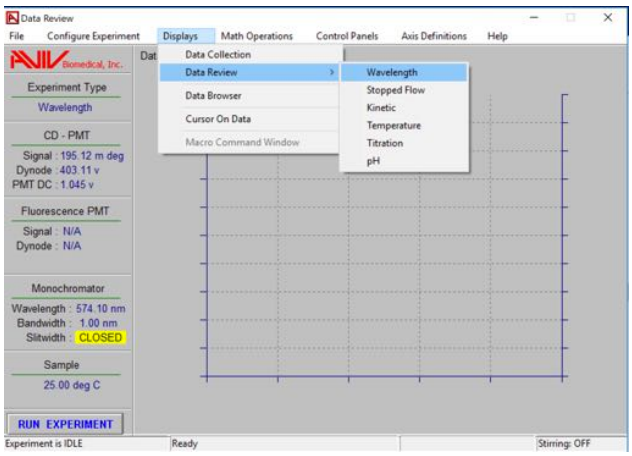
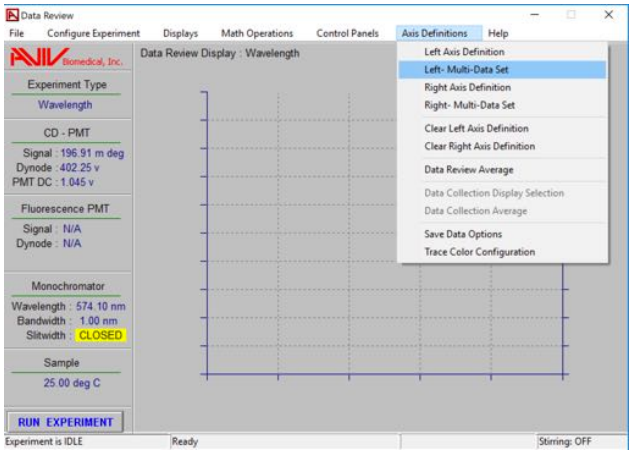
Processing CD wavelength data using the AVIV software

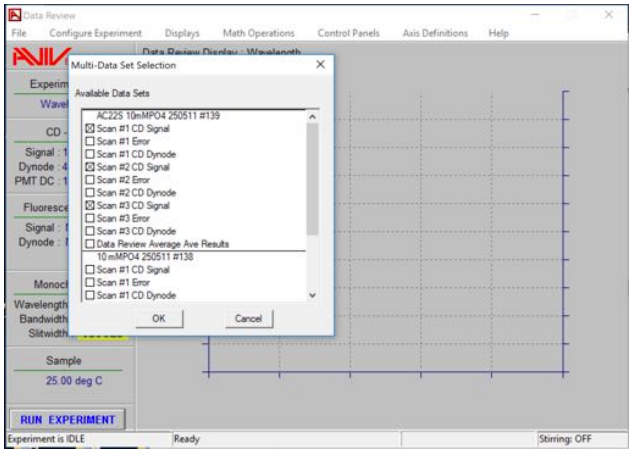
1) After data collection, navigate to the Data Review & Wavelength window:



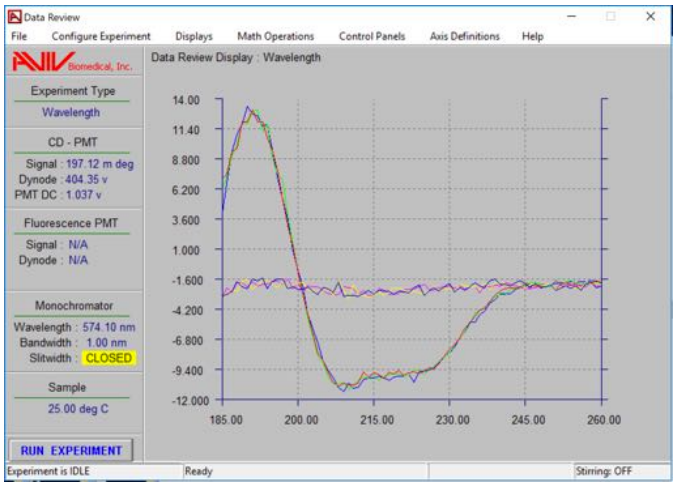
Navigate to the multi-data set view:



