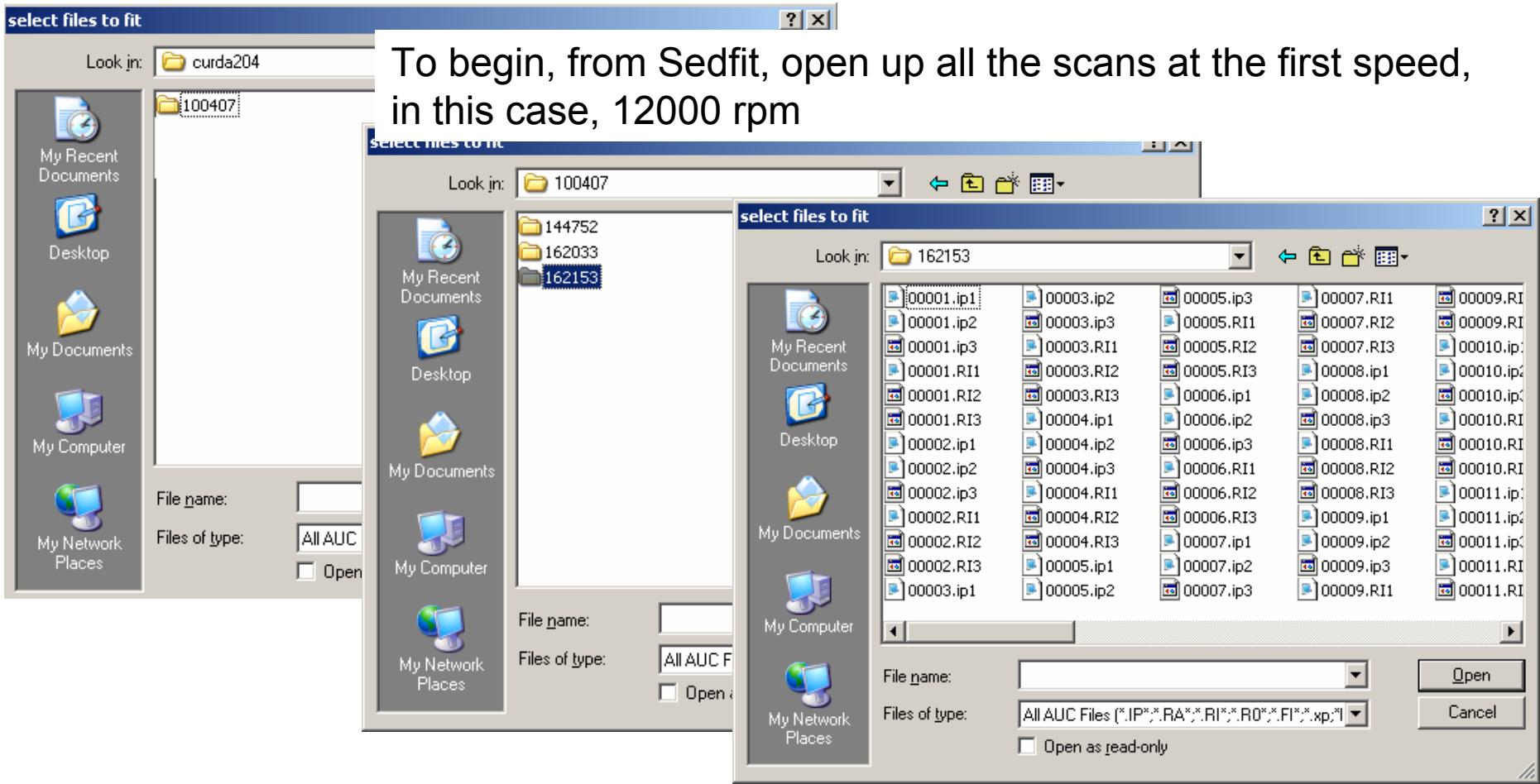
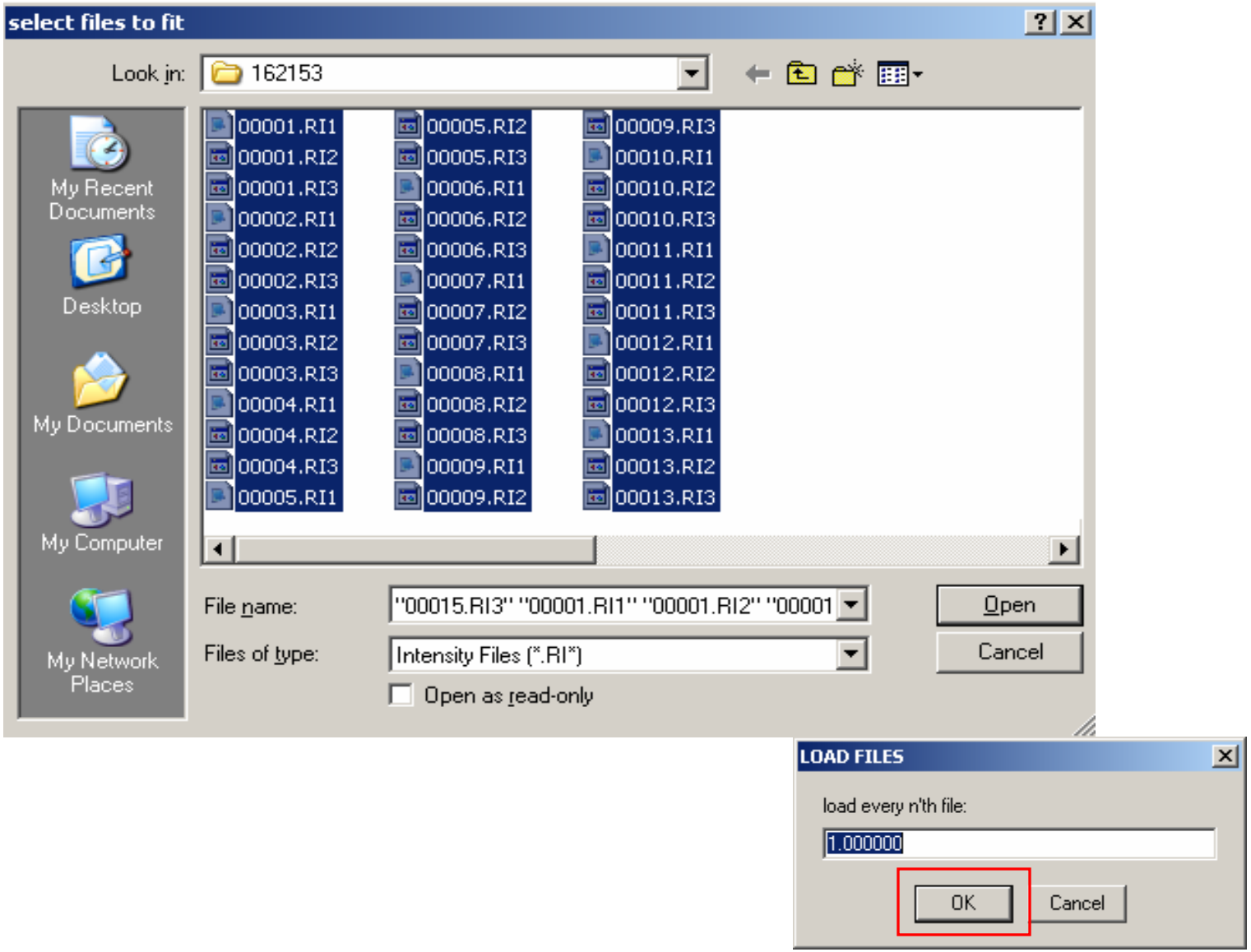
This tutorial will demonstrate how to use SEDFIT to sort interference and absorbance data from a Sedimentation Equilibrium experiment to determine if scans are in equilibrium.

