POLYMERS and BIOMATERIALS LABORATORY

Manual for experiment:

Dynamic Light Scattering application in size detection of molecules and molecular aggregates

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The aim of experiment is the size of macromolecules and/or micelles measurement using **Zetasizer Nano Malvern** apparatus.

Chemicals:
synthetic polymers, surfactants, organic solvents
synthetic polymers solutions
surfactants solutions

Warning! Students must read the **Health and Safety** information before starting this experiment. It is absolutely necessary because if the instrument is misused, sample and instrument may be hazardous.
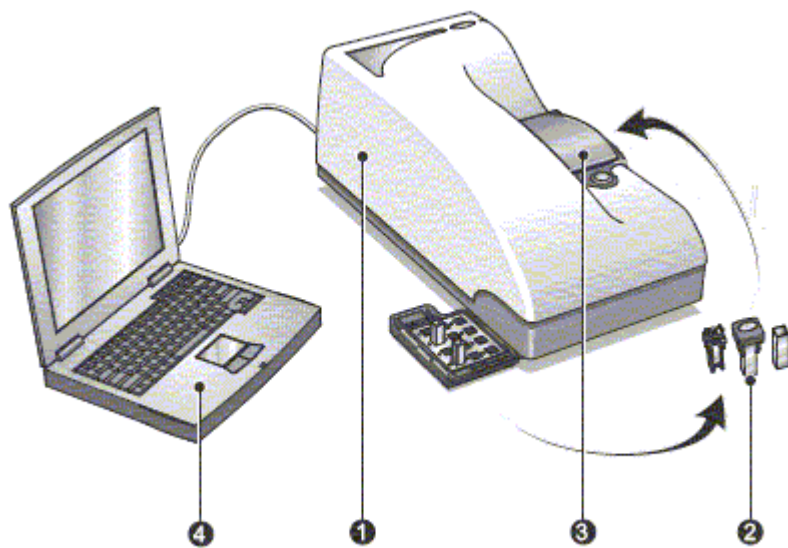Zetasizer Nano installation is presented below in Figure 1.

Figure 1. Zetasizer Nano installation. 1 – optical unit; 2 – celles; 3 – cell area; 4 – computer running the Zetasizer softwear.
Computer controls the optical unit as well as processing and presenting the measured data to give either the size, molecular weight or zeta potential for the sample measured.

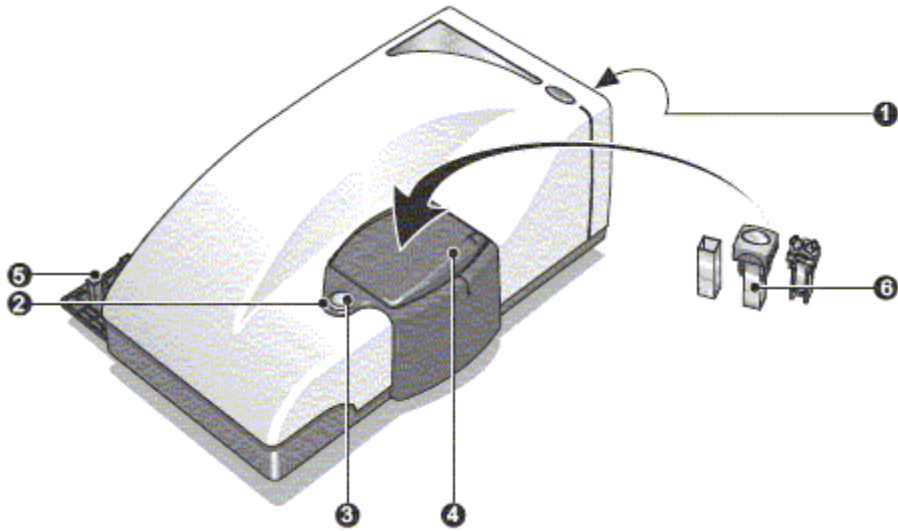The optical unit components are shown in Figure 2.

Figure 2. Optical unit elements. 1- rear panel; 2 – status indicator; 3 – cell access button; 4 – cell area; 5 – cuvette holder; 6 – celles and cuvettes eamples.

