Basic Protocol for Dynamic Light Scattering

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General Rules

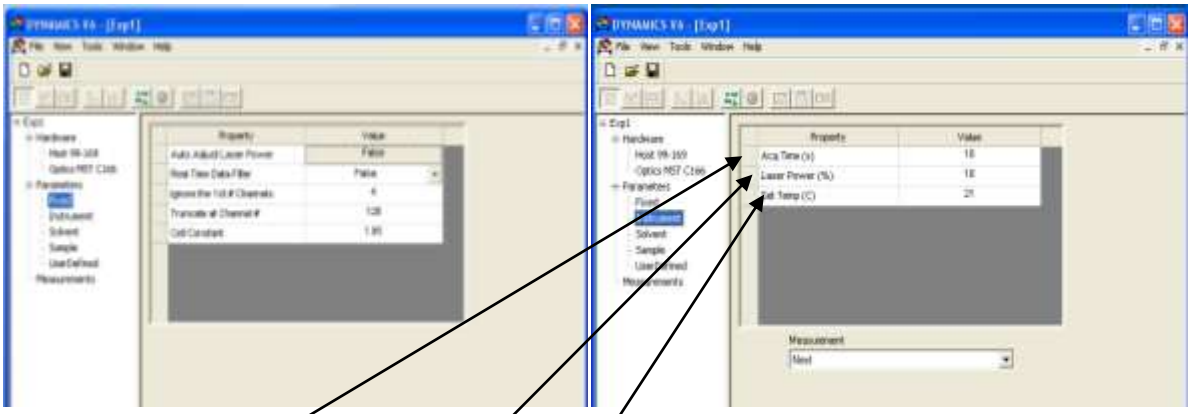
- This protocol provides step-by-step instructions for preforming a basic DLS experiment assuming you have been trained on the instrument by Dr.Erbse or Nicole Kethley. **Reading the protocol does not replace the training with Dr.Erbse or Nicole Kethley.**
- **All new users must complete a short training session with Dr.Erbse or Nicole Kethley** (Contact info above) before they are allowed to use the DLS instrument independently.
- After training you will receive access to the DLS Google Calendar. All users should use the DLS Google Calendar to reserve instrument time and should log into the logbook at the beginning of each use.
- Most cuvette manufactures have stopped making 12 µl cells. Users should treat the shared 12ul cuvettes with **extreme care**. Failure to clean and handle the cuvettes correctly will result in losing the privilege to use them. Broken cuvettes must be replaced by the user.

Experiment

1. **Switch on the host and the temperature controlled sample unit. Set the desired temperature (range 4-60 °C). Give the laser at least 20 min to warm up.**
2. Start the software (Dynamics V6.3.40) 
3. The Dynamics window opens.
4. Create a new experiment (or load an old one if you have one with the right parameters).
5. Check the parameters:

   Under “Hardware” you should read:

Under Parameters:

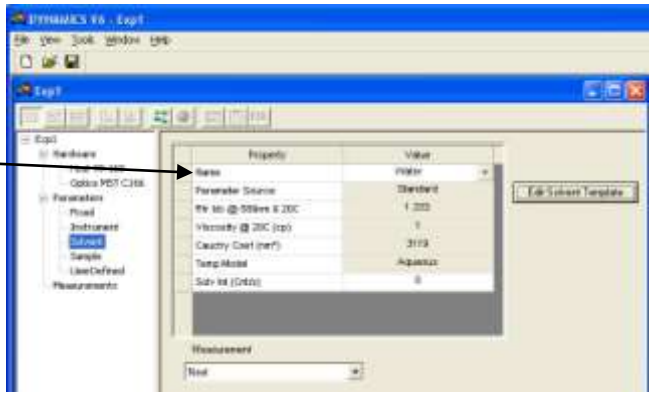


You can change AcqTime, Laser Power and Set Temp here. Good start values are Acq Time 10, Laser Power 10, and Set Temp (C) 21, they can be changed as required later.

Under Solvent:

Choose your buffer here,

Rfr Indx. and viscosity <u>should</u> change as you choose your buffer conditions. If your conditions are not included, you should add them to the list.

