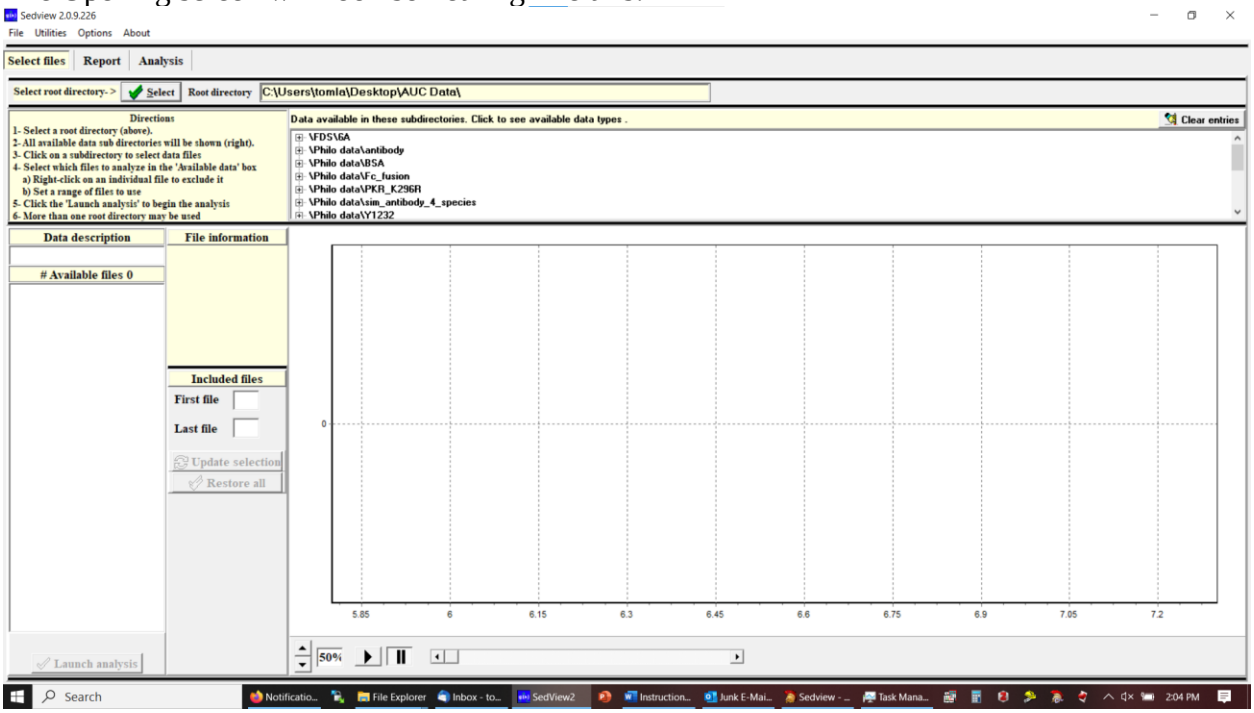
**Instructions for using Sedview 2**

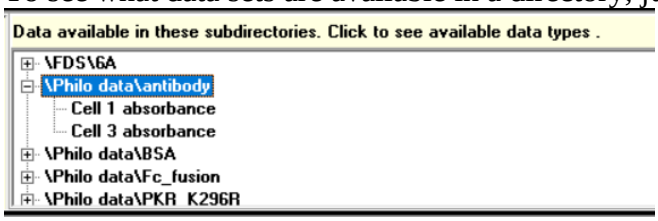
1. Download the Sedview x.xx.zip file
    a. Navigate to the GitHub repository page that contains the Sedview installer: https://github.com/TomLaue/Sedview-download
    b. Click on “Sedview x.xx.zip” where x.xx will be the most recent version of the software (currently 2.09). This will open the file in GitHub.
    c. On the file page, click either the **Raw** or **Download** button.


    d. The Zip file will be downloaded to your computer.
2. Install Sedview 2
    a. Right-click on the Sedview x.xx.zip file and choose ‘Expand all’
    b. You will be given a choice of where to put the unzipped files.
    c. There are three files- Sedview.exe, Sedview.cfg and FileTypes.cfg. Only the Sedview.exe is absolutely required, the program will re-create the two configuration files if they are missing.
3. Run Sedview
4. The Opening screen will look something like this:
5. 

    a. Click the “Select” button to find a Root directory. This can be a directory that has several subdirectories that each contain AUC data sets. Sedview sorts the

directories by cell number and file type. The subdirectory structure shown above is for the directories and files found in the AUC Data.zip file that may be downloaded from the same GitHub location.