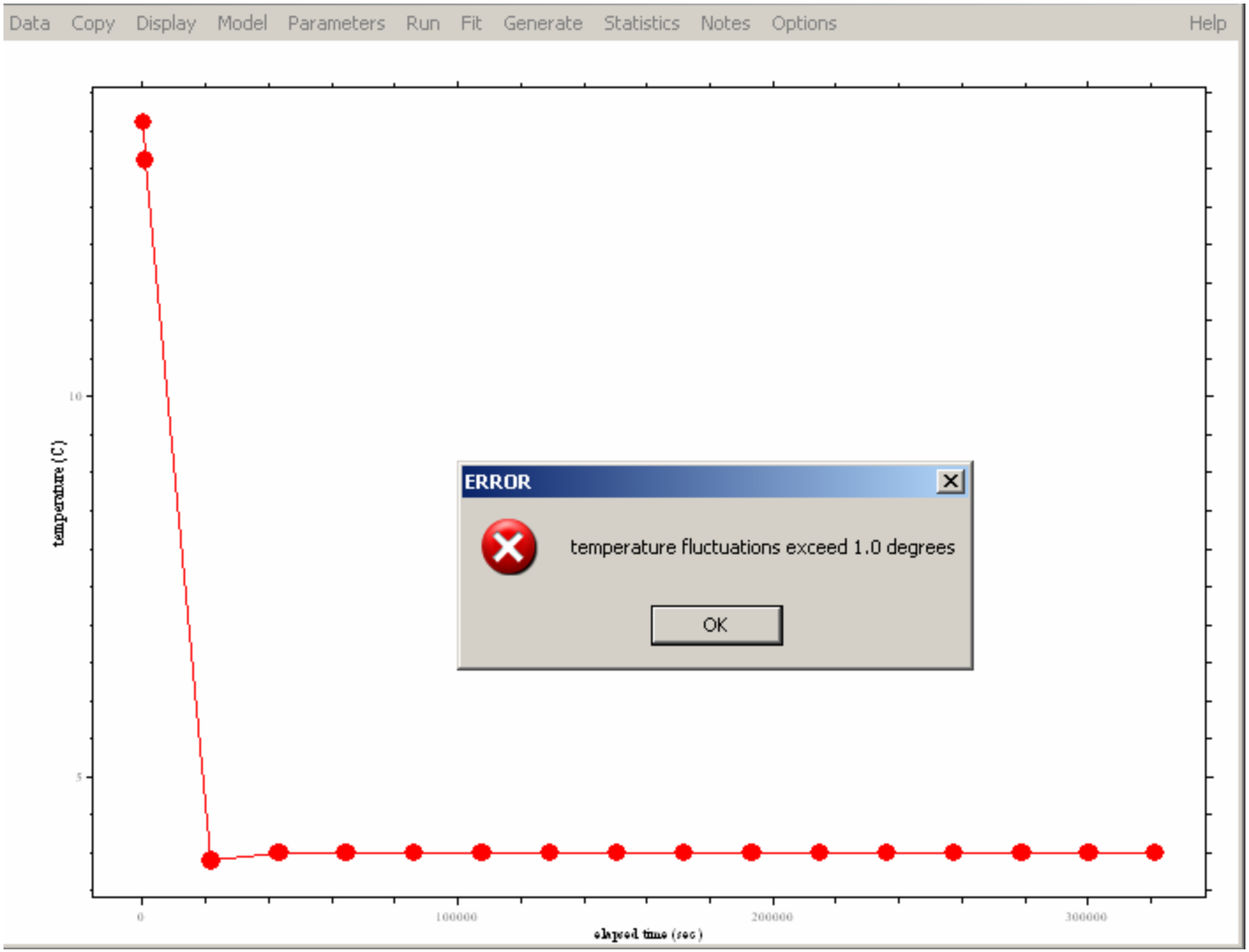
This SE run contains 3 cells, all scanned by interference optics plus absorbance optics at 280nm and 250nm using the option of collecting Intensity (RI) data instead of Absorbance (Ra). The initial speed was 12000 rpm. We will check to see if these scans are in equilibrium before we change to the next higher speed.



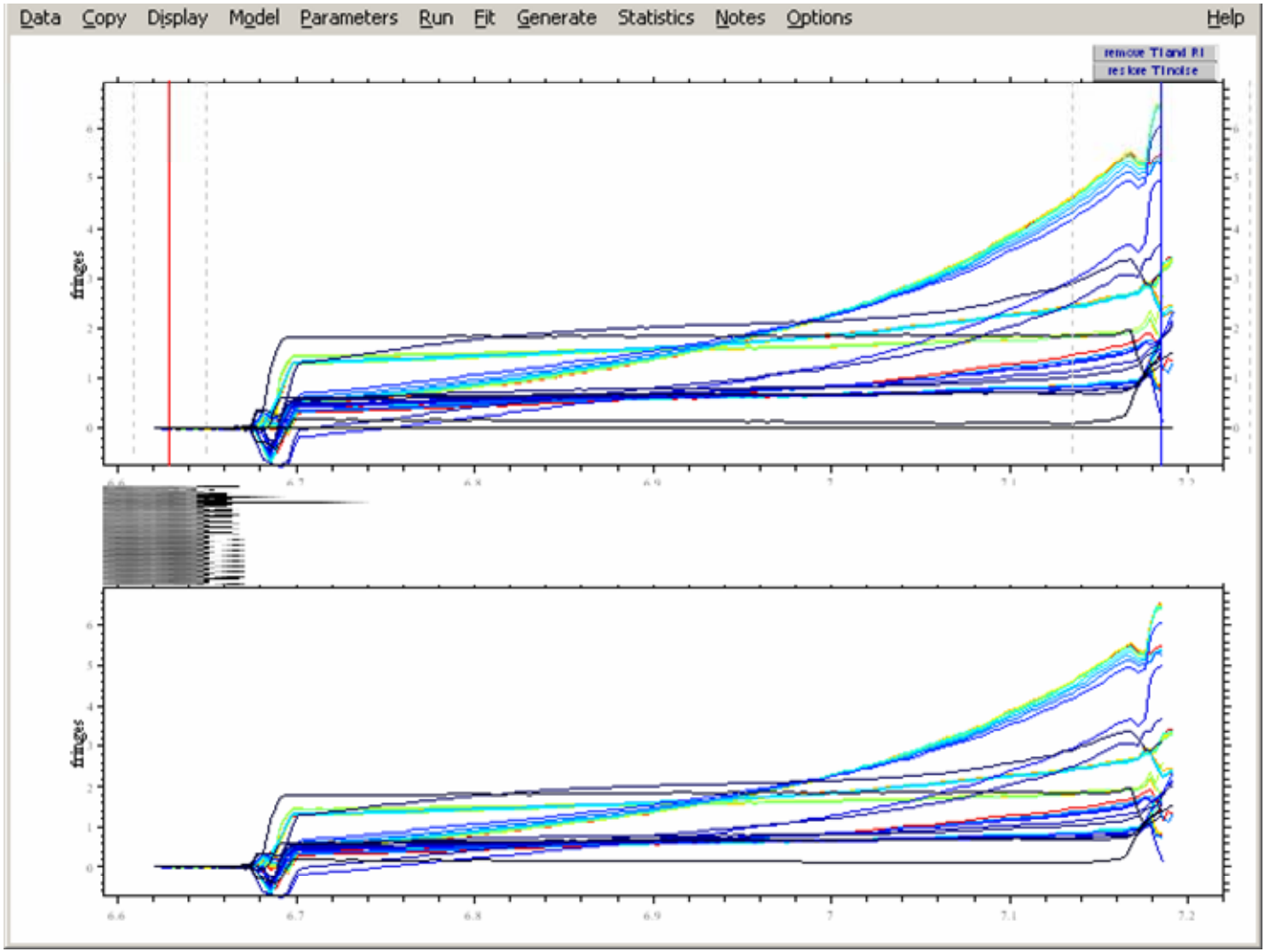
Then, open up all the Interference scans for all the cells in the run.