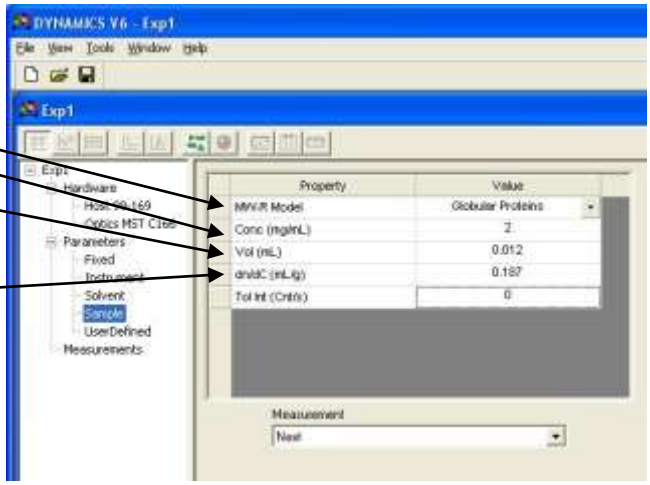


Under Sample:

Choose the model you will use here (Globular Protein for protein work), and your concentration

Make sure the volume is set to .012mL (12ul).

If you want to calculate molar mass enter your dn/dC (dn/dC (mL/g) = 0.187 is right for protein).

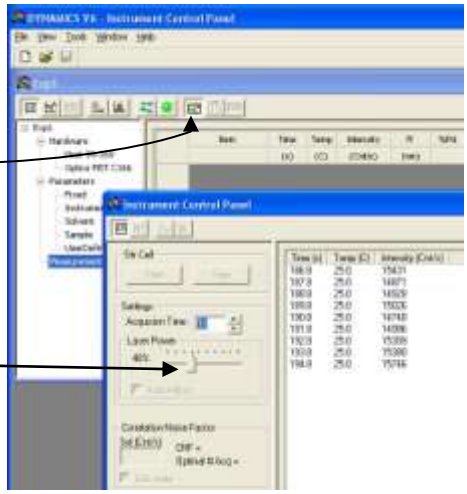


Under User Defined:

You can add additional infromation.

6. Click the connect to the instrument button. 
7. The round button next to it should turn to green. 
8. The instrument should now ramp up to the set temperature.

9. Check the readings coming in by clicking the Instrument Control Panel. A new window opens displaying the incoming readings. <u>You can use this window to control laser power and acquisition time</u>. **Without a cuvette in place, readings should be 20,000 cts/s with the laser power at 10%.** If they are higher, there is a glitch in the system. Reslect the laser power by clicking on the slider and set the laser power to 10% again.



Prepare Your Samples

10. A protein concentration of 1 – 2 mg/ml is a good starting range. For smaller proteins, better readings are obtained with higher concentrations.

11. It is extremely important that your samples are free of any particulates like dust, free of unwanted aggregates, and free of bubbles.

12. Filter your samples, buffer, 100% EtOH, and MQ H_2O through <u>0.1 µm filters</u> (spin filters for small sample volumes, syringe filters for intermediate volumes of filter discs or for filtering large volumes of buffer). Also make sure that all your sample vials and buffer bottles are dust free.

13. When filtering is not possible due to adverse effects of the filter on the sample (shear forces and binding problems) spin the sample for 15 min in an ultra-centrifuge and only take the top third.

14. To avoid the formation of air bubbles, degas your buffer and give the samples time to reach the desired temperature. This is especially important for samples on ice or samples to be tested at temperatures higher than room temperature.

Buffer Control and Water Control

15. Prepare the cuvette. Again, the cuvette must be clean and dust free. Shared cuvettes should be stored clean, dry and closed on the shelf above the DLS. <u>Don't rely on them being clean.</u> You will measure a water and buffer baseline before you measure your sample. To be sure that the cuvettes are clean and dust free you can rinse them first with filtered water (3 times), then with absolute EtOH (3 times). Dry them by holding them upside down and **gently** blowing into them using compressed air from a can. **Do not use house compressed air**; it can contain oil/dust from the compressor and will contaminate the cuvettes strongly. Once the cuvette is dried keep it closed to prevent dust from forming.