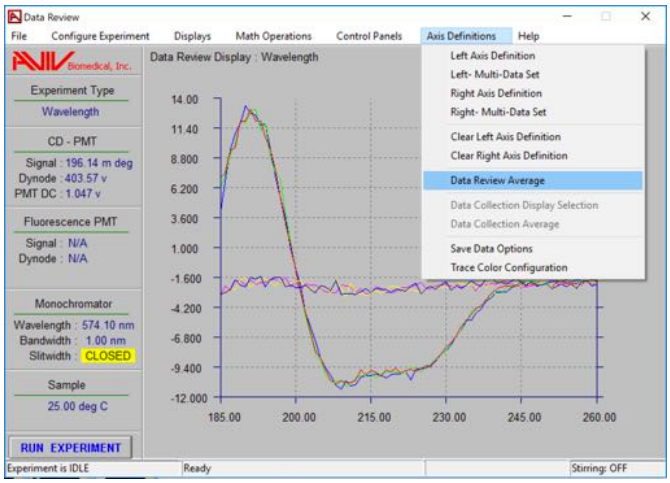
Select the “CD Signal” scans for averaging, for the sample and the buffer:



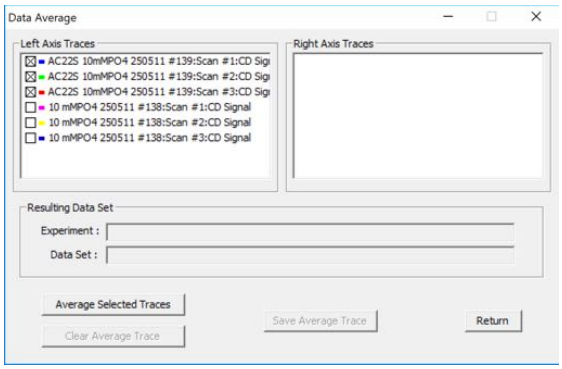
Click “OK”. They should now be displayed:



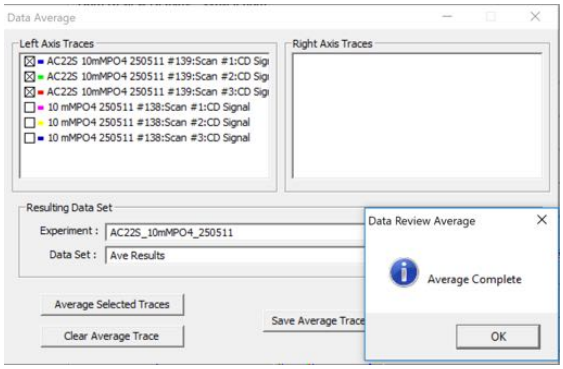
2) These sets of three scans need averaging to produce a single, mean scan for each of the samples, and the buffer. To do this, navigate as follows:



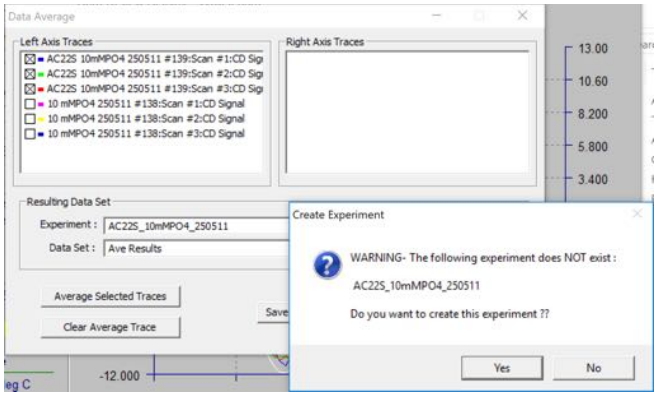
Select the three scans for the sample:



Click “Average Selected Traces”:

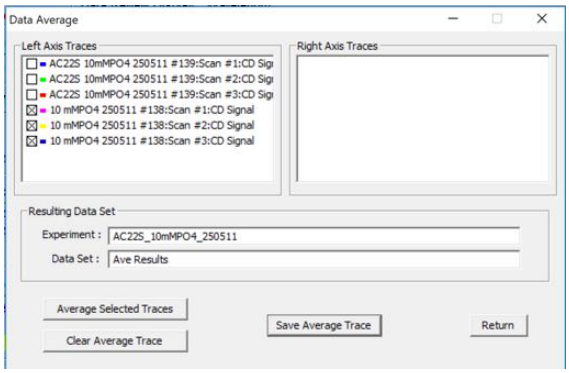


Dismiss the notification pop-up by clicking “OK”. Click “Save Average Trace”. The software will ask if you want to create a new experiment with the same name, but missing the # suffix. Click “Yes”:



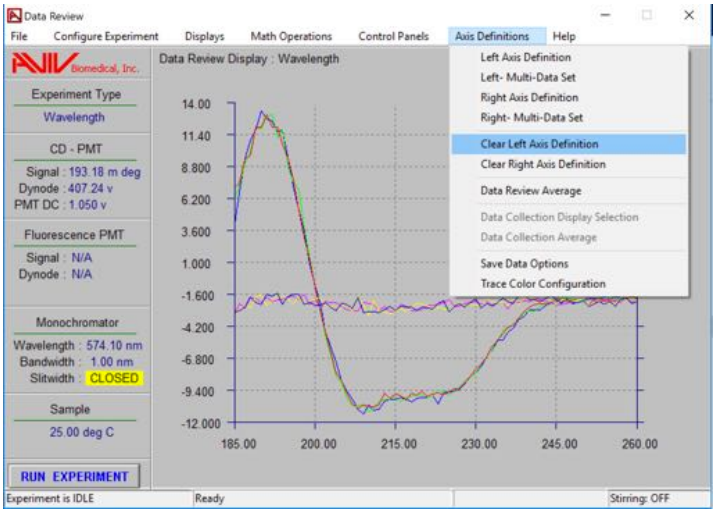
And dismiss the confirmation window that follows (“OK”).

Now, as necessary, deselect the first set of scans and select the next for averaging:

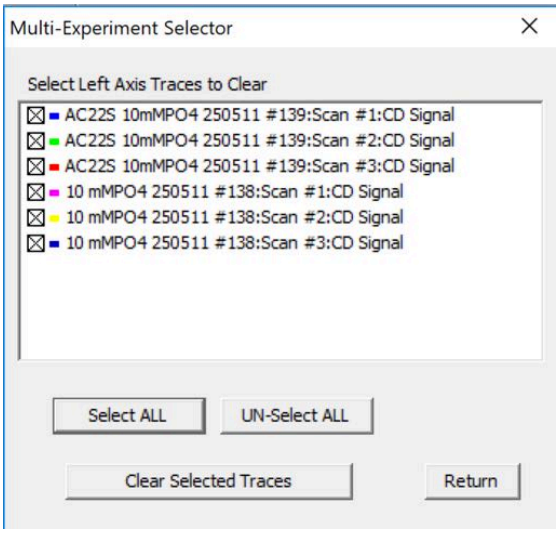


Repeat the averaging and saving as necessary until you have average scans saved for all your experiments.

