NANOSTAR DLS notes

<u>Samples</u>

The DLS can distinguish between particle populations only if their radii differ by a factor of 5. You won't see monomer/dimer/trimer distinction, but you will see if there are large aggregates of your protein, even if these represent only 1% of the particles present.

If you have a population of particles whose radii are 1 and 1.5, the instrument will report a single radius value somewhere between those two values but will indicate a polydispersity of >15% which indicates heterogeneity. Two peaks of widely different radii will have low polydispersities if they are both homogeneous.

Doing a buffer blank may reveal that a component (like DTT) has a peak. You just need to know it's there. You can't subtract it. DTT will add some viscosity, so your particle size may be apparently larger than you were expecting.

There will be a 1% increase in viscosity per mg/ml concentration increase. There will be a 1% increase in translational diffusion per degree of temperature increase.

Having the y axis on % intensity highlights aggregates (emphasizes species producing strongest scattering signal) while having it on % mass highlights monomer (emphasizes most prominent species mass-wise) and having it on % number emphasizes the most abundant species number-wise. Wyatt recommends that you use % intensity. You can switch from % intensity to % mass and % number after the data has been collected.

There is an optimization calculator under Tools. If you know the molecular weight of your particles, it will give you an R value dependent upon the model you choose so you can try on the different models before you do an experiment.

Organic solvents scatter light more than aqueous media.

If you work at higher temperatures, the DLS will compensate for your solvent's viscosity only if it's aqueous. If it's Fixed, you can enter the higher temp. viscosity value (if you know it) by entering that data as a separate solvent.

Doing a series of dilutions can give you information on your aggregation state. See the LSU training notebook, section 5, page 19.

Laser

The laser has auto-attenuation to adjust itself to 1-2 million counts. If it can't attenuate and the green light comes back on, that means you need to spin, filter or dilute your sample.

With the laser off you will see 100-1,000 counts.

The laser in the DLS is at 658 nm.

Cuvettes

The area being read will be the same for either type of cuvette so the readings should be equivalent in both.

The quartz cuvette holds 2 ul in its tiny chamber so run 5 ul or so of sample down the side of the cuvette to load it. Check carefully for air bubbles each time. The quartz cuvette can be used for either regular DLS or static light scattering. (See Static Light Scattering Notes.) The temperature maximum for the quartz cuvette is 150°.

The plastic UVettes hold 50 ul minimum volume but you can add up to 2 ml of sample. The can be used for DLS measurement only. Their temperature limit is 70°.

The special small plastic Wyatt cuvettes hold 4-70 ul sample and will have about 20K counts when clean. They are optically clearer that the UVettes so may work better for problem samples.

Acquisition time and Autocorrelation Curves

A good acquisition time to start with is 5 sec. Longer readings make it more likely that you'll hit dust particles. Four suddenly low readings mean that dust came by and shut down the laser.

If you have aggregates, shorter acquisition times will help you miss them.

Autocorrelation measures how similar the light intensity fluctuations are to themselves over time. It's a general measure of how good your data is. Autocorrelation curves for larger particles take longer. If the autocorrelation curve doesn't reach 1, you have aggregates.

Data and Models

Red values may not show up during live acquisition but after all acquisitions are collected. That's random.

To see a curve instead of a histogram, uncheck Plot Histogram on the R of the screen.

To zoom in on a data area, hit Ctrl/L mouse button. To undo, R click, then Autoscale.

To print any or all of the graphs produced, right click on them to get the Copy function. You can paste them into Word, Excel or PowerPoint and print that. You also have the choice of making a PDF of them.

To export raw or processed data, R click on the view you want, then choose Export. Exporting the raw data will give you 14 pages so copy/paste that instead. .csv files can be read by Excel.

You can change the experiment name by R clicking on Measurement 1 in the tree to the left. To overlay experiments, go up higher than both of them in the tree then click on the Autocorrelation Curve icon or the Regularization View.

In the datalog grid view, all acquisitions and the lower table are fit to a single exponential. (cumulants fit, can get skewed) In the regularization fit view (with the histogram), those values have been fit to multiple exponentials.

Polydispersity is standard deviation.

To remove a single acquisition, go into the raw data table and R click on the number of the bad acquisition. This should turn the whole line blue. Then mark it (R click on it and choose from the drop-down menu) and its font should turn red. This removes it from the data set for the group histogram.

When you close a data file, the bad data gets incorporated into the statistics, so something that looked good in summary (with the red values ignored) will look crummy when you re-open the summary. (different R values, for example) So the trick is to save the good summary to PPT before closing the software. Or when you re-open the software, go back into the raw data and take out the red values again.

To see the diffusion coefficient of your sample, go to the datalog grid view, R click and select Table Settings. Highlight "D", click the Add button, then OK. This should add a diffusion coefficient column to the table.

If you have large particles (>50 nm) like liposomes or viruses, the model-dependent fit will be % mass and choose Isotropic spheres rather than Rayleigh.

To see the different R_h factors used for the different models, see the LSU training notebook, section 7, page 25.

Software-related
To add a new solvent to the drop-down list, you need to know its refractive index and viscosity at 20°. Then go to Tools, Parameters, and Solvents to add those values. Click on the Add button then enter the solvent's name. Double click in the RI box to change

the value. Do the same with the Viscosity. The Temperature Model should be changed to Fixed if the solvent is not aqueous. Then click OK.

All versions of the Dynamics software are compatible so old data can still be loaded.

The NanoStar User's Guide is on the desktop. The Dynamics User's Guide can be found under Programs, Wyatt Technology, Dynamics 7.1.4.3, Dynamics User's Guide. (PDF)

You're welcome to take the Dynamics 7.1.9.3 software with you on a flash drive so that you can work on your data elsewhere. Take the .exe file and the autorun file. (in the WTC folder under Program Files x 86) Get the Activation Key from Debby.

You cannot change the x-axis in the histogram view to be linear.

To save default instrument settings after changing them, go to File, Save settings.

Troubleshooting
To see the correlation curve even if all values are red, after acquisition is complete:
- Click on the plain grey box in the upper left corner of the data table. Things turn blue.
- Right click on the table and choose Unmark All. This turns the values black.
- Now you can click on the correlation curve or histogram icons to see your data.

If you get a peak of 0.1 nm, 0 MW, 100% mass, it might be:
- water. The instrument tries to fit something. An after-pulsing of the detector causes what you see. To remove it, move the L hand truncating line on the correlation curve to the R some.
- salts from your buffer. It can see the salt particles themselves. Since there are more of those than protein molecules, it tries to fit them. To remove that peak, just uncheck it in the summary table and everything will adjust.

If there are no values at all in the Raw Data window that means the sample is so messy, the DLS can't resolve anything and has given up. Spin or filter your sample.

Unstable readings giving baseline values of 1.4 – 1.5 were once the result of something gray and blotchy on the inside of the cuvette walls. Cleaning the cuvette with detergent and a tiny sponge-on-a-stick solved the problem.

If connection fails between the DLS and computer, close the software and restart the computer. If that doesn't work, see the DLS Connection sheet hanging from the shelf.

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