The Dyna Pro NanoStar
(Batch DLS/SLS Experiments)

1. Use disposable cuvettes for samples dissolved in water – only for DLS measurement. Quartz cuvette should be used for SLS measurement and DLS measurement in organic solvents.
2. Clean and dry the NanoStar quartz cuvette- both inside and outside.
3. Quartz cuvette has an anti-reflection coating on each of four windows. Avoid touching the lower windows so as not to scratch or dirty them, in addition cuvette should not be subjected to extreme shocks, either mechanical or thermal. The outside windows should be only wiped with lens cleaning paper wetted with a few drops of solvent. For organic and oily deposits 99.9% hexanes is recommended. For other dirt 99.9% methanol is recommended.
4. Inside cuvette- never let sample dry out without cleaning it. If the cuvette is not perfectly clean between uses, store it in a solvent bath to prevent sample from drying on the surfaces. When cuvette needs to be cleaned it is important to determine the residual materials that must be removed and solvents. After cleaning cuvette rinse it with methanol and nitrogen blow dry.
5. Ultrasonic cleaners can operate at frequencies that a resonant with the cuvette, which can cause to break. Don't use ultrasonic cleaners.
6. If the cap is dirty- clean it with detergent solution, followed by a water rinse, alcohol rinse, and nitrogen blow dry.
7. Check the clean water count rate. The clean water (after filtration) count rate is expected to be equal to the value of water count rate shown on instrument's COP, within 15%. The variability of clean water over the 10 acquisitions is expected to be less than 5%. If it is more – cuvette is dirty. Clean and run again.
8. Open Dynamics.
    - File, New
    - Click the Connect to Hardware button. The Record button on the Experiment Window tool bar will turn green.
    - Open Control Panel to see count rate.
    - To start recording data, click the green button. The button will change to a flashing red.
    - To stop recording data manually, click the flashing red Record button. The Record button face will then change to green.

Measuring the Solvent Offset

- The next step is to determine the scattering from "solvent", without analyte present.
- The solvent scattering will be subtracted from the total sample scattering to determine the excess Rayleigh ratio, from which MW will be determined.
- Select Parameters, Solvent.
- Select the solvent from the down list.

- Defining New Solvent – Select Tools - Parameters - Solvents- select New in the solvent list box. Enter parameters- a name for the new solvent, temp., viscosity, refractive index, Temp. Model- fixed for organic solvents or aqueous for water). Click Save to save the new solvent to the Solvent Database.
- Filter solvent, load ~ 100 µl of solvent into clean and dry quartz cuvette.
- Insert the cuvette into the NanoStar, and close the lid.
- Select Parameters, Cuvette.
- Select your cuvette from the drop down list (for example JC-021).
- Press "Measure Offset".
- The static scattering detector response will be displayed.
- Set the despiking filter such that the standard deviation as a % of the mean scattering is less than 1% then click on OK.
- If the despiking Filter is located at position 5 or lower (refer to the left), we recommend that you clean the cuvette and try again.
- Accept or run again, save, update. Without saving, update is not valid!
- Record the DLS data for the solvent. Take note of the ACF determined for the Solvent to confirm it is particle free. If the Solvent is not particle free, the particles may influence both DLS and SLS results and interpretations.

Measuring Sample

- Concentration: down up to 0.2-0.1 mg/ml. Centrifuge 10-15 min at 3000 RPM or filter. Molecular weight of the sample: M_w from 1000 – 1 mln.
- Using the standard technique, determine the concentration of your sample. The concentration should be determined after filtration for example by UV.
- Insert cuvette into the instrument.
- **Parameters:**

- Fixed: Don't change.
- Instrument – Acq Time = 5. Number Acq. = 10, LASER POWER = 100% or Auto-attenuation = Yes, Attenuation Level – 0%, Set Temp on Connection- Yes, Set Temp = 25, Temp Ramp Enabled – No, Temp Range Rate – 0, DLA ONLY – No.
- Solvent: choose correct solvent. If you need to add solvent go to Tools-Parameters-Solvents- The Edit Solvents window is displayed. Select New option in the Solvent list box. Enter parameters for your solvent. Click Save.
- Sample: Rg Model; globular- proteins, PS (linear polymers) etc, concentration (mg/ml), dn/dc ml/g (polystyrene = 0.11 or proteins = 0.185). dn/dc can be measured by Optilab rEX. A2 (second virial coefficient) high conc. > 10 mg/ml (aggregation), choose Rg model – spheres, random coil or star.
- Don't change – User Defined, Names.
- Choose cuvette and solvent. See Offset Measurement. Always use disposable cuvette with a holder. Don't use a holder for the quartz cuvette.

- Press the RECORD button.

Results

- Review data.
- Are the majority of acquisitions unmarked?
- Are the results consistent among all acquisitions (%S- standard deviation less than 10% for Normalized Intensity, R, MW-S)?
- Is the Average ACF valid? If the ACF indicates a dirty cuvette or sample, clean the cuvette and filter sample or spin and try again).
- Is the Mass-weighted size distribution monomodal and homogenous?
- The DLS data indicate a homogenous size distribution, with %PD (the size distribution algoritm) less than 15% as determined by the cumulant algorithm. The size distribution algorithm detects a trace amount of large non-specific aggregation (0.1% estimated % mass).
- The results are consistent with the estimated MW-R determined from the measured Radius.
- Save you measurement into DLS_SLS Results- into yours file.