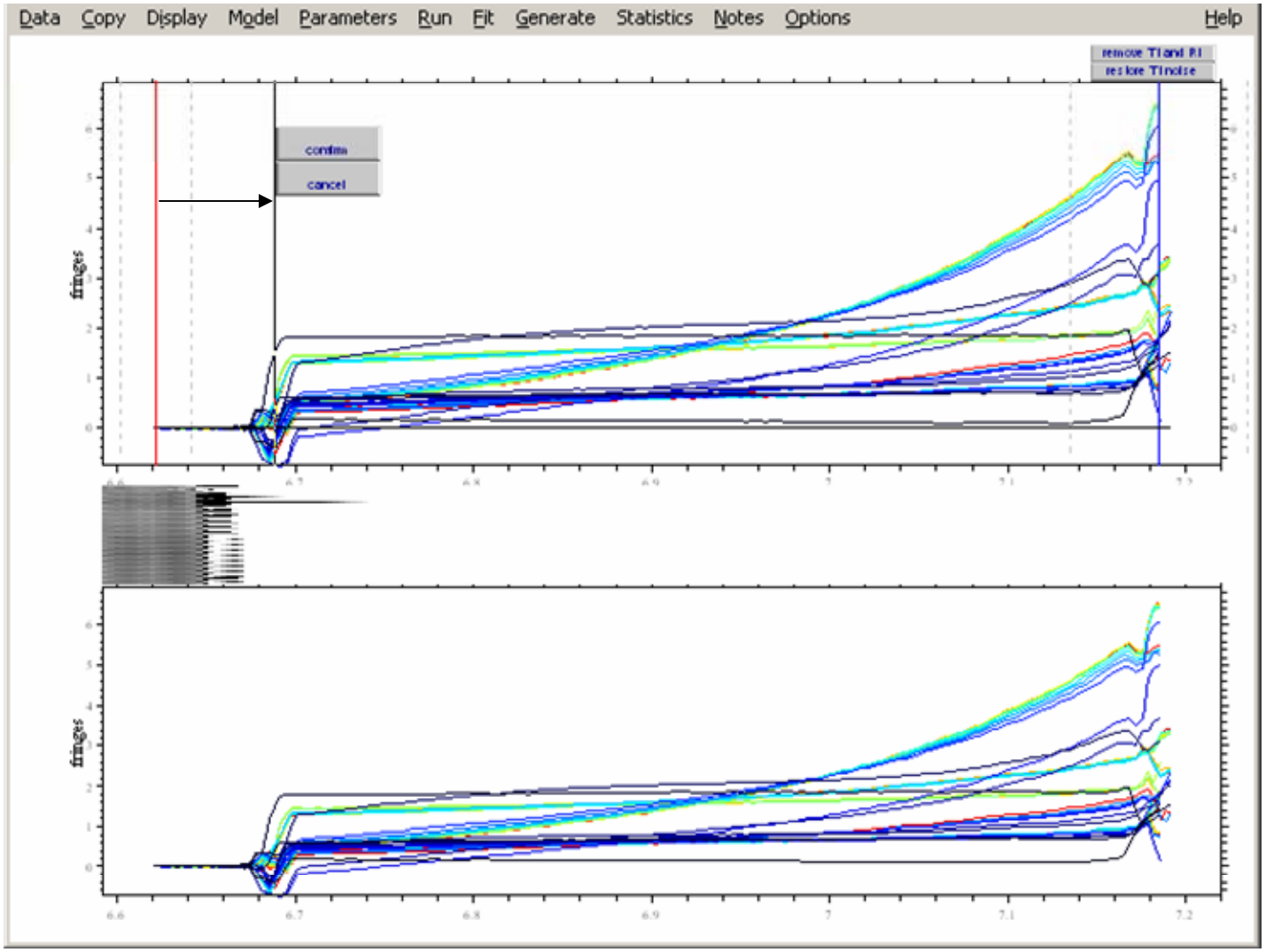
Since it isn't necessary to begin SE runs at thermal equilibrium, the temperature vs time plot is show. These initial temperature fluctuations are irrelevant here, so, just hit OK.



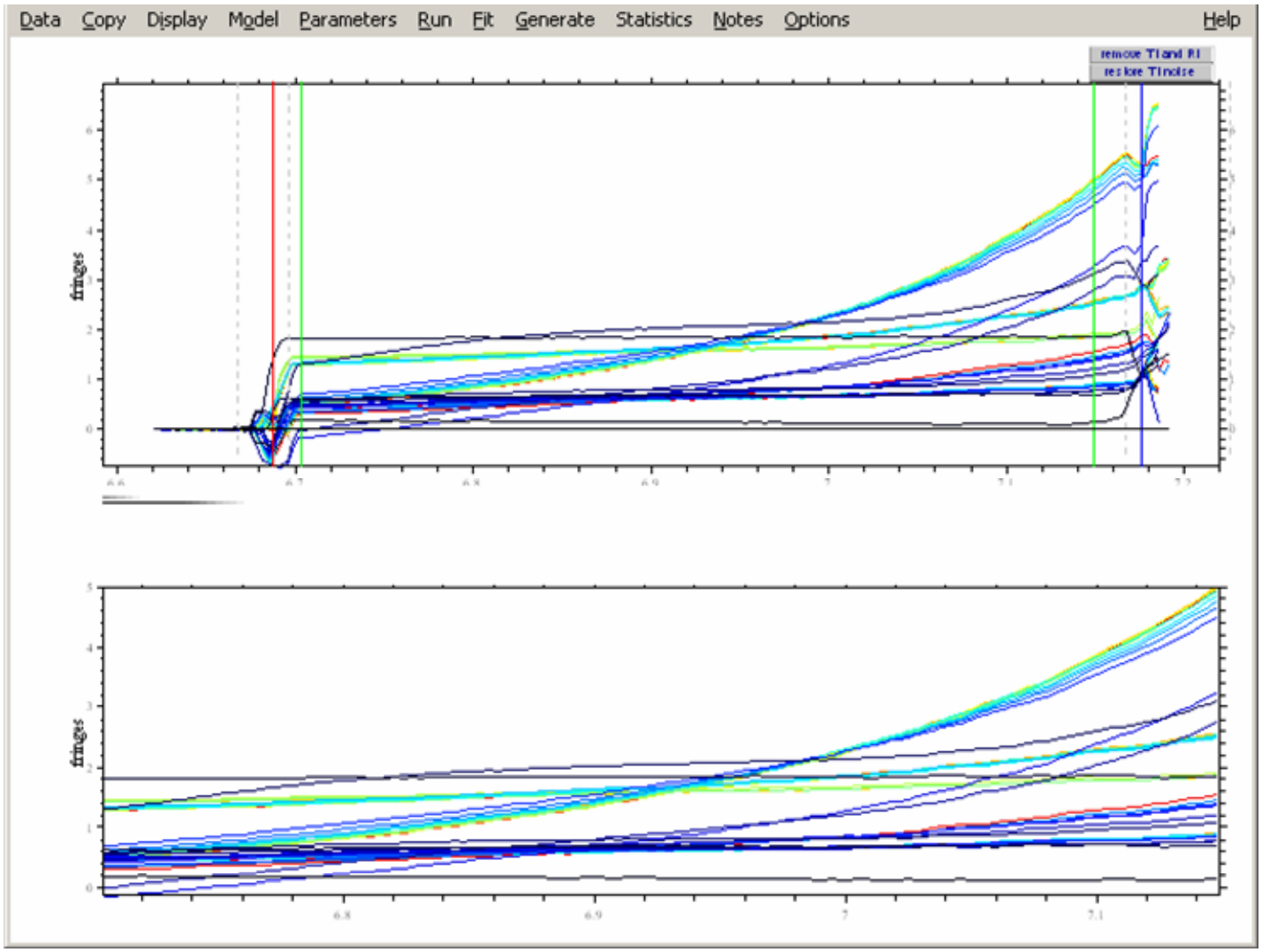
Interference scans from all 3 cells at 12000 rpm are shown. Next, set the meniscus, the bottom and the data limits.