3) OPTIONAL: If you wish to check these look OK, you can display them in the main window. First, remove the current, non-averaged, scans from the display:

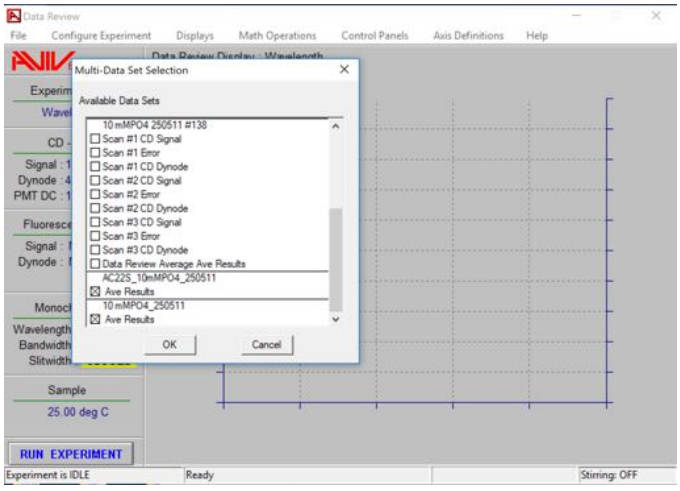


Then “Select ALL”:

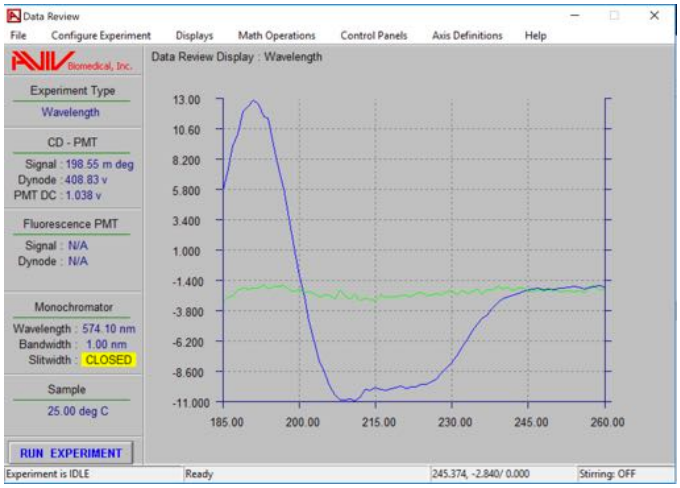


And “Clear Selected Traces” (this doesn’t delete them, just removes them from the display). Return to “Axis Definitions” -> “Left Multi Data Set”…

Scroll to the bottom of the list to find the new averaged data sets you created:

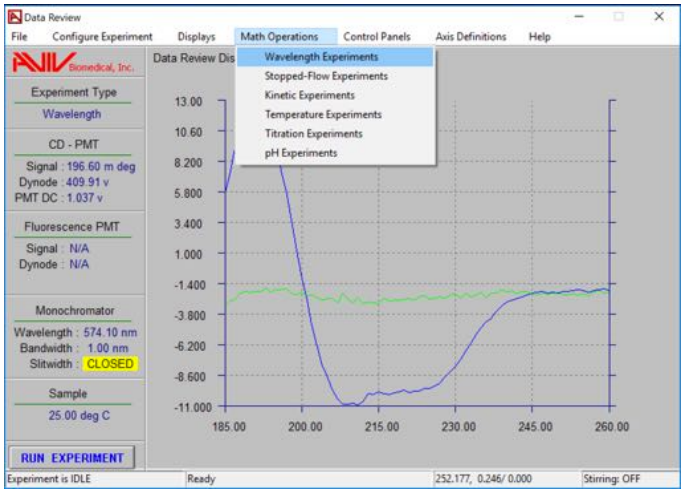


And check all looks well with the data:

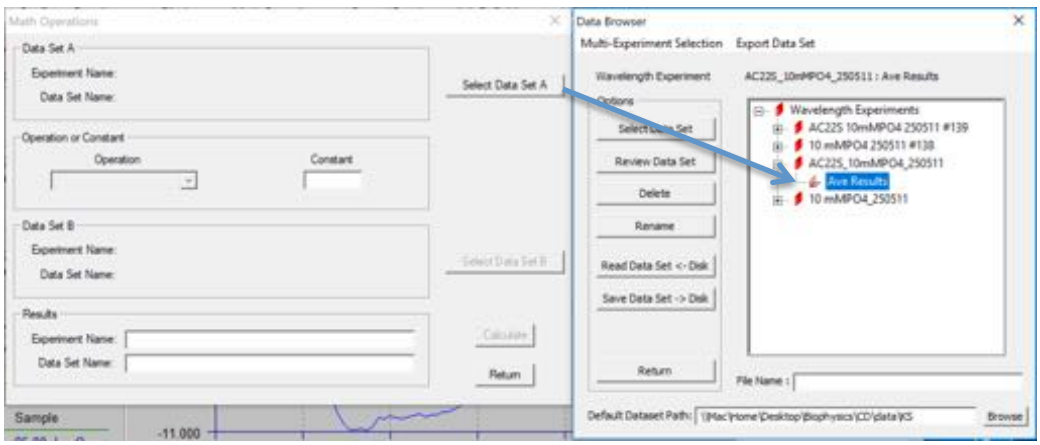


NB: A bug in the software generates an extra white trace that can be safely ignored, or deleted from view by double-clicking on it directly.

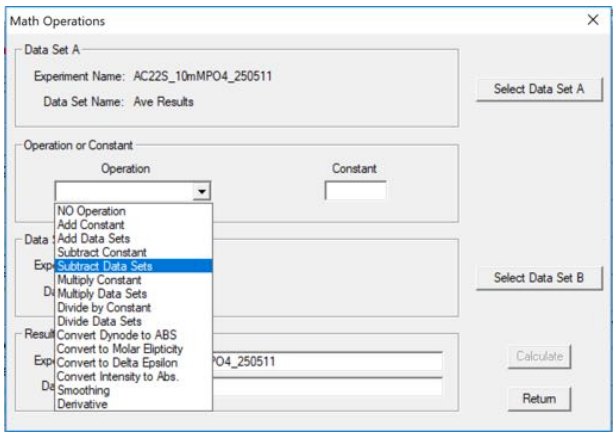
4) Now we have to subtract the buffer signal. Navigate as follows:



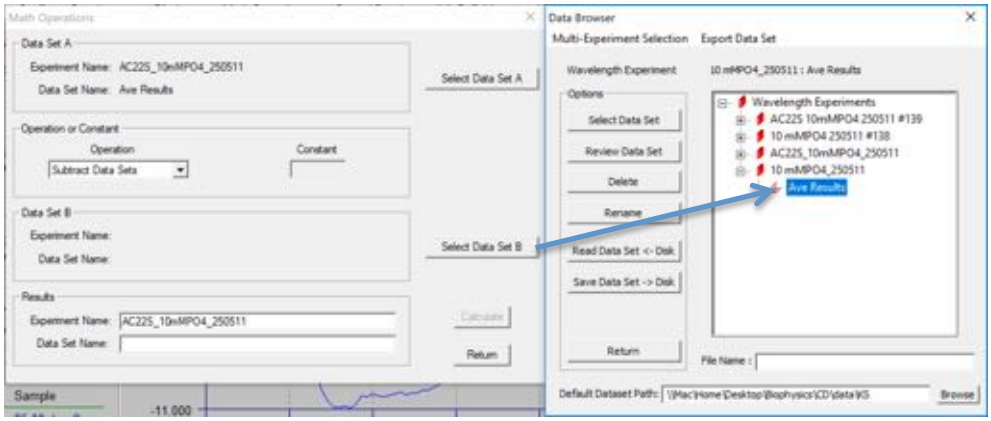
Click “Select Data Set A” and find your averaged sample scan:



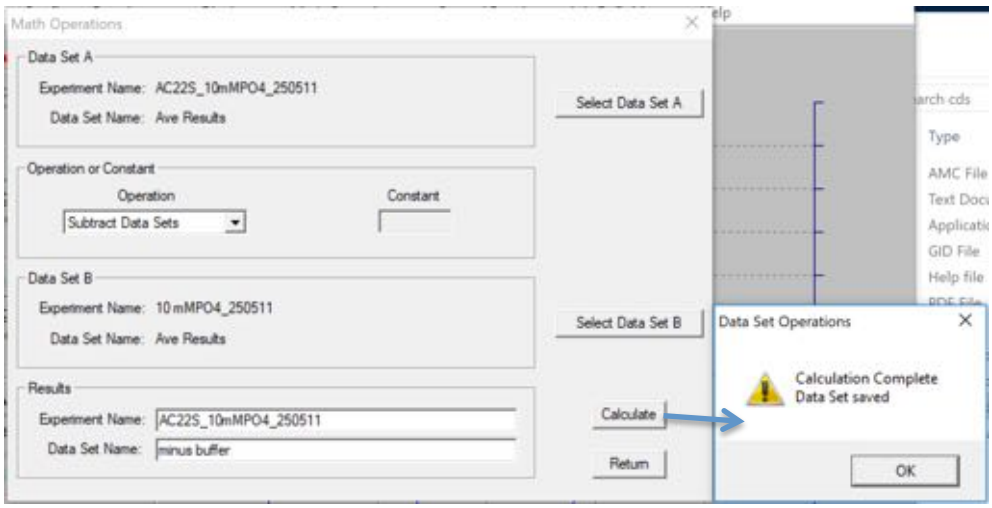
Click “Select Data Set”. Find “Subtract Data Sets” in the “Operation” drop-down menu:



“Select Data Set B” (buffer) using the same procedure as for A:



“Experiment Name” is automatically taken from A. The “Data Set Name” will be the column heading within the file. Name it something explicit like “minus buffer”, and click “Calculate”.

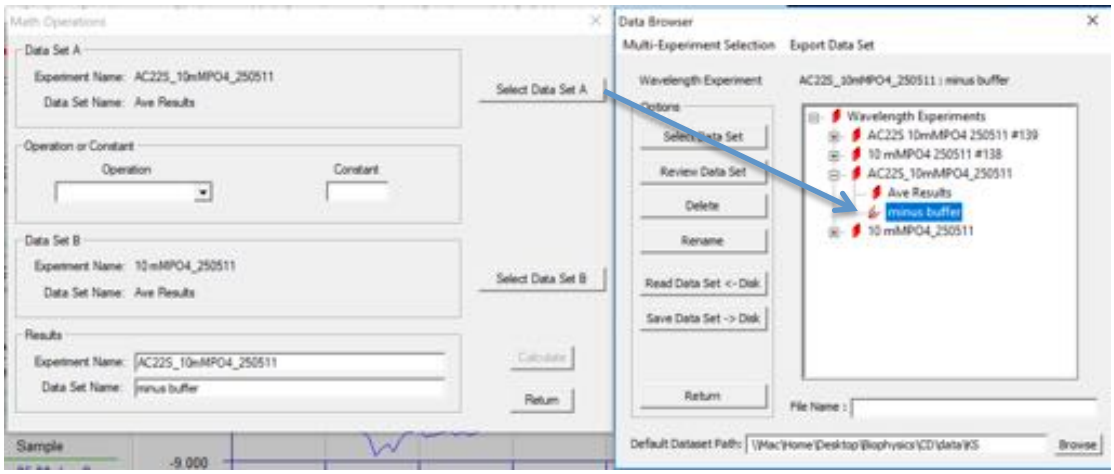


The data are saved automatically (dismiss the notification pop-up). Repeat as necessary, changing Data Set A as appropriate for all your samples. When you have subtracted the buffer from all your sample scans, you can dismiss the Math Operations window by clicking "Return", or continue straight to 5).

