RESEARCH INSTRUMENTATION STANDARD OPERATING PROCEDURE FOR THE MALVERN ZETASIZER PRO BLUE DYNAMIC LIGHT SCATTERING INSTRUMENT (OPDLS-1) AT NUCS FULMER

REVISION 0.0, 8/3/2022

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OPERATING PROCEDURE OPDLS-1: OPERATION OF THE MALVERN ZETASIZER PRO DYNAMIC LIGHT SCATTERING INSTRUMENT

1 Background

Dynamic light scattering (DLS) is a non-invasive scientific technique that can be used to determine the size distribution profile of small particles in suspension or polymers in solution. DLS can also be used to probe the behavior of complex fluids such as concentrated polymer solutions. Stability studies can be done conveniently using DLS. Periodical DLS measurements of a sample can show whether the particles aggregate over time by seeing whether the hydrodynamic radius of the particle increases. If particles aggregate, there will be a larger population of particles with a larger radius. In some DLS machines, stability depending on temperature can be analyzed by controlling the temperature in situ. DLS is used to characterize size of various particles including proteins, polymers, micelles, vesicles, carbohydrates, nanoparticles, biological cells, and gels. If the system is not disperse in size, the mean effective diameter of the particles can be determined. This measurement depends on the size of the particle core, the size of surface structures, particle concentration, and the type of ions in the medium.

To determine the size of particles, a monochromatic light source, usually a laser, is shot through a polarizer and into a sample. The scattered light then goes through a second polarizer where it is collected by a photomultiplier and the resulting image is projected onto a screen. All of the molecules in the solution are being hit with the light and all of the molecules diffract the light in all directions. The diffracted light from all of the molecules either interfere constructively (light regions) or destructively (dark regions). This process is repeated at short time intervals and the resulting set of patterns are analyzed over time. Since DLS essentially measures fluctuations in scattered light intensity due to diffusing particles, the diffusion coefficient of the particles can be determined. DLS software of commercial instruments typically displays the particle population at different diameters. If the system is monodisperse, there should only be one population, whereas a polydisperse system would show multiple particle populations.

It is important to note that the size determined by dynamic light scattering is the size of a sphere that moves in the same manner as the scatterer. So, for example, if the scatterer is a random coil polymer, the determined size is not the same as the radius of gyration determined by static light scattering. It is also useful to point out that the obtained size will include any other molecules or solvent molecules that move with the particle. So, for example, colloidal gold with a layer of surfactant will appear larger by dynamic light scattering (which includes the surfactant layer) than by transmission electron microscopy (which does not "see" the layer due to poor contrast).

The Malvern Zetasizer Pro Blue is also capable of measuring zeta potentials of colloids. Zeta potential is a scientific term for electrokinetic potential in colloidal dispersions. The usual units are volts (V) or, more commonly, millivolts (mV). Zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. The zeta potential is widely used for quantification of the magnitude of the charge. The zeta potential is an important and readily measurable indicator of the stability of colloidal dispersions. The magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly

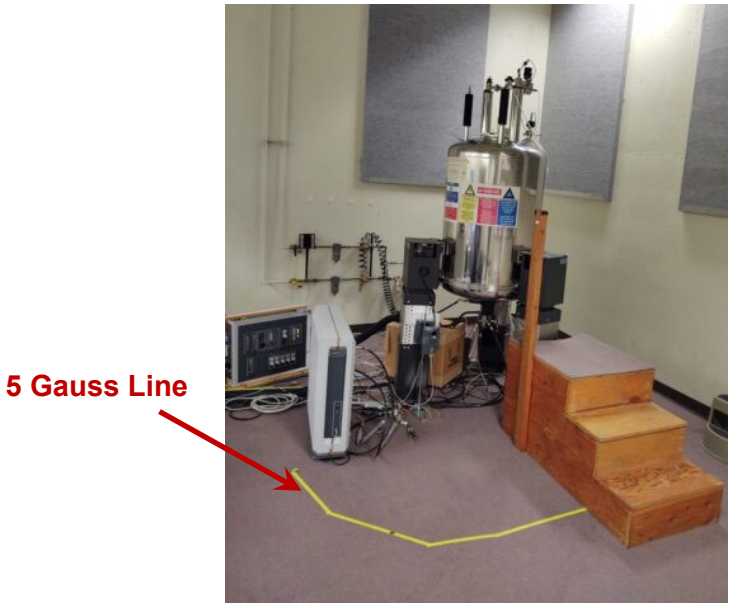
charged particles in a dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability, i.e., the solution or dispersion will resist aggregation. When the potential is small, attractive forces may exceed this repulsion and the dispersion may break and flocculate. So, colloids with high zeta potential (negative or positive) are electrically stabilized while colloids with low zeta potentials tend to coagulate or flocculate.

1.1 Monthly Calibrations/QA/QC Checks

Monthly calibrations are undertaken to verify the functionality of the instrument and to head off any problems or safety concerns before they become larger problems that could result in instrument failure. A commercial powdered milk sample is used in the monthly calibration (see Section 3).

2 Safety Requirements

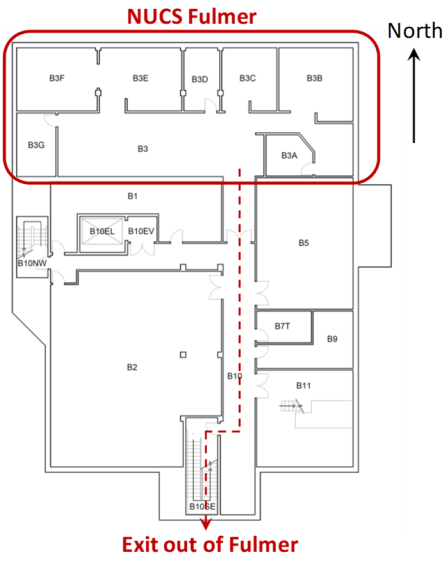
The Malvern Zetasizer Pro DLS is located in the Nuclear and Chemical Science (NUCS) Core Facility, which contains strong magnets for nuclear magnetic resonance (NMR) spectroscopy. Electronic, electrical, or mechanical medical implants may be affected or even stopped in the presence of a static or changing magnetic field. For your own safety, if you have a pacemaker or other medical implant that could be adversely affected by strong magnetic fields, do NOT enter the NMR labs. Magnets can exert large attractive forces on equipment or other ferromagnetic objects when brought. Please do not approach the magnets beyond the 5-gauss line (outlined with yellow tape) or within 10 feet of the magnets.

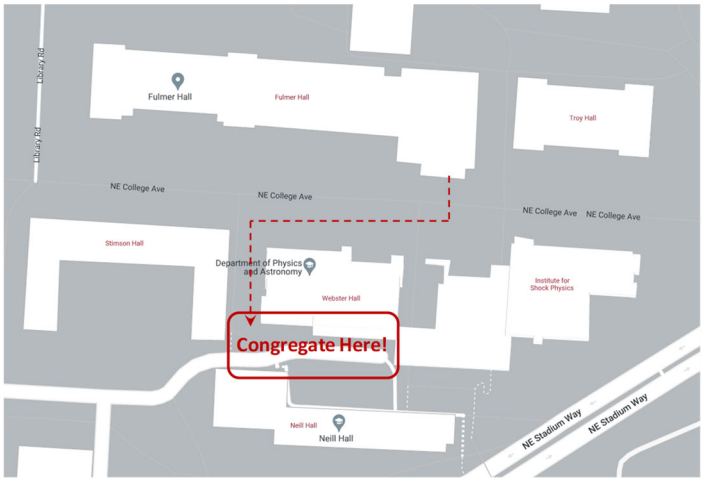


All of the NMR spectrometer magnets are superconducting, which means they are kept in a cryostat filled with liquid helium. A concentric dewar of liquid nitrogen is placed around the helium cryostat in order to keep the helium boil-off rate low. Additionally, liquid nitrogen may be kept in portable dewars around the facility. Cryogens can pose several risks including: asphyxiation, frostbite, and chemical explosions. In the event of a magnet quench (see picture below), the superconducting wire inside the instrument transitions to a normal conducting state. This would

boil off all of the liquid helium very quickly. The rapid expansion of helium as it vaporizes can displace the oxygen in the NMR lab and cause asphyxiation. The facility contains an oxygen meter, which causes an audible alarm if the oxygen concentration drops below 20%. If you observe a sudden exhaust of gas from a magnet (and NMR staff are not performing a cryogen fill) or hear an audible alarm due to low oxygen levels, exit the NMR lab immediately following the same procedure as the occurrence of a fire alarm.

In the event of an audible alarm, due to a fire alarm or an air alarm from low oxygen levels, or an instrument quench, please exit the facility through the main door to the NUCS Fulmer Core Facility and go up the stairs at south end of the building (see left picture below). The building exit at the south stairs will lead to College Avenue. Please congregate behind Webster until the fire alarm has ended (see right picture below).





The same principles of research safety apply in instrumentation laboratories when you are handling samples. Research samples, glassware, chemical storage, spills, and waste disposal must be properly handled. You must wear long pants (or equivalent) and closed-toed shoes. No food or beverages are allowed in the NMR lab. The NMR lab is not a wet lab. All sample prep should be done in your lab. Do not prep samples at spectrometers. Do not bring your lab coat or gloves into the NMR lab. Keeping a shared lab clean requires the cooperation of everyone. Please do not leave KimWipes, paper towels, etc. laying around. If you believe any sample may have spilled into or onto one of the instruments, please notify the NMR facility staff immediately. Place a written note on the keyboard to inform the next user.

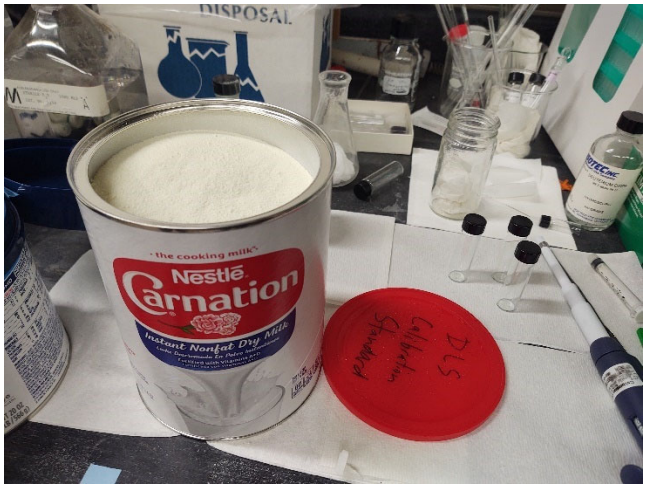
Some users are approved to analyze radioactive samples in the NUCS Fulmer Core Facility. If an instrument is blocked off with a barrier (see below picture) for the analysis of a radioactive sample, do not cross this barrier and enter the instrument bay until the barrier has been removed (indicating that the instrument bay is free of radioactive material).

3 Monthly Calibration Procedure

Materials: Carnation Instant Nonfat Dry Milk, Malvern Zetasizer pro Blue, Disposable Polystyrene Cuvette

3.1 Turn on the instrument as indicated in Step 6.3. While the instrument is warming up, prepare the calibration standard.

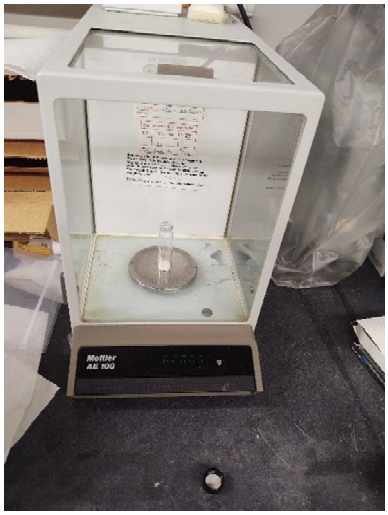
3.2 To prepare the calibration standard, find the Carnation Instant Nonfat Dry Milk, which can be found in Fulmer 40A.



3.3 Find two 2 dram vials.

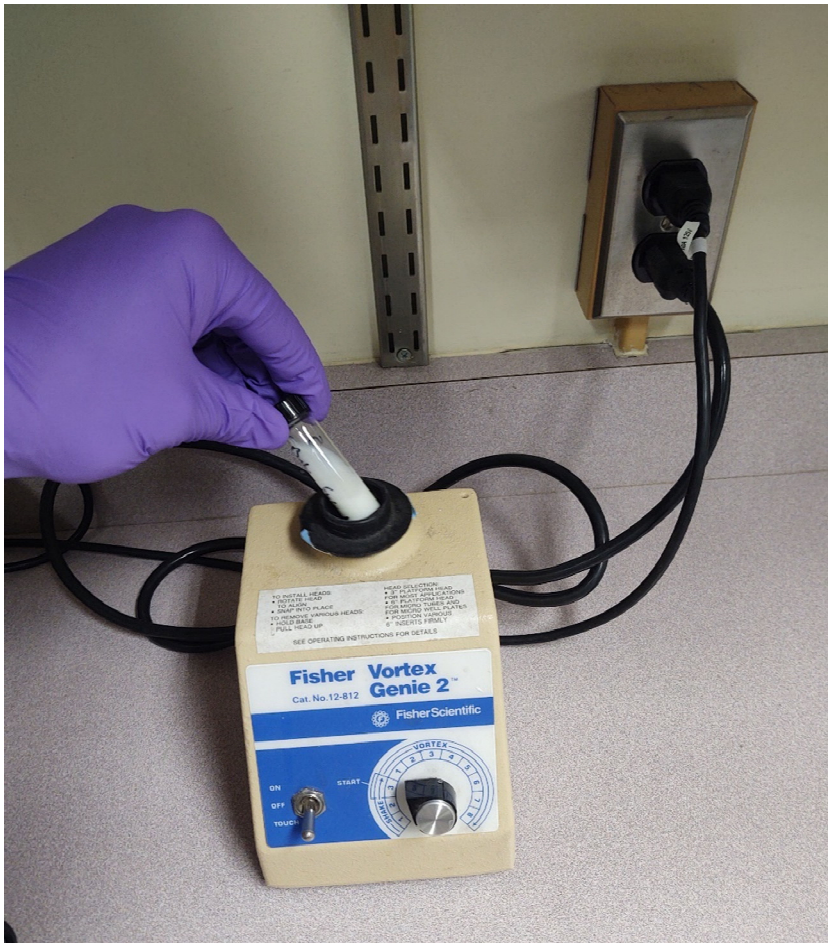
3.4 Fill one of the 2 dram vials with deionized water.

3.5 Weigh out 200 mg (± 20 mg) of Carnation Instant Nonfat Dry Milk in a 2 dram vial.



3.6 Add 2.0 mL of deionized water to the 2 dram vial containing the powdered milk, and homogenize the slurry by shaking for 30 seconds. It should look similar to the picture seen below.

3.7 Bring the vial to NUCS Fulmer and vortex the mixture for 30 seconds. The vortex mixer can be found on the bench in the Varian 500 instrument bay.



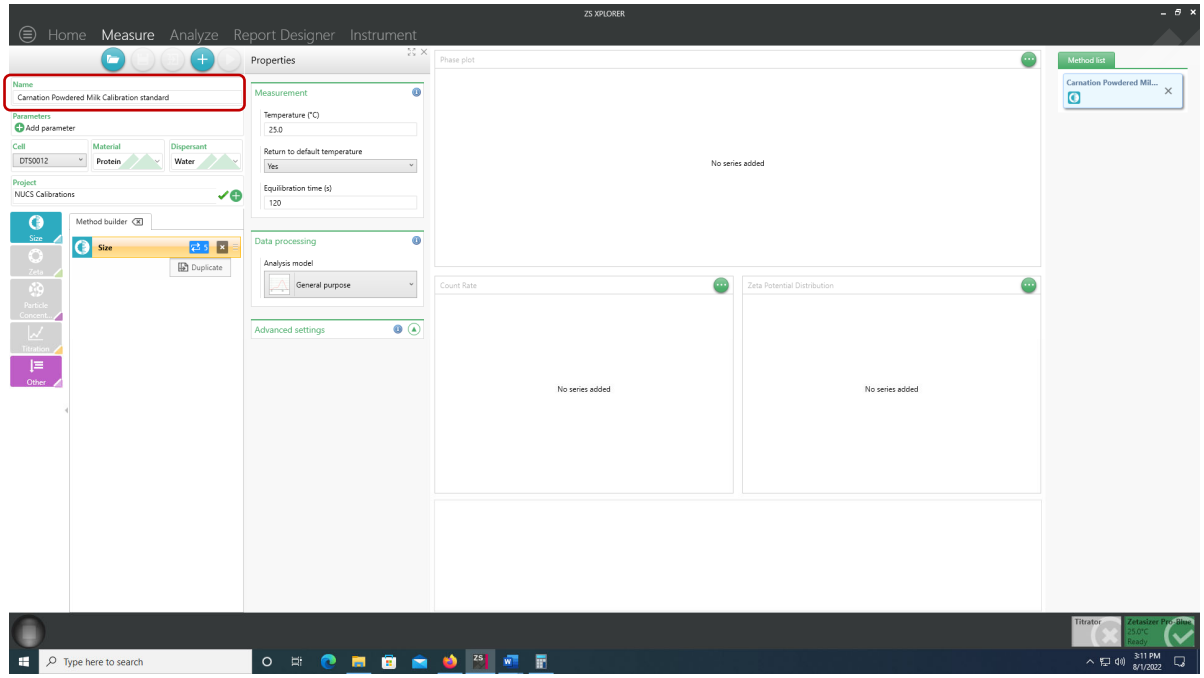
3.8 Transfer 1 mL of the slurry into the disposable cuvette. The slurry level should be in between the min and max level of the sample cell (shown on right).

3.9 Double check the liquid level prior, making sure it is between the minimum and maximum levels before placing the sample into the sample holder of the DLS.

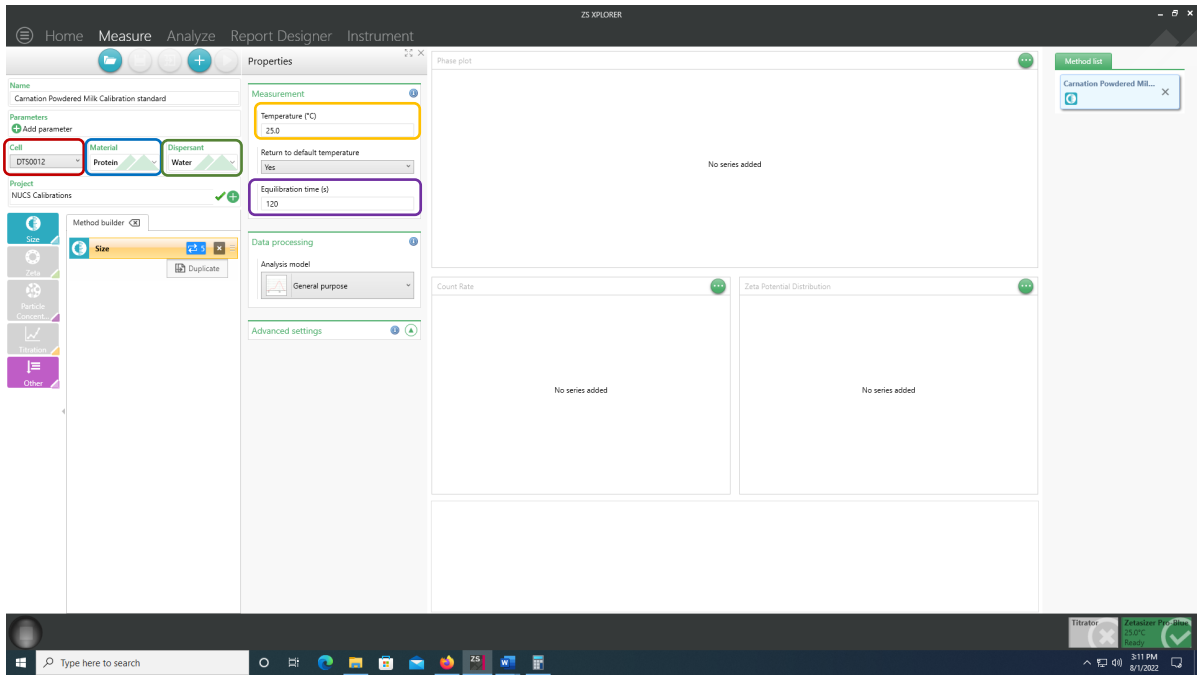
3.10 Place the sample in the sample holder and place the thermostat cover (highlighted in red) on top of the sample before closing the instrument cover. A green light (highlighted in blue) indicates the instrument is ready for data collection.

3.11 Open the ZS XPLORER software as described in Steps 6.6 – 6.8 if the software is not already open.

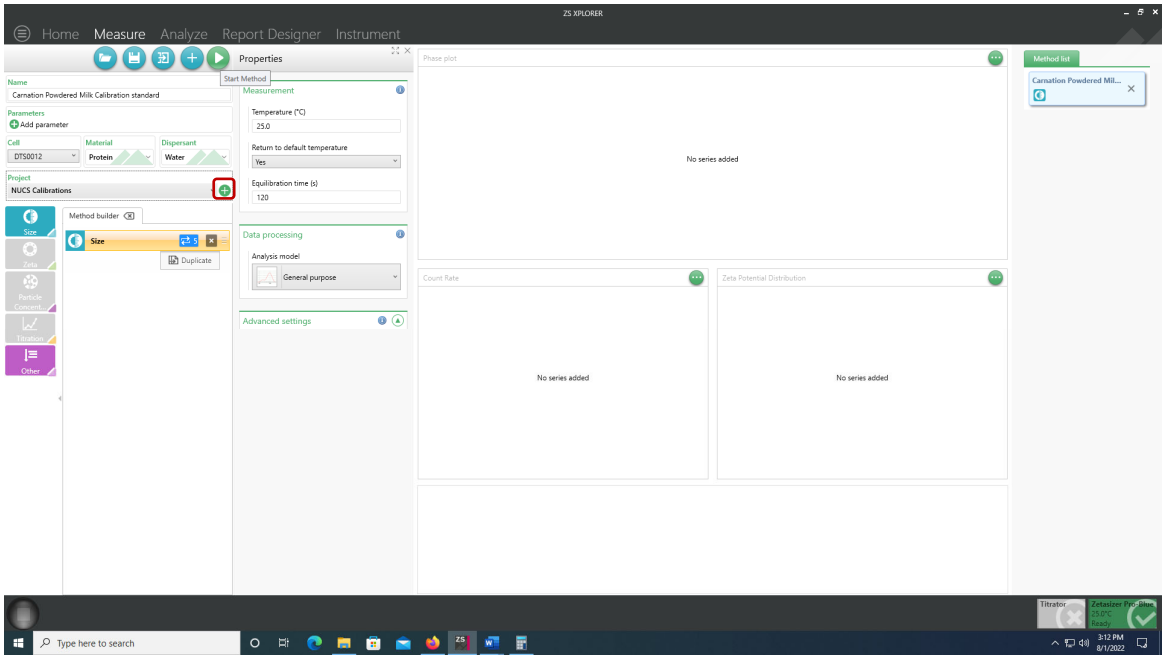
3.12 Title the calibration sample: Carnation Powdered Milk Calibration Standard (highlighted in red).



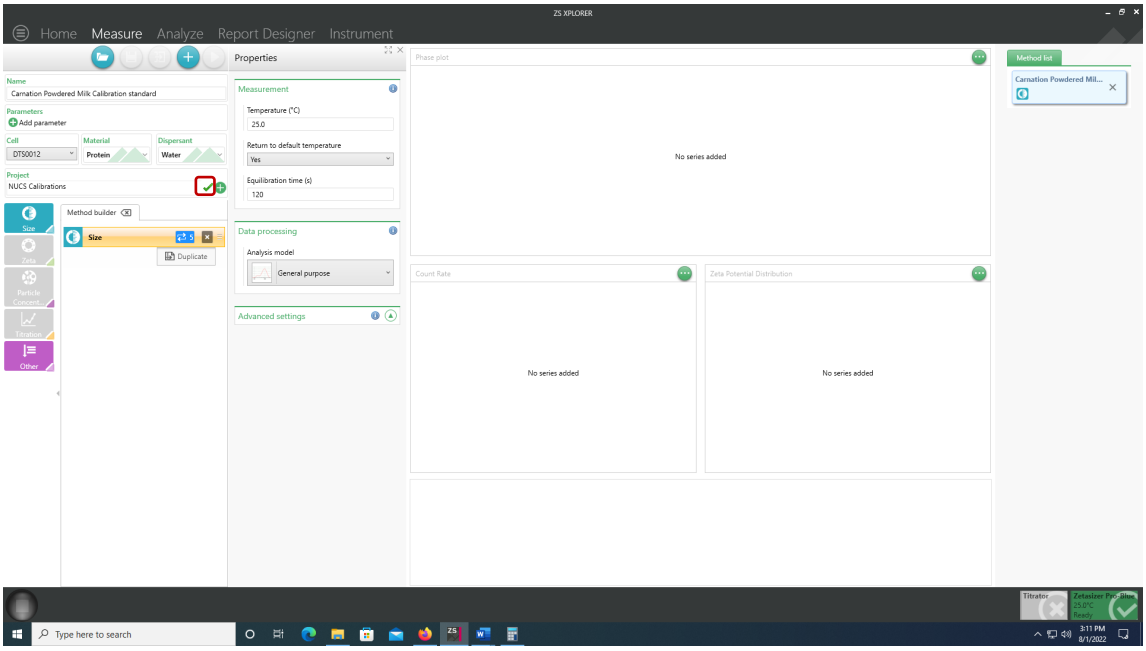
3.13 Set the Cell to DTS00012 (highlighted in red), the Material to Protein (highlighted in blue), and the Dispersant to Water (highlighted in green), the Temperature to 25.0 °C (highlighted in orange) and the equilibration time to 120 seconds (highlighted in purple).