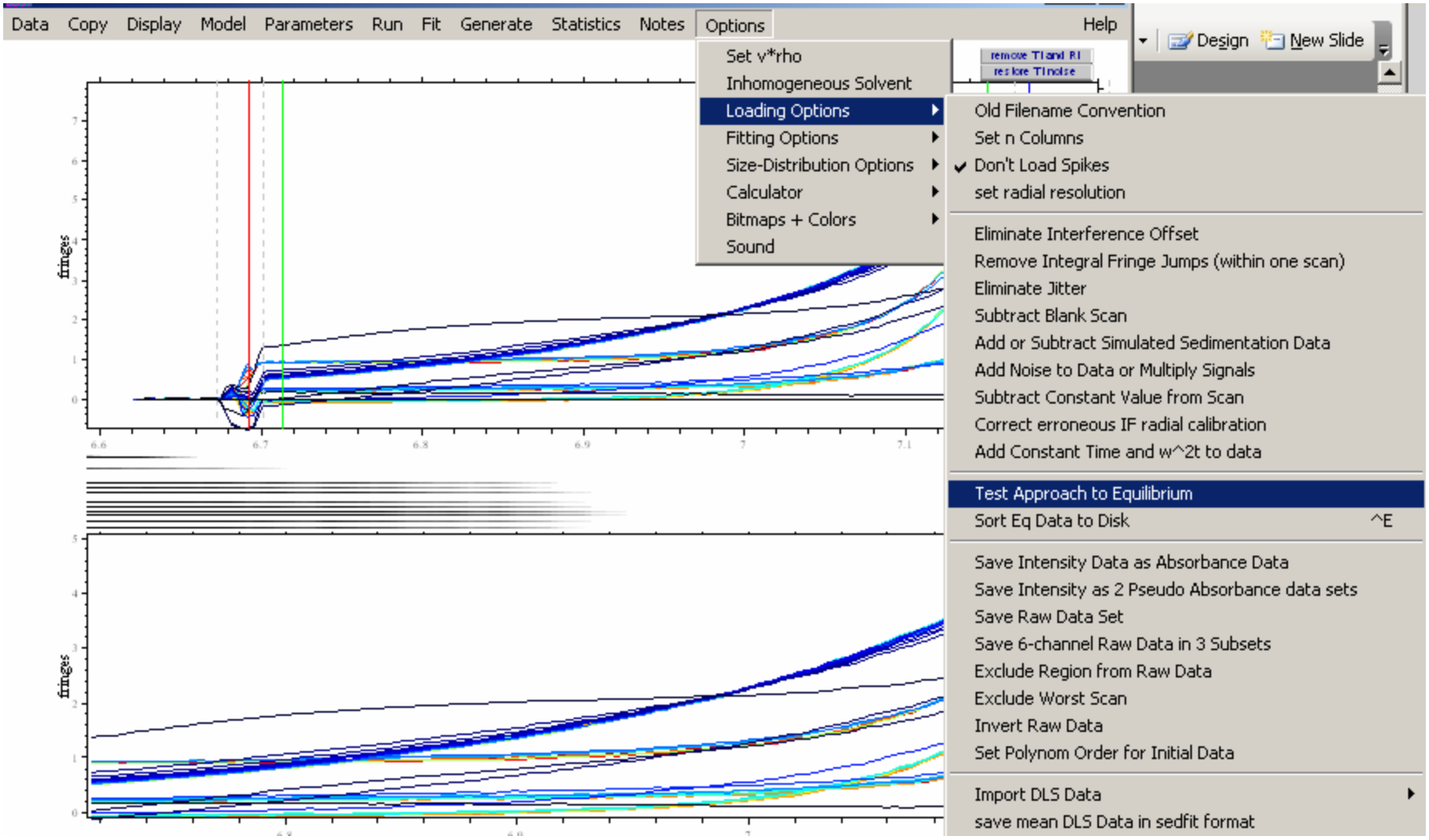
Set the meniscus by dragging the red line to the proper position and hit confirm.
Do the same for the bottom and the data limits.



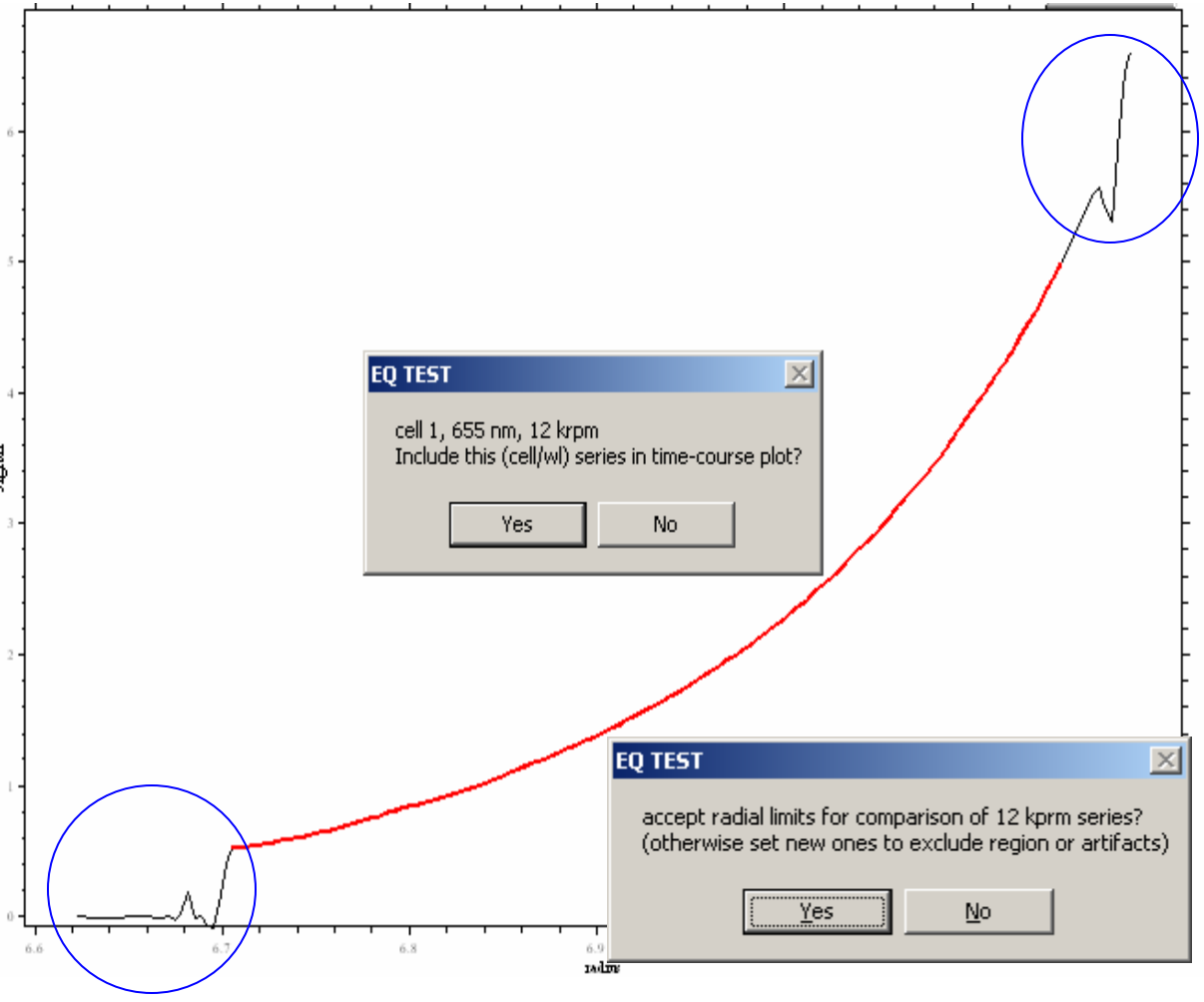
Set the green fitting limits so that they are between the meniscus and bottom.