Warning! **green colour** of status indicator means that the instrument is functioning correctly and is ready to start a measurement
Green-flashing occurs when the instrument is performing a measurement.
Red colour of status indicator informs that the instrument has detected an error. In this case the measurement will be stopped.

The sets of studied solutions (polymer and/or surfactant) should be prepared gravimetrically and stirred carefully. The solutions concentrations for studied systems are given by instructor. <u>Before DLS measurement transparency of each solution should be checked.</u> If necessary the solution should be filtered to remove dust particles.

Remarks regarding the sample concentration

Each type of sample material has its own ideal range of sample concentration for optimal measurements. Because of that, sample concentration must be selected between the specific ranges to make the measurement optimal.
- If your sample's concentrations is too low; there may not be enough light scattered to make your measurement.
- If your sample is too concentrated; light scattered by one particle will be scattered by another (this is known as multiple scattering).
- So that it is important to prepare your sample in the range of concentration like given in the table below:

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

Warning! As it was mentioned DLS technique is very sensitive to dirt or dust in the sample and so that it requires a careful sample preparation.
Prepared sample may require standing for a while, this time may be even over 24 hours.
All glass materials and apparatus must be clean and glass materials shouldn't have any scratches.

Not to provide the conditions above will last with poor or wrong results.

Selecting and preparing the measurement cell

This instrument has a range of cells and cuvettes to perform measurements with the Zetasizer system. The choice of cell or cuvette is dependent upon the type of measurement being performed and the sample which will be measured.
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Sample type	Cell used	Conductivity	Voltage applied
Aqueous	Folded capillary cell	< 5mS/cm	150V
		> 5mS/cm	50V
		> 30mS/cm	10V
Aqueous	Universal 'Dip' cell	< 10mS/cm	5V
		> 10mS/cm	3V
Aqueous	High concentration cell	< 5mS/cm	40V
		> 5mS/cm	10V
		> 30mS/cm	2.5V
Non-aqueous	Universal 'Dip' cell	Negligible	40V

Note, generally for water solutions the plastic cells can be used in a case of organic solvents quartz quvette has to be used.
General information (following instrument manual) about Zetasizer Nano softwar