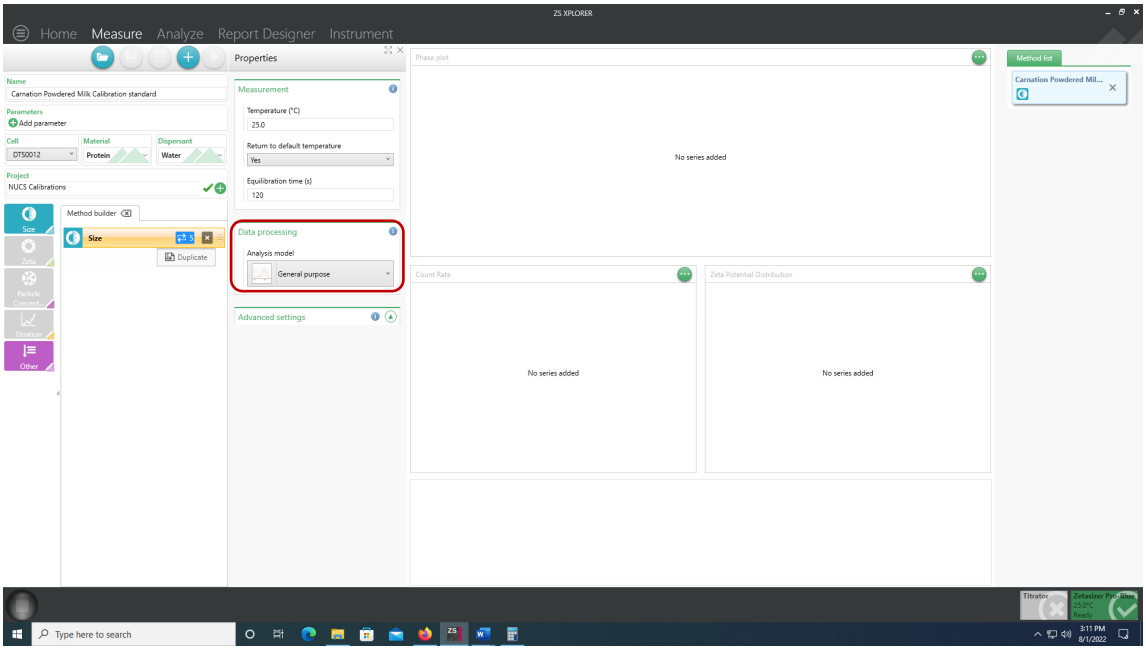
3.14 To create the Project name, click on the plus sign in a green circle (highlighted in red).



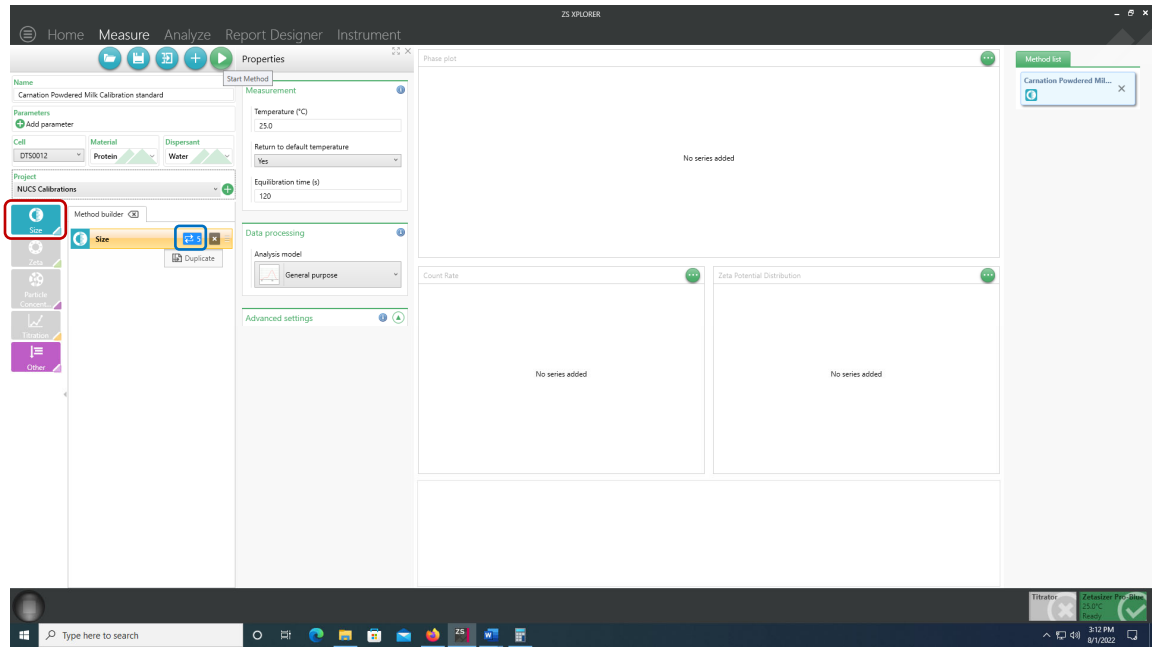
3.15 Name the Project NUCS Calibrations Month Year (e.g. NUCS Calibrations August 2022), and click on the checkmark (highlighted in red) to accept the project name.



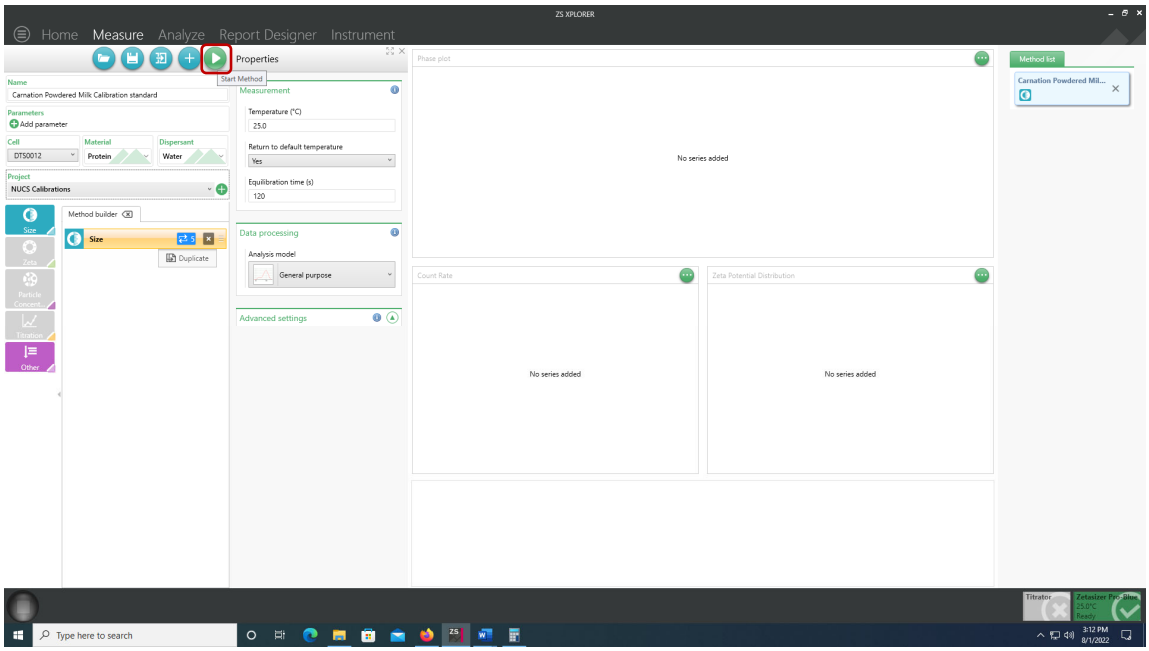
3.16 Check that the Data Processing Analysis model is General purpose (highlighted in red). If it is not, select General purpose from the dropdown menu.



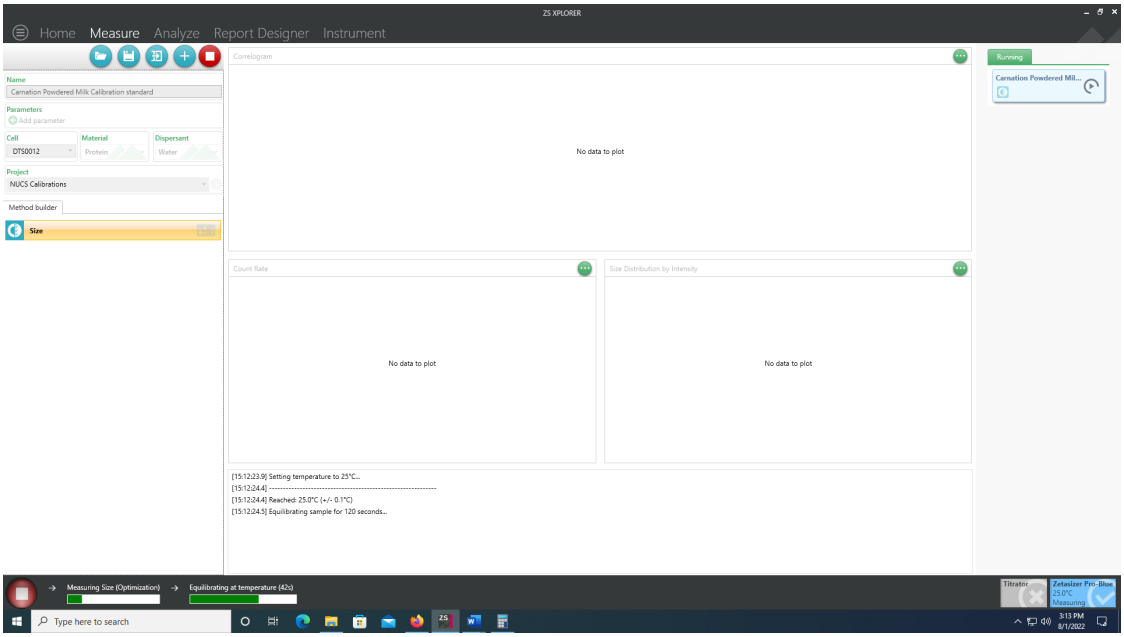
3.17 To add the size measurement experiment, click on the blue Size icon (highlighted in red) and then select a reproducibility of 5 by clicking on the icon highlighted in blue and selecting 5.



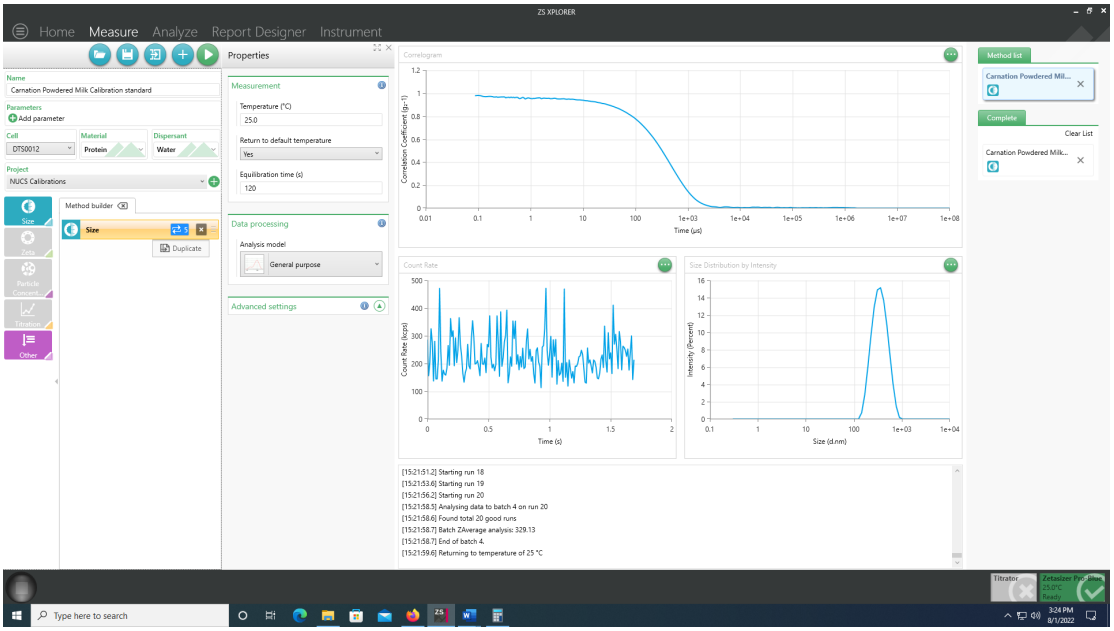
3.18 Click on the icon of green circle with a triangle in it (highlighted in red) to start the data collection.



3.19 When the instrument is collecting data, the software will look like the following picture. The data collection should take about ten minutes.



3.20 When the data collection has completed, the software will look similar to the picture below.



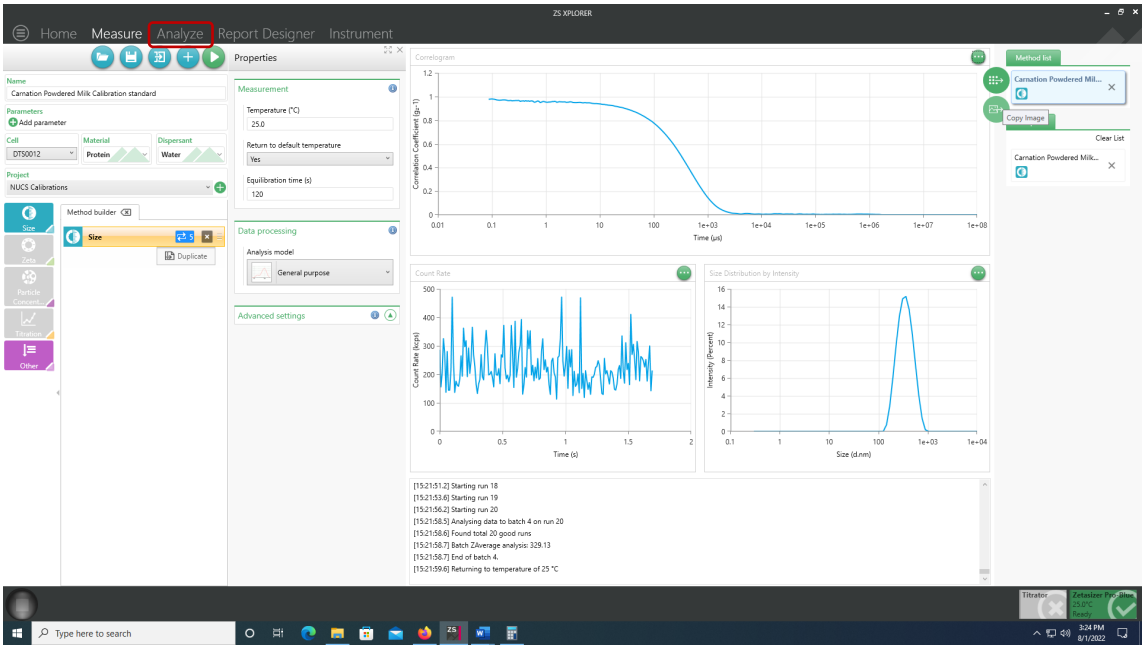
3.21 After the data collection has completed, the sample, and any remaining material, can be disposed of in the trash or glass waste and the instrument can be turn off, as indicated in Section 8.

3.22 The collected data is analyzed and a calibration report is generated as indicated in Section 4.

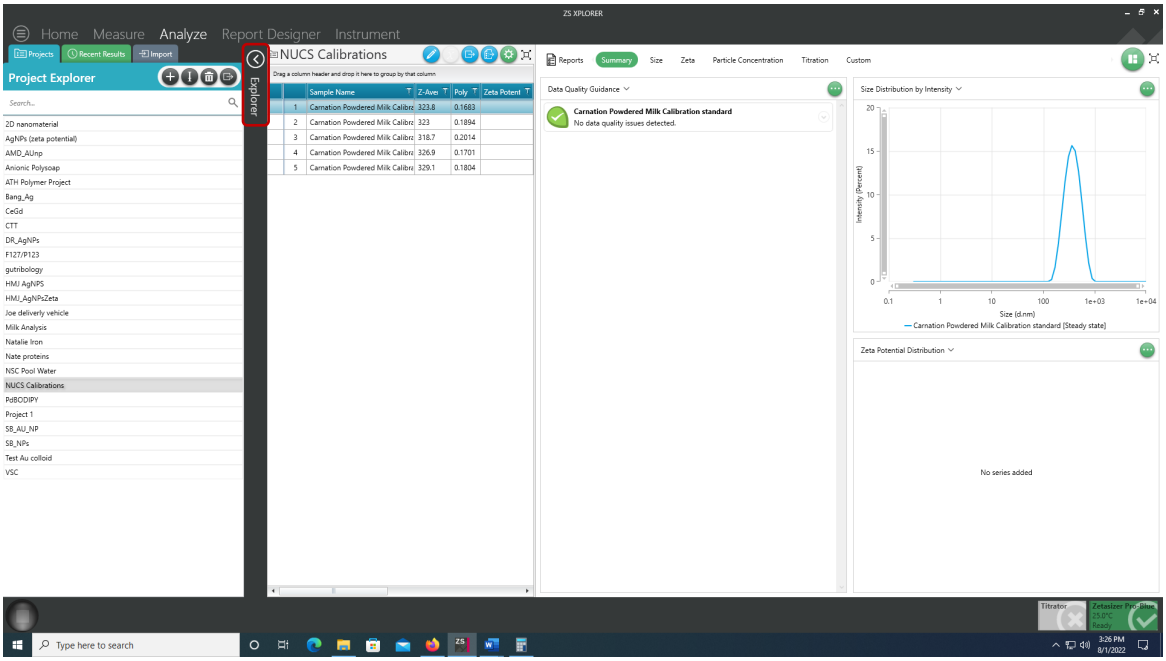
4 Reporting Monthly Calibration

4.1 Collect size determination data on the Carnation Instant Nonfat Dry Milk sample as indicated in Section 3.

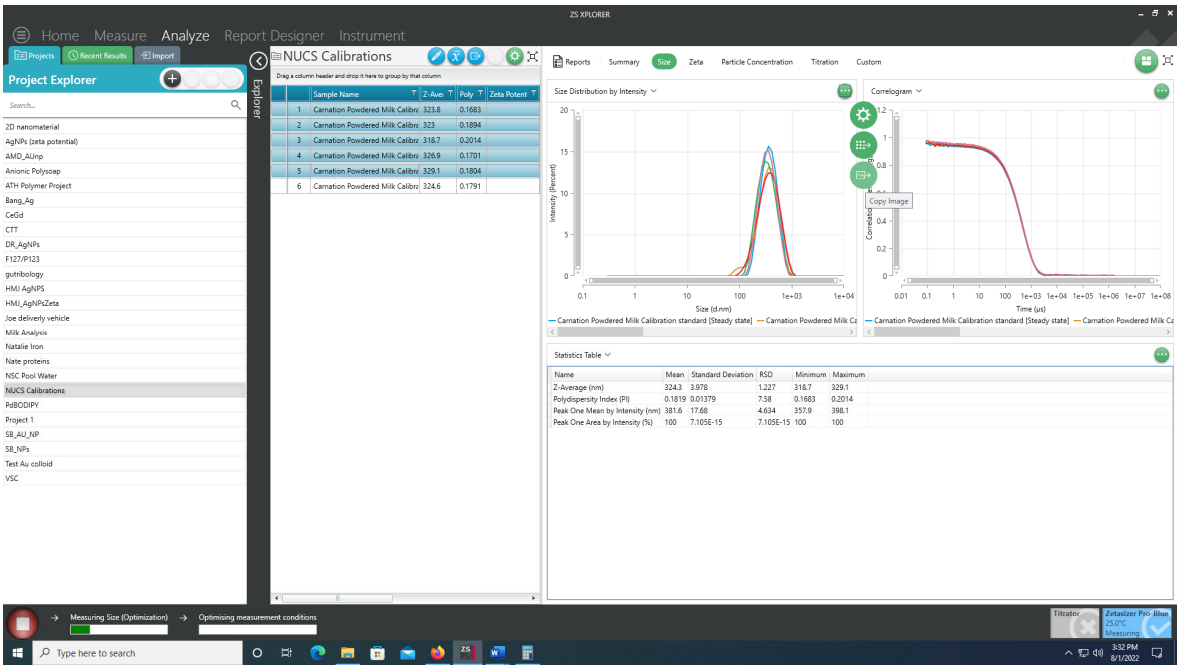
4.2 Before filling out a report on the monthly calibration, the size data needs to be analyzed. Click on the Analyze Tab on the top of the screen (highlighted in red).



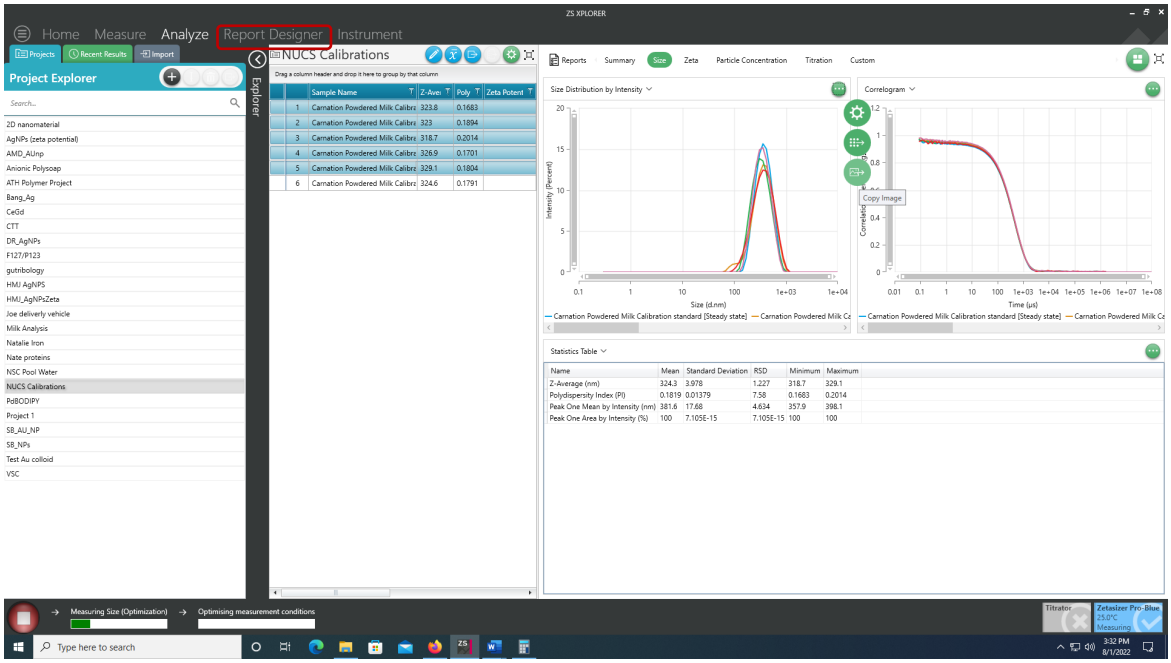
4.3 Click on the Explorer icon (highlighted in red) and select the Project created in Section 3. The project should be titled NUCS Calibrations Month Year.