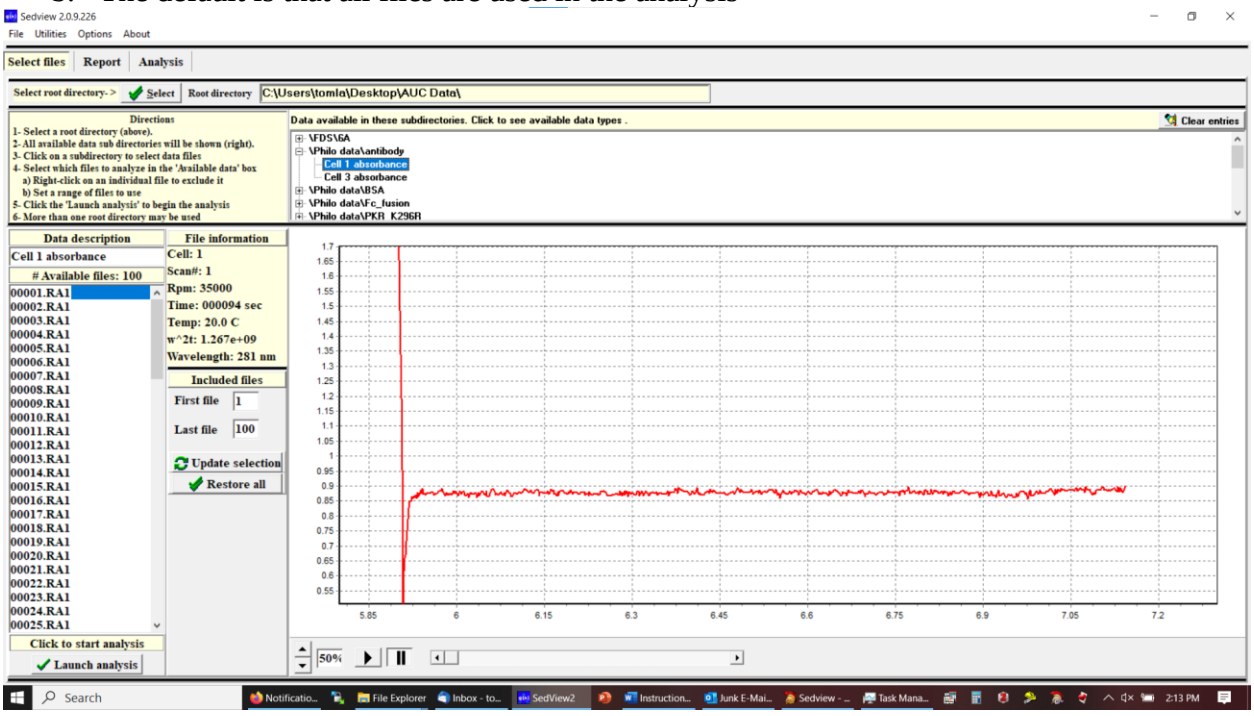
b. To see what data sets are available in a directory, just click on it. For example:

c. 


d. Shows that the "\Philo data\antibody" directory contains absorbance data sets for Cell 1 and Cell 3

e. Click to select one of these data sets (Cell 1 shown below)

6. Editing data
   a. Once a data set is selected, you may select which files will be included/excluded from further analysis
   b. The default is that all files are used in the analysis

7. 


   a. You may view the files in the graph to the right by simply moving the cursor over the file list.
   b. Alternatively, you can run a 'movie' of the experiment by clicking the button below the graph or by sliding the 'thumb' on the bar below the graph.
   c. If there is a bad scan, find it in the file list and right click on it. You will be given the option to exclude it from the analysis. Excluded files will have their names grayed out. Right-click on it and choose "Include" to re-include it in the analysis.
   d. One simple, convenient analysis to do with Sedview is to determine how many files are needed to provide a complete analysis (useful to limit the number files used in fitting programs, thus speeding up the analysis). To select a range of files, enter the number of the first and last files to include, then click the "Update

selection" button. Files that are not to be included in the analysis will be grayed out.