The Malvern Zetasizer Nano software controls the system during measurement and after processes the measurement data to produce size, zeta potential or molecular weight results. Moreover it displays the results and allows reports to be printed.
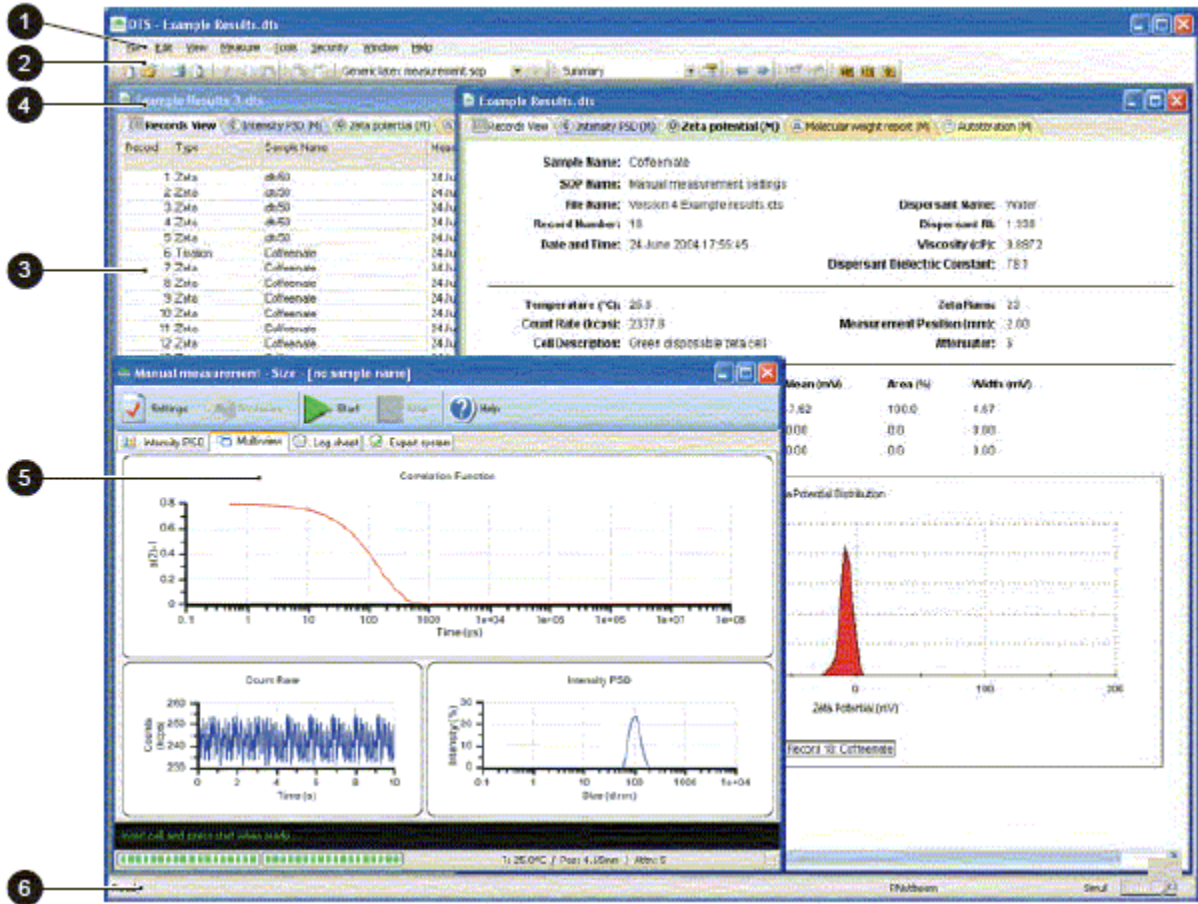A typical screen view is shown in Figure 3.

Figure 3. The Zetasizer Nano software application. The features and their function are described in Chapter 2 User manual.

Before each measurement the settings should be defined and put into an input file containing physicochemical parameters about the sample.

The measurement progress is observed on the screen. The Measurement display (Figure 4) shows results depending on the type of measurement being performed and the view tab selected.
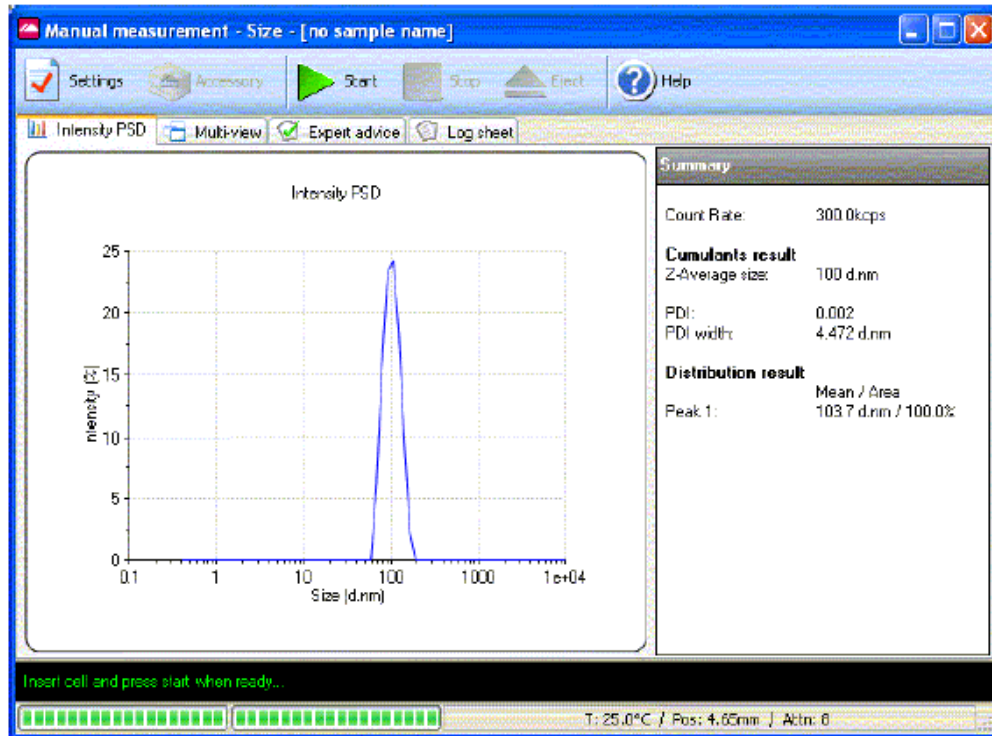
Figure 4. An example of Measurement display.