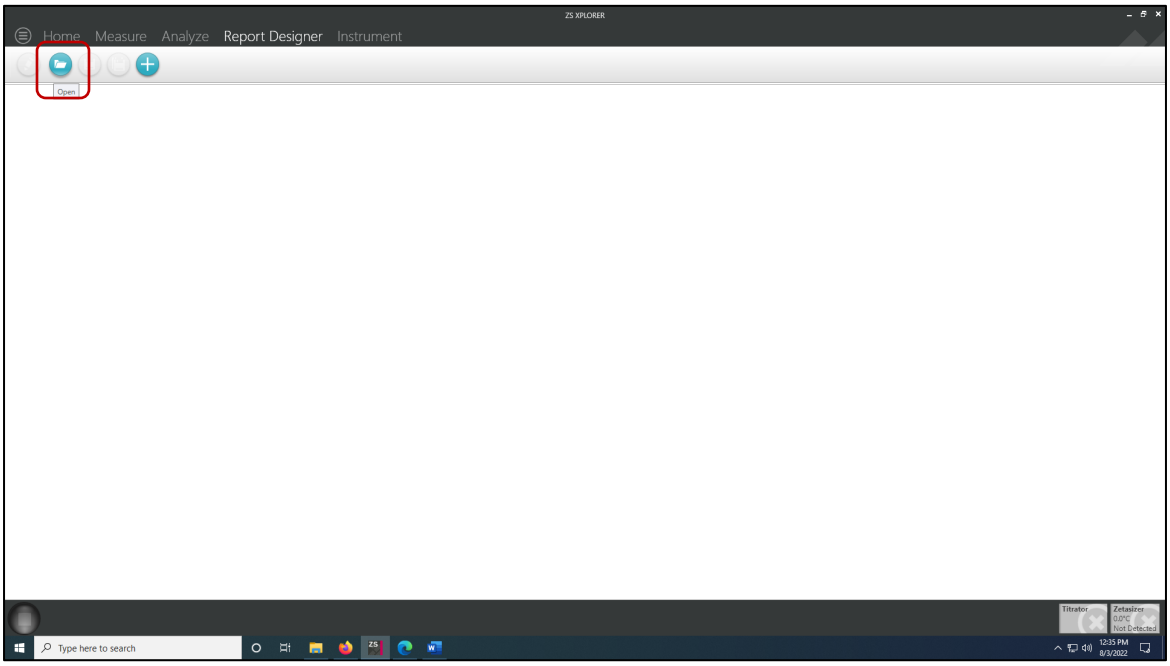
4.4 Highlight all the five data collections, by clicking and dragging so that all of them are highlighted or clicking on the first data set and holding the Shift key and clicking on the fifth data set. The screen should look similar to the picture below.



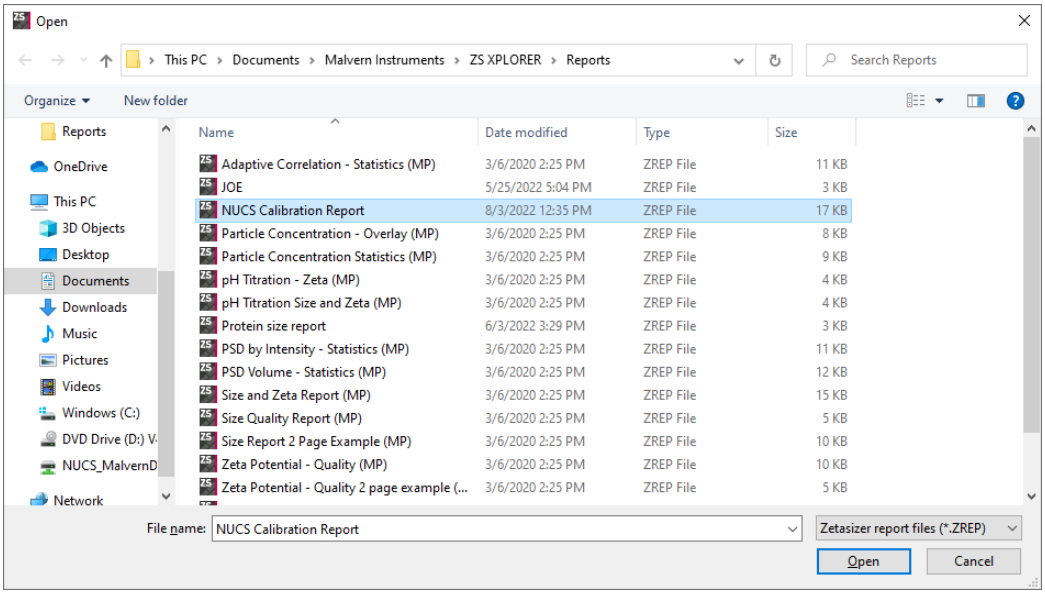
4.5 Then click on the Report Designer Menu at the top of the screen (highlighted in red).



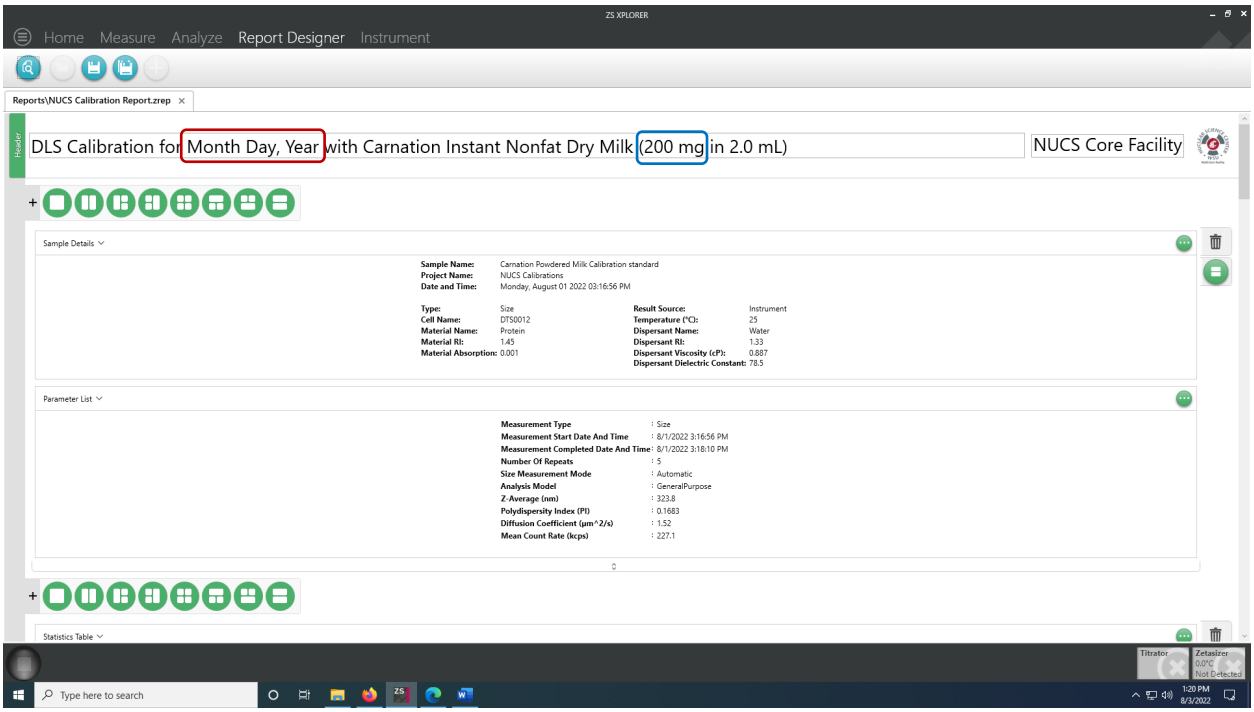
4.6 Click on the teal folder icon (highlighted in red) to select a template for the software generated report.



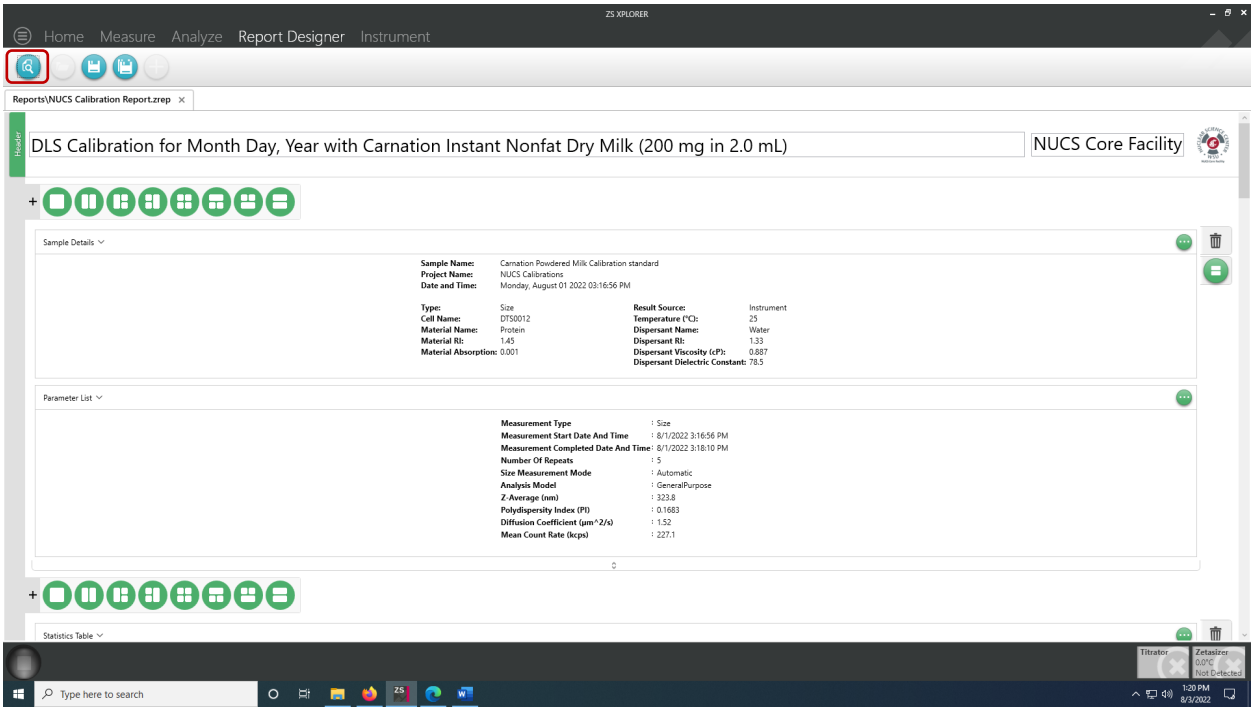
4.7 A window will open, select the NUCS Calibration Report and click Open.



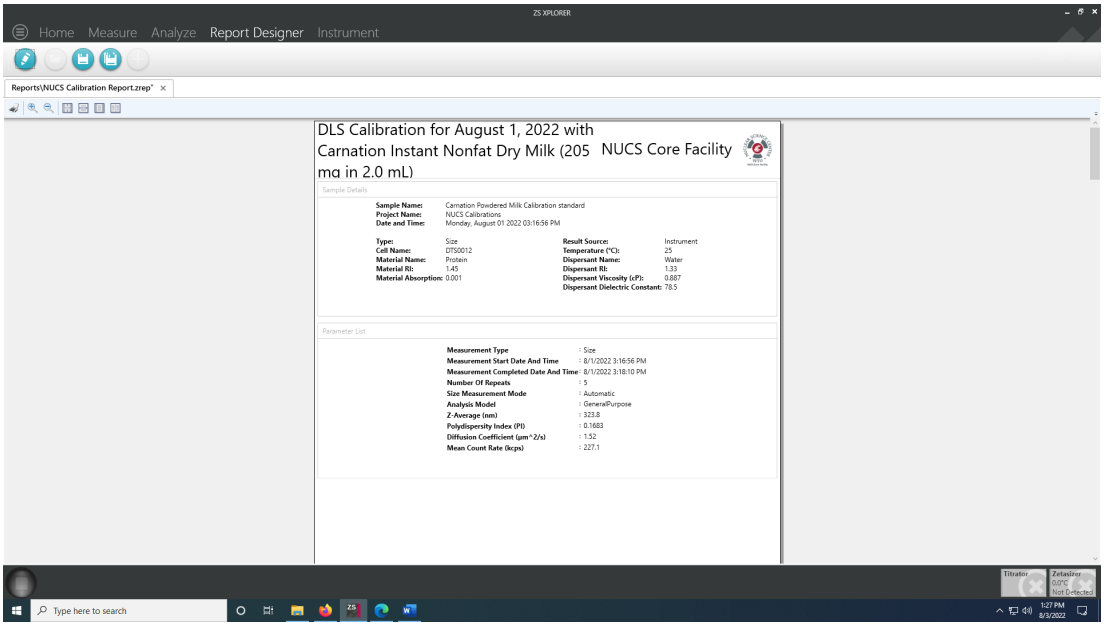
4.8 A report will be generated, as seen in the picture below. Change the Month Day and Year (highlighted in red) to the date of the calibration, and change 200 mg (highlighted in blue) to the mass of the calibration weighed out.



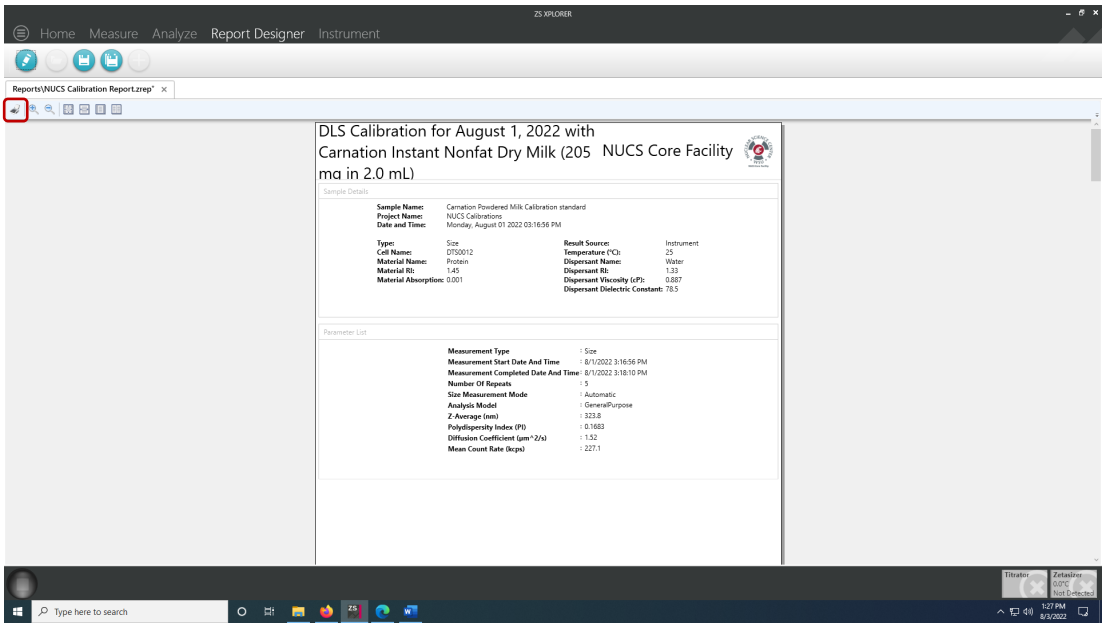
4.9 Once the title has been saved, to create the pdf of the report, click on the magnifying glass icon (highlighted in red).



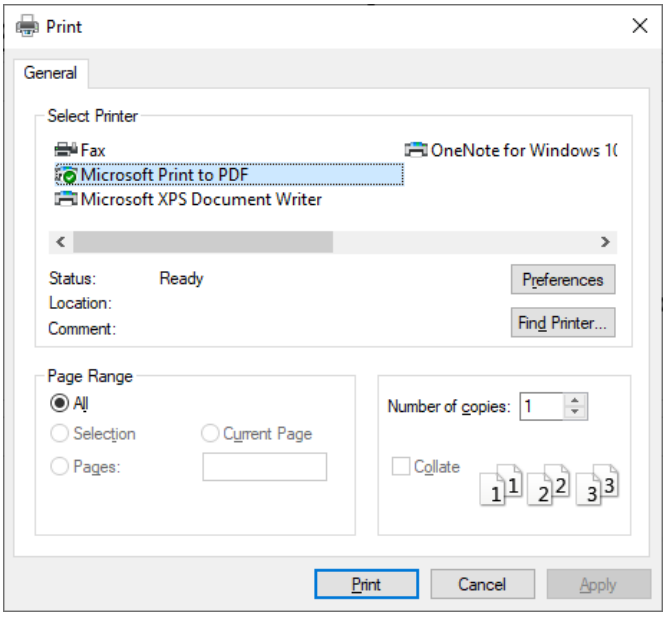
4.10 A preview of the pdf report will result. An example of the report generated in August 2022 is shown below



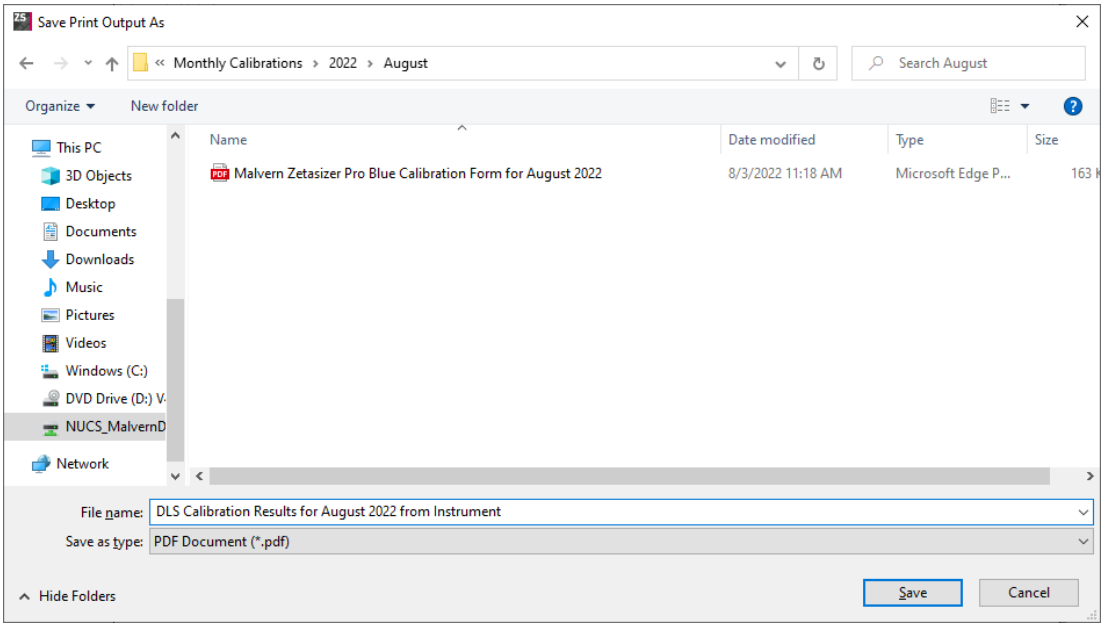
4.11 To save the pdf, click on the printer icon (highlighted in red).



4.12 A window will appear, select Microsoft Print to PDF, followed by clicking the Print button.



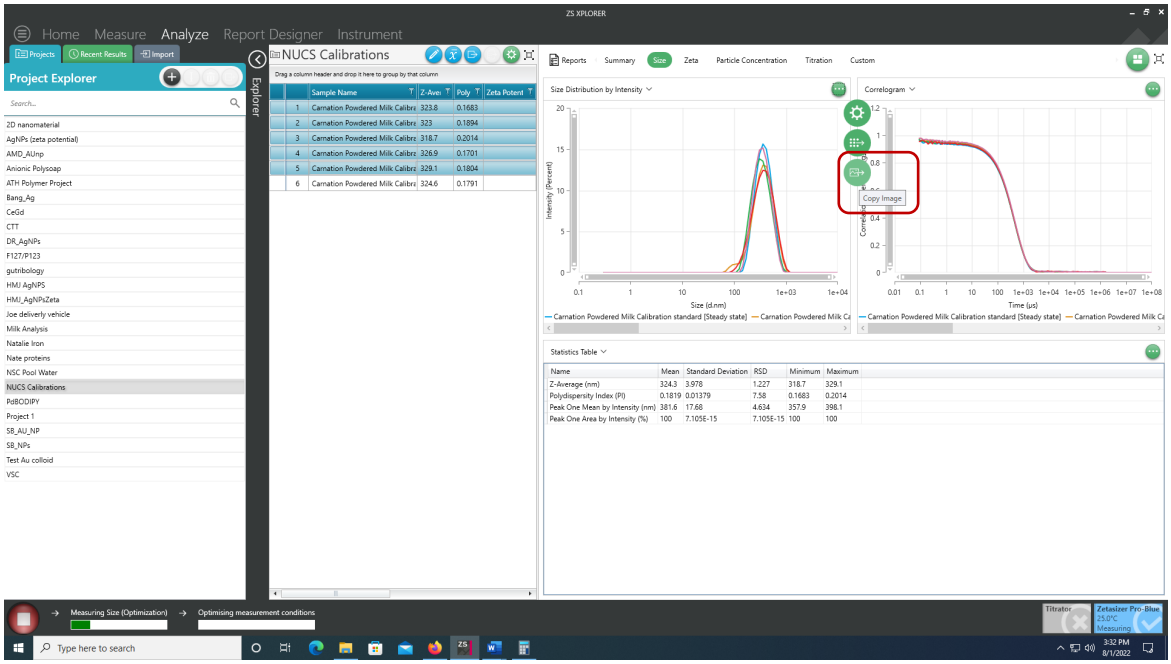
4.13 Save the pdf file in the Z:\Malvern DLS\Monthly Calibrations\Year\Month directory (e.g. Z:\Malvern DLS\Monthly Calibrations\2022\August) as DLS Calibration Results for August 2022 from instrument.pdf.



4.14 The NUCS Calibration Report for monthly calibrations can be found on the NUCS Instruments drive at the following file location: Z:\Malvern DLS\Monthly Calibrations\

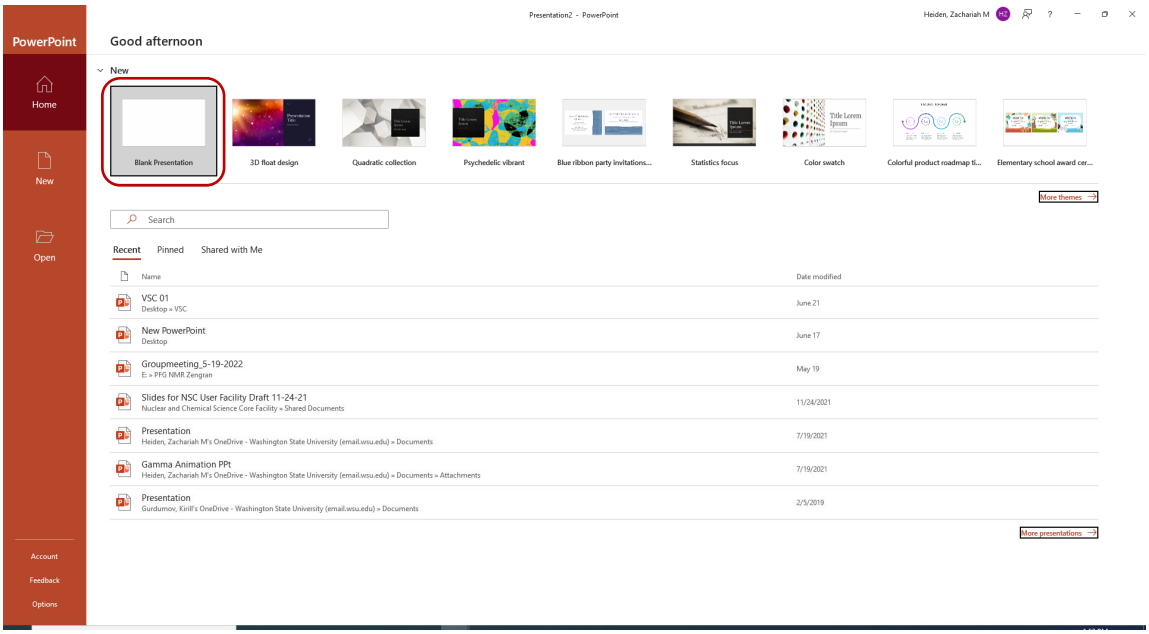
4.15 Enter the name of the NUCS Core Facility staff that collected the data and date of the data collection. Also, include the instrument parameters on the Monthly Calibration Form, which can be found on the instrument generated report.

4.16 To add the pictures of the Size Distribution to the report, click on the Analyze menu option, followed by the green circle with three dots, then the bottom circle to copy the image (highlighted in red).



