

SLS Manual

1. Prepare 1 mL of the solution: ~40 mg/mL (must be purified same way as for DLS- see DLS manual).
2. Prepare 2 cells- with your solvent and with benzene.
3. Before measurement turn ON laser for 10 min. Open SLS program on the computer.
4. **'A' Dark Count Rate: - Experimental Parameters** command button in the lower part of the screen. Block the light to the PMT. Enter 10 seconds in the **Duration/Repeat** field. Click on the **Dark Count Rate** command button. The dark count rate is the total photons counted divided by the duration in seconds. The units are counts/second, abbreviated cps. Click **OK** command button and notice that the measured dark count rate **is automatically** transferred to the appropriate field. Since the dark count rates are normally small, the fluctuations are large. Therefore, counting for ten seconds yields smaller fluctuations, and is easier to estimate the true dark count rate (359 cps is ok). If it is too high - turn off light.
5. **CORRECT PINHOLE:** Click on the **Set Detector Angle**. Turn arm to 90 degree. Try pinhole 1, 2 and 3 with duration = 0.1 s for your concentration. Click on the Dark Count Rate should be ~ 100 000. No more than 500 kps.
6. **Open Experimental Parameters-** system has inserted **'A' Dark Count Rate** which was measured before- for example~ 359 cps, **'B' = 0**, **Duration/Repeat = 0.1 s** (for measurement and 10 s for calculation of the dark count rate), Number of Repeats = 10, Dust Rejections Ratio = 1.33. Dust Rejections Multiplier = 3, Pinhole = 2 (you should check it), **Polarization Analyzer = None**, **Interference Filter = OUT**. Click **OK**.
7. **Sample Parameters** command button in the lower part of the screen. Fill in the **Sample Identification, Operator Identification, and Notes fields**. Select a liquid from the pull down box or select Unspecified and then fill in the refractive index for the Sample Liquid. Fill in the values for the Sample Cell (typically ~ 1.5, Mark- 1.331) and the Refractive Index of **Vat Liquid = 1.4740 (for decalin)**.
8. If you click on APPLY REFLECTION CORRECTIONS, then the refractive indices are used to correct for the fact that a measurement at any angle also includes a small fraction of the intensity measured at the supplement of the angle. This is due to the light being reflected at the cell/liquid and cell/vat-liquid interfaces. When the sample liquid and vat liquid are both organic liquids, with refractive indices in the range of 1.45 to 1.55, the reflection correction is typically insignificant. When the sample liquid is water (ref. index 1.33), the reflection correction is small, but worthwhile making. Because the reflection correction is small, it is not worth the effort to correct the refractive indices for wavelength or temperature. Likewise, unless the temperature of the measurement is many tens of degrees different than room temperature, it is not worth making a correction. Use the refractive index at room temperature. **Enter Refractive Index (dn/dc)** for your sample. Check literature. Enter **633 for laser**. Click on **Autosave** Depolarization Ratio = 0.

9. **Measurements: Duration/Repeat** should be = 0.1 s. **Pinhole size** = 1-3 the number which you measured before. You can increase pinhole to larger number or increase the duration/repeat number if signal is weak. It may be better to accept 50,000 to 20,000 or even 10,000 total counts rather than increase the duration/repeat. This will result in an increase in the total experiment duration; it will also increase the probability that the measurement will be ruined by DUST. Experience will help you judge the best alternative.
10. **The strongest scattering will generally come from sample at the lowest angle of interest.** Set Detector Angle at, the lowest angle. Click the Intensity command button in the main screen or from within the Experimental Parameters window. The Total Measured Counts are shown in the upper part of the main screen. Adjust the pinhole or the duration/repeat until a suitable total measured count is achieved as described before. **IN ANY CASE, IT IS THE BEST TO KEEP THE MAXIMUM COUNT RATE < 1.5 Mcps.** All others counts, the Total Measured Counts will generally be lower (the exception is when the scatterer to be near a minimum in the scattering pattern. In this case try a few different angles).
11. **Number of Repeats-** if the sample is clean, the pinhole is maximum 3 mm, select 3-5 repeats. If the pinhole is set at 1-2 mm, select 5-10 repeats and 1 second duration to achieve 100,000 total measured counts.
12. **Dust Rejection Ratio-** values from 1-10 allowed. Small numbers are best to clean samples. Larger numbers (up to 2-3) may be used if dust is difficult to remove. Larger number may reduce accuracy, especially at low angles, but may be necessary if dust cannot be removed. **When fitting the data, look for abnormally high and erratic intensities at the lower angle, delete them if necessary during fitting.**
13. **List of Measurement Angles.** Click Angles at the top of the screen. Click Edit Measurement Angles List. Click the Set List Range command button. Enter the First and Last Angle (25-135 degree maximum). Click on the Increment Between Angles. Enter the Increment in degrees (5-10 deg). After creating a specialized list of angles, click on **Save List As and enter a filename up to 8 characters.** Click on Load List to reload a list of angles. **Sample- Random coil or rod. Random coil for proteins. Wavelength= 633 nm.**
14. **Click on Start-** to initiate measurement. You will be asked if you want to calibrate instrument (for example benzene). Insert cell with benzene. **Calibrate-** system will enter calibration constant.
15. You will be asked if you want to measure **the liquid without sample.** Insert cell with solvent. Click Yes.
16. Enter first concentration (40 mg/ml -1 ml in the cell). Insert cell with first sample. OK.
17. Enter second concentration (20 mg/ml – adding 1 ml with pipette and wait 10 min. OK and measure.
18. Enter third concentration (13.33 mg/ml adding 1 ml more and waiting 10 min before measurement.
19. Enter fourth concentration (10 mg/ml)
20. Enter fifth concentration (8 mg/ml). **DONE**
21. File- **Save AS**

22. If you get **an Error: save experiment**. Close program and open it again. You can add more points to your experiment at any time. **Calculate** using Zimm Plot or another eq.. You can delete some points from low angles if they are incorrect.
23. File- **Print Report**.