Zetasizer Nano software involve on change the units used to define the parameter i.e. Temperature has the option to use either °C (Celsius) or K (Kelvin). For all other parameters employed in calculation detected results the units can be selected in the SOP (Standard Operating Procedure) and other windows throughout the software. The parameter unit chosen in SOP windows will be displayed in all results and reports.

Experiment steps
I. Powering up the Zetasizer Nano system

- Close lid and turn on the optical unit.
- Provide electricity to the instrument by plugging it to electricity socket
- Turn on the power from the switch which is on the instrument. At this moment you should hear a "beep" it means instrument's routine has started. Next, you should hear a second "beep" which means instrument's routine has finished. After a while you should hear 2 beeps. This means instrument has reached to default temperature which is 25°C
Warning! After these steps, students must wait around 30 minutes for laser stabilization. This procedure is required to prevent any thermal equilibrium problems which can affect the measurement results.

II. Turn on computer and start the Zetasizer Nano softwear

1. Double click on ![Zetasizer icon] the icon on the computer screen to start the software.

2. Make an SOP measurement.
3. If necessary **Open or create a new measurement file**.
4. Select **Measure-Start SOP** from the Zetasizer software.
5. Select the **SOP** required and select **Open**.
6. Follow any onscreen instructions that are displayed.

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

Next, choose the cell appropriate for the sample and measurement type.
- Fill the cell with the prepared sample.
- Insert the cell into the instrument right place and wait a few minutes for the temperature stabilizing.

7. Turn back to the computer and click **Start.**
The measurement will be made and the results displayed will be shown on the screen and saved to the measurement file that was selected when the SOP was started.

8. Print out results and discuss.

Ad.3. Opening or creating a Measurement File.
Each time a measurement is made, the measurement data will be saved to a measurement file. The measured files can be managed in different ways (details are given in Zetasizer Malvern user manual.

Making a Manual experiment

Making a manual measurement is essentially the same as making an SOP measurement, except that in the measurement options all will be necessary to set them immediately before.
Instead of starting an SOP, is necessary to select **Measure-Manual**. This will open the **Manual measurement** editor allowing any measurement types to be chosen and the settings to be configured.
The windows are virtually identical to those in the SOP editor.
Once all settings have been made, if required, select the **Save as…** icon or **File-Save as…** from the menu to store the settings. Click the **OK** button to close the **Manual measurement** editor and return to the measurement display.