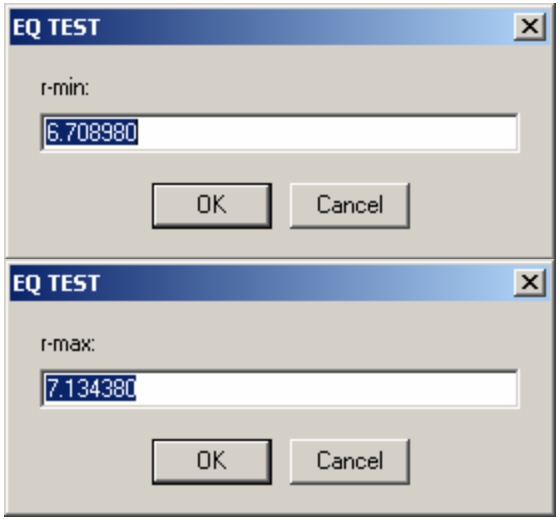
From the Sedfit top menu Options, open Test Approach to Equilibrium.



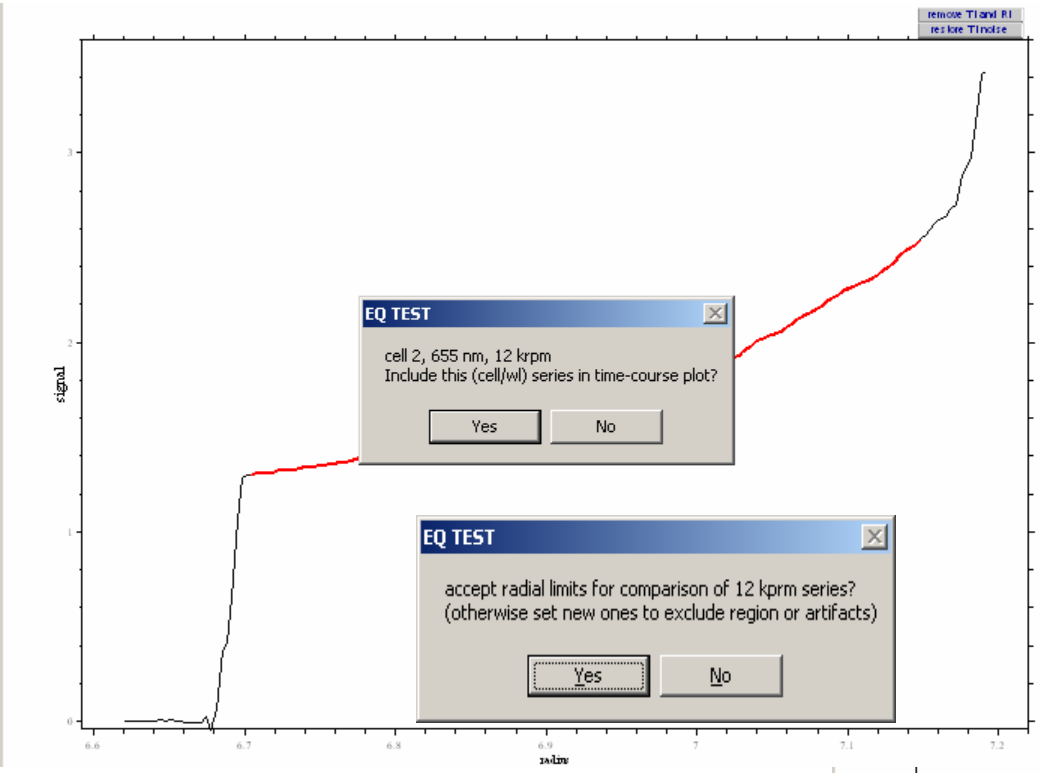
One-by-one Sedfit will display the Interference scan from each cell at 12000 rpm and prompt us to accept the scan and the radial limits, which are made visible with a red line. Make sure the radial limits are set so that neither the meniscus nor the bottom (area within blue circles) are included. If you are not happy with the radial limits, first accept the cell in the plot, then hit NO to the second window and do not accept the radial limits. Sedfit will permit you to re-set the limits with the next two windows.



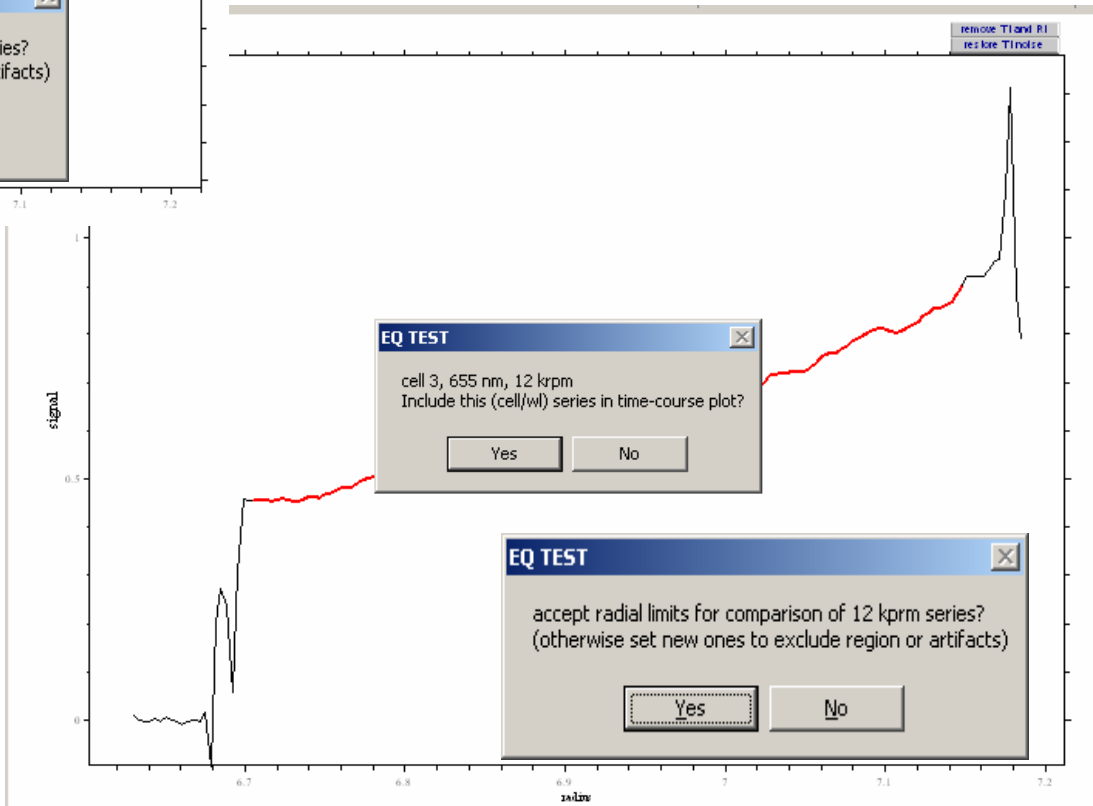
Re-set radial limits with these 2 windows, then again include the cell in the plot and accept the new limits.