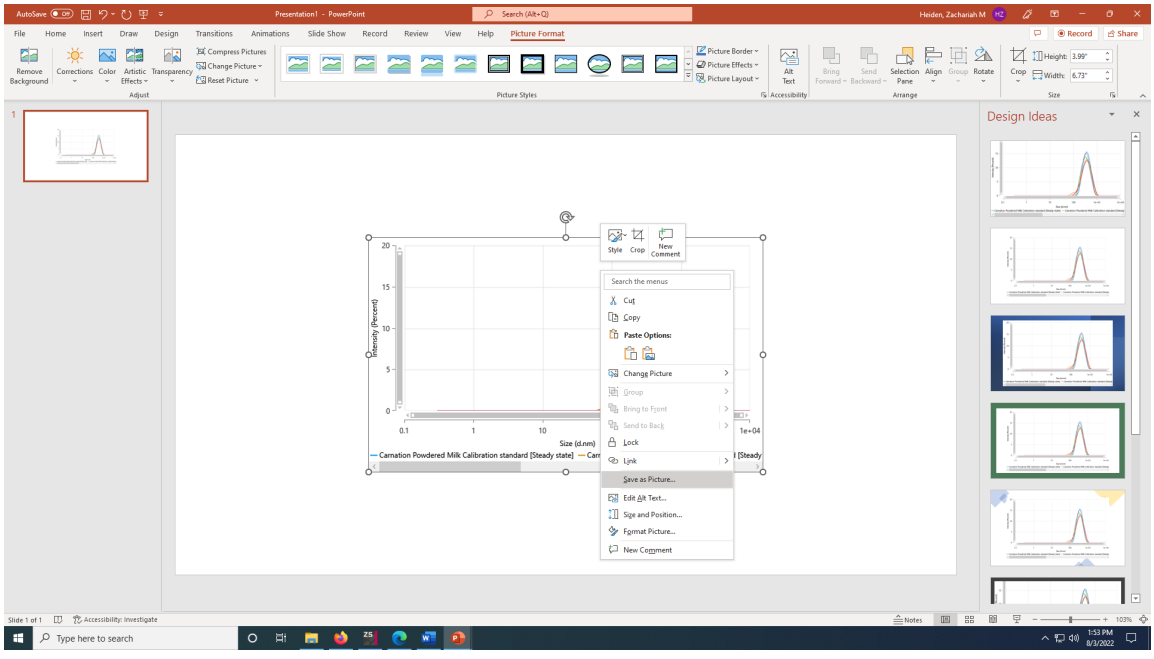
4.13 Open Microsoft PowerPoint.

4.14 Create a blank presentation (highlighted in red).

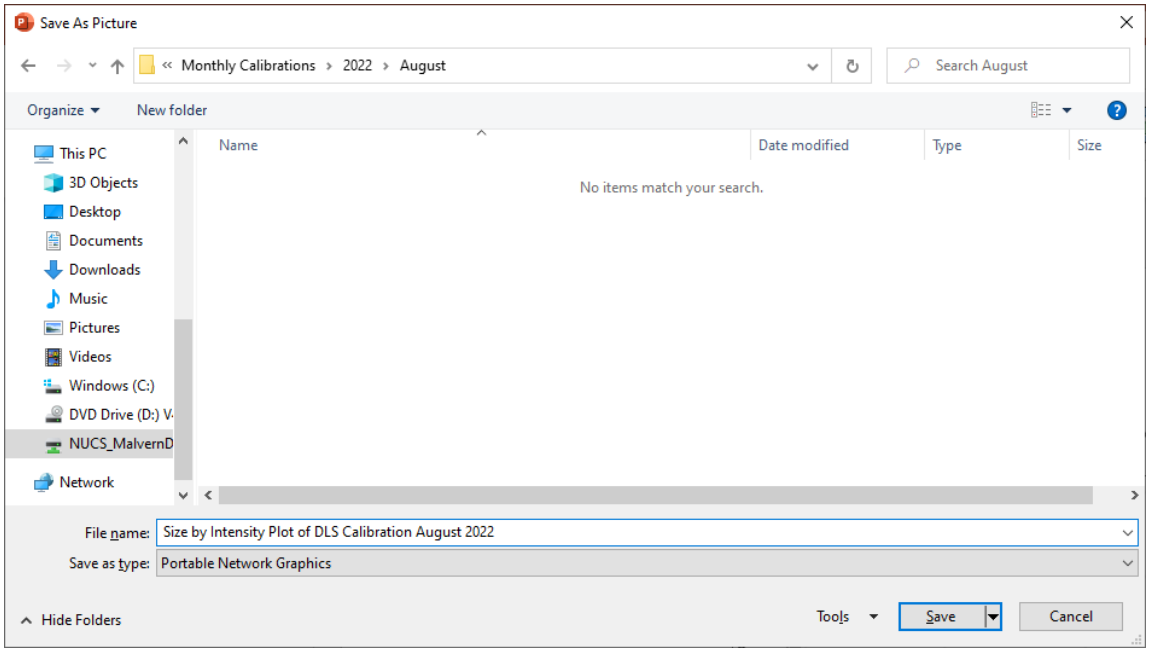


4.15 Paste (control + v) the image in PowerPoint.

4.16 Right click on the image and select Save as Picture to save it as a .png file.



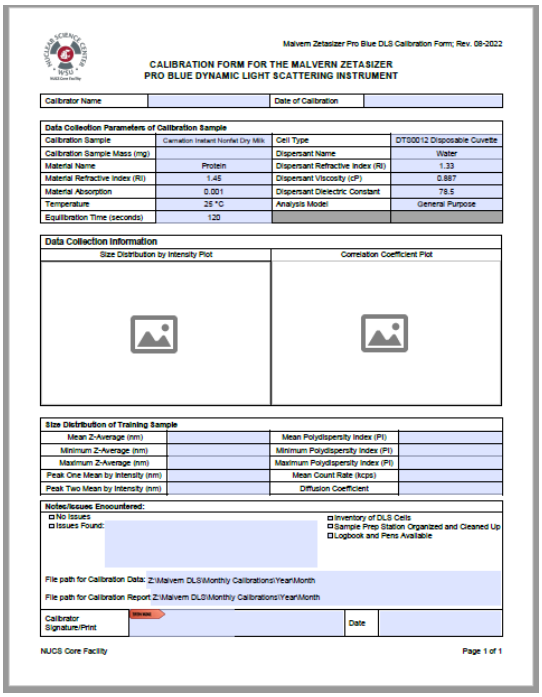
4.17 Save the image as: Size by Intensity Plot of DLS Calibration Month Year.png in the Z:Malvern DLS\Monthly Calibrations\Year\Month folder.



4.18 Add the picture to the NUCS Calibration Report.

4.19 Repeat Steps 4.12 and 4.18 for the correlogram.

4.20 Please save the data and the report on the NUCS drive using the following file location: Z:\Malvern DLS\Monthly Calibration Data and place in the respective year and folder for the data and report. Please ask the NUCS Core Facility staff if you have any questions. A sample blank (left) and completed (right) report for August 2022 can be seen below.



Malvern Zetasizer Pro Blue DLS Calibration Form; Rev. 08-2022

CALIBRATION FORM FOR THE MALVERN ZETASIZER PRO BLUE DYNAMIC LIGHT SCATTERING INSTRUMENT

Calibrator Name		Date of Calibration	

Data Collection Parameters of Calibration Sample

Calibration Sample	Carnation Instant Nonfat Dry Milk	Cell Type	DTS0012 Disposable Cuvette
Calibration Sample Mass (mg)		Dispersant Name	Water
Material Name	Protein	Dispersant Refractive Index (RI)	1.33
Material Refractive Index (RI)	1.45	Dispersant Viscosity (cP)	0.887
Material Absorption	0.001	Dispersant Dielectric Constant	78.5
Temperature	25 °C	Analysis Model	General Purpose
Equilibration Time (seconds)	120		

Data Collection Information

Size Distribution by Intensity Plot	Correlation Coefficient Plot

Size Distribution of Training Sample

Mean Z-Average (nm)		Mean Polydispersity Index (PI)	
Minimum Z-Average (nm)		Minimum Polydispersity Index (PI)	
Maximum Z-Average (nm)		Maximum Polydispersity Index (PI)	
Peak One Mean by Intensity (nm)		Mean Count Rate (kcps)	
Peak Two Mean by Intensity (nm)		Diffusion Coefficient	

Notes/Issues Encountered:

☐ No Issues
☐ Issues Found:

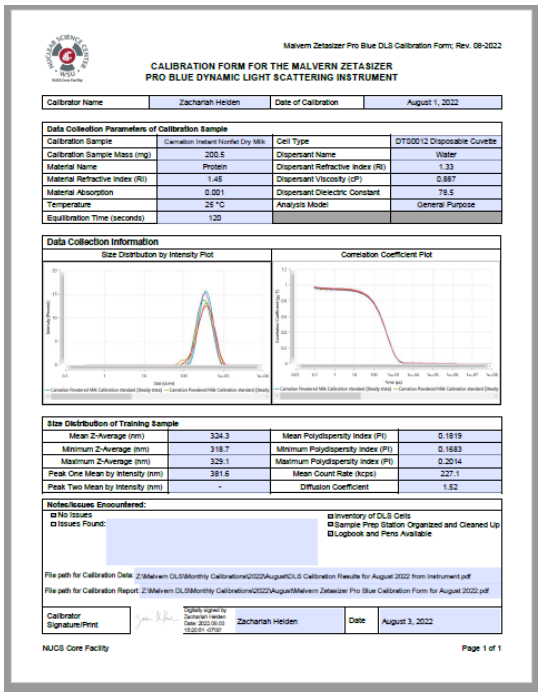
☐ Inventory of DLS Cells
☐ Sample Prep Station Organized and Cleaned Up
☐ Logbook and Pens Available

File path for Calibration Data: Z:\Malvern DLS\Monthly Calibrations\Year\Month

File path for Calibration Report: Z:\Malvern DLS\Monthly Calibrations\Year\Month

Calibrator Signature/Print		Date	

NUCS Core Facility Page 1 of 1



Malvern Zetasizer Pro Blue DLS Calibration Form; Rev. 08-2022

CALIBRATION FORM FOR THE MALVERN ZETASIZER PRO BLUE DYNAMIC LIGHT SCATTERING INSTRUMENT

Calibrator Name	Zachariah Helden	Date of Calibration	August 1, 2022

Data Collection Parameters of Calibration Sample

Calibration Sample	Carnation Instant Nonfat Dry Milk	Cell Type	DTS0012 Disposable Cuvette
Calibration Sample Mass (mg)	200.5	Dispersant Name	Water
Material Name	Protein	Dispersant Refractive Index (RI)	1.33
Material Refractive Index (RI)	1.45	Dispersant Viscosity (cP)	0.887
Material Absorption	0.001	Dispersant Dielectric Constant	78.5
Temperature	25 °C	Analysis Model	General Purpose
Equilibration Time (seconds)	120		

Data Collection Information

Size Distribution by Intensity Plot	Correlation Coefficient Plot

Size Distribution of Training Sample

Mean Z-Average (nm)	324.3	Mean Polydispersity Index (PI)	0.1819
Minimum Z-Average (nm)	318.7	Minimum Polydispersity Index (PI)	0.1683
Maximum Z-Average (nm)	329.1	Maximum Polydispersity Index (PI)	0.2014
Peak One Mean by Intensity (nm)	381.6	Mean Count Rate (kcps)	227.1
Peak Two Mean by Intensity (nm)	-	Diffusion Coefficient	1.52

Notes/Issues Encountered:

☐ No Issues
☐ Issues Found:

☐ Inventory of DLS Cells
☐ Sample Prep Station Organized and Cleaned Up
☐ Logbook and Pens Available

File path for Calibration Data: Z:\Malvern DLS\Monthly Calibrations\2022\August\DLS Calibration Results for August 2022 from Instrument.pdf

File path for Calibration Report: Z:\Malvern DLS\Monthly Calibrations\2022\August\Malvern Zetasizer Pro Blue Calibration Form for August 2022.pdf

Calibrator Signature/Print	Digitally signed by Zachariah Helden Date: 2022.08.03 15:20:01 -07'00'	Zachariah Helden	Date	August 3, 2022

NUCS Core Facility Page 1 of 1

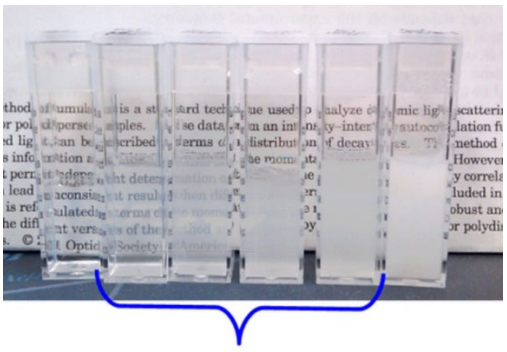
5 Sample Preparation

5.1 Samples can be prepared using the cells provided next to the Malvern Zetasizer Pro Blue.

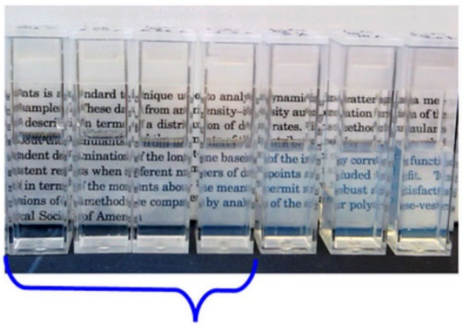
5.2 When preparing and handling samples, gloves should be worn to avoid transferring oils from a user's hands to the cells, which can influence the measurements.

5.3 For size analysis, a box of polystyrene disposable cuvettes are available.

5.4 For the best size analysis, the sample should be slightly cloudy, as seen below. Sometimes multiple samples with varying concentrations are needed to obtain acceptable results.

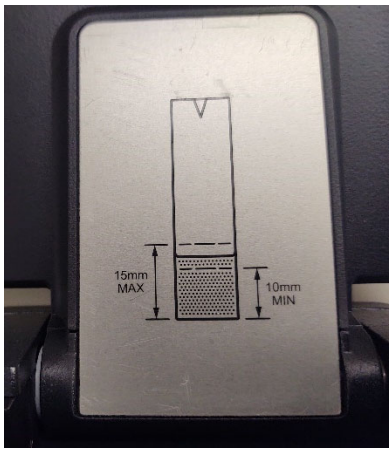


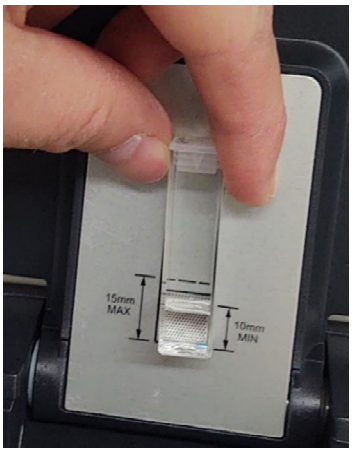
Best range of concentration for measuring this ~ 1 micron diameter particle system by dynamic light scattering.



Best range of concentration for measuring this 36 nm diameter nanoparticle system by dynamic light scattering.

5.5 The height of the sample should be between 10 and 15 mm. The cover of the sample chamber contains a picture that the sample holder can be placed next to check that the sample quantity is within the instrument limits.

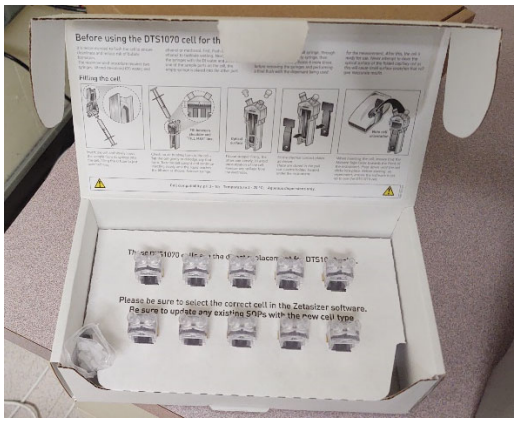




Particle size and molecular size

Measurement principle	Non-Invasive Back Scatter (NIBS) Dynamic Light Scattering
Measurement angle	173°, 13°
Measurement range	Diameter: 0.3 nm - 10 µm
Minimum sample volume	12 µL
Concentration range	**Minimum sample concentration:** Blue Label: 0.2 mg/mL 15 kDa Protein Red Label: 0.1 mg/mL 15 kDa Protein **Maximum sample concentration:** (3) 40% w/v

5.6 For a zeta potential analysis, a box of disposable sample cells are available next to the Malvern Zetasizer Pro Blue.



5.7 To fill the cell for a zeta potential analysis, fill a syringe with the solution to be analyzed and inject it into the cell using the ports at the top of the cell. A diagram for filling the cell can be seen on the inside cover of the Zetasizer capillary cell box.

5.8 Upon filling the cell, attach the caps, as seen in the picture seen below.

Zeta potential

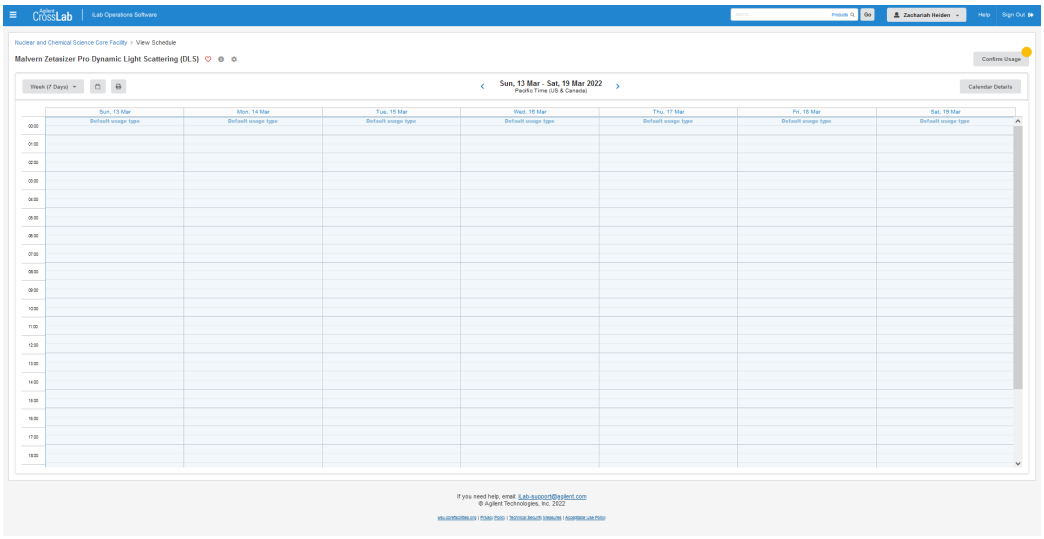
Measurement principle	Mixed-Mode Measurement phase analysis light scattering (M3-PALS)
Size range	Diameter: 3.8 nm – 100 µm [2]
Minimum sample volume	20 µL [4]
Concentration range	Red Label: 1 mg/mL [5] to 40% w/v [6] Blue Label: 10 mg/mL [5] to 40% w/v [6]
Sample conductivity range	Maximum: 260 mS/cm

6 Data Collection on the Malvern Zetasizer Pro Dynamic Light Scattering Instrument

This section is to be completed after a weekly calibration (Sections 3 & 4) has been performed.

Materials: Sample for size or zetapotential analysis, Malvern Zetasizer Pro Blue DLS

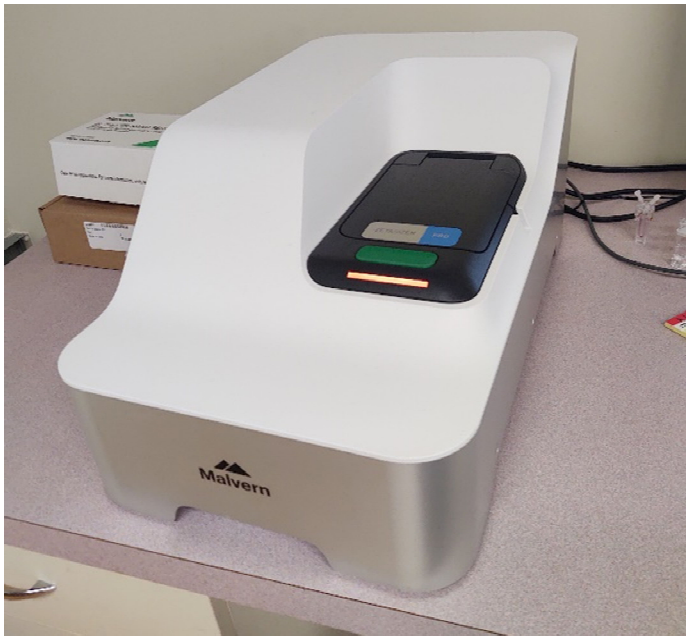
6.1 Instrument time on the Malvern DLS are made on iLab (https://wsu.corefacilities.org/), prior to the user's instrument time. Reservations are based on service, not time. Please indicate the number of data collections obtained during the reserved time. If you have questions regarding booking instrument time, please ask the NUCS Core Facility Staff.



6.2 Start by writing the date, starting time, and your name in the instrument logbook.

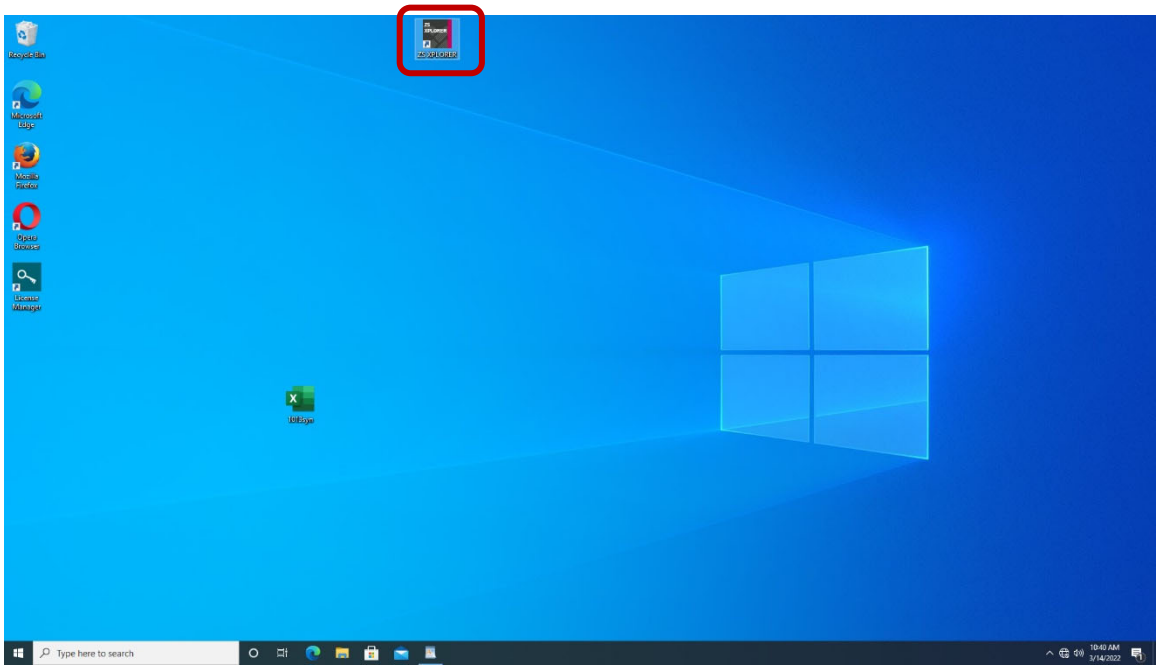
6.3 Check to see if the Malvern is on. The default state is to have the system powered down. The power switch can be found on the back of the instrument on the right side, when facing the instrument. To turn on, push the black button, highlighted in red in the below picture.

6.4 The light on the front of the DLS will be orange as the laser warms up. It will take about 5 minutes to turn green in color to be ready to go. It is recommended that you wait at least 15 minutes after turning on the instrument before using.

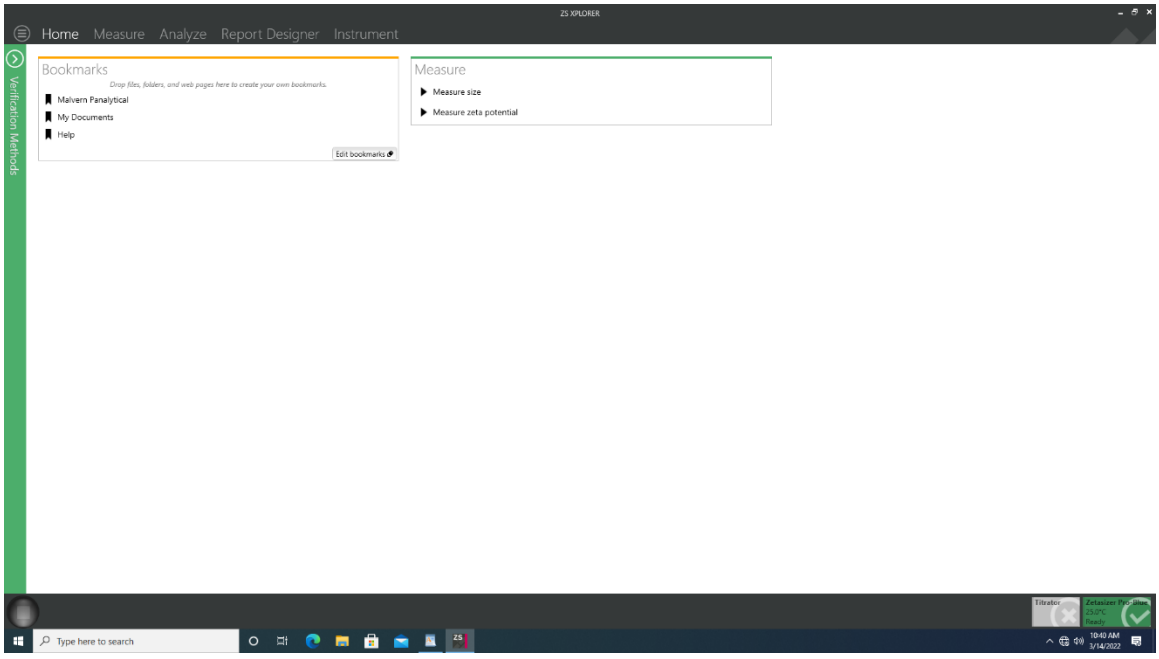


6.5 When the instrument is ready to go, the light on the front of the instrument will be green in color, highlighted in red in the below picture.