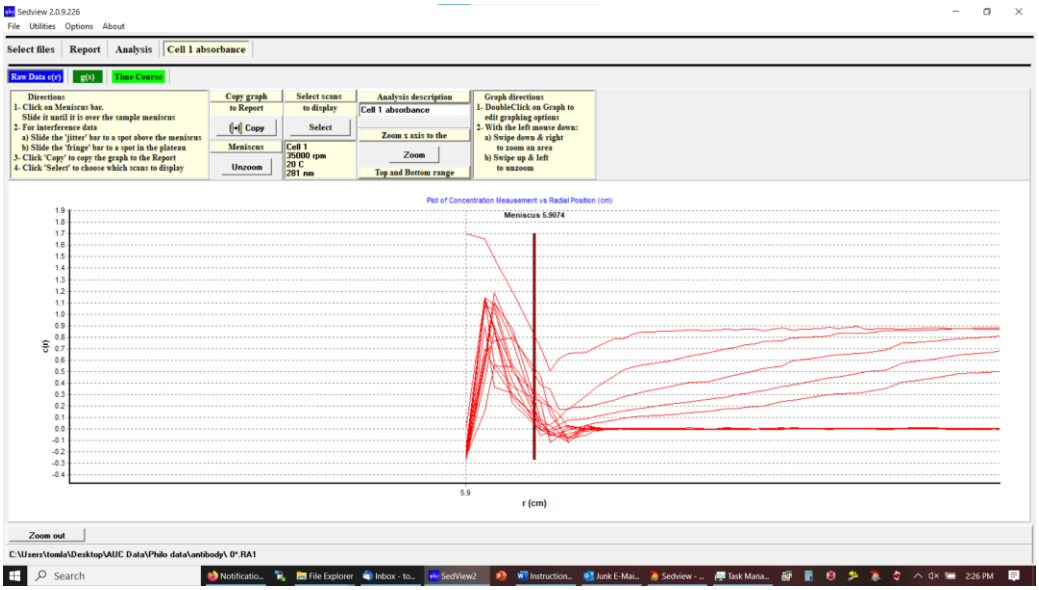
i. By 'Launching' the analysis on data sets that include an increasing number of files (e.g. 1-20, 1-30, 1-40, etc.), you will be able to see how the g(s) distribution changes as more files are added. Using the Analysis tab (below), the g(s) distributions will be superimposed. With increasing number of files used in the analysis, you may find there is a point at which including more files (e.g. 60 versus 80 files) does nothing to the g(s) distribution. This result suggests that there you do not need to include more than 60 files in any subsequent analyses.

e. You can cancel the effects of the editing by clicking on the "Restore all" button.

f. Once you have determined which files are to be included in the analysis, you are ready to start an analysis.

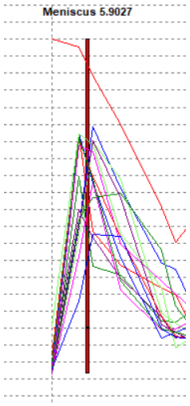
8. Click the "Launch analysis" button to begin.

a. The files are read in and a new window appears showing the portion of the data near where the meniscus should be:




b.

