

Lab 2, Dynamic Light Scattering  
September 15/16, 2008

**Objective:** The goal of this lab is to use Dynamic Light Scattering (also called Photon Correlation Spectroscopy) to measure the diffusion coefficient(s) and size distribution for several polystyrene systems.

**Safety:** The main risk in this experiment comes from the laser light source. It can damage eyes and vision, so preventing any direct exposure is crucial. A second risk is exposure to nanoparticles from the dispersed polystyrene spheres. Follow these precautions for a safe lab experience:

- Do not look directly at the laser beam.
- Do not position your head in the plane of the laser.
- Do not wear anything metallic, such as a watch, ring, bracelet, earrings, belt buckle, etc. Metallic objects could inadvertently intersect the laser path and reflect the light in a random direction.
- Close the lab door when the laser is on. This prevents any accidental beams from reaching the hallway. Post the “laser light in use” sign on the door.
- Wear laser safety goggles if possible.
- Wear rubber gloves and safety glasses when preparing the polystyrene solutions/dispersions.
- Dispose of the used polystyrene solution in the small waste bottle, which will be indicated.

### Procedure to perform Dynamic Light Scattering

#### *Prepare the instrument*

1. Open the cooling water valve (near the wall) so the valve is parallel to the water pipe.
2. Turn on the temperature controller using the rear switch **and** front switches (3 switches in all).
3. Set the temperature controller value (e.g. 25°C)
4. Turn on the decalin filter and let it run for about 10 minutes. This removes dust from the decalin, which serves as a contrast matching fluid around the sample.
5. Turn off the decalin filter before taking measurements.

#### *Prepare the sample*

6. Rinse the test tube using a small amount of high purity water. Good water is available in the Bose lab (plastic container above the sink) and excellent water is available in the Bothun lab. *Don't use tap water.* Test tubes and lids are available in a far right drawer in the Bose lab.
7. Add about 2 cm of water to the test tube.

8. Add about 6 to 7 drops of the polystyrene solution. Use the standards as directed below.
9. Add a lid to the test tube.
10. Mix the water/PS solution very gently. **Don't** shake vigorously, since foam will disrupt the measurement.
11. Wipe the test tube using a chemwipe. Fingerprints or dirt will impact the measurement.
12. Remove the cap on the sample holder and place the sample in the instrument. The sample will go down so only the lid is visible.
13. Allow time for the sample to equilibrate. At 25°C, 2 to 3 minutes should be sufficient. For a temperature such as 37°C, 10 minutes is more appropriate.

*Choose the instrument settings*

14. The default angle is 90°. We will use this angle unless mentioned otherwise.
15. The laser operates at 514 nm. Choose this setting on the detector.
16. Set the detector aperture to 100 as a starting point. The larger apertures help for samples with poorer signal-to-noise ratios.

*Start the laser*

17. Move the Line lever to up (i.e. on)
18. Turn the key control to On
19. Confirm the sign is on the door and goggles are on
20. Press the power on switch (under INTLK)
21. Wait for the Ready light, above the power-on switch.

*Start and control the software*

22. Turn on the monitor (if necessary)
23. Run the program "bi9" at the "C:>" prompt. (The computer uses DOS, not any version of windows.)
24. Use "set calc" on the number pad to set the experiment parameters. This includes the scattering angle, temperature, viscosity, wavelength, and refractive index.
25. Use "DUR" to enter the length of the data collection period (60 seconds, 90 seconds, etc.)
26. Use "DLY" (delay) and "CHANnel" to modify the range of decay times that are measured by the correlator. The first time is set using the arrow keys (from a limited number of values). The last time is changed by hand.

*Make a measurement*

27. Move the laser control switch from "current" to "light".
28. Turn off the room lights to decrease stray light noise.
29. Hit "start" on the keypad.

30. Watch the kilocount level. The target is 250-300 kCount/s. Use the laser intensity and aperture size to increase or decrease the count rate.
31. If you change the level, stop the experiment (“stop” key) and start again.
32. The experiment will stop automatically after the sampling time has elapsed.
33. Turn the laser from “light” to “current” to not waste laser power.
34. Note the baseline percent difference (between calculated and measured values). If the difference is smaller than 0.10, use the calculated baseline in the data interpretation. Otherwise use the measured baseline.

#### *Interpret and save the data*

35. Hit “calc” on the number pad for data analysis.
36. The decay time, diffusion coefficient, and diameter are provided. Record these.
37. Use **S**ave to save the correlation function results.
38. Use **D**istribution analysis to process the data further. Some of the techniques we will use include Cumulant analysis and NNLS (nonnegative constrained least squares)
39. Note the sizes, size distributions, etc. View the **R**esiduals to view the difference between the measured correlation function and the fit.
40. Hit “clear” to prepare for the next measurement. Hit “func” to choose an option to reload data from a saved file.