16. If you use a 12 µl cuvette fill it with your filtered H_2O, using a long gel loading tip. Do not use glass transfer pipettes or steel needles to avoid scratching the quartz windows of the cuvette. If you use a syringe filter system be **extremely** careful. Use no more than 14 µl and go slow observing through the window to avoid trapping bubbles in the active cell

volume. 14 µl is enough to fill the sample compartment and to avoid bubbles at the top of the window. Using more increases the chances of washing dust from the top part of the cuvette into the active volume of the cuvette.

17. Close the cuvette to prevent dust from getting in.

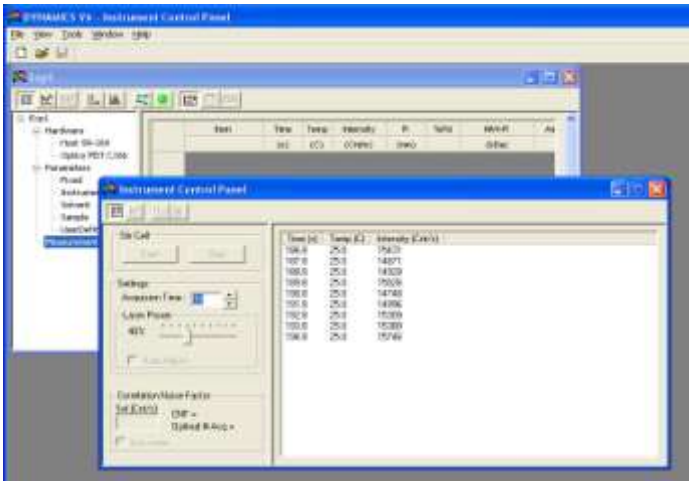
18. Wipe the cuvette from the outside with lens paper to remove any dust**. Do not use Kimwipes** it can scratch the quartz windows of the cuvette.

19. Insert the cuvette into the sample holder with the frosted side to the left!

20. Give the sample at least 5 minutes to equilibrate.

21. After equilibration check the readings that come in by clicking the Instrument Control Panel  (icon to the right of the green light).

22. A new window opens displaying the incoming readings:



You can use this window to control laser power and acquisition time. Slowly increase laser power to 40%. **If the values are higher than 2,000,000 cts/s disconnect immediately, these readings are high enough that they can damage the detector (avalanche photodiode)!** A clean cuvette filled with filtered, bubble free H_2O should have readings between 15,000 and 20,000 cts/s.

23. Repeat steps 15-22 with your filtered buffer.

24. If the values look consistent start data acquisition by clicking the green icon  . It will turn red and blinking as the data acquisition is starting.

25. Do at least 10 acquisitions for the buffer control. If the values look good you can stop by clicking the now red icon.

26. If the data is good you can rinse the cuvette with filtered water and absolute EtOH and dry it as before. You are now ready to measure your sample.

27. If you plan to use more than one cuvette, test them all.

28. If the data looks suspicious and you are sure your buffer is dust and contamination free, clean the cuvette as described and try again. If you suspect your buffer is the issue, test with filtered miliQ water.

Sample

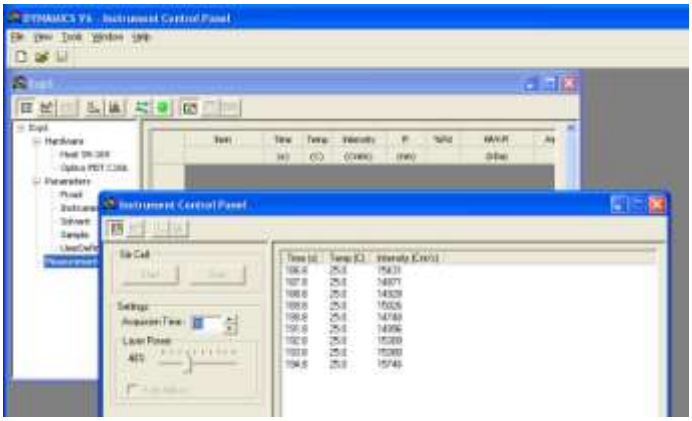
29. For best results use the same cuvette for buffer and samples that you want to directly compare.

30. Load your sample in a dried, clean cuvette using a gel loading tip, avoiding bubbles as described above.

31. Close the cuvette insert it into the sample holder with frosted side to the left and give it 5 minutes to equilibrate.