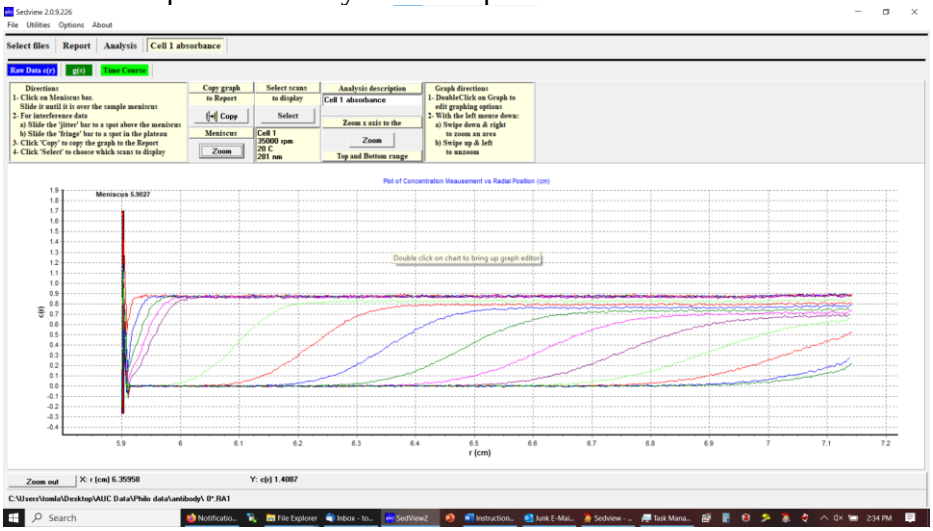
c. Click on the red bar marked "Meniscus" and while holding the left mouse button down, slide the bar until it is over the meniscus:




d.

e. The meniscus peak may be spread over a small range. You can always come back to the blow up of the meniscus by clicking on the "Zoom meniscus" button.

f. Once you release the mouse button, the graph will expand to show you the raw data. At this point the analysis is complete.



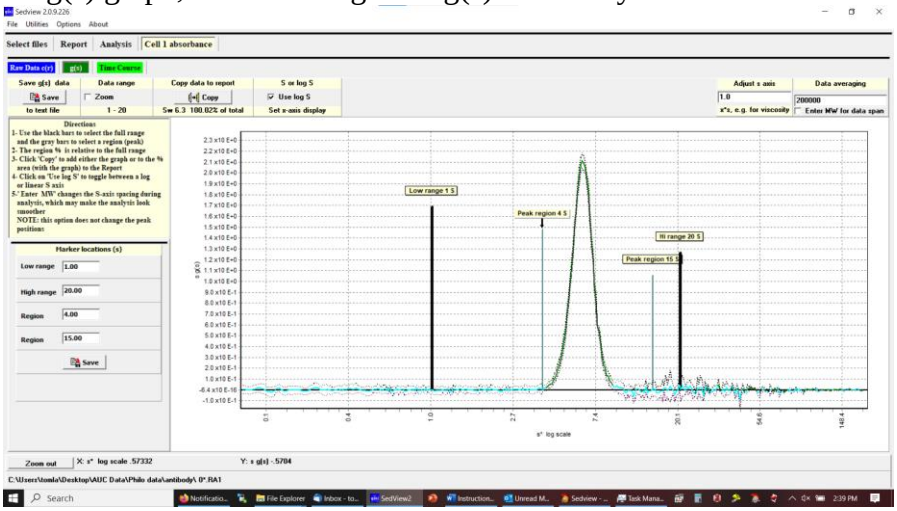
g.

h. Only a selection of scans is displayed. To select more or fewer scans, click the "Select scans to display" button. A grid is presented that will allow you to turn the display of particular scans on and off and turn on/off the display of all scans, etc. NOTE: selecting/deselecting the display of scans does no impact the data analysis.

i. The "Copy" button will copy the graph to the Report (discussed separately below). NOTE: Sedview automatically copies the graph to the report when you first start the analysis.

j. You may wish to enter a more descriptive label for this analysis (e.g. 'Cell 1 scans 10-60') to distinguish these data when comparing them with other data sets. You can change the description at any time.

9. To see the g(s) graph, click on the green "g(s)" tab and you will see:



a.

10. You will notice that the g(s) data are shown in different colors. During the analysis Sedview determines the signal-to-noise ratio (S/N) of g(s) at each value of s using the formula S/N (in decibels) = 20*log[signal/noise]. When the signal amplitude is greater than the noise, S/N > 0. When the signal amplitude is less than the noise, S/N < 0.