The manual measurement settings can be viewed and subsequently saved
by selecting **Edit-Extract SOP** from each record in the **Records view.**
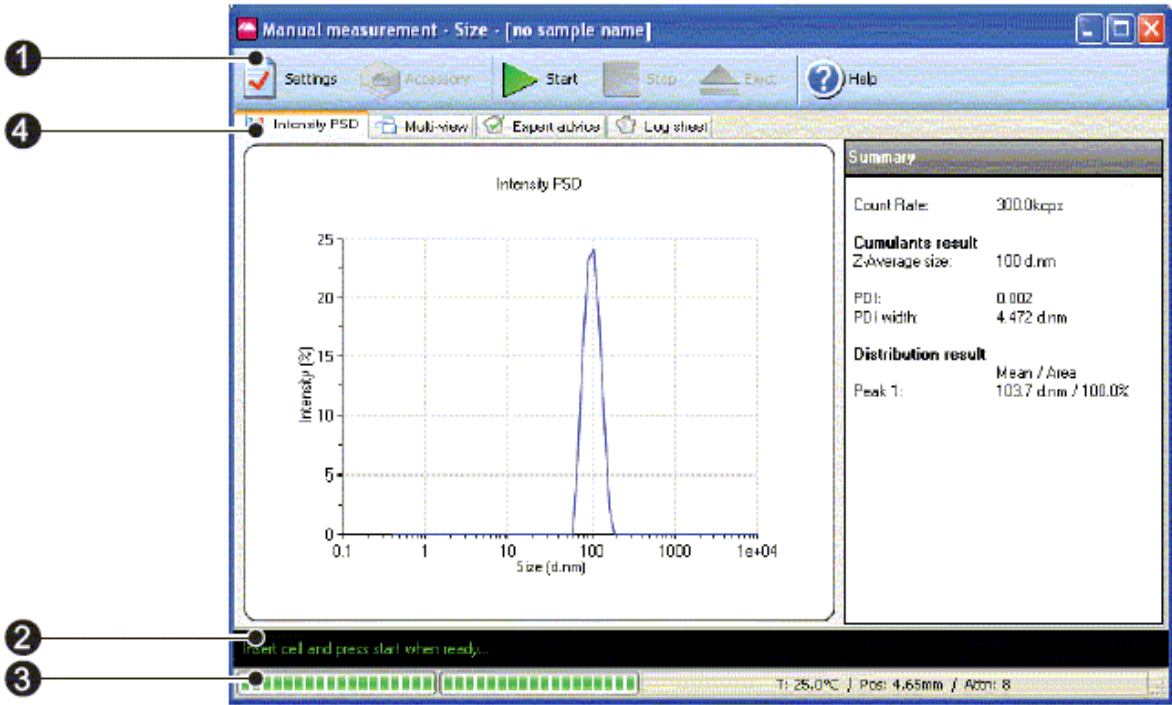In Manual measurement option the Measurement display shape is shown in Figure 5.

Figure 5. The Measurement display in manual measurement option.

In this option of measurement the Measurement display shows the progress of the measurement as in SOP case.
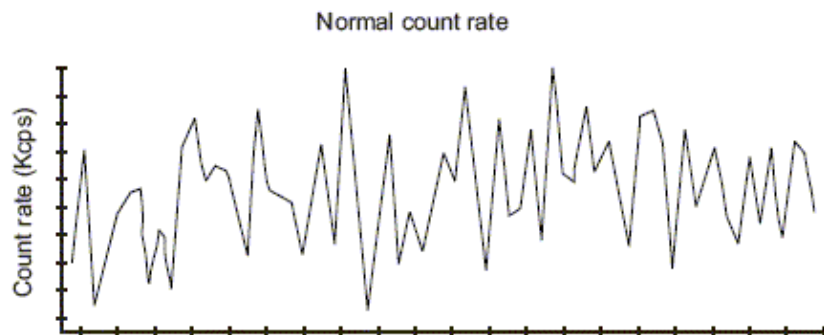
Size measurements