Column Heading Options

#Peaks - The total number of regularization view peaks, including those that may be excluded by setting the upper and lower limits for regularization view peak display.

#Peaks in Range - The number of peaks within the exclusion range, which will be the number of peaks displayed in the regularization view.

%Pd - The polydispersity divided by the estimated hydrodynamic radius from the cumulants fit of the autocorrelation function multiplied by 100. %Pd should be less then 15%. Detects a trace amount of large non-specific aggregation (0.1% estimated max).

% Acquisitions Unmarked - The percentage of acquisitions that are unmarked in a measurement. This provides a quick view of the quality of the sample. Samples that are homogeneous and stable typically will have 100% or nearly 100% unmarked acquisitions.

A2 - For the DynaPro NanoStar only, the second virial coefficient for the sample (in mol-mL/g2). This is a thermodynamic term which is indicative of solvent-solute interactions. Positive A_2 indicates a high affinity for the solvent.

Acq Time - The integration time for each correlation function in the measurement.

Amp - The amplitude of the correlation function at zero delay time.

Attenuation Level - For the DynaPro Plate Reader Plus only, the attenuation of the signal seen by the beam collector.

Baseline - The measured value of the normalized intensity autocorrelation curve at the last channel used. Values of 1.000 indicate that the measured correlation curve has returned to the baseline within the time encompassed by the defined number of channels. Deviations from the theoretical value of 1.000 indicate either a noisy baseline or a range of correlator channels that is too small.

Col - The column position of the Plate Reader well is logged. The positions begin counting from zero rather than 1. For informational purposes only.

Conc - The concentration of the sample. For informational purposes only.

D - The translational diffusion coefficient.

Date - Lists the date when a measurement was taken.

Diam - The diameter of the particle in nm, determined by doubling the hydrodynamic radius estimate from the cumulants fit of the autocorrelation function.

Disposable Cuvette - Records that a measurement was taken using a disposable cuvette.

dn/dc - The refractive index increment for the sample.

Forward Monitor - For the DynaPro NanoStar only, the voltage reading of the detector directly across the sample from the laser. When compared with the Laser Monitor signal, it can provide a measurement of light absorbance by the sample.

Intensity Std. Dev. - The standard deviation (Cnts/s) in the measured intensity.

Lambda - Fit parameters from Cumulants analysis (1/sec). For details, please refer to Equations (3), (6), and (7) in Analysis Methods.

Laser Monitor - For the DynaPro NanoStar only, the voltage reading of the detector reading the laser intensity prior to entering the sample cuvette.

Laser Power - The percentage of maximum laser power used for the measurement.

MW-R - The molar mass estimated upon the particle conformation, size, and density. From DLS measurement.

MW-S - The mass derived from the static light scattering sensor in the DynaPro NanoStar From SLS using root mean square radius-radius of gyration – Rg).

Normalized Count Rate - For the DynaPro Plate Reader Plus only, it is the intensity divided by the laser power which is multiplied by the attenuation level.

Normalized Intensity - The intensity (counts/sec) after correcting for variations in laser power and attenuation.

Normalized Static Scattering Detector - For the DynaPro NanoStar only, the static scattering detector voltage, corrected for variations in laser power.

Pd - The polydispersity, or width of the distribution, in nm determined using a Cumulants analysis.

Pd Index - The polydispersity index based on a Cumulants analysis. This is comparable to the distribution width divided by the mean.

Peak # - The regularization Radius calculation for a user-defined peak range.

Peak # %Int - The light scattering signal intensity of the specified peak divided by the total signal intensity of the measurement multiplied by 100.

Peak # %Mass - The estimated total mass of the particles in solution corresponding to the user-specified peak divided by the estimated total mass of all particles in solution from the regularization data.

Peak # %Pd - The percent polydispersity within a user-defined peak.

Peak # MW-R - The estimated mass for the peak based on the estimated hydrodynamic radius, particle density, and conformation model from the regularization fit.

R - The estimated hydrodynamic radius based on the cumulants fit of the autocorrelation function. (nm). From DLS measurement.

Row - The row position of the Plate Reader well is logged. The positions begin counting from zero rather than 1. For informational purposes only.

RMS Error- The root-mean-square error in the cumulants fit of the correlation function.

Set Temp - The set temperature for the temperature-controlled MicroSampler.

Sigma - Fit parameters from Cumulants analysis ($1/sec^2$). For details, please refer to Equations (3), (6), and (7) in Analysis Methods.

Solv Int - The count rate from the solvent alone. For informational purposes only.

Solv Rfr Idx - The solvent refractive index. For informational purposes only.

Solv Visc - The solvent viscosity in centipoise.

Solvent - Lists the solvent used for each measurement

SOS - The sum-of-squares from the correlation function fit. Value < 20 indicate reasonable agreement.

%S – Standard deviation (should be less than 10%).

Temp - The temperature of the measurement in Celsius.

Temp Ramp Rate - The speed at which temperature changes in time (C/s).

Temp Std. Dev. - The standard deviation (C) in the measured temperature.

Time - The time at which the correlation function was measured from the start of the measurement.

Time Stamp - The time at which a measurement was taken.

Viscosity Temp - The temperature (C) for which the solvent viscosity property is valid. If collecting at a different temperature while using the Aqueous model, the viscosity in calculations will be adjusted for the difference in temperature.

Vol - The sample volume in milliliters. For information purposes only.