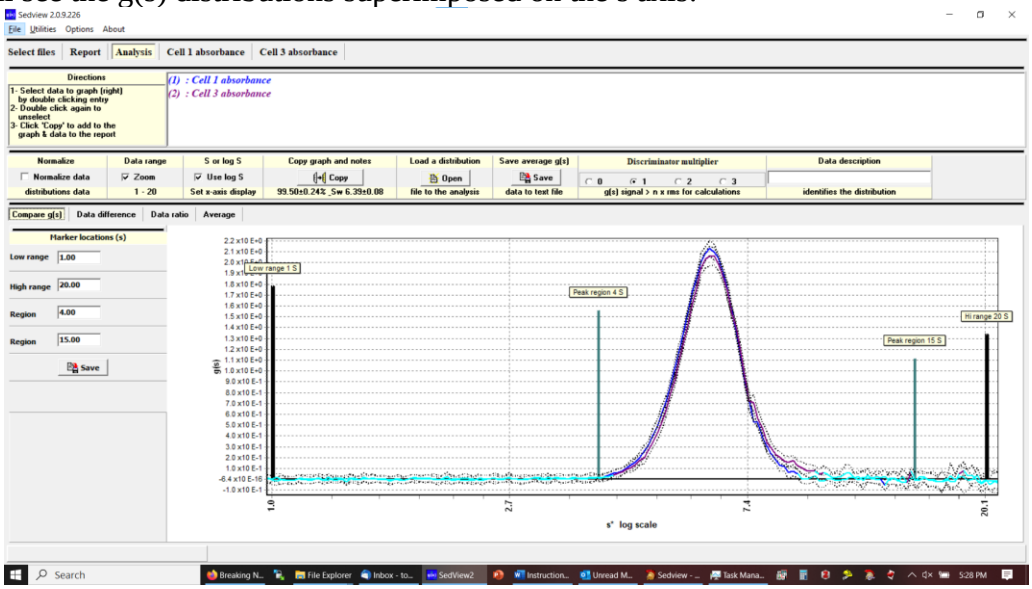
a. For S/N less than or equal to zero, the g(s) values on the graph are aqua colored. For S/N > 0, the g(s) data are green. Hence, just by eye you can see where the data can be trusted for interpretation and comparison.
b. There are four bars on the g(s) graph. The two black "Range" bars are used to encompass the range of s over which all subsequent analyses are performed.
c. The two gray "Peak" bars may be used to select a region in the range for calculation of two values: the % of the total signal between the bars and the weight-average sedimentation coefficient enclosed by the bars. These two quantities are displayed below the "Copy" button.
d. The positions of the bars may be changed by clicking on them and sliding them. As each is moved, its position is updated in the "Marker location" box to the left and the % and Sw values are updated.
    i. The exact positions of the Range and Peak bars may be specified by entering their values in the boxes to the left. These values are saved from run-to-run which can save time between Sedview sessions.
e. Initially, the g(s) graph is shown for the entire sedimentation coefficient data range. If you wish to zoom to view just the region between the two Range bars, click on the "Zoom" button above the graph. Clicking on the button a second time will "Unzoom" the data display.
f. Notice that the S axis is a log scale. This is because the wide-distribution analysis algorithm used in Sedview (Hayes DB, Stafford WF. (2010) "SEDVIEW, real-time sedimentation analysis." Macromol. Biosci. 10:731-735. doi:10.1002/mabi.201000075) yields g(s) distributions that cover orders of magnitude in S.
g. Click on the "Use log S" checkbox to switch the S axis between linear and log scales.
h. The g(s) distribution data may be saved in an ASCII (text) file with three columns of numbers separated by a space: s, g(s) and the standard deviation. Just click on the "Save Data" button and provide a file name. By default, the file is given a ".dat" extension. The resulting file may be read in for comparison with other analyses (discussed later).
i. To the right at the top, there are two other places where values may be entered.
j. The "Adjust s axis" is provided if you want to compare data acquired under different conditions or in different solvents. The number to be entered should be the ratio of the desired density and viscosity (e.g. pure water at 20 $^{o}$C) to the actual density and viscosity as:

$$s_{20,w} = s_{T,b}\frac{(1-\bar{v}\rho)_{20,w}\eta_{T,b}}{(1-\bar{v}\rho)_{T,b}\eta_{20,w}}$$

k. The second provides a way for the user to alter the number of scans used in determining g(s). If too many scans are used a boundary may have moved sufficiently to 'flatten' the g(s) graph a bit. The algorithm developed by Stafford and Hayes does an excellent job of avoiding this problem (see the reference above). Ordinarily, leave this box unchecked. If you do want to enter a value, check the box and enter a molecular weight. You will see immediately the effect of changing the algorithm. Sometimes entering a value results in a smoother g(s)

distribution. The value you enter, and whether the box is checked or not, is saved between runs.

