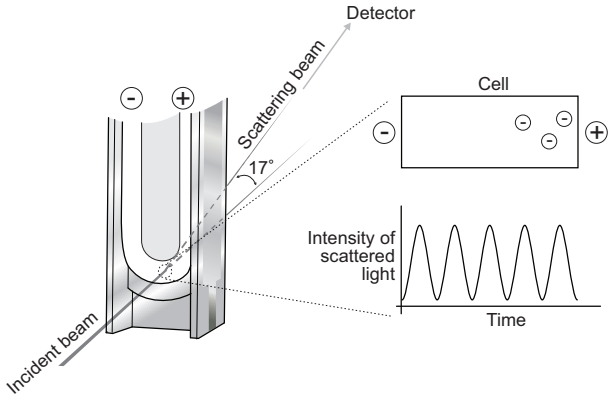


Figure 8.14 Laser Doppler Velocimetry

By receiving this scattered light and measuring the frequency of the intensity fluctuation relative to the reference, the Doppler shift can be found. This is used to calculate the velocity of the particles in the sample.

Electroosmosis

The walls of the capillary cell carry a surface charge. When the electric field used to stimulate electrophoresis is applied, this field also acts on the surface charge. So the liquid adjacent to the walls will undergo electroosmotic flow. The capillary is a closed system, so the flow along the walls must be compensated for by a reverse flow down the centre of the capillary. These additional flows change the velocities of the particles in the fluid.

There is a point in the cell at which the electroosmotic flow is zero, where the two fluid flows cancel. If the measurement is performed at this point, the particle velocity measured will be the true electrophoretic velocity. This point is called the stationary layer.

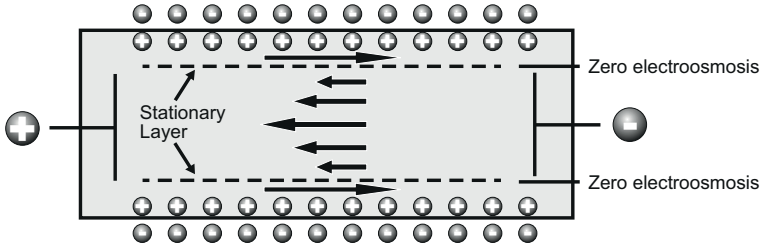


Figure 8.15 The stationary layer technique

The stationary layer technique described above is commonly used. But because of electroosmosis, the measurement can only be performed at a specific point within the cell.

The M3-PALS technique

To perform measurements at any point within a cell and obtain the electrophoretic mobility, Malvern Panalytical has developed its patented M3-PALS technique. This is a combination of Malvern Panalytical's improved laser doppler velocimetry method - the M3 measurement technique, and the application of PALS (Phase Analysis Light Scattering).

M3

M3 consists of both Slow Field Reversal (SFR) and Fast Field Reversal (FFR) measurements, hence the name 'Mixed Mode Measurement'. An M3 measurement is performed in the following manner:

- A Fast field reversal measurement is made at the cell center. This gives an accurate determination of the mean particle velocity.
- A Slow field reversal measurement is made. This gives higher 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.

All systems that measure mobilities using LDV (Laser Doppler Velocimetry) traditionally just use the slow field reversal mentioned below. However, M3 consists of two measurements for each zeta potential measurement, SFR and FFR.

Slow Field Reversal (SFR): The field is usually reversed about every one second to allow the fluid flow to stabilize. This reversal is applied to reduce the polarization of the electrodes.

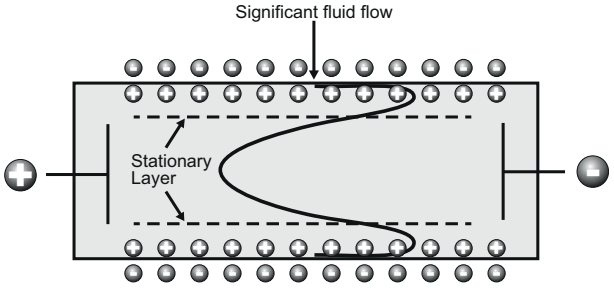


Figure 8.16 *Slow Field Reversal*

Fast Field Reversal (FFR): When the field is reversed much more rapidly, the particles reach terminal velocity, while the fluid flow due to electroosmosis is insignificant. This means that the mobility measured is not affected by electroosmosis. The mean zeta potential that is calculated by this technique is very reliable, but as the velocity of the particles is sampled for such a short period of time, information about the distribution is degraded.

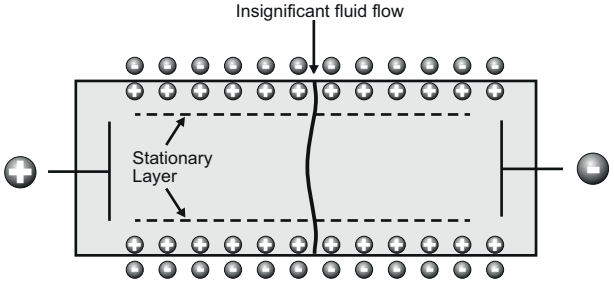


Figure 8.17 *Fast Field Reversal*

Benefits of M3

Using M3 the entire zeta potential measurement is simplified. It is not necessary for the operator to select any system parameters for the measurement, as the appropriate settings are calculated as part of the M3 sequence. By reducing the number of measurement variables, the measurement is more accurate and repeatable.