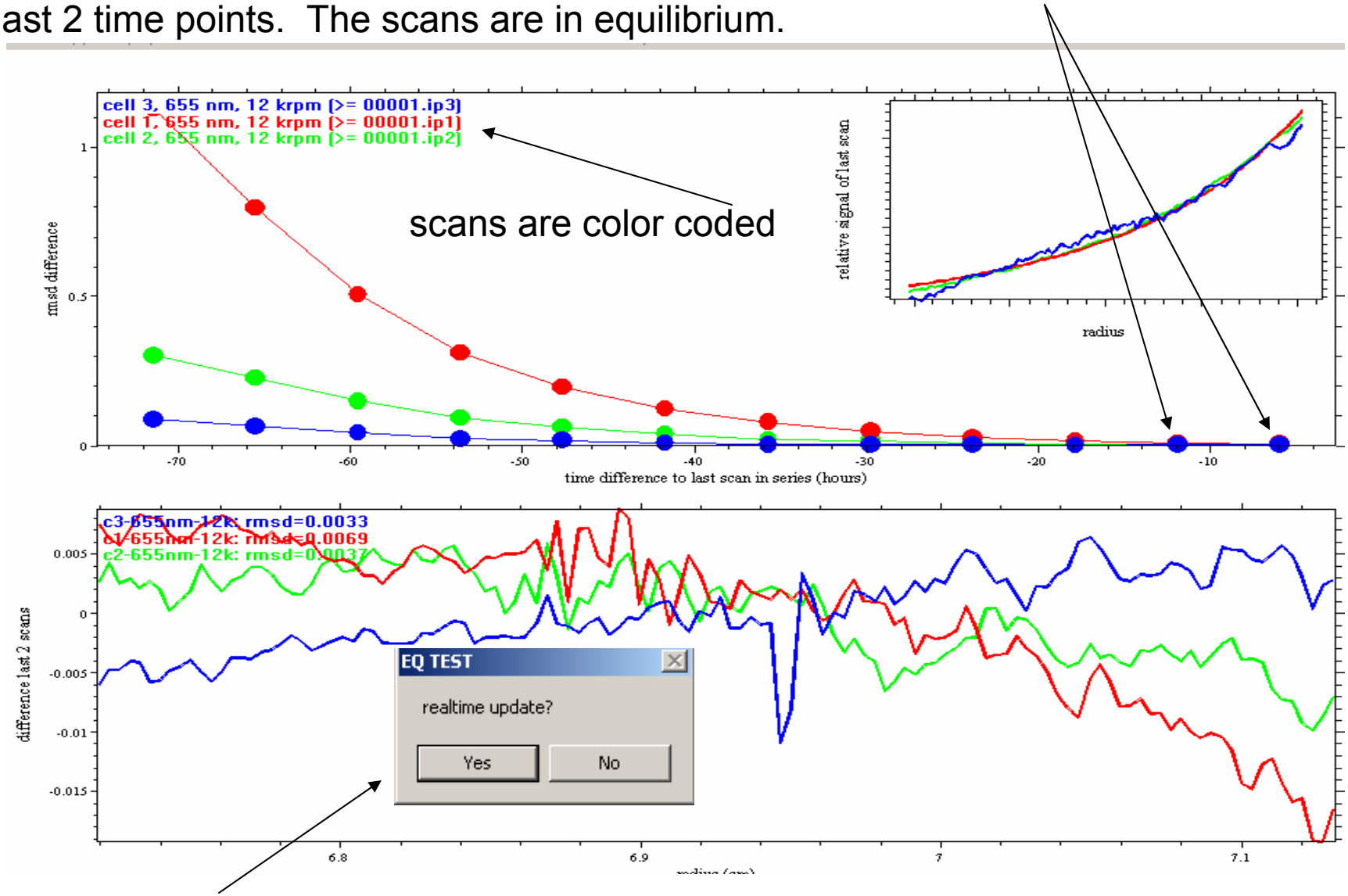
As a alternative, you can reload all the scans and reset the data limits taking care not to drag the lines too close to the meniscus or the bottom.



Check the remaining cells and include each in the time-course plot.

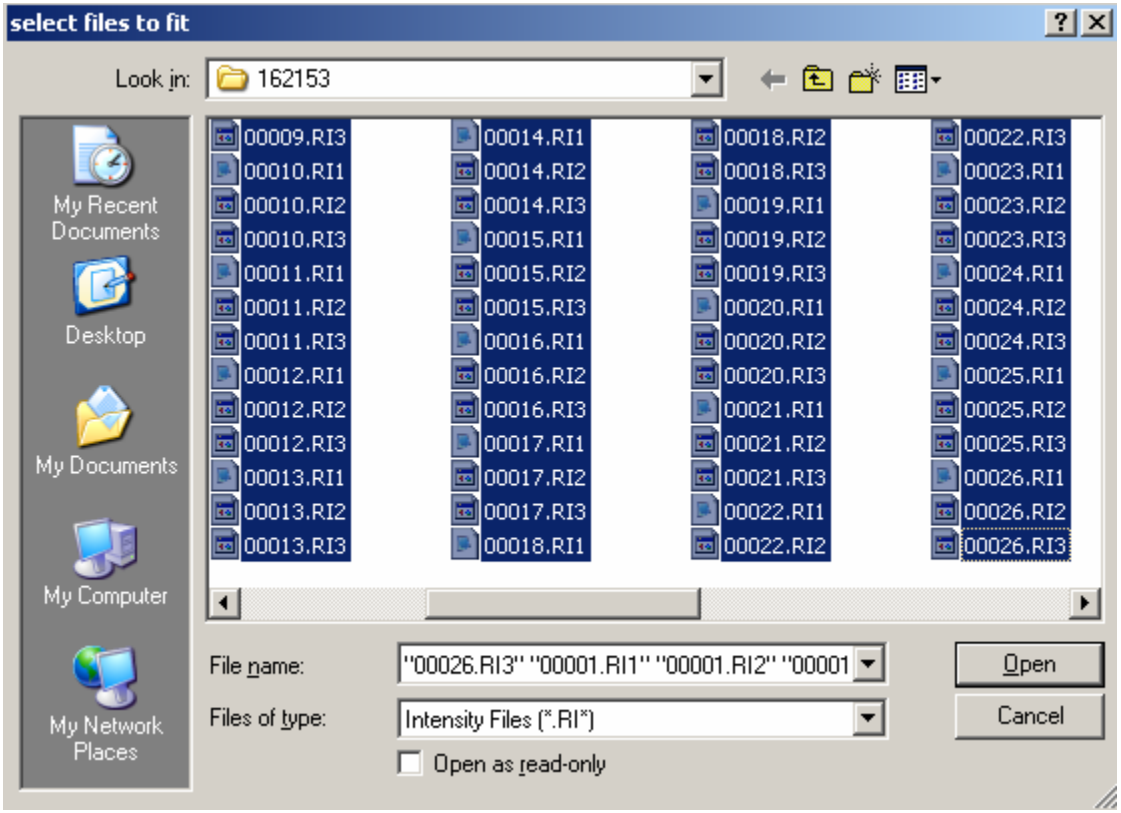


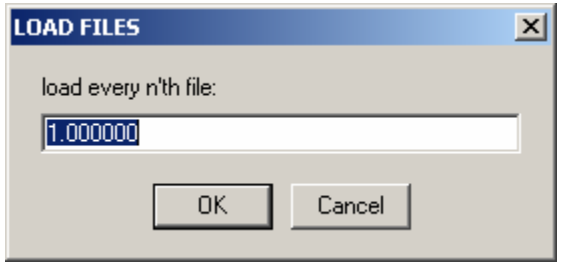
A plot of rms difference over time will be displayed comparing each scan with the previous scan. As you can see here, the rms difference is unchanged between the last 2 time points. The scans are in equilibrium.