The result table will show:
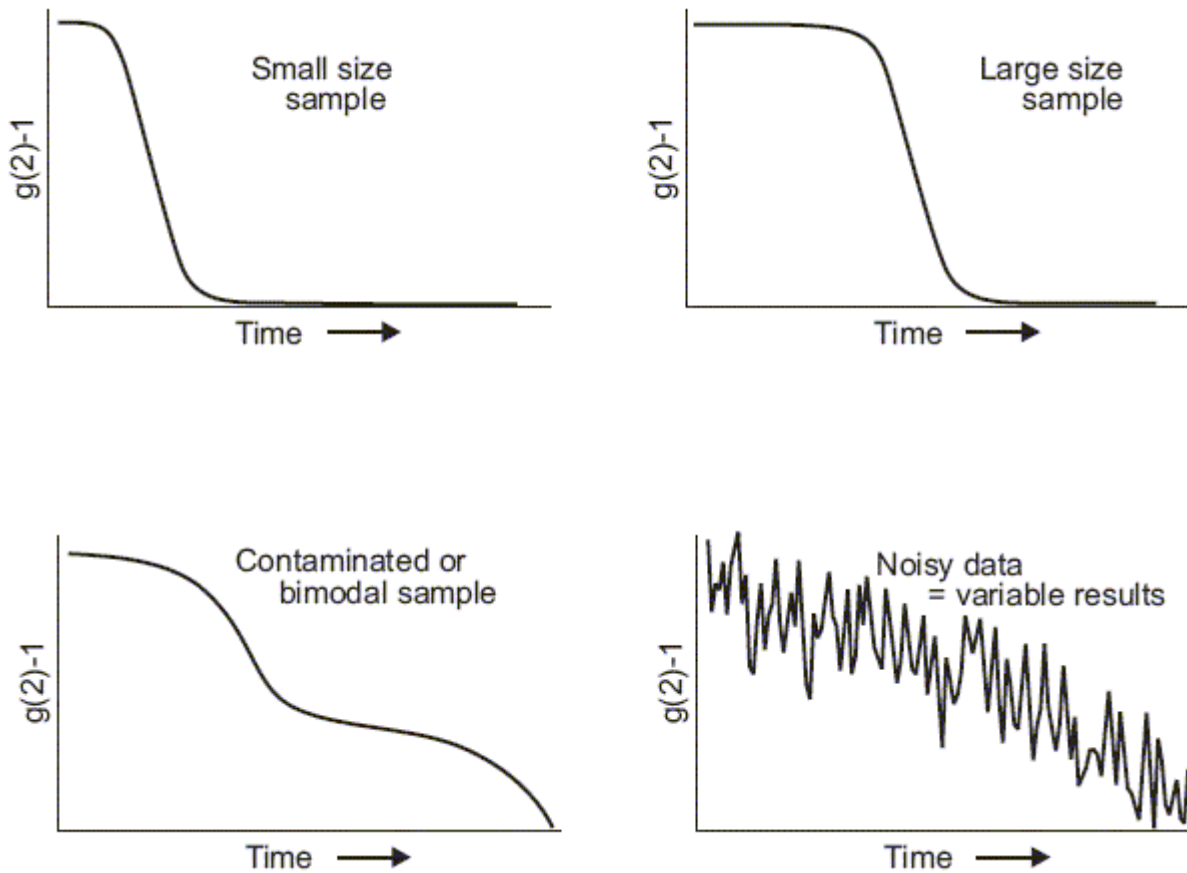Count Rate, Correlation Function, Intensity PSD, Volume PSD, and Number PSD.

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

Normal count rate display.

Correlation function - the correlation function helps the experienced user to interpret any problems with the sample (see figures below)**.**

Intensity PSD - displays the result as an Intensity based particle size distribution. A summary table will displayed alongside the graph.

Volume PSD - displays the result as a Volume based particle size distribution. A summary table will displayed alongside the graph.

Number PSD - displays the result as a Number based particle size distribution. A summary table will displayed alongside the graph.