Additionally, alignment is not so critical as the measurement is performed in the center of the cell. Using the center of the cell also reduces the chance of flare from the nearby surface, and enables the calculation of charge on the cell wall.

PALS (Phase Analysis Light Scattering)

PALS is a further improvement on traditional Laser Doppler Velocimetry and the M3 implementation described in this section.

Overall, the application of PALS 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 particle velocity, Phase Analysis Light Scattering 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.

Electrophoretic mobility and consequently the zeta potential is then determined by summing the phase shifts measured during the FFR part of the measurement.

Zeta potential optical configuration

In common with a size measurement, a zeta potential measurement comprises six main components. First of all a laser [1] provides a light source to illuminate the particles within the sample; for zeta potential measurements this is split to provide an Incident (A) and Reference beam (B). The modulation on the reference beam gives rise to a Doppler shift, but this is additional to the Doppler shift from the measured particles. The modulation is applied to improve the signal to noise ratio of the measurement, as phase analysis against a reference of known phase is more precise than compared with a stationary reference.

The laser beam passes through the center of the sample cell [2], and the scattering beam (C) at an angle of 17° is detected.

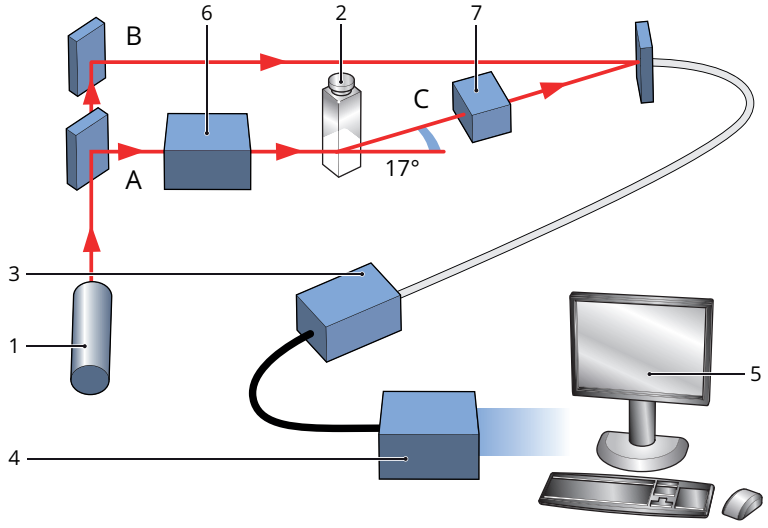


Figure 8.18 Zeta potential optical configuration

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 ZS XPLORER produces a frequency spectrum from which the electrophoretic mobility and hence the zeta potential is calculated.

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 the detector response will become non-linear. To overcome this an attenuator [6] reduces the laser intensity and hence the intensity of the scattering.

For samples that do not scatter much light, such as very small particles or samples of low concentration, the amount of scattered light must be increased. 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.

Theory glossary

Adaptive correlation - automatic size measurement process which makes more informed choices about data classification (especially for contaminants such as dust) and reduces measurement time.

Brownian motion - the random movement of particles caused by collisions with surrounding solvent molecules.

Colloid - a system where one state of matter is finely dispersed in another. They typically have dimensions of less than 1 µm resulting in a very high surface-to-volume ratio.

Count rate - the number of photons detected per second.

Cumulants analysis - simple method of analysing the autocorrelation function generated by a DLS experiment. Z-average and polydispersity index values are derived from this.

Diffusion coefficient - defines the Brownian motion of the particles being measured. It is calculated by fitting the correlation function to an exponential decay.

Dynamic Light Scattering (DLS) - a non-invasive technique used to measure the size of particles in suspension. The intensity fluctuation of scattered laser light is used to find the velocity of the Brownian motion of the particles, which can be used to find size.

Electroosmosis - the movement of a liquid relative to a stationary charged surface under the influence of an electric field.

Electrophoresis - the movement of a charged particle relative to the liquid it is suspended in under the influence of an applied electric field.

Electrophoretic mobility - the velocity of a particle in an electric field.

Hydrodynamic size - the size of a hypothetical hard sphere that diffuses at the same speed as the particle being measured.

Intensity correlation - the degree of similarity between the intensity of two signals received at different points in time.

Isoelectric point - the pH at which the zeta potential of a particle is equal to zero.

Laser Doppler Velocimetry - a technique used to study the velocity of particles traveling in a fluid stream.

Multi Angle Dynamic Light Scattering (MADLS®) - a fully automated process that measures the correlation function in three scattering directions. This data is then combined to create a higher resolution particle size distribution measurement than possible with single angle measurement.

Phase - the position of a particular point on a waveform.

Phase shift - the difference in phase between two signals.

Polydispersity index - value generated by cumulants analysis which describes the distribution of particle sizes found in a sample.

Rayleigh theory - theory of light scattering applicable to small particles and molecules whose diameters are less than 10% of the laser wavelength. Rayleigh theory states that particles of this size scatter light equally in all directions.

Slipping plane - the boundary dividing which ions surrounding a particle will travel with the particle when it moves.

Stern layer - the inner layer of ions surrounding a particle in suspension. They are strongly bound to the particle.

Z-average - the intensity weighted mean hydrodynamic size of the collection of particles measured by dynamic light scattering (DLS). The Z-average is found from a Cumulants analysis of the measured correlation function.

Zeta potential - the electric potential at the slipping plane, an indicator of the stability of a colloidal system.

INDEX

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