11. The “Analysis” tab is used to compare g(s) distributions. To use the Analysis functions of Sedview, load another data set.
    a. Click on the “Select files” tab at the top of the window and select another set of data. For example, John Philo’s sample data includes data from replicate samples (Cell 1 and Cell 3). Click on the Antibody Cell 3 (or any other data set) then click the Launch analysis button.
    b. Determine the meniscus position for these scans
12. Now click on the “Analysis” tab at the top of the window. For the replicate antibody data you will see the g(s) distributions superimposed on the s axis:




    a.
    b. The list at the top shows which data sets are being compared. The color of the data set description corresponds to the color of the graphed data.
        i. If you click once on one of the listings, you can change its description in the “Data description” box. NOTE: both the description in the list and the title of the corresponding data tab are changed.
        ii. If you click twice on a listing, its listing is switched from italic to normal text, and that analysis is removed from the comparison. Double click again to return the data to the comparison.
    c. The data may be normalized by clicking on the far-left checkbox labeled “Normalize.” Normalizing the data is particularly useful when comparing data acquired using different optical systems, different sample concentrations or data acquired at different wavelengths.
        i. The g(s) data are normalized for each data set, with the sum of its signal between the “Low range” to the “Hi range” bars constituting 100%
        ii. For the analysis page, the data are zoomed by default.
    d. The option to switch the s axis between log(s) and s functions the same as for the individual data sets.
    e. The “Copy” button will copy the current graph, whether from the “Compare g(s)”, “Data difference”, “Data ratio” or “Average” page to the Report (below), along with the relevant information about which data sets are contributing to the graph.

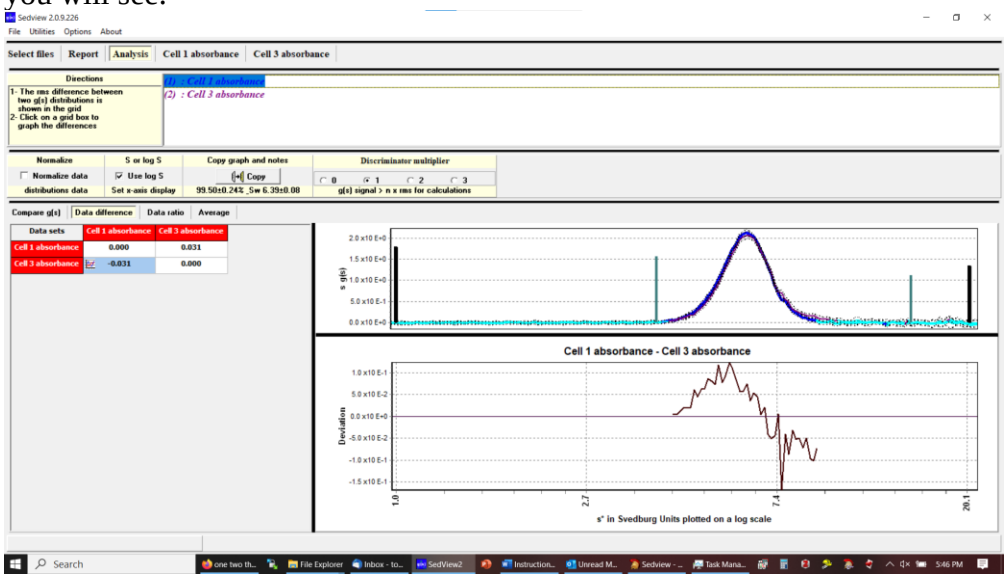
i. The average % ± std, and the average S and ± std are provided below the "Copy" button. For the "Compare g(s)" page, both these averages and the individual % and average S values for the contributing data sets are copied to the Report.

f. Fitted g(s)/c(s) distributions from saved from Sedfit, Ultrascan and Sedanal may be included in the Analysis. Clicking on the "Open" button provides a dialog box that will allow you to select the file.
   i. NOTE: Sedfit uses a "*.dat" file extension, UltraScan uses a "*.csv" file extension and SedAnal uses a "*.txt" extension. You may select which file type to look for in the dialog box.
   ii. Sedview also allows g(s) distribution data to be saved from either individual data sets or from the Analysis/Average page. By default the files are given a "*.dat" extension.

g. The S/N ratio used for discrimination of which data to use in the "Data difference," "Data ratio" analyses may be adjusted using the "Discriminator multiplier." The selected multiplier is applied to the rms noise. Values range from 0 (no noise), 1, 2 or 3 standard deviations (corresponding to a 68, 95 and 99.7% certainty that the true signal lies within the noise envelope).