Size measurement - standard report is Intensity PSD (M) (Particle Size Distribution) is shown in Figure 6.
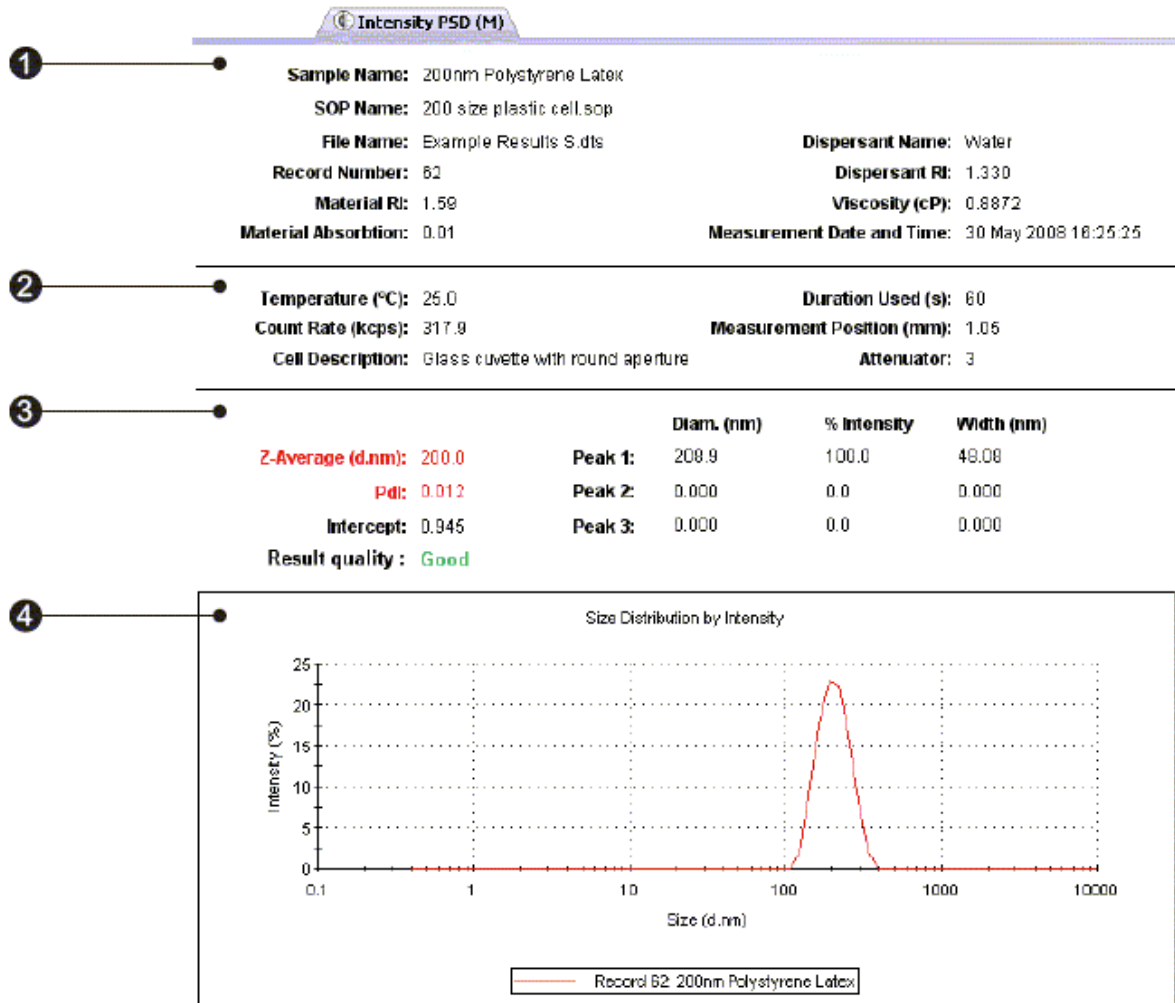
Figure 6. Standard report for size measurement.

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

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

2 - system details. This section gives details on instrument settings for this particular measurement. Specifically, these are: **Temperature - m**easured temperature at the start of the measurement; **Count rate - a**verage count rate for the measurement; **Duration used(s) - d**uration of measurement, used in the analysis of the result; **Cell type - t**his displays the cell type selected.

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

Z-average size (also known as the "cumulants mean"). In **D**ynamic **L**ight **S**cattering (**DLS**) this is the most important and stable number produced by the technique. This is the size to use if a number is required for quality control purposes. It will only be comparable with other techniques if the sample is monomodal (i.e. only one peak), spherical and monodisperse (i.e. no width to the distribution), and the sample is prepared in the correct dispersant. In any other case, the Z-average size can only be used to compare results with

samples measured in the same dispersant, by the same technique.

The cumulants analysis only gives two values, a mean value for the size, and a width parameter known as the **Polydispersity Index** (**Pdi**). It is important to note that this mean size (often given the symbol Z or z-average) is an intensity mean. It is not a mass or number mean because it is calculated from the signal intensity. The cumulants analysis is actually the fit of a polynomial to the log of the g1 correlation function.

$$Ln\ [g1] = a + bt + ct^2 + dt^3 + et^4 + \ ....$$

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 2c/b2 is known as the polydispersity index (PdI). The calculations for these parameters are defined in the ISO standard document 13321:1996 E and 22412.