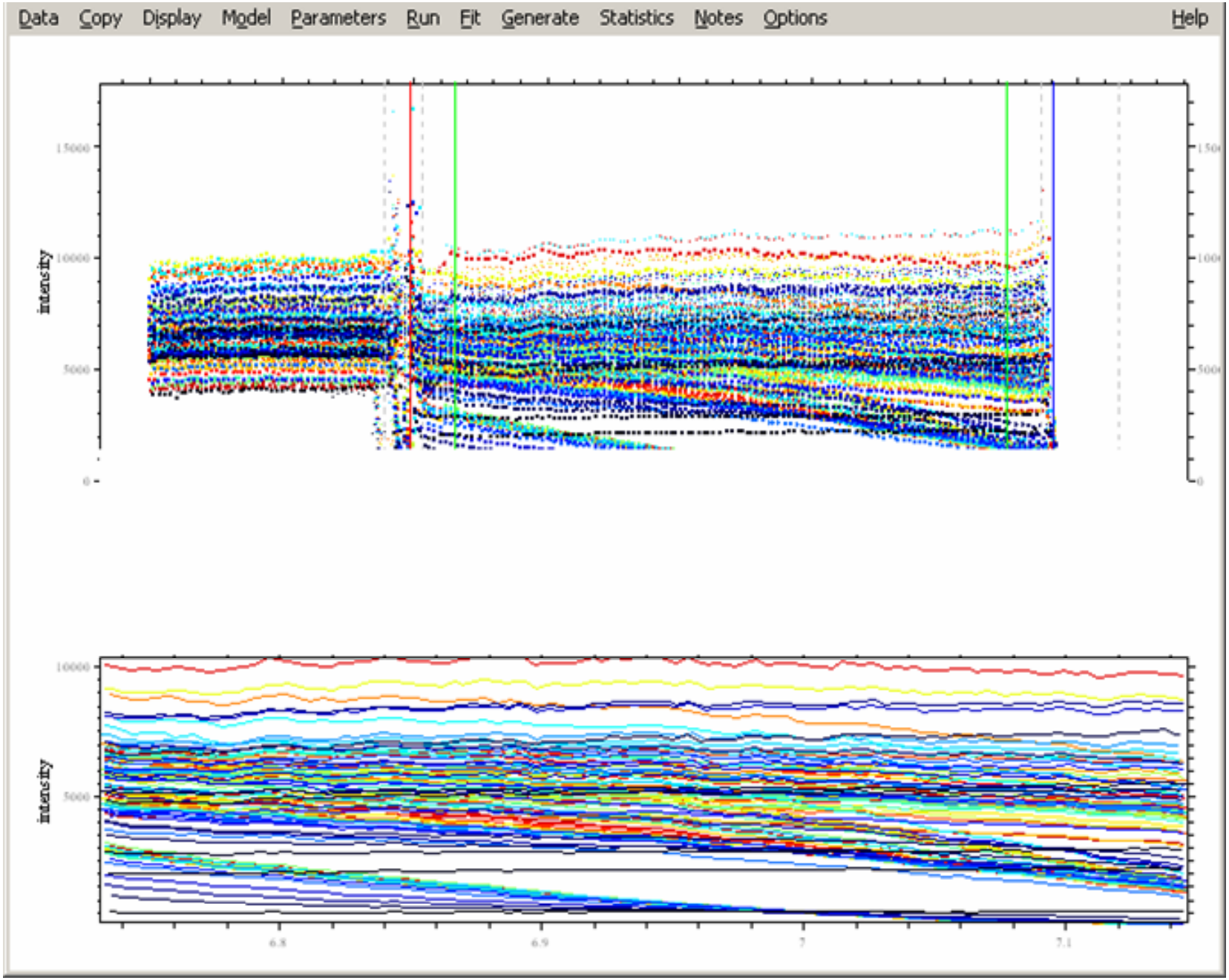
Sedfit gives us the option to view this time plot in real time as the SE run is progressing

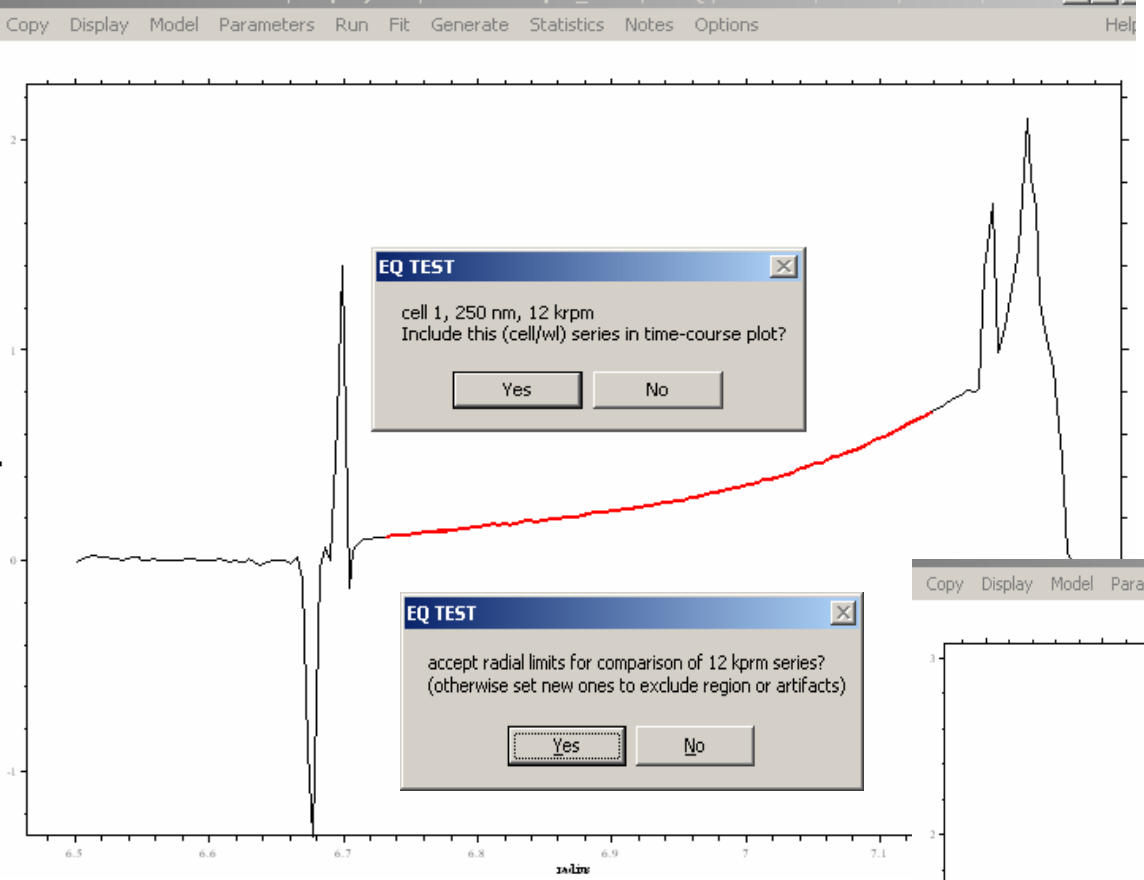
Even though it is very likely that the absorbance scans will also be in equilibrium, we will take a look anyway. Open all the absorbance scans for all 3 cells. Remember, there will be twice as many, 13 scans at 280nm and 13 scans at 250nm to total 26 scans.