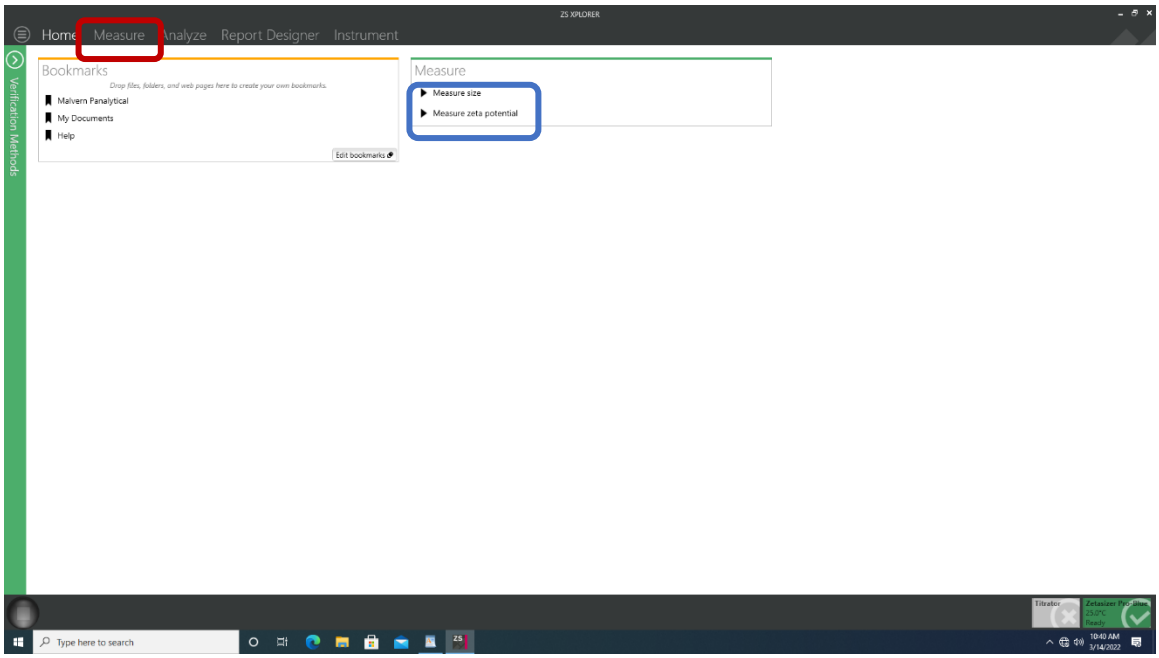
6.6 At the computer, double click the ZS XPLORER icon, highlighted in red, which will open the software to control the DLS.



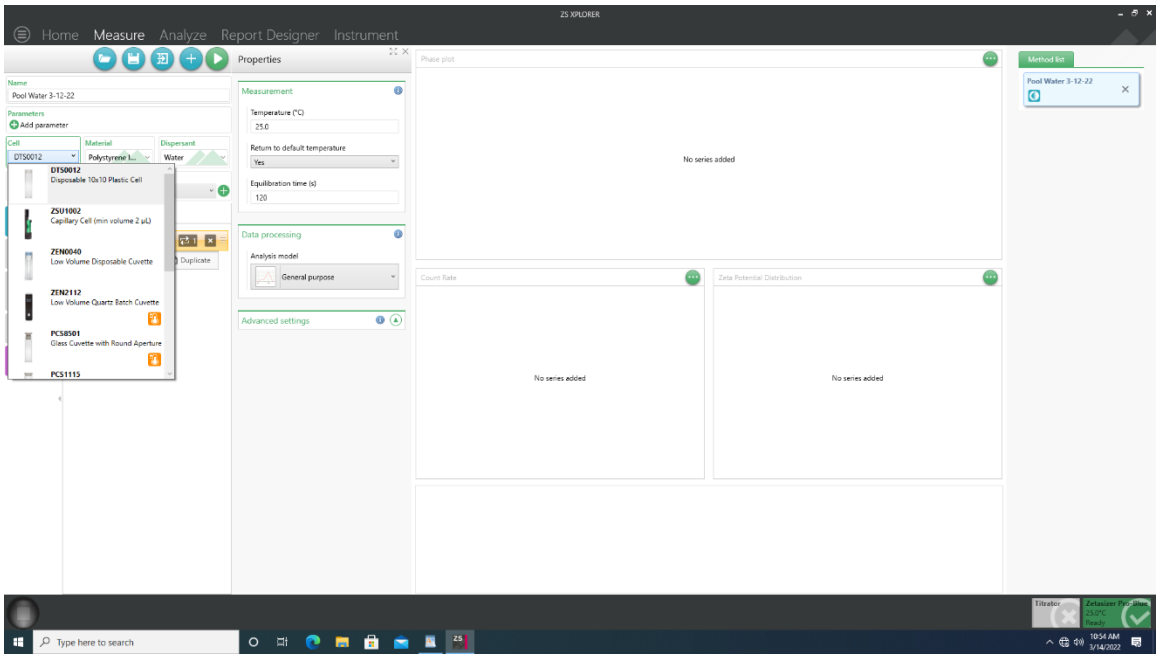
6.7 When the software has loaded, you will see the following image.



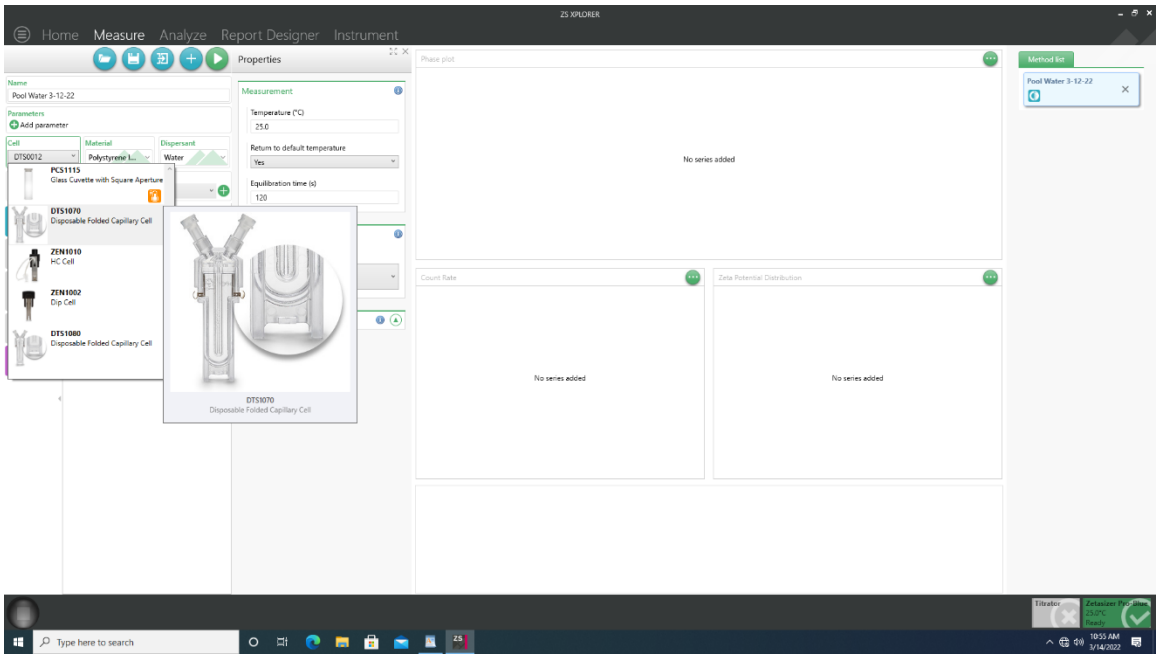
6.8 To make a measurement, there are two options, the Measure menu option (highlighted in red) can be clicked or the Measure size or Measure zeta potential option (highlighted in blue) under the Measure option can be clicked.



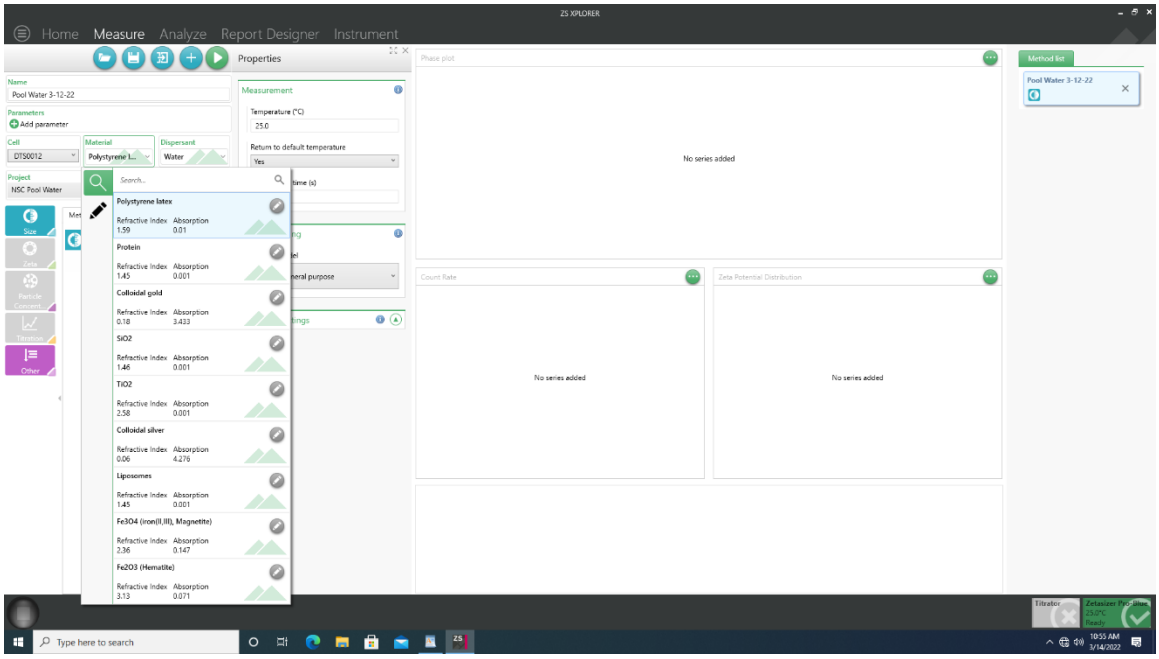
6.9 If the desired experiment is to measure the size of a dissolved particle, then select a cell containing the sample, for example a polystyrene disposable cell (provided).



6.10 If the desired experiment is to measure the zeta potential of a dissolved particle, then select a cell containing the sample, for example a disposable capillary cell (provided). Skip to Step 6.22 for additional steps for the analysis of the zeta potential of a solution.



6.11 Then, the user will want to select the material that is most similar to the material that the user wants to measure the size of. The options are: polystyrene latex, protein, colloidal gold, SiO_2, TiO_2, colloidal silver, liposomes, Fe_3O_4 (magnetite), and Fe_2O_3 (hematite).



6.12 If a new material needs to be added, listed below is a table of common materials.

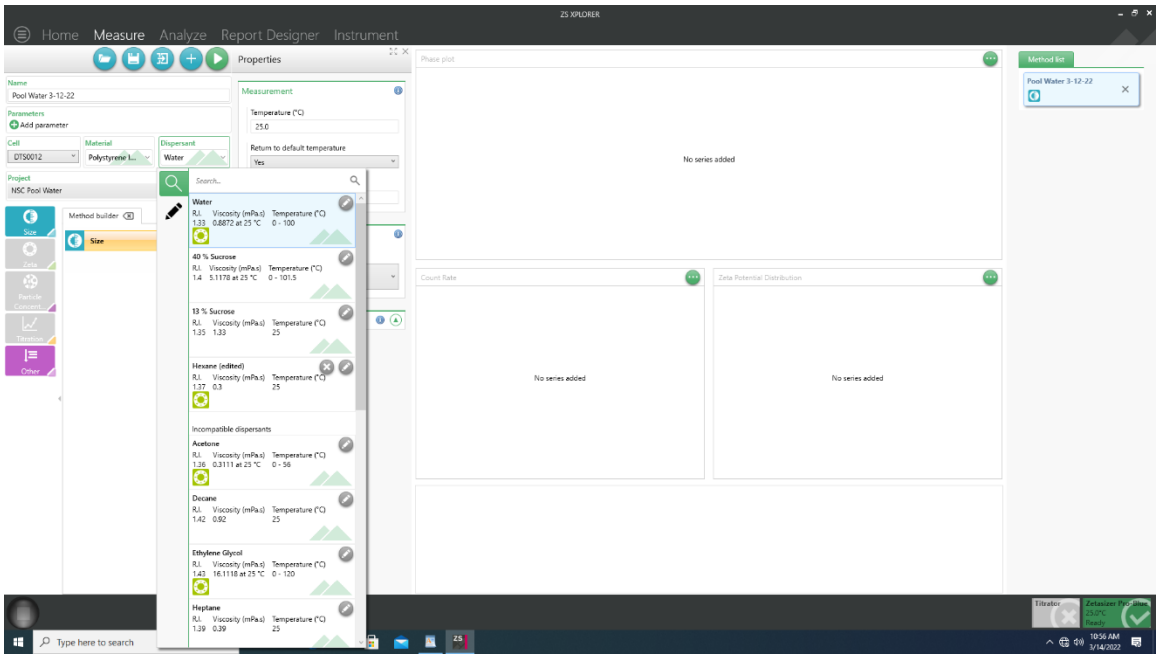
Table of select material properties		
Sample material	**refractive index**	**absorption**
Liposomes #		
Phospholipids	n=1.45	k=0.001
Exosomes	n=1.37 – 1.39*	k=0.01
Microvesicles (> .2µm)	n=1.40*	k=0.01
Nanoparticles and Colloids		
Gold [Au]	n=0.20	k=3.32
Silver [Ag]	n=0.135	k=3.99
Platinum [Pt]	n=2.32	k=4.16
Palladium [Pd]	n=1.77	k=4.29
TiO2	n=2.41	k=0.001
SiO2	n=1.54	k=0.00
PFOB emulsions	n=1.305	k=0.10
Nanodiamonds	n=2.42	k=0.00
Macromolecules		
Proteins	n=1.45	k=0.001
Polystyrene	n=1.59	k=0.01

"Optical characterization of liposomes by right-angle light scattering and turbidity" Biochimica et Biophysica Acta (BBA) – Biomembranes 1467, 1, 219-226 (2000)

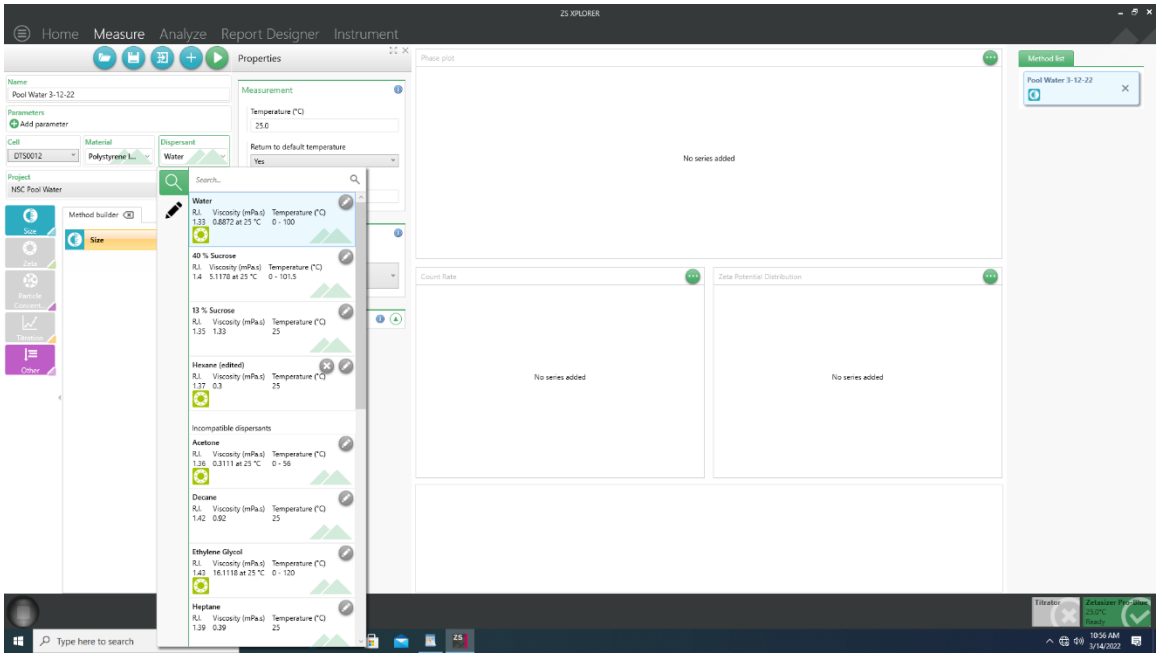
* "Measurement of refractive index by nanoparticle tracking analysis reveals heterogeneity in extracellular vesicles" Journal of Extracellular Vesicles 2014, 3:25361 DOI: 10.3402/jev.v3.25361 (2014)

+ In addition, the "Refractive index of amorphous polumers" at polymerdatabase has a relevant related list.

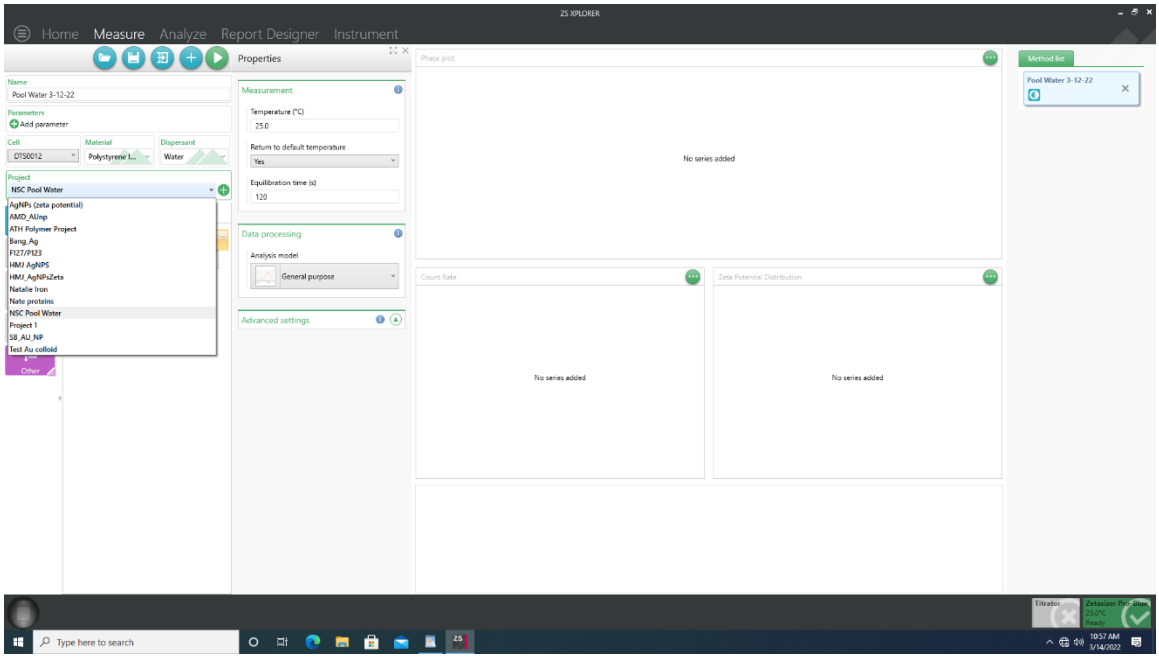
6.13 Then, the solvent needs to be chosen. Options are: Water, 40% Sucrose, 13% Sucrose, Hexane, Acetone, Decane, Ethylene Glycol, Heptane, Isopar P, Kerosene, Methanol, Methyl Methacrylate, Propan-2-ol, THF, Toluene, and Ethanol.



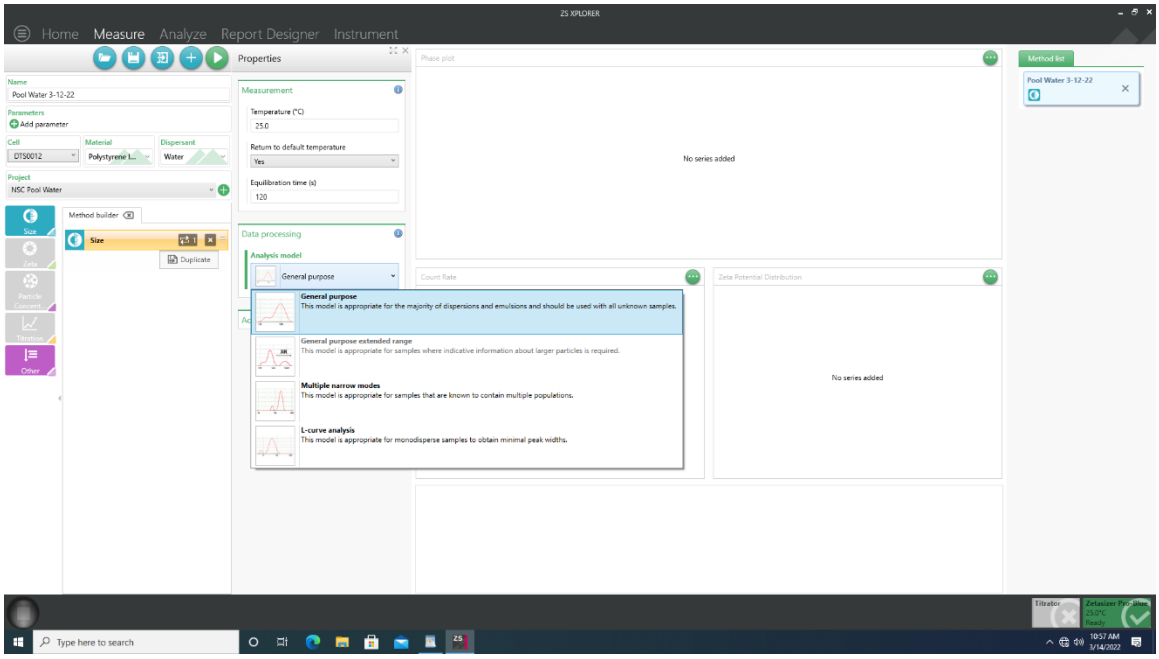
6.14 A user can also define parameters, if known, such as concentration, pH, and batch.



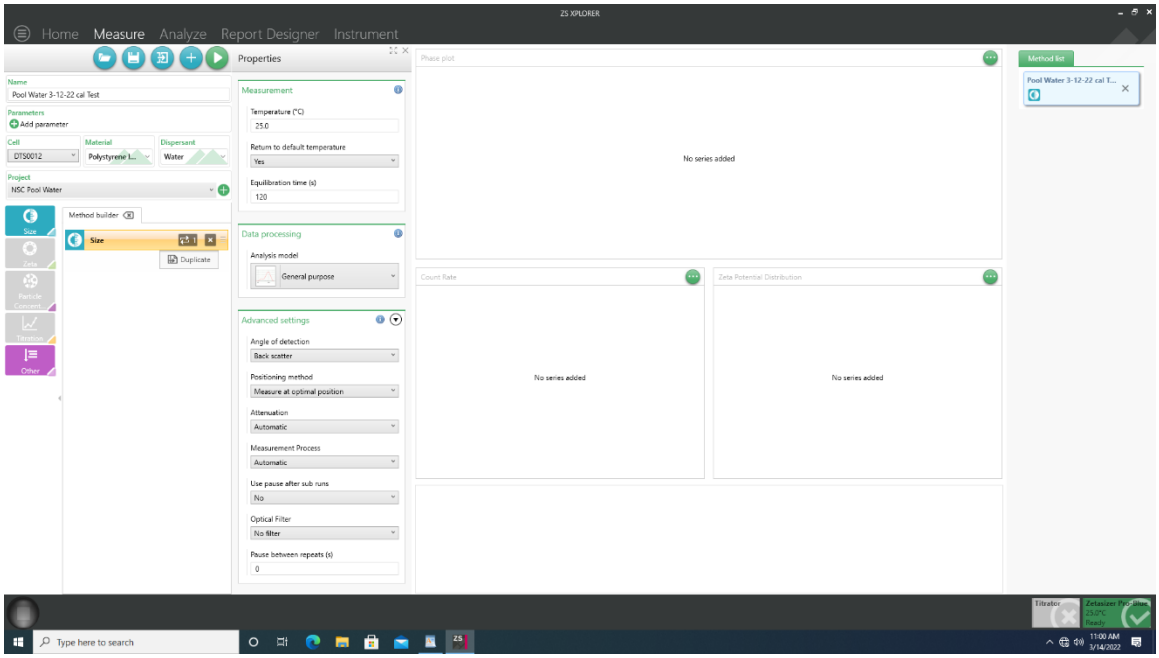
6.15 After setting the parameters, a project on where to save the date needs to be selected. If a new project is needed, the green plus sign is used to create a new project.