13. Data difference analysis

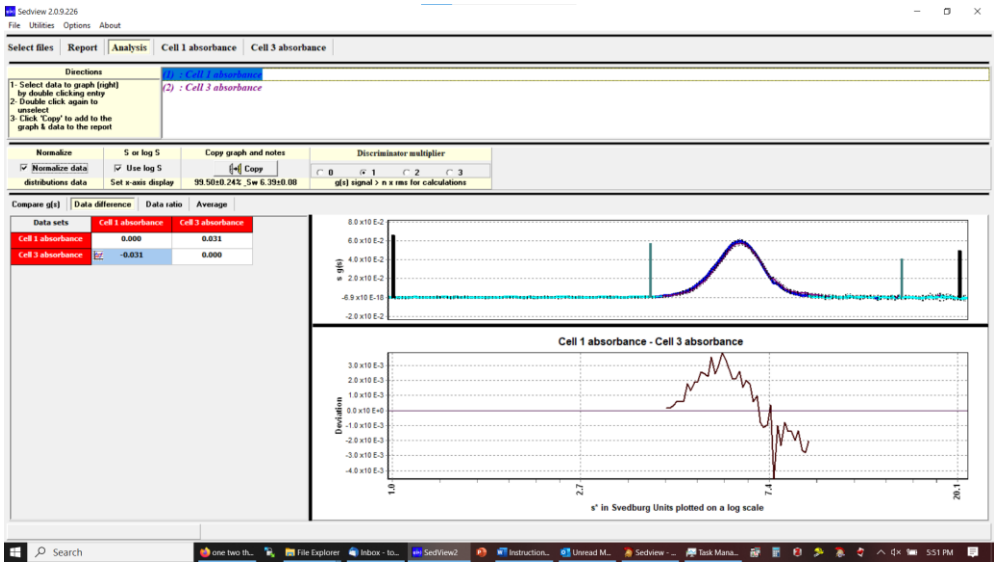
a. If you click on the "Data difference" tab below the row of buttons for these data, you will see:




b.

c. The grid allow the selection of which data sets are subtracted from one another. The data shown above are the difference between the 'raw' g(s) data.

d. For the normalized data, the Data difference graph looks like:

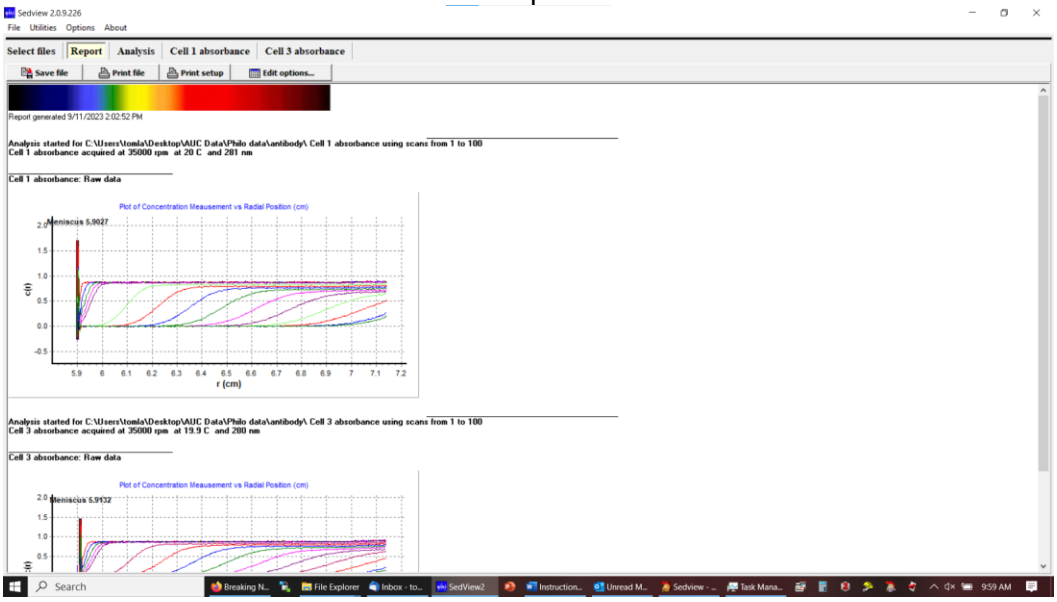



e.

f. In this case, the normalized and non-normalized data difference look nearly identical.

14. The “Data ratio” analysis divides one g(s) distribution by another. For these data, the ratio turns out to be 1.0 across the entire g(s) distributions.
    a. The data ratio is useful when comparing data acquired at two wavelengths. For example, 230 nm and 260 nm data for a nucleoprotein complex like a virus.
    b. Full interpretation of the ratio data requires consideration of the extinction coefficients of the composite material’s components as well as their relative abundance in a complex.
15. The “Average” analysis determines is most useful for analyzing replicate samples. In this analysis, the average g(s) and its std is determined for all of the selected distributions in the list.
    a. The “Save average g(s)” button allows the average data to be saved in a text file as three space separated columns, s, g(s) and std. The average data distribution file may be read in (above) for comparison with other data.

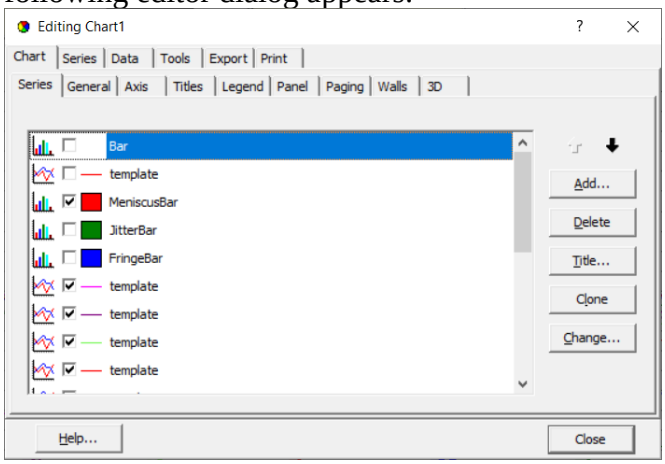
16. The “Report” tab provides a way to save the graphs and information from a Sedview session. For the data sets used here the report looks like this to start with:




   a. Sedview automatically copies the Raw data graph to the report immediately after the meniscus position has been determined.
   b. Clicking on any “Copy” button will copy the current graph along with some pertinent information to the report.
   c. The report is fully editable- you can add to it, delete from it, or modify it in any way.
      i. If you wish to copy text from another program (e.g. Word), you can use the Ctrl-C/Ctrl-V shortcuts
      ii. For copying images, however, you can use the Ctrl-C command for the copy, but you must use the Edit options…/Paste image menu commands. This is a peculiarity of the Microsoft Rich Text Edit component… sorry.
   d. Clicking the “Save file” button produces a dialog box that will allow you to select the output location and file name.
      i. The Report uses Rich Text Format, a format recognized by all common word processors (Word, OpenDocument, Word Perfect, etc.).
      ii. The output file extension will be “*.rtf” by default
   e. You also may select and copy the entire file contents using the Ctrl-A/Ctrl-C shortcut sequence.
      i. The copied contents may then be pasted into a word processor using the Ctrl-V shortcut.

17. There are some common features to all charts.
   a. You may zoom the view by clicking at the top-left corner of the region to be zoomed while holding down the left mouse button and sweeping the cursor down and to the right to enclose the zoom region.
   b. To unzoom the chart, click the chart and while holding down the left mouse button, sweep the cursor up and to the left.
   c. To scroll the chart, drag the cursor while holding down the right mouse button.

d. A chart editor is available from any graph. Simply double-click on a chart and the following editor dialog appears:




e. This editor allows a wide range of options… too many to enumerate here. Have fun with it, but be careful, too.