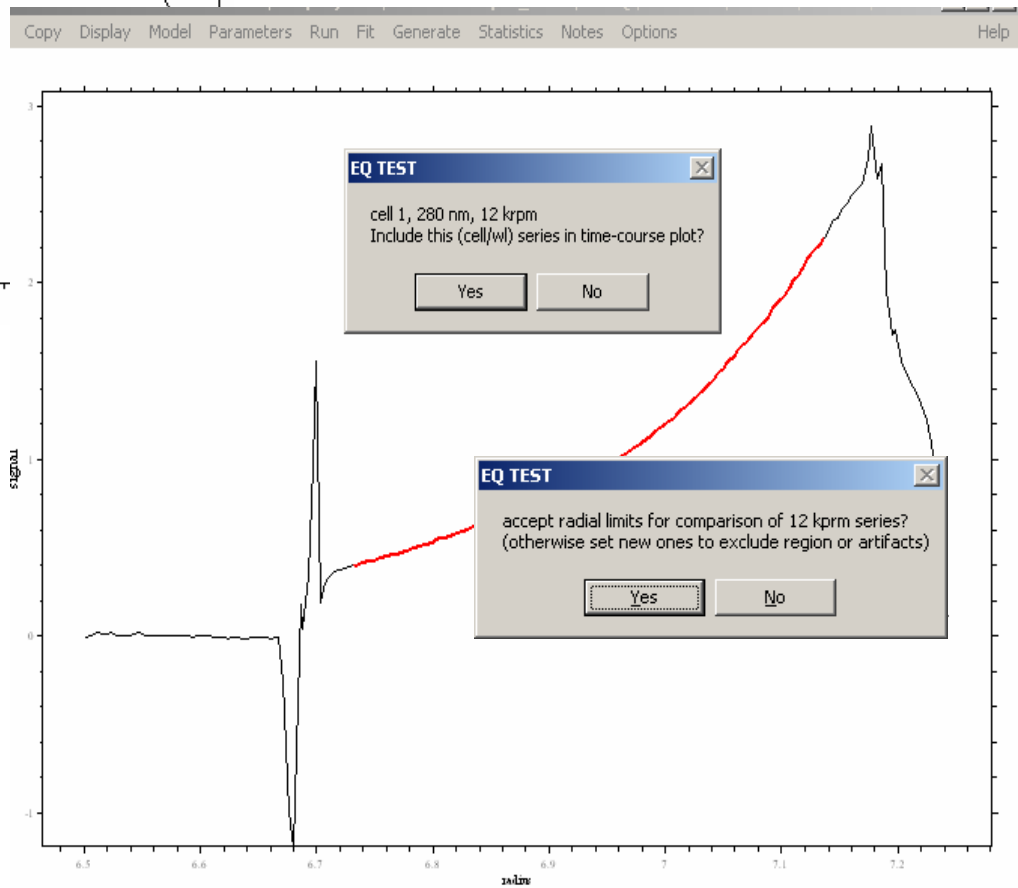


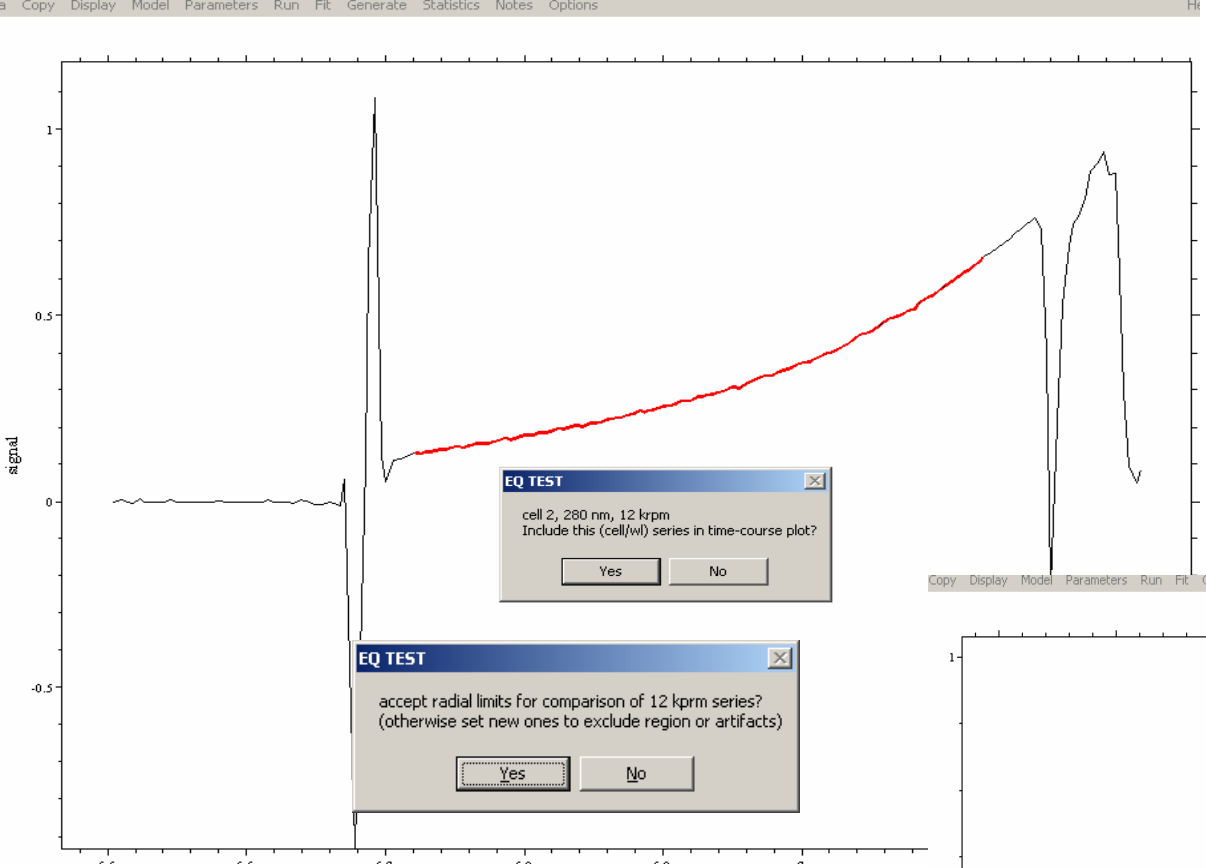
Set the meniscus and bottom. Then set the data limits between them.



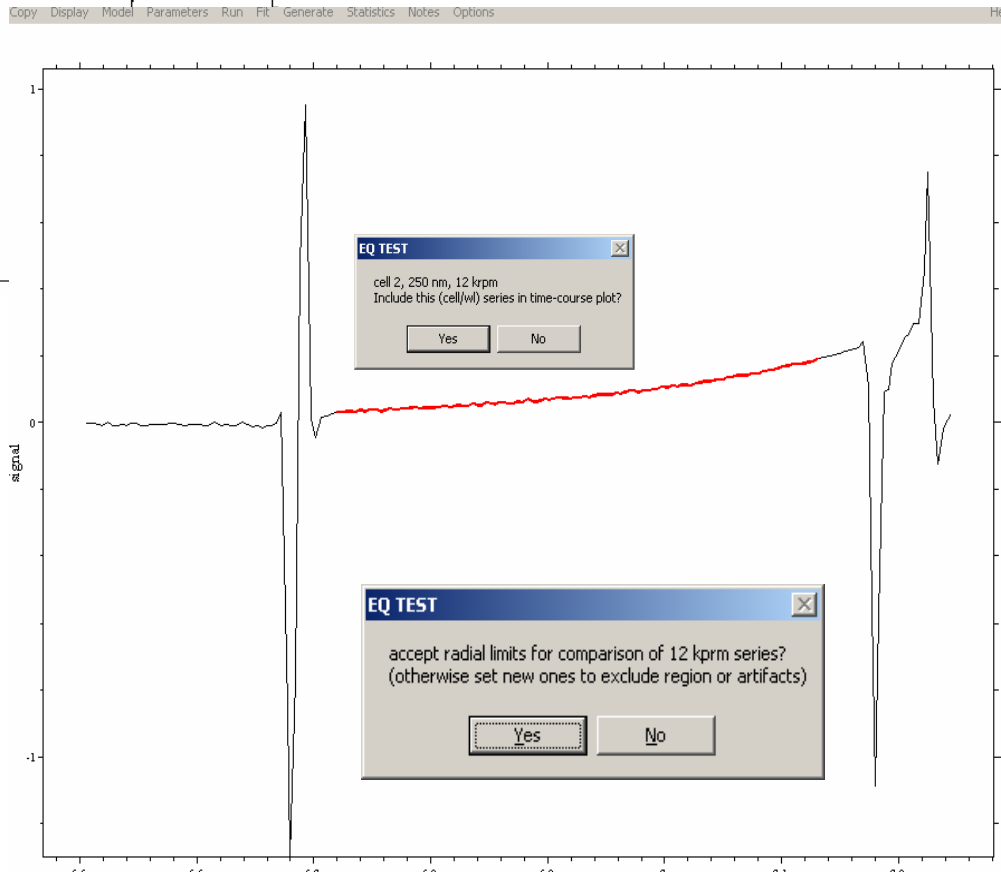