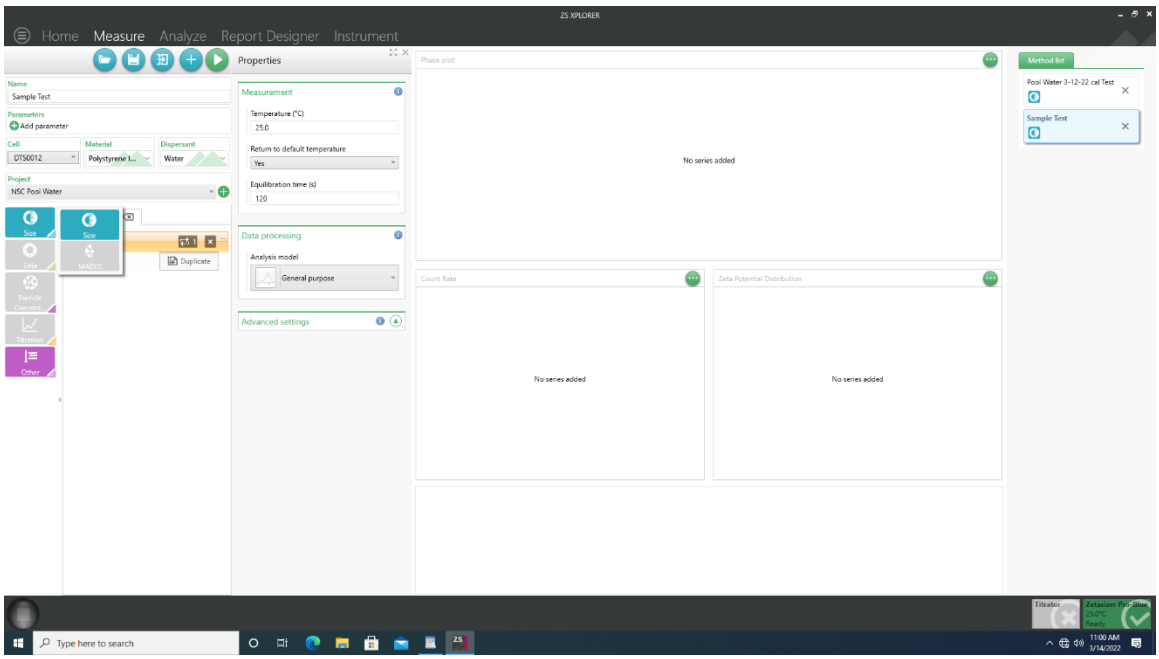
6.16 Then to set up the data processing routine, a user can select between general purpose, narrow modes (used for multiple peaks), and L-curve analysis. For most samples, the general purpose mode is the best option.



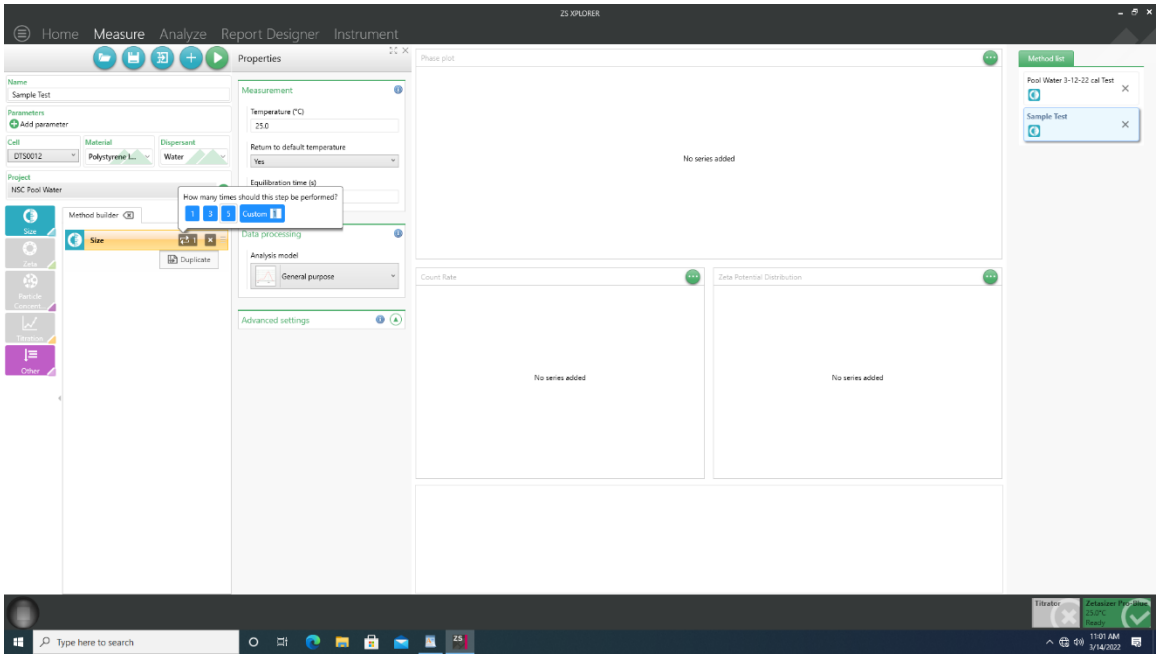
6.17 If desired, the advanced settings can be adjusted, but for most data collections, the default values will provide satisfactory results.



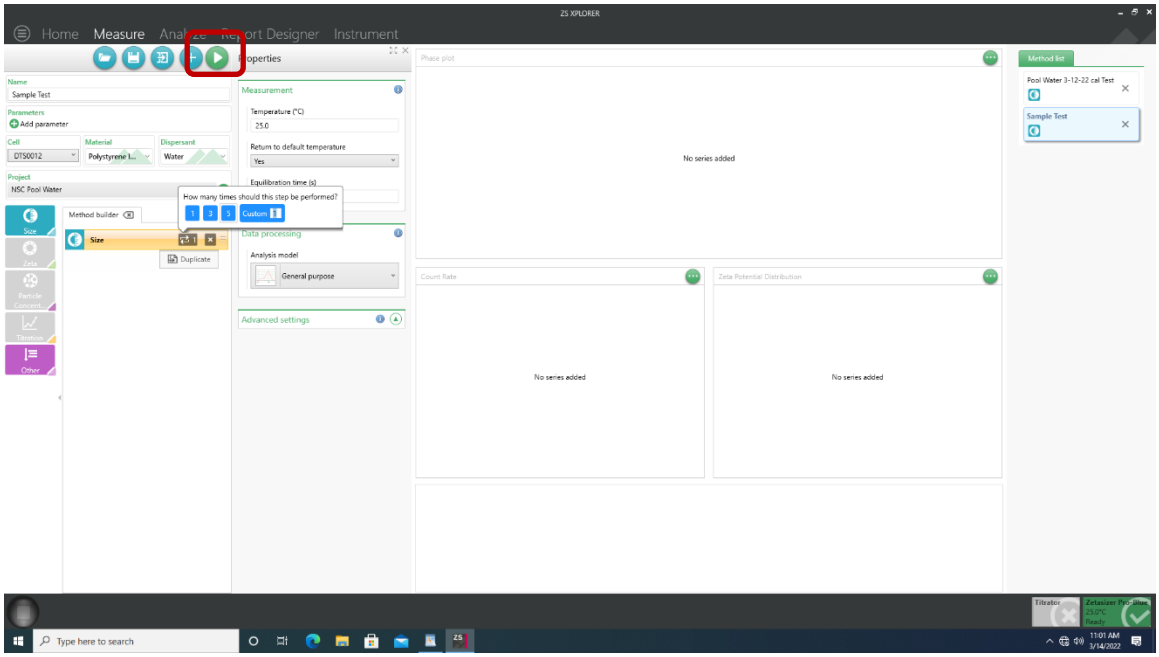
6.18 If a cell that is chosen in Step 6.9 is only capable of size analysis, the Size option will be the only option highlighted.



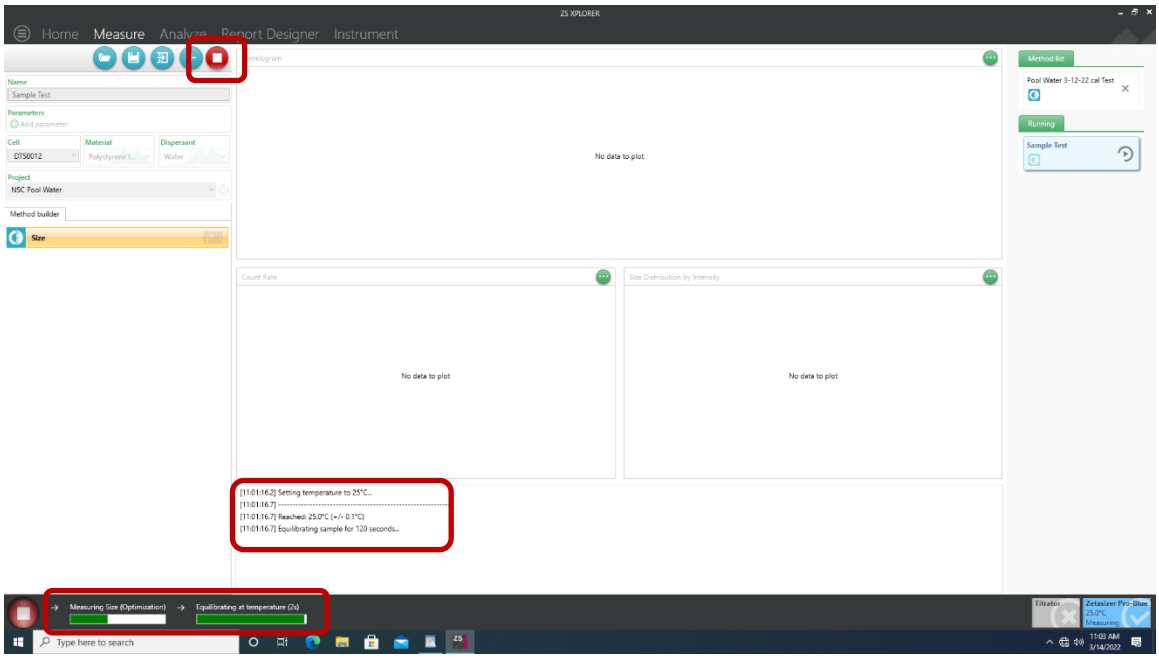
6.19 In the method builder section, a user can choose how many times the data collection is repeated. Collection of the data at least three times is recommended.



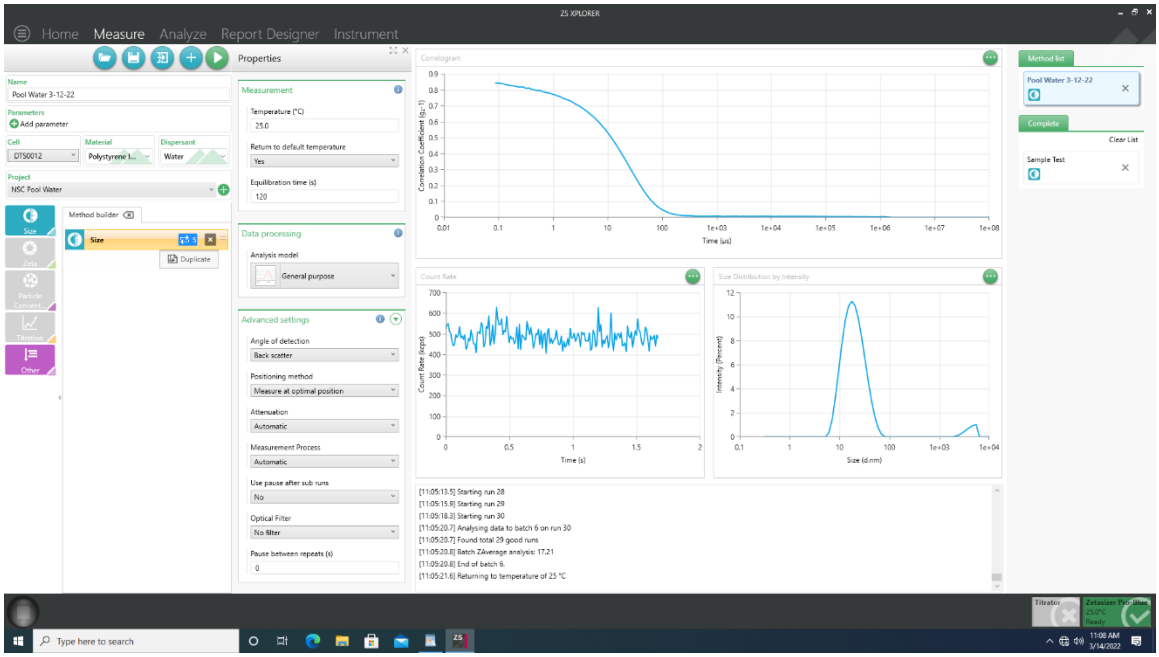
6.20 When the experiment is set up, the user can click on the green circle with a triangle (highlighted in red) to start the data collection.



6.21 Start of the collection of data will be indicated by the green circle changing to a red circle (highlighted in red) with a square inside of it. The text output at the bottom of the screen will also indicate the experiment status.

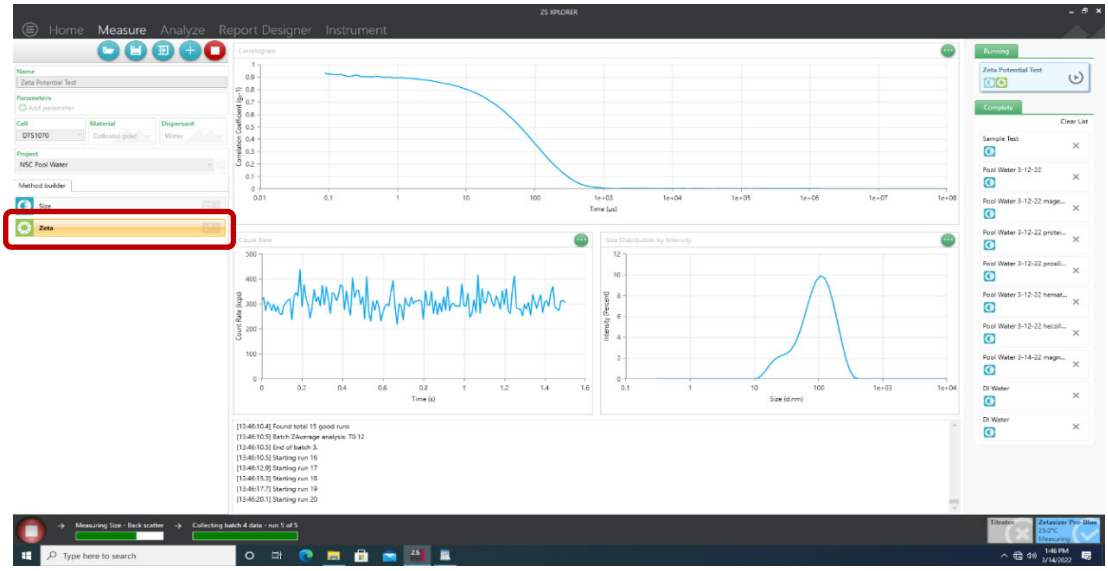


6.22 After the data collection has completed (5-10 minutes), the data will appear on the screen.



6.23 To measure the zeta potential for a sample, the cell chosen in Step 6.10 must be capable of zeta potential measurements. Cells capable of zeta potential measurements can be used to also measure the size of particles in solution.

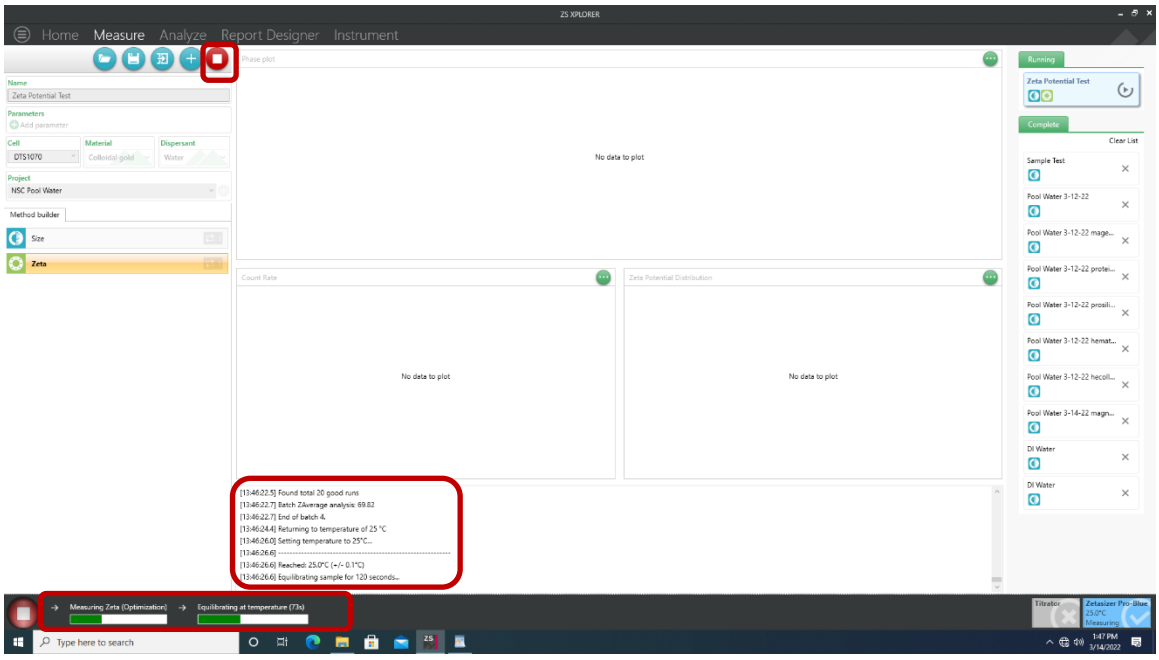
6.24 Select Zeta under the Method Builder Option.



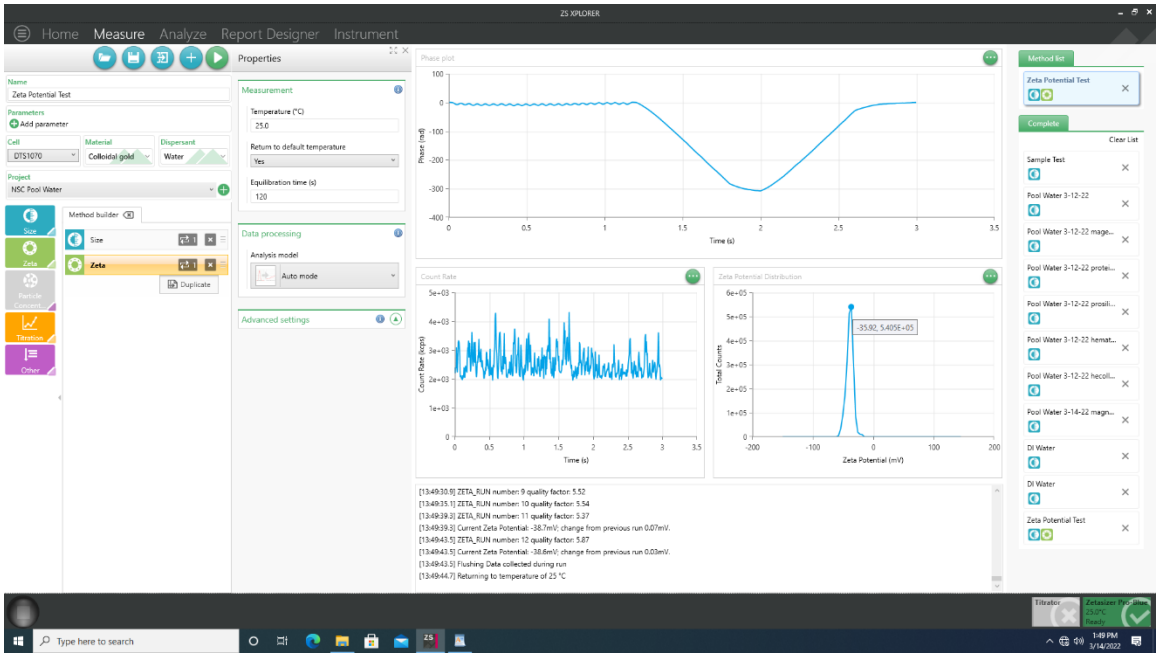
6.25 In the method builder section, select how many times the zeta potential data collection is repeated. Collection of the data at least three times is recommended.

6.26 To start the data collection, click on the green circle with a triangle to start the data collection.

6.27 Start of the collection of data will be indicated by the green circle changing to a red circle (highlighted in red) with a square inside of it. The text output at the bottom of the screen will also indicate the experiment status.



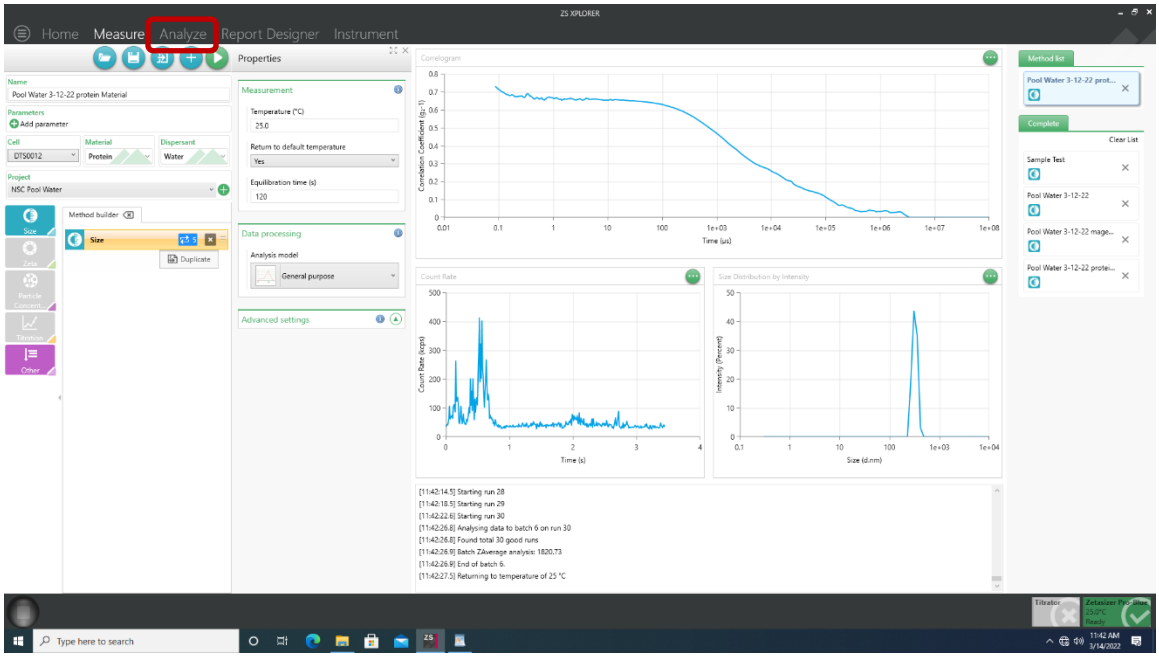
6.28 After the data collection has completed (5-10 minutes), the data will appear on the screen.