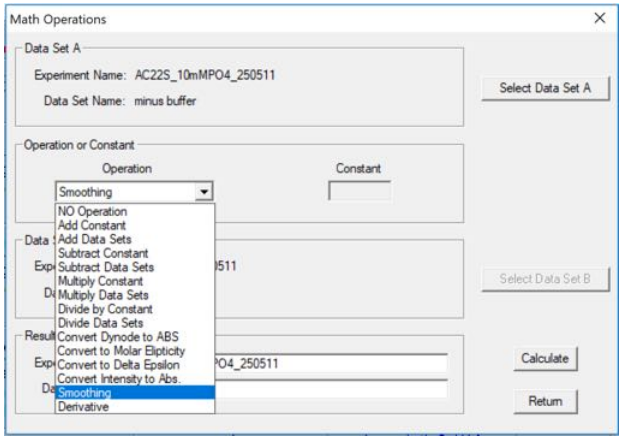
If you want to check these scans look OK, you can repeat step 3, this time selecting the "minus buffer" scans for display.

5) Almost all published CD wavelength data has been subjected to a smoothing operation (usually a sliding polynomial) and therefore we recommend you do the same. It doesn't improve the data quality *per se* but does remove visually-distracting high-frequency noise components.

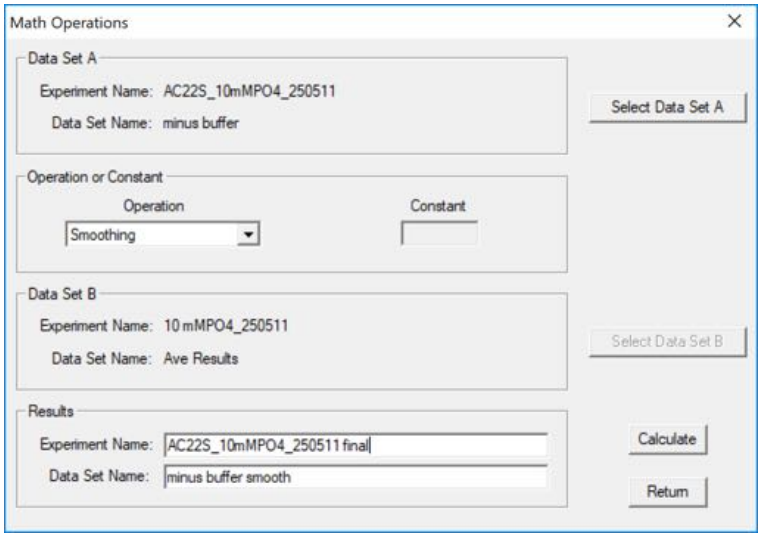
Return to "Math Operations" -> "Wavelength", and select your "minus buffer" data for A:



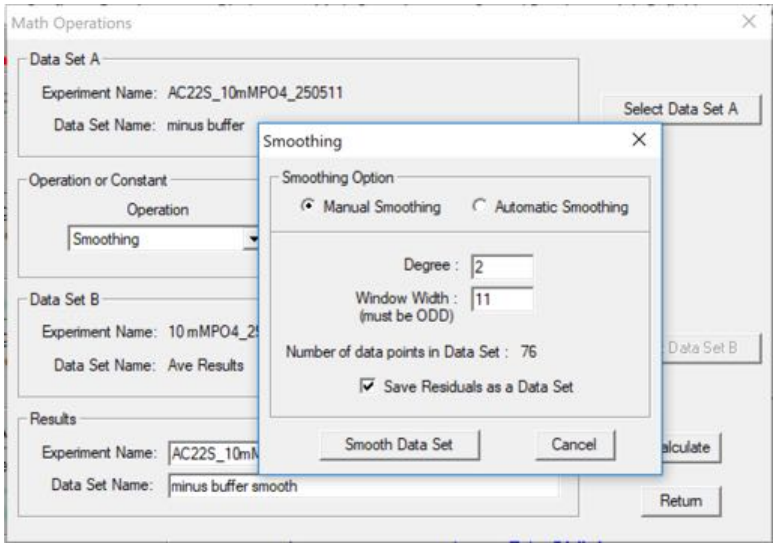
This time select "Smoothing" from the drop-down menu:



For the Data Set Name, choose something explicit such as "minus buffer smooth". I also like to rename the Experiment to create a new one with the suffix "final" so that I end up with a single column of *y* values, which is easier for e.g. importing into Dichroweb or Excel:



Clicking "Calculate" will then ask if you wish to create a new experiment. Click "Yes", and a new window describing the smoothing operation will be displayed:

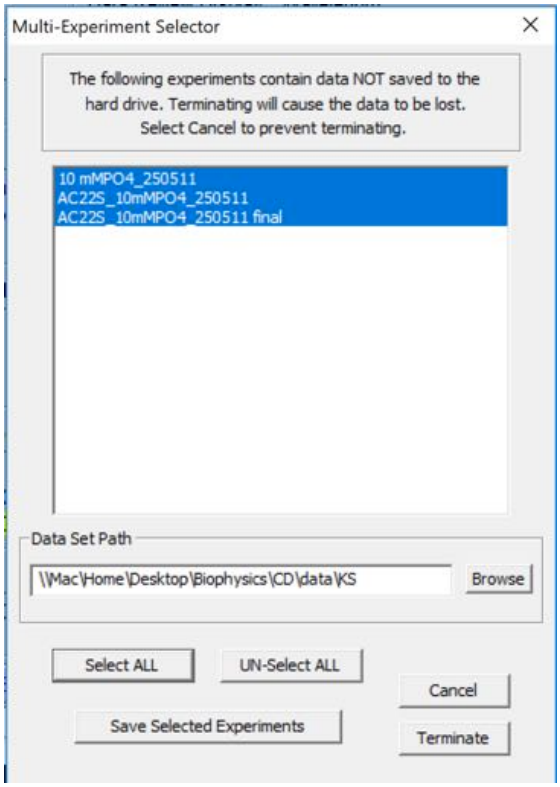


The options displayed are the default, and work well in the majority of cases so there is no need to change them. Click "Smooth Data Set" and dismiss the next two pop-ups that have the D_2/S_2 value§, and the saved notification.

§D_2/S_2 is a measure of the randomness of the residuals and should be close to 2; use "Automatic Smoothing" to optimise the smoothing parameters using this criterion.

Repeat step 3 if you wish to view your final, averaged, buffer-subtracted and smoothed data.

6) Your processed data is NOT SAVED AUTOMATICALLY, but it is easy to do on exiting the software. Go to File -> Terminate CDS Program, and click "OK". A list of your unsaved processed data will appear. Click "Select ALL" and make sure everything you wish to save is highlighted in blue before clicking "Save Selected Experiments":



Click "OK" when the various notifications appear. All your processed data will now be saved in the same directory as your original experiments (the difference being the raw data has a # suffix). The software will terminate automatically a few seconds after this process has completed.

Further points to note:

- "Math Operations" also has functionality for converting your acquired data in millidegrees (the unit produced by Aviv) to molar ellipticity, and for taking the derivative of thermal melt curves, which is a quick method to estimate T_m.

- To compare CD spectra of different proteins, they are usually normalized to the number of peptide bonds present in each sample (mean residue ellipticity, Θ):

  $\Theta_{mre}(\lambda)$ (usually expressed in deg . cm^2 . $dmol^{-1}$)
  = millidegrees / {(AAs – 1) * concentration (M) * cell pathlength (cm) * 10}

- Further fitting and post-processing (including the conversion above) can be carried out using Dichroweb, for which you need to apply for a personal user account:
  http://dichroweb.cryst.bbk.ac.uk/html/home.shtml

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