#### *Changing the sample*

41. Turn the power off to the laser (middle button).
42. Turn the room lights on for convenience. Remove the sample and put it in the waste bottle.
43. Prepare the new sample and put it in the instrument, as directed above.
44. Turn on the laser and check the kCount/s, as directed above.
45. Conduct the experiment as directed above.

#### *Finishing the experiments*

46. Turn the power off to the laser (middle button).
47. Turn the key control off and move down the Line lever.
48. Turn off the temperature controller (3 switches).
49. Turn off the cooling water.
50. Use “ESC” to leave the software program if there are not any more measurements or interpretations to do.

## Experiment 1: Results for a known polystyrene standard

The goal of this experiment is to obtain experimental results for a fresh polystyrene standard of known dimensions (30 nm spheres).

Prepare the sample as directed above. Use the 30 nm standards. These have been purchased recently and are in good condition. Measure the dynamic light scattering (i.e. the photon correlation function) at  $90^\circ$ . Record the results (relaxation time, diffusion coefficient, size) that are provided immediately after the experiment. Save the results so you can use them later on your own.

Next, analyze the results using the cumulant analysis and NNLS methods; the latter provides a distribution of sizes. Look at the residuals after each calculation. They should appear random if the calculated fit is good. Clear patterns in the residuals indicate experimental artifacts such as convection, poor heat transfer away from the laser beam (perhaps the beam is too intense), or otherwise poor matches between the fitting function and the experimental data.

Repeat the measurement on the same sample, but now use two other sampling times (one shorter and one longer). Save the data in each case. How do the results depend on the length of the experiment?

In your lab report, you will show how the scattering correlations consistent with your interpreted results (i.e. the molecule size) compare with the data reported by the instrument:

$$\langle I(0)I(t) \rangle = \langle I \rangle^2 + (\langle I^2 \rangle - \langle I \rangle^2) \exp(-\Gamma t) \quad (1)$$

where the relaxation time  $\Gamma = -Dq^2$ . The magnitude of the scattering vector  $q$  is calculated from the wavelength  $\lambda$ , index of refraction  $n$ , and scattering angle  $\theta$  as  $|q| = (4\pi n/\lambda) \sin(\theta/2)$ .

## Experiment 2: Effects of instrument parameters

The goal of this experiment is to learn about how instrument parameters affect the measurement.

Continue to use the same sample of diluted 30 nm polystyrene spheres in water. Use the method of experiment 1, but use several scattering angles other than  $90^\circ$ . At each angle, record the relaxation time, diffusion coefficient, and size that is reported initially. Save the data in each case. It may be necessary to tune the laser power and/or aperture size to maintain the desirable count frequency.

How does the signal intensity (kilocounts) and signal-to-noise ratio change with scattering angle? Are longer or shorter experiments required to achieve comparable noise levels? In your lab report, interpret the relaxation time results according to the expected scaling between the relaxation time and scattering angle:

$$\Gamma = Dq^2 \quad (2)$$

How does the value of  $D$  calculated in this manner compare to the result obtained in experiment 1 when using only the  $90^\circ$  scattering angle?

### **Experiment 3: Polydispersity, part I**

The goal of this experiment is to learn how polydispersity in the sample affects the measurement and results.

Choose a different polystyrene standard for making your sample. The other standards are older and are no longer necessarily at the sizes claimed on the packaging. Prepare the sample using the method above, and measure the photon correlation function at  $90^\circ$  scattering. Tune the count frequency and experiment sampling time as necessary. Save the data so you can also analyze it outside of the light scattering software.

Perform the same kinds of data analysis as in experiment 1. How do the size results compare with the nominal size of the polystyrene? How does the size distribution compare to what you measured for the 30 nm sample? Are the residuals random for each analysis method? Try out other analysis methods if time permits. Do they improve the agreement? Record the size distribution (from NNLS).

### **Experiment 4: Polydispersity, part II**

The goal of this experiment is to learn further how polydispersity in the sample affects the measurement and results.

Prepare a new sample for which half of the sample is 30 nm sphere standards and the other half is the size you used in experiment 3. Repeat the experiment 3 procedure using this new sample. How do the analysis results compare to the results you would expect based on the sizes that you measured for the two individual samples? Save the experimental results so you can compare the fits determined in this experiment with the fits you would obtain using a combination of the fits based on the distributions in experiments 1 and 3.