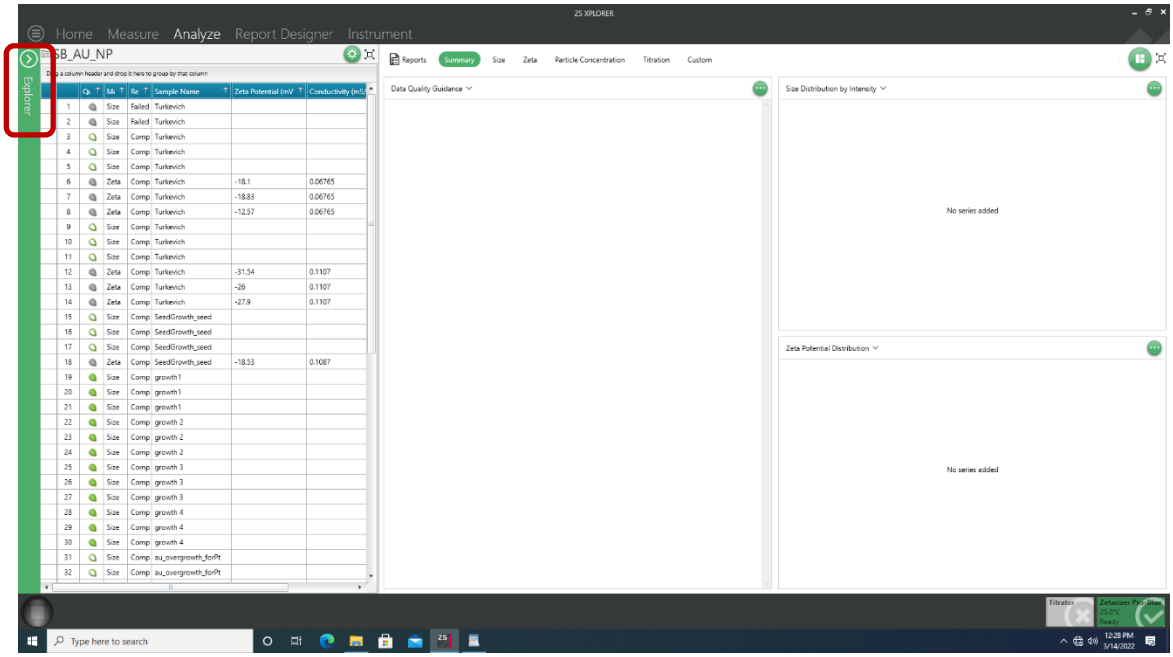
6.29 Upon completion of the data collection of the samples, the default state of the system is to be powered down when not in use. See section 8 for instructions on powering down the instrument.

7 Data Workup on the Malvern Zetasizer Pro Blue DLS

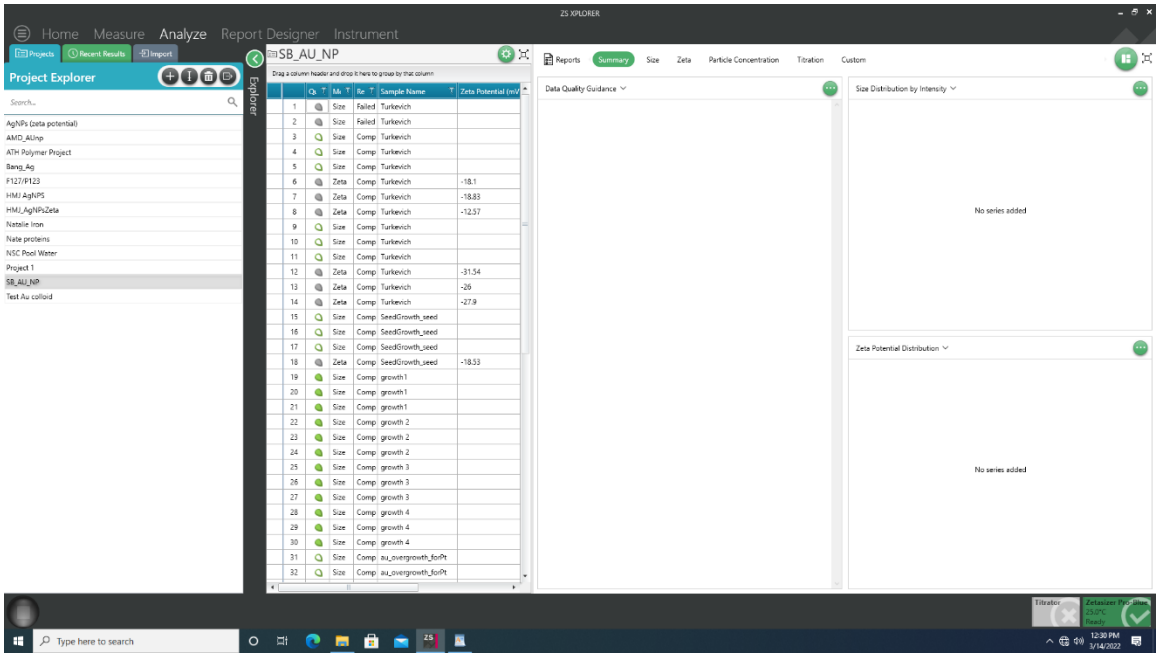
7.1 To analyze the data, select Analyze in the menu options at the top of the screen (highlighted in red).



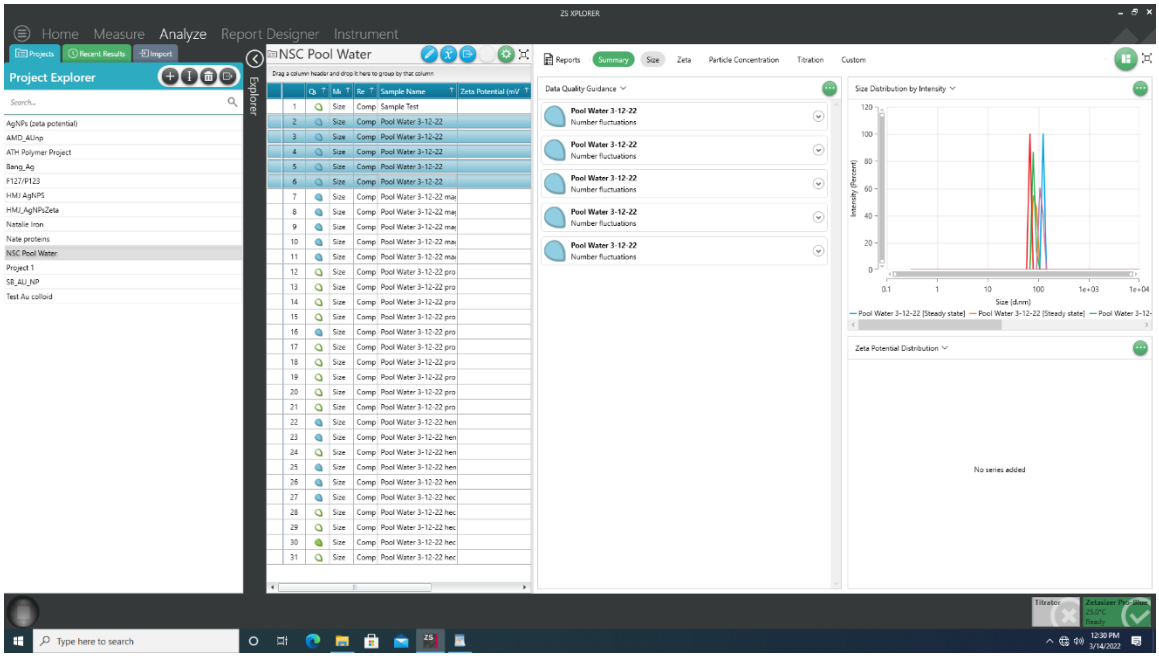
7.2 The analyze option will show the data for the data from the last user that used the analyze option. To access the data that was just collected, click on the green explorer arrow (highlighted in red).



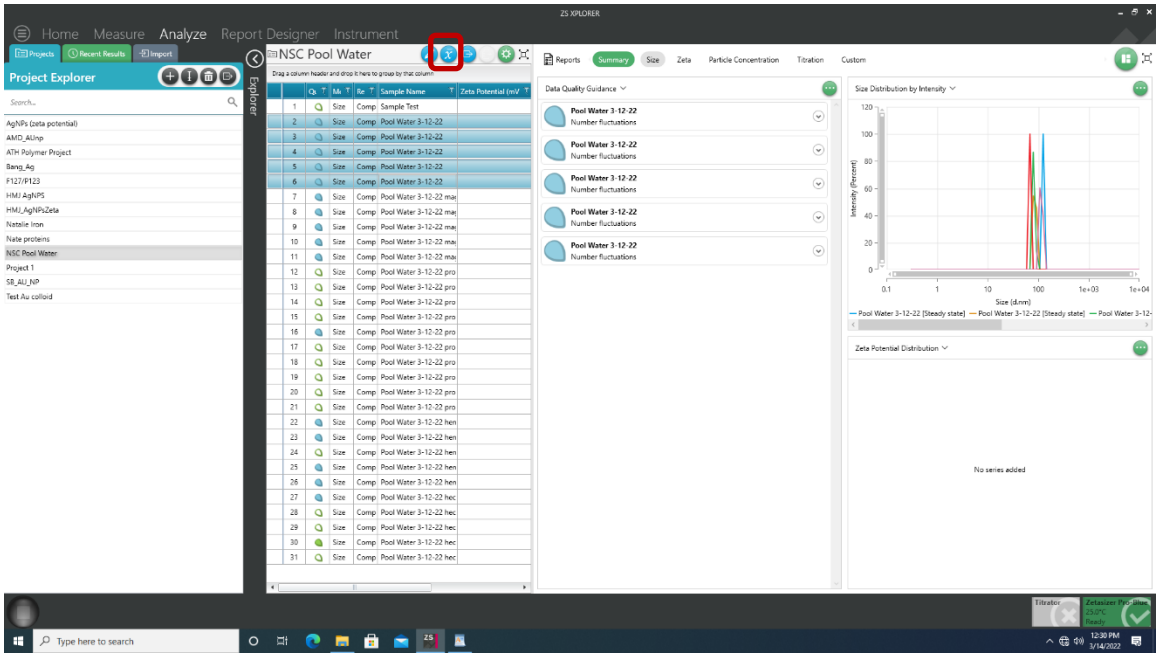
7.3 The Project Explorer will open and allow the user to select the location of the data collection. Select the desired folder and click back on the green arrow to close it.



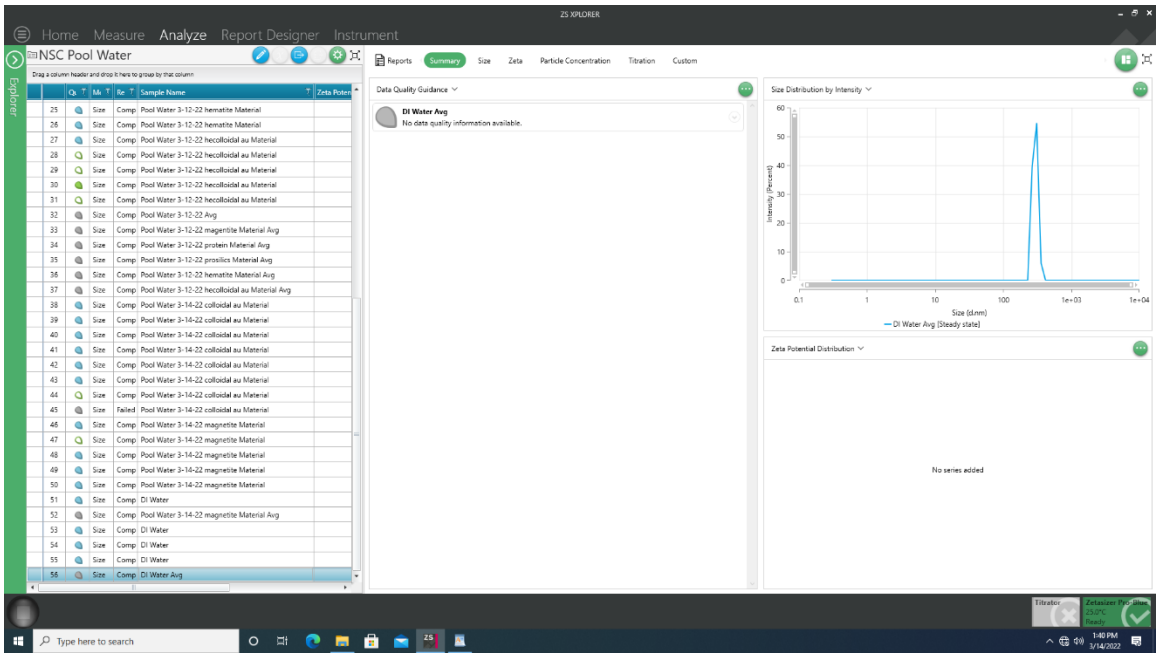
7.4 To view a selected data set, highlight the desired data sets. The highlighted data sets will automatically be plotted on the screen.



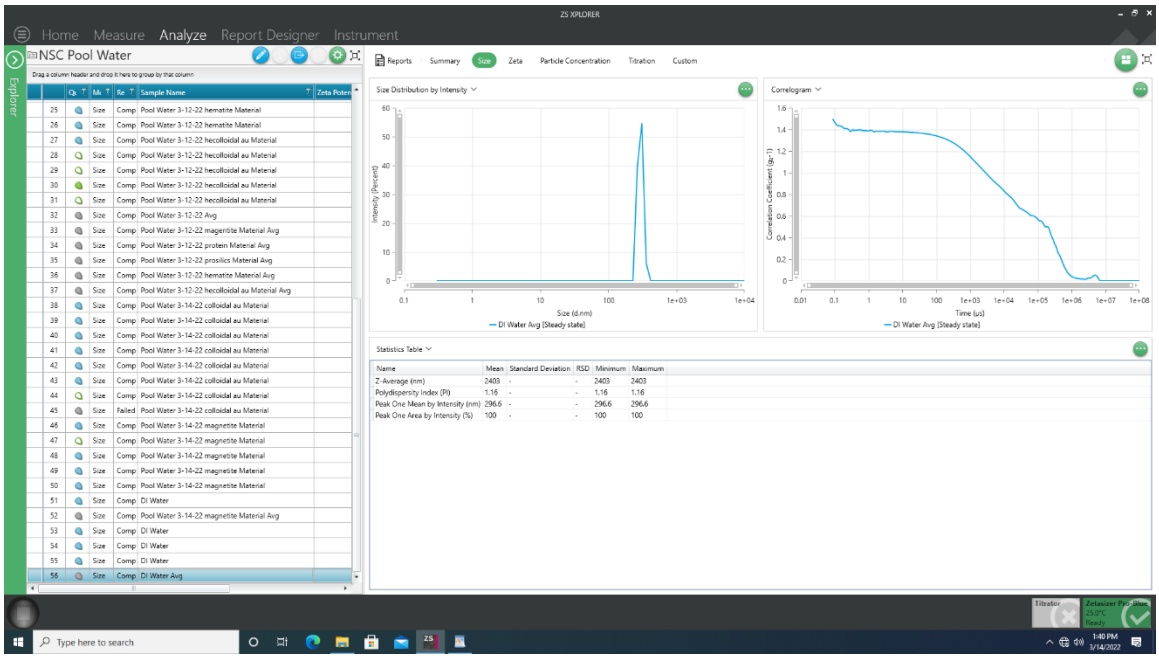
7.5 To average a data set, select the data to be averaged and then click on the average button (highlighted in red)



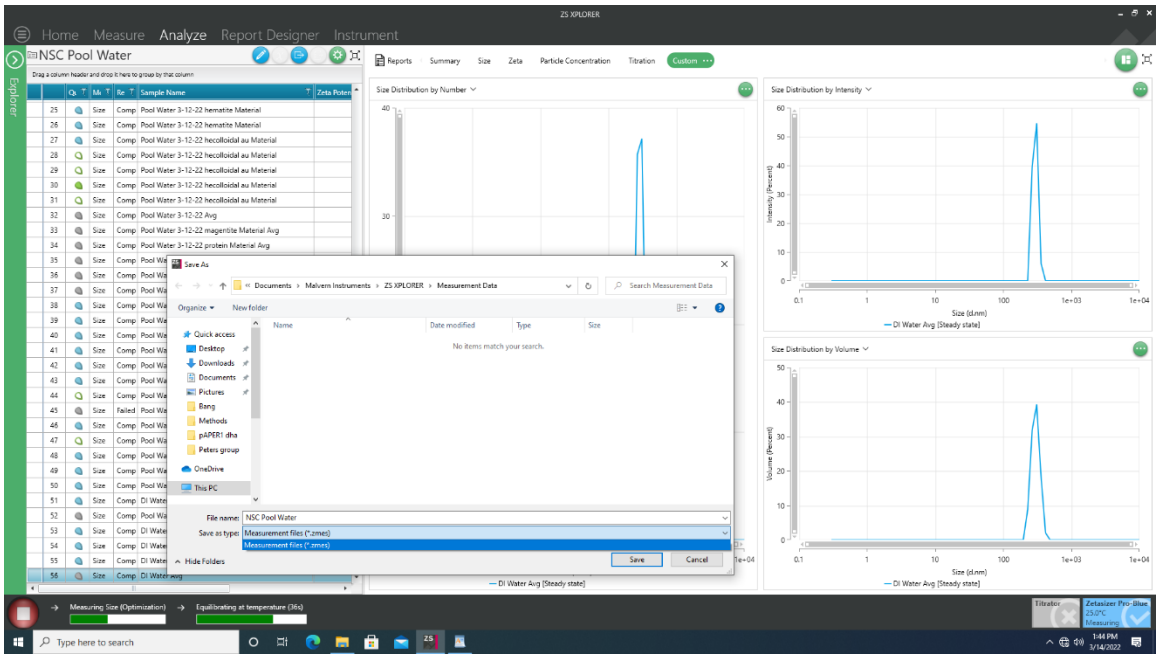
7.6 To view the averaged data set, select the average data set, at the bottom of the list.



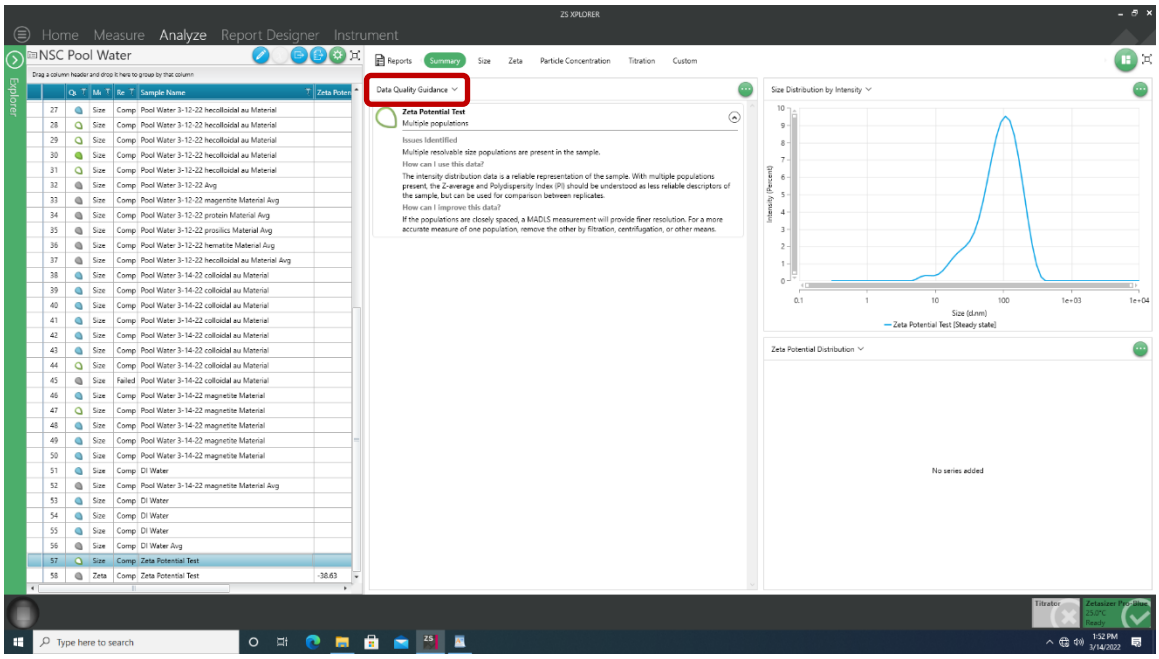
7.7 You will then see another window show up behind the 1D View window. The window will be called the Peak Column View. Click on this window to see information on the peaks found in the spectrum.



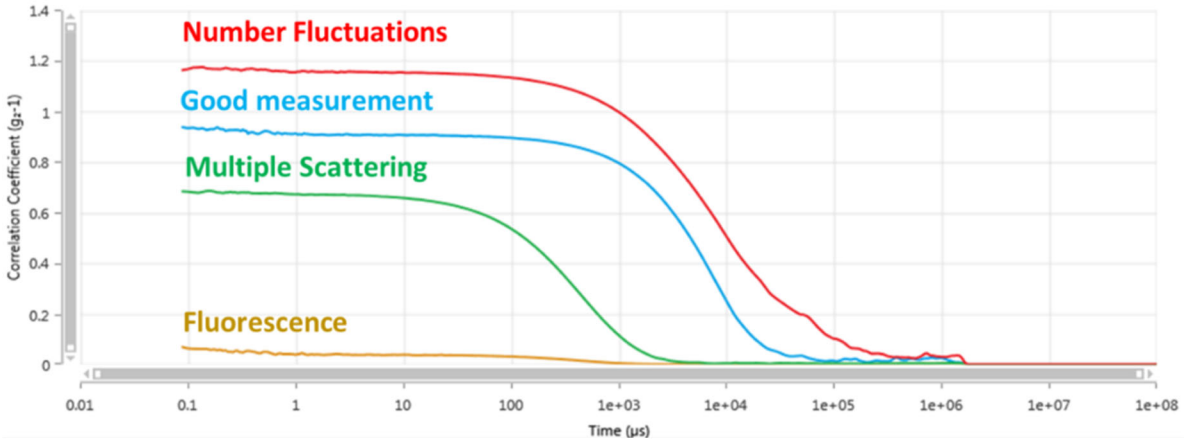
7.8 To save the data, right click and select Save As. A window will show up and the data can only be saved as a Measurement File (.zmes) in the Malvern software.



7.9 The quality of the data can be examined by Selecting the Data Quality Guidance from the drop down menu (highlighted in red).

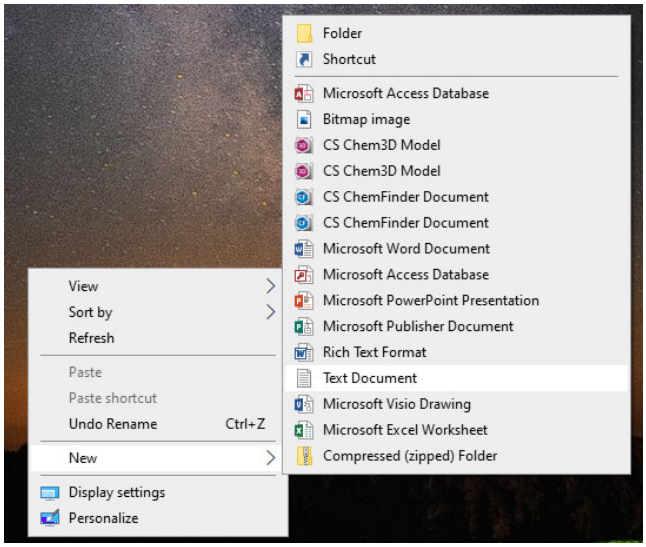


7.10 In addition to examining the data quality guidance, analysis of the correlograms can provide insight into the system. A low correlogram intercept indicates fluorescence, where the sample emits its own light, which is detected additionally to the scattered light. A higher intercept indicates that the number of particles within the laser beam is changing significantly over time. This means that the average scattering intensity is not stable.

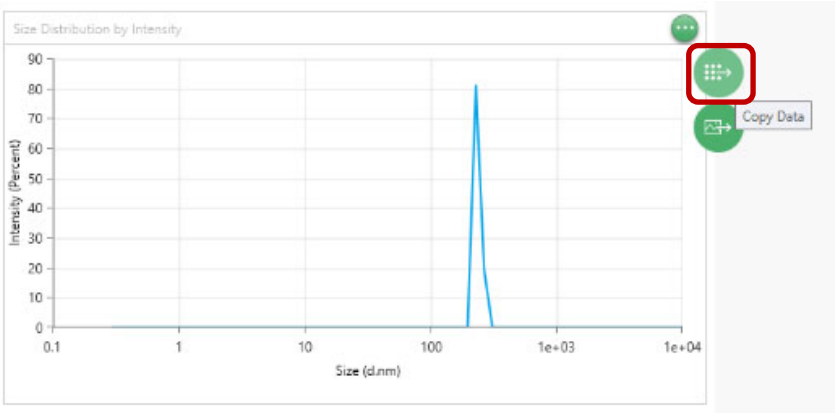


7.11 To manipulate the data outside of the Malvern software, the data will need to copied to a text file.

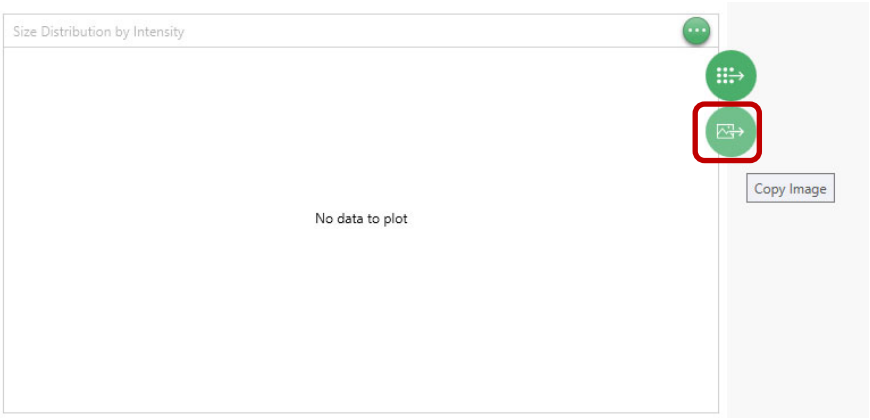
7.12 Create a new text file by right clicking on the Desktop and selecting New -> Text Document.