Intercept - this is the amplitude of the g1 correlation function at time 0. For a good measurement it will be between 0.85 and 0.95. Lower values will be obtained for measurements on weaker scattering sample, or in turbid sample where background scattering is significant. Values >1 will only occur for samples with dust that makes the sample count rate fluctuate wildly.

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

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

4 – graph. The results are also shown in graphical form. The format of the graph can be altered by moving the cursor over the graph and right clicking the mouse. The **Graph control properties** window is then displayed. This window allows the following attributes to be altered: **Display –** The **Display** tab allows a choice of graph type and how it is to be displayed. i.e. Either as a histogram or curve, or particular statistics shown. The **Options** tab allows the graph key position to be chosen. A graph tips option can also be set, this allows the setting of pop up tips (flags showing data points on the graph) on the report itself. **Axis settings –** allows both the X and Y axis settings to be defined. Whether **logarithmic** or

linear axis are required and the axis scales - user defined or auto-scaling. **Graticule**, or grid lines can also be shown on the graphs. **Font – a**llows the font style to be altered. The setting will apply to all annotations used
on the graph. **Upper/Lower limits – f**urther tabs allow the setting of **Upper** and **Lower** warning or action **limits** so the value shown can be checked to be within desirable limits.

The graph below (Figure 7) has been configured using the window to display a size distribution by Intensity graph as a histogram, with logarithmic x-axis and linear y axis settings. Graticule, or grid, lines have been included and upper action and warning limits have been set.
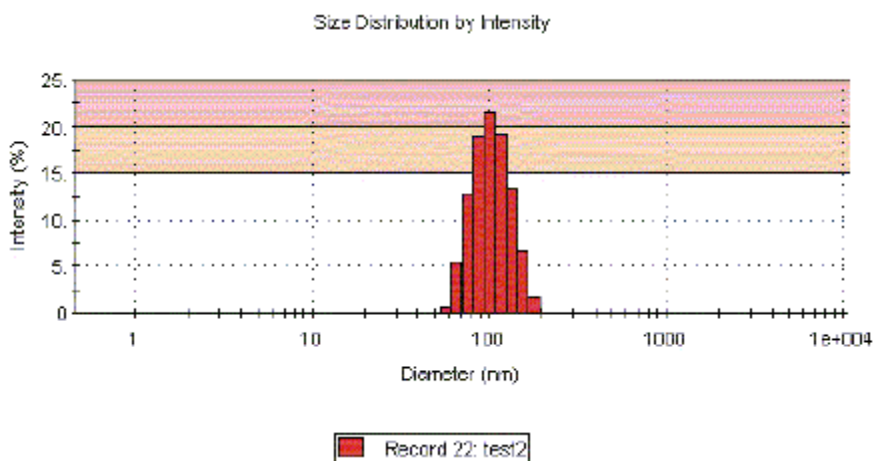

Figure 7. Example of histogram.

Other Yetasiyer Nano Malvern views available for size measurements include:
Intensity Statistics (M)
Volume PSD (M)
Volume Statistics (M)
Number PSD (M)
Number Statistics (M)
Size diagnostics report (M)
This report shows six graphs: The size distribution by intensity and volume, size and cumulants residuals by time, and the data and cumulants fit. These graphs are helpful for more experienced users to determine the quality of their measurement data.
Size quality report (M). This is a summary report detailing whether the result meets the Malvern Instruments quality criteria for a size measurement. The report indicates **Result meets quality criteria**, or **Result does not meet quality criteria** in which case a list of parameters that do not meet the criteria, and probable reasons will be displayed. Some of these parameters will only be displayed if the research software has been used for the measurement. Details are given in Yetasiyer Nano user manual.

Collected data should be present in the following table:

Solution	Concentration [mol/L]	Hydrodynamic radius [nm]	Diffusion coefficient	File name

Measurement setting parameters:
Sample 1:……….
………..