32. Check the readings that come in by looking at the Instrument Control Panel.

33. A new window opens displaying the incoming readings. Remember, You can use this window to control laser power and acquisition time.



Values of around 150,000 to 600,000 cts/s are good. You can adjust the laser power to increase or decrease the values. Do not go below 10% laser power.

If the values are higher than 2,000,000 cts/s and reducing the laser power to 10% does not reduce them under 2,000,000 cts/s, disconnect immediately, these readings are high enough, that they can damage the avalanche diode!

34. If changing the laser power does not give you a good range for the readings, you have to decrease (to high values) or increase (to low values) your sample concentration.

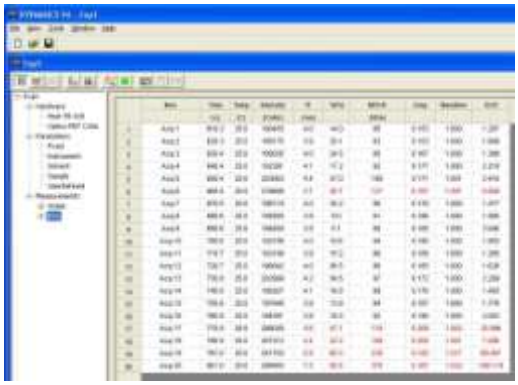
35. If the readings look good, start data acquisition. Do at least 20 acquisitions. Sometimes tiny bubbles can diffuse through the light path and skew some of the acquisitions. You can exclude those from the data evaluation later.

36. If you experience difficulties with your sample but a water control was fine, try re-filtering or re-centrifugation. Make sure your buffers are degased to avoid bubbles.

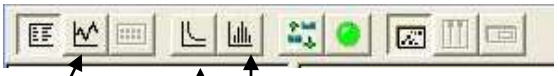
37. Sometimes it helps to simply tap the cuvette gently a couple of times on the bench to dislodge tiny bubbles.

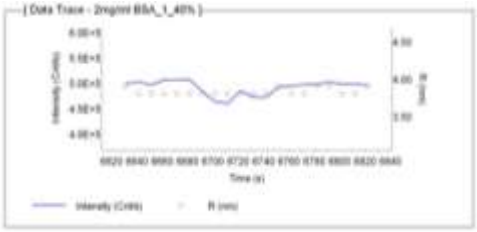
38. Before you shut everything off, do atleast 10 aquisitions of the cuvette after cleaning. Fill the cuvette with filtered H_2O to obtain 10 data acquisitions proving you left the cuvette clean **and** save it as “Water_After_Cuvette #”

39. Below is a typical result measured with 2mg/ml BSA, 40% laser power at 20 ^{o}C.



40. The red marked readings were excluded from evaluation. The high values indicate an air bubble.
Right click outlier acquisition.
Select Mark.
Selected acquisition will turn red, signifying exclusion.

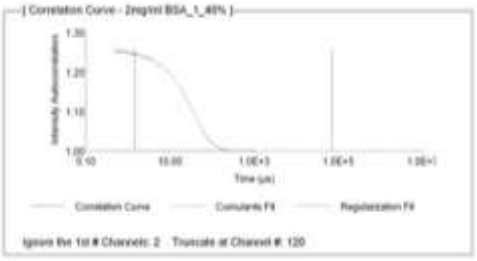


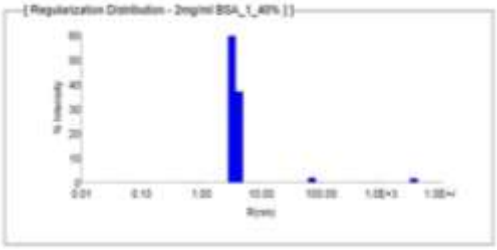


40. View the Scattering Intensities

41. View Correlation Function

42. View Size Distribution





Item	R (nm)	%Pd	MW-R (kDa)	%Int	%Mass
> Peak 1	3.7	14.0	72	98.4	100.0
> Peak 2	71.3	0.0	72997	1.9	0.0
> Peak 3	3580.7	0.0	696206000	1.7	0.0

43. If you suspect the instrument or that you are doing something wrong setting it up, try a BSA standard. You can buy a good BSA standard (2mg/ml) free of particulates from Pierce (http://www.piercenet.com/browse.cfm?fldID=02020108).