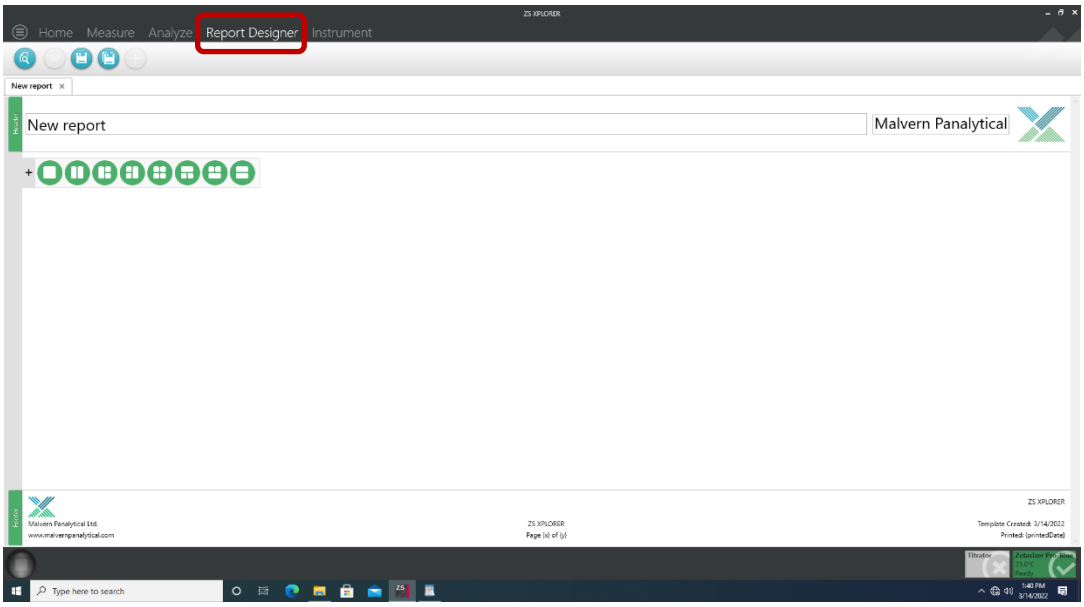
7.13 In the Malvern software, click on the circle with three dots on the plot of interest. You will be given two options, select the top green circle (highlighted in red) to Copy the Data. The data can be pasted in the text file and the text file can be saved and opened in other data analysis software (e.g. Microsoft Excel, Origin, etc.)



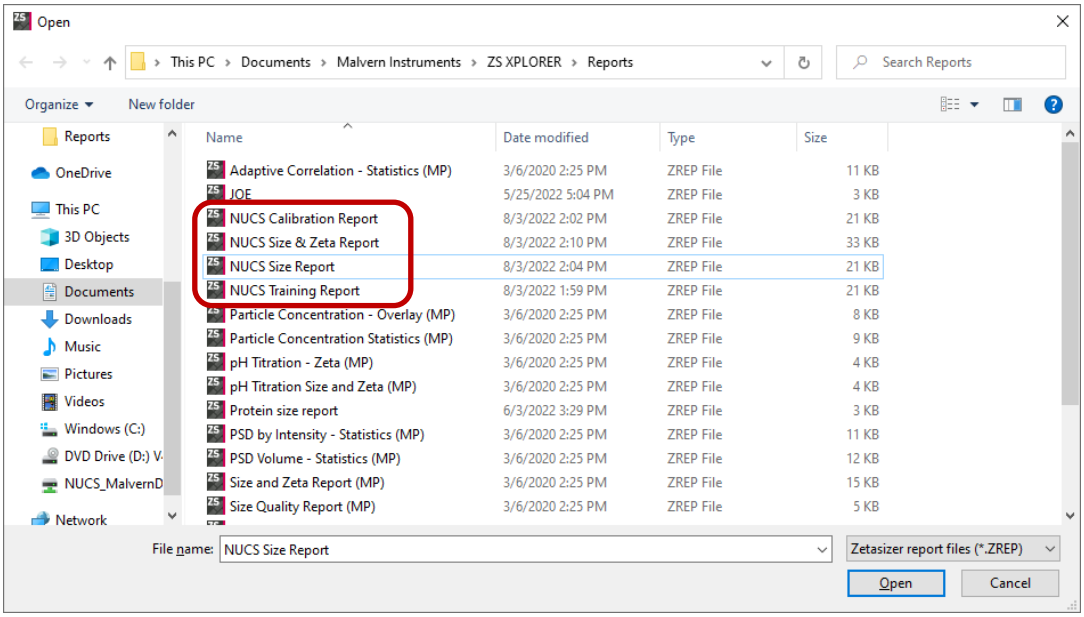
7.14 To copy the image, click on the green circle with three dots in the figure to be copied. Click on the bottom green circle (highlighted in red) to copy the figure, which can be pasted to a different location.



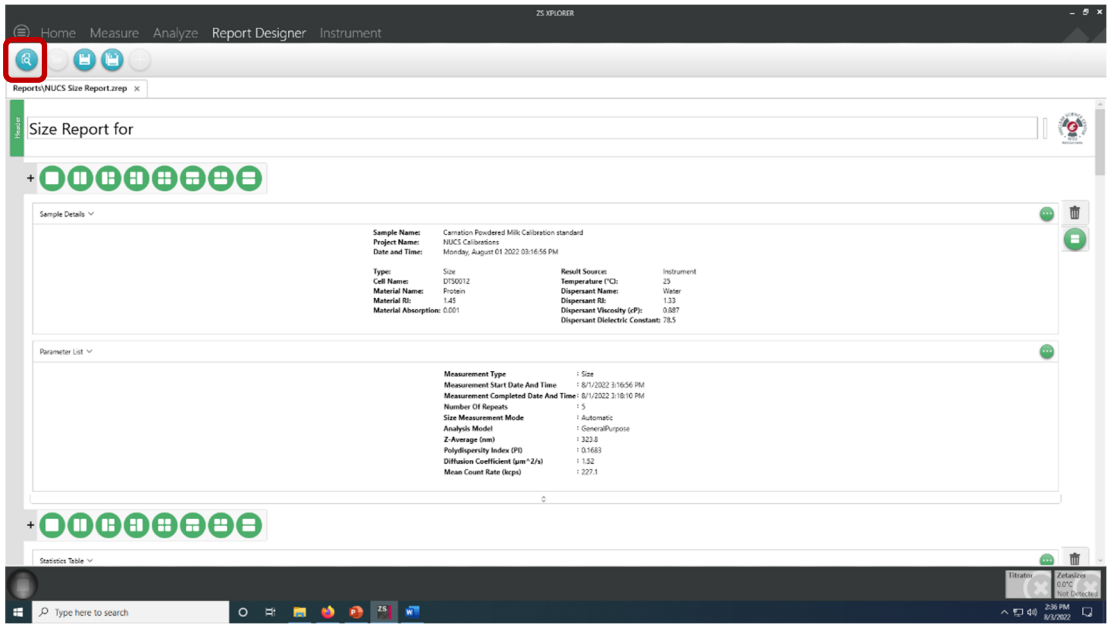
7.15 Custom reports can be designed using the Report Designer tab.



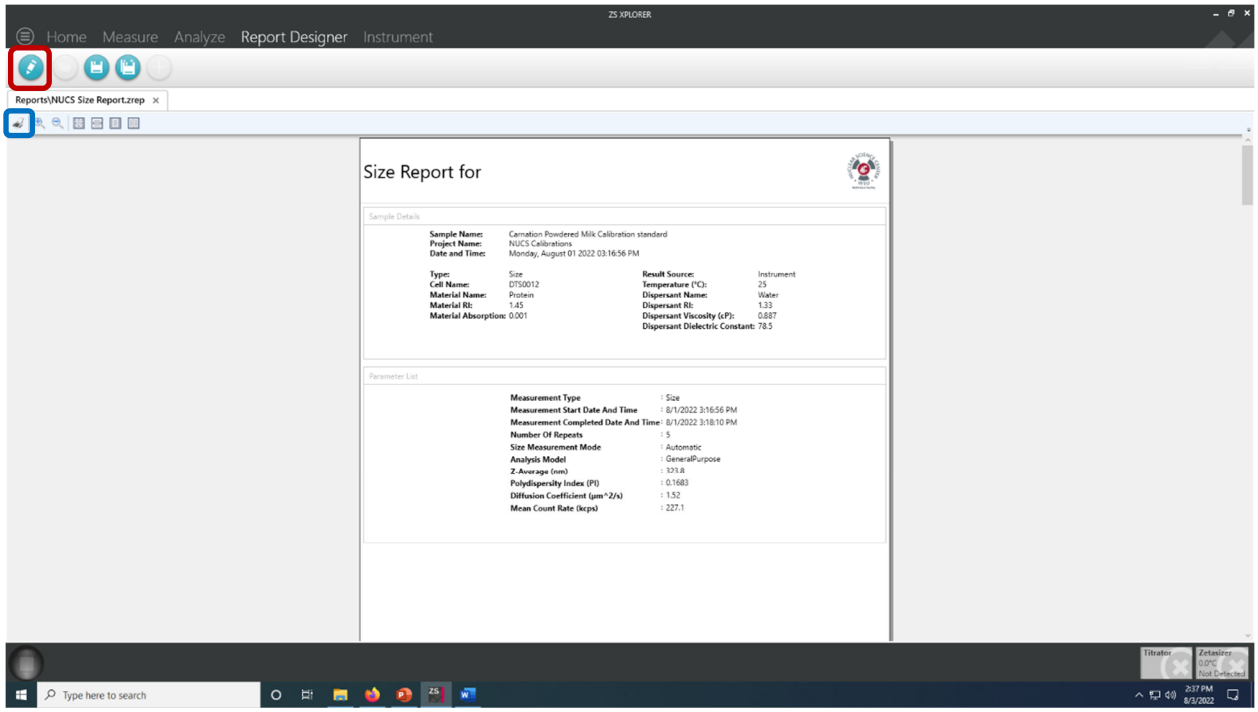
7.16 The NUCS Core Facility has developed a couple of reports to be utilized in data analysis (highlighted in red).



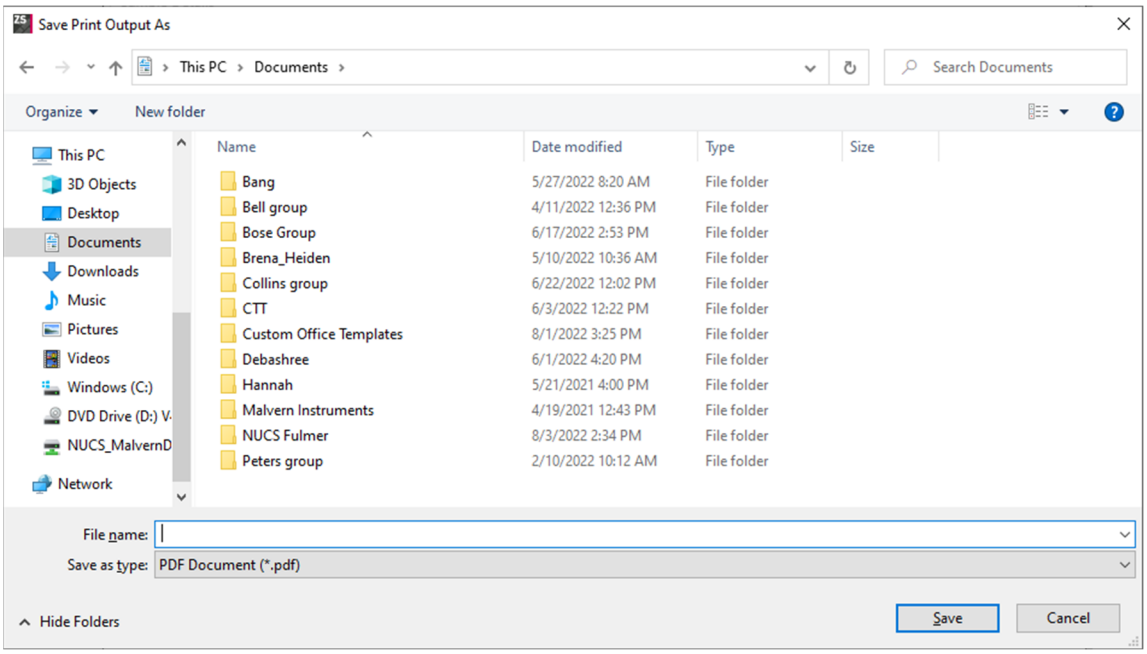
7.17 Opening one of the Report options, when a desired data set is selected, results in the report seen below. The title can be edited to add a sample title. After adding a sample title, click on the magnifying glass icon (highlighted in red).



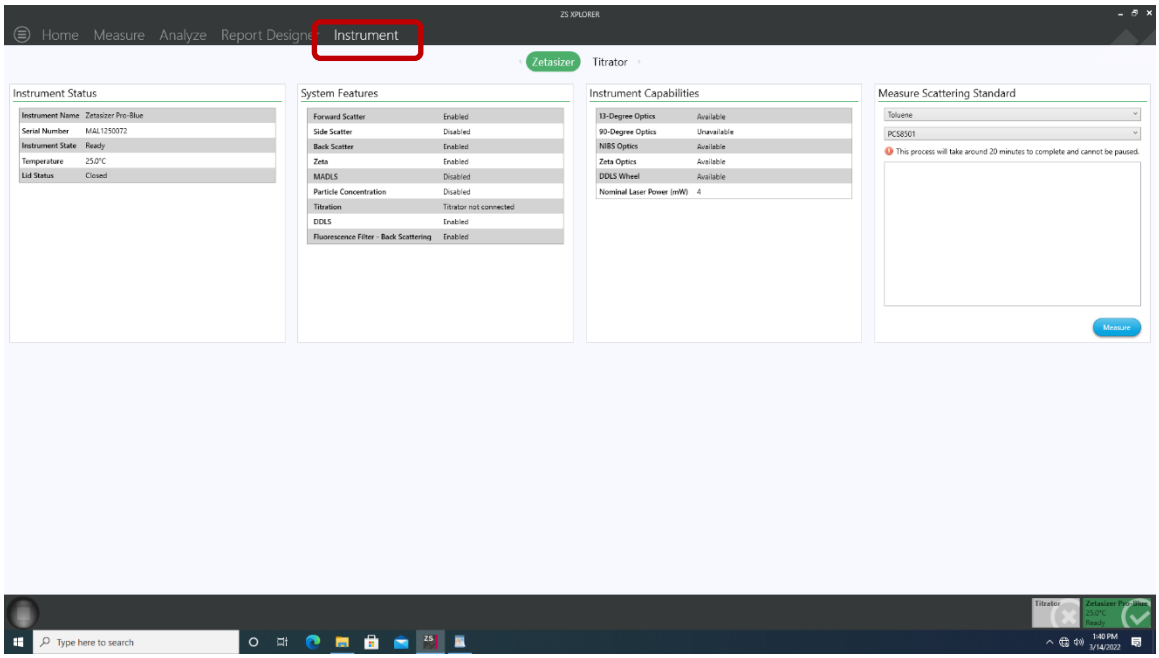
7.18 A sample pdf of the report will be generated. If the report is to be edited, click on the crayon icon (highlighted in red). If the report is to be saved as a pdf, click on the printer icon (highlighted in blue).



7.19 A window will appear to save the pdf. Please save it in the respective file location for the user.



7.20 Clicking on the instrument option provides the information on the instrument status (instrument name, serial number, instrument status, temperature, and the lid status). This section also indicates what features are enabled, available capabilities, and an option to measure the scattering standard.

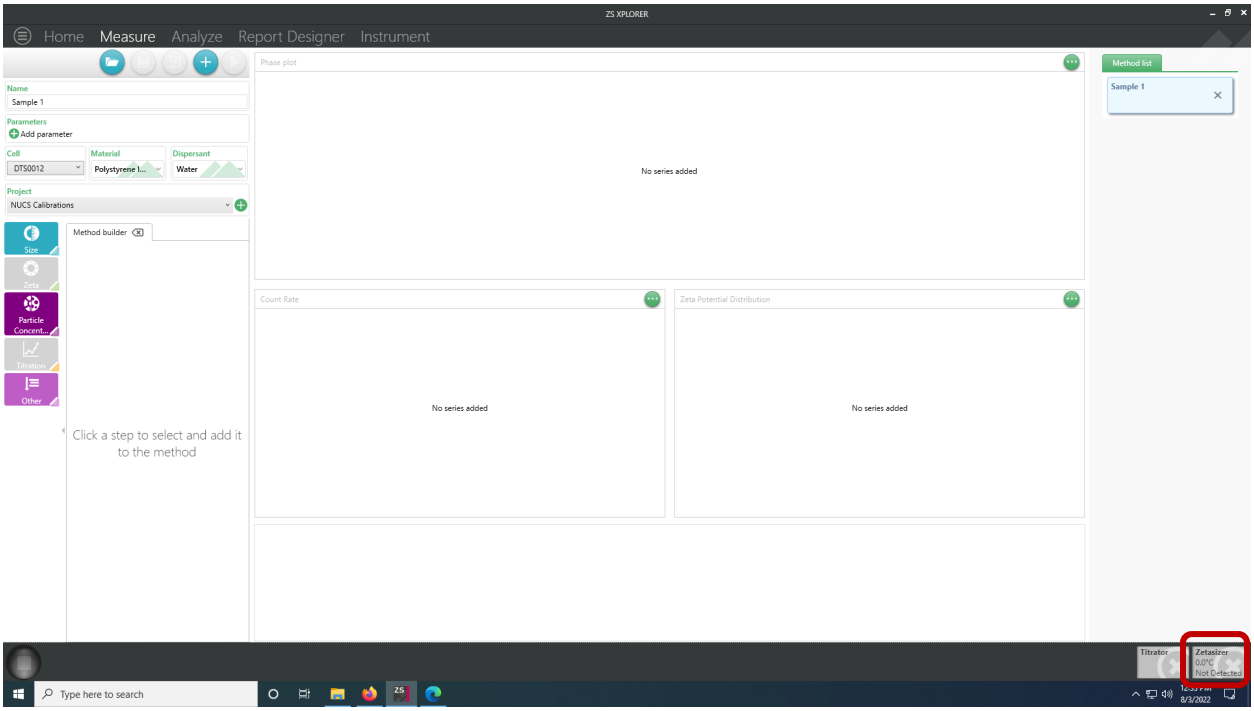


8 Powering Down the Malvern Zetasizer Pro Blue DLS

8.1 To shut down the instrument, press the power switch on the back of the instrument (highlighted in red) on the right side, when facing the instrument.

8.2 The light on the instrument (highlighted in red) will turn off when the instrument is off.

8.3 The software will indicate that the instrument is no longer detected, as indicated by a grayed-out instrument status (highlighted in red).



8.4 Close the software for the Malvern Zetasizer Pro Blue.

9 Training

All users of the Malvern Zetasizer Pro Blue must be trained by NUCS Core Facility staff and have documented training, signed off by a designated trainer for the Malvern Zetasizer Pro Blue, prior to using the instrument. Records of trained users can be found here: Z:\Malvern DLS\Training Records

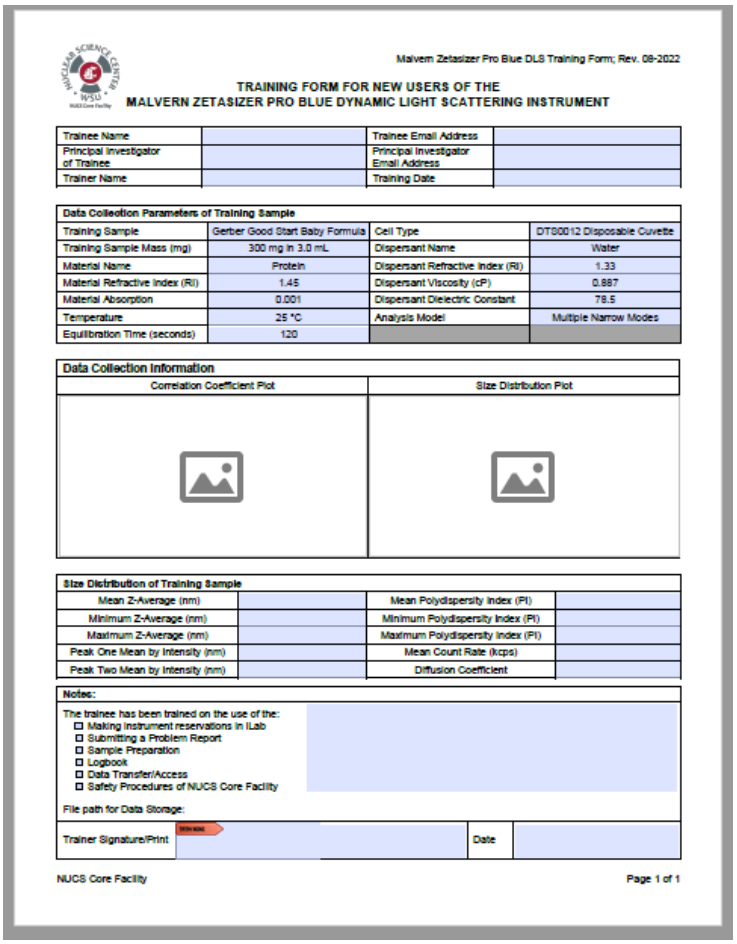
Designated NUCS Core Facility Instrument Trainer for the Malvern Zetasizer Pro Blue

Zach Heiden, NUCS Core Facility, 509-335-0936

Nuclear Science Center Emergency Line: 509-335-0004

Training on the Malvern Zetasizer Pro Blue Dynamic Light Scattering Instrument consists of the safe use of the instrument, collection of a single DLS size dataset on a Gerber Good Start Baby Formula (300 mg in 3.0 mL), data analysis using the DLS software, generating reports using the DLS software, discussion on how to collect a zeta potential measurement, making instrument reservations in iLab, submitting an instrument problem report, powering down the instrument, keeping records of use in the instrument logbook, and data transfer/access.

The form that is completed during a training session is seen below:



Malvern Zetasizer Pro Blue DLS Training Form; Rev. 08-2022

TRAINING FORM FOR NEW USERS OF THE MALVERN ZETASIZER PRO BLUE DYNAMIC LIGHT SCATTERING INSTRUMENT

Trainee Name		Trainee Email Address	
Principal Investigator of Trainee		Principal Investigator Email Address	
Trainer Name		Training Date	

Data Collection Parameters of Training Sample			
Training Sample	Gerber Good Start Baby Formula	Cell Type	DTS0012 Disposable Cuvette
Training Sample Mass (mg)	300 mg in 3.0 mL	Dispersant Name	Water
Material Name	Protein	Dispersant Refractive Index (RI)	1.33
Material Refractive Index (RI)	1.45	Dispersant Viscosity (cP)	0.887
Material Absorption	0.001	Dispersant Dielectric Constant	78.5
Temperature	25 °C	Analysis Model	Multiple Narrow Modes
Equilibration Time (seconds)	120		

Data Collection Information

Correlation Coefficient Plot	Size Distribution Plot

Size Distribution of Training Sample			
Mean Z-Average (nm)		Mean Polydispersity Index (PI)	
Minimum Z-Average (nm)		Minimum Polydispersity Index (PI)	
Maximum Z-Average (nm)		Maximum Polydispersity Index (PI)	
Peak One Mean by Intensity (nm)		Mean Count Rate (kcps)	
Peak Two Mean by Intensity (nm)		Diffusion Coefficient	

Notes:

The trainee has been trained on the use of the:
- ☐ Making instrument reservations in iLab
- ☐ Submitting a Problem Report
- ☐ Sample Preparation
- ☐ Logbook
- ☐ Data Transfer/Access
- ☐ Safety Procedures of NUCS Core Facility

File path for Data Storage:

Trainer Signature/Print		Date	

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