Data Reduction with IgorPro and the NCNR Packages
Quick Start Guide for the Lysozyme SANS data reduction

Software Required

Follow the link to the Igor Pro 8 and the NCNR SANS packages webpage for documentation and instructions to download and install the software. If you do not own an Igor Pro license, you can use the free 30-day evaluation mode. NOTE: if you are installing the NCNR SANS packages on a Mac running Catalina or later, there is an issue with XOPS used in some of the SANS packages, resulting in error messages saying an XOP cannot be opened. More information and a workaround can be found at https://www.wavemetrics.com/node/21088.

SANS data

Download the scattering data collected for Lysozyme solutions from the shared drive. You will need the 86 data files (SANS001.SA4_NG7_F### to SANS086.SA4_NG7_F###), a MASK file (DEFAULT.MASK), and a sensitivity file (Plex_16JUL2020_NG7.DIV).

Data reduction

Go to the SANS and USANS reduction video tutorials page, where you will find the *quick start guide for SANS reduction* video. The first 20 minutes of the video demonstrate Igor reduction procedures using a simpler demo silica data (you can skip the last 2 minutes of the video, which cover data analysis using Igor). Follow the same procedures demonstrated on the video to reduce the Lysozyme SANS data, along with the information from steps below.

1. **File catalogue and getting familiar with the data:** The sample labels on the lysozyme data were chosen by the user at the time of data collection. The following examples illustrate the naming convention chosen:
    *   Open Beam: same as *Empty Beam* in the video tutorial
    *   EC1: empty cell measurement, 0.1 cm sample thickness
    *   EC2: empty cell measurement, 0.2 cm sample thickness
    *   **5Lys**NaCl: 5 mg/mL Lysozyme solution in 150 mM NaCl buffer.
    *   2p5**Lys**D2O: 2.5 mg/mL Lysozyme solution in D_2O (no salt).
    *   **Buff**NaCl**EC2**: 150 mM NaCl buffer solution, measured in a 0.2 cm cell.
    *   **Buff**D2O**EC1**: D_2O measured in a 0.1 cm cell.

    You will see in the *File catalogue* that the sample thicknesses are listed. Note that some samples were measured at 0.1 cm (EC1 and lysozyme higher concentrations 45 to 100 mg/mL), others at 0.2 cm (EC2 and lysozyme concentrations from 1 to 15 mg/mL). The two buffer solutions were each measured at both thicknesses.

2. **Transmissions:** calculate the transmissions for all samples, as shown on the video tutorial.[1] You will not need to calculate transmissions for the blocked beam measurements (these samples have, by definition, transmission zero).

3. **Reduction:** the video tutorial demo silica data was collected using only 2 SDDs, and all samples had the same exposed thickness. The lysozyme SANS data were collected using 3 SDDs (see experimental section
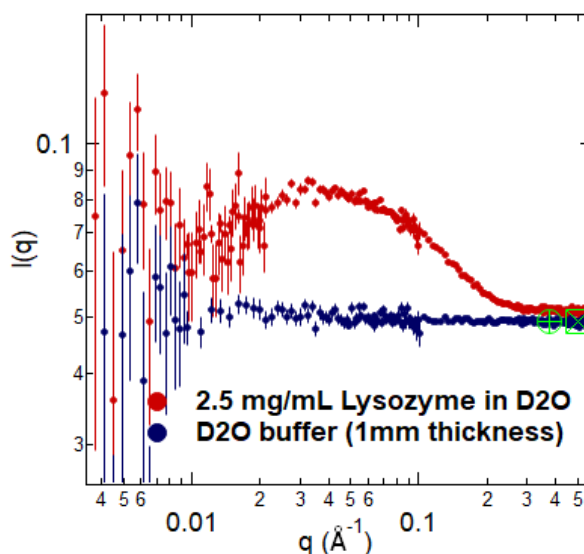
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[1]For the demo silica, and for this practical lysozyme data, transmissions were all measured at a single Sample-Detector-Distance (SDD) since the scattering data was all measured at the same wavelength.

of the practical handouts), and some samples were 0.1 cm while others 0.2 cm thick. You will therefore need to build 6 reduction protocols (2 sample thicknesses for each of the 3 SDDs)[2].

4. **Plotting and Sorting:** at this point you will follow the instructions on the video to plot and combine, for each sample, the 3 SDD configurations measured. Note that the sort panel allows for 4 configurations, but you will use only three: *Low Q* (SDD = 13 m), *Medium* Q (SDD = 4 m) and *High Q* (SDD = 1 m).

5. **Incoherent Background subtraction:** this step is not shown in the video tutorial but will be demonstrated during the live tutorials. There are 2 options to subtract the incoherent neutron scattering background:

    5.1. Buffer SANS data subtraction (recommended for dilute samples): SANS data was collected for a sample containing buffer only, using the same configurations as used for the corresponding sample(s). The buffer data is then subtracted from the sample data:

    ● In the *1-D ops* tab, click on *1D Arithmetic*. For each sample: *Load 1D Data Set 1* (Sample) and *Load 1D Data Set 2* (buffer). Use a Log I(q) scale.
    ● Drag the A and B cursors (shown here in green) to define the high q range where the two curves are expected to match. Click on *Get Matching range*, and *Calculate* the subtracted curve.
    ● *Save the result* in a file: samplename-sub.dat

    5.2. Flat background subtraction (recommended for concentrated samples): If the buffer scattering at high q is too different from the sample scattering, or the scaling seems unreliable for some samples, it may be preferable to apply a flat incoherent background subtraction to all samples, for consistency:

    ● Click on the option *Data Set 2=1?* and plot the Data Set 1 (sample).
    ● Inspect the data to find the value for the highest q reliable intensity.
    ● Copy this value to the *Scale factor (f)* field, and press *Calculate*.
    ● *Save the result* in a file: samplename-sub.dat

6. **Plotting and visual inspection of the reduced data**
    You can use the Plot option (1-D Plot tab) to activate the plot manager and produce plots of the various scattering profiles and compare them. This is useful to spot any errors during the SANS data reduction, and to start planning the analyses steps.

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[2]If you are using the free demo version of Igor, after the 30 trial days, when you close the Igor session it will not save the reduction protocols you built. To keep the protocols between sessions, save them (*Save* button in the build protocol data reduction window) AND export them as text files to your data folder. You can then import them back in a different session if needed.

SUGGESTED DISCUSSION

A. Empty cell and Buffer measurements were carried out using sample thicknesses of 0.1 cm and 0.2 cm, effectively doubling the number of background measurements carried out and the reduction protocols necessary. Discuss the need for these measurements.

B. Use the File Catalogue window to inspect the count rates and exposure times used for the Lysozyme solutions measured at various concentrations. How would you optimize the data collection?

C. In the print screen image shown for 2.5 mg/mL Lysozyme in D2O, why are the intensities at high q higher than those for the D_2O buffer?

D. Compare the I(0) intensities that you previously calculated using the SASSIE contrast calculator with the experimental values. Are there discrepancies? If yes, what are possible reasons for these differences?

E. What is the meaning of "dilute" vs "concentrated"? How do we know if we have a dilute solution?

DISCLAIMER

Certain software is identified to foster understanding. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the software identified are necessarily the best available for the purpose.

RECOMMENDED READING

28th CHRNS School BioSANS Practical Handout (2022). S. Krueger, S. Teixeira

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A. Shukla, E. Mylonas, E. Di Cola, S. Finet, P. Timmins et al. (2008). *PNAS* 105 (13), 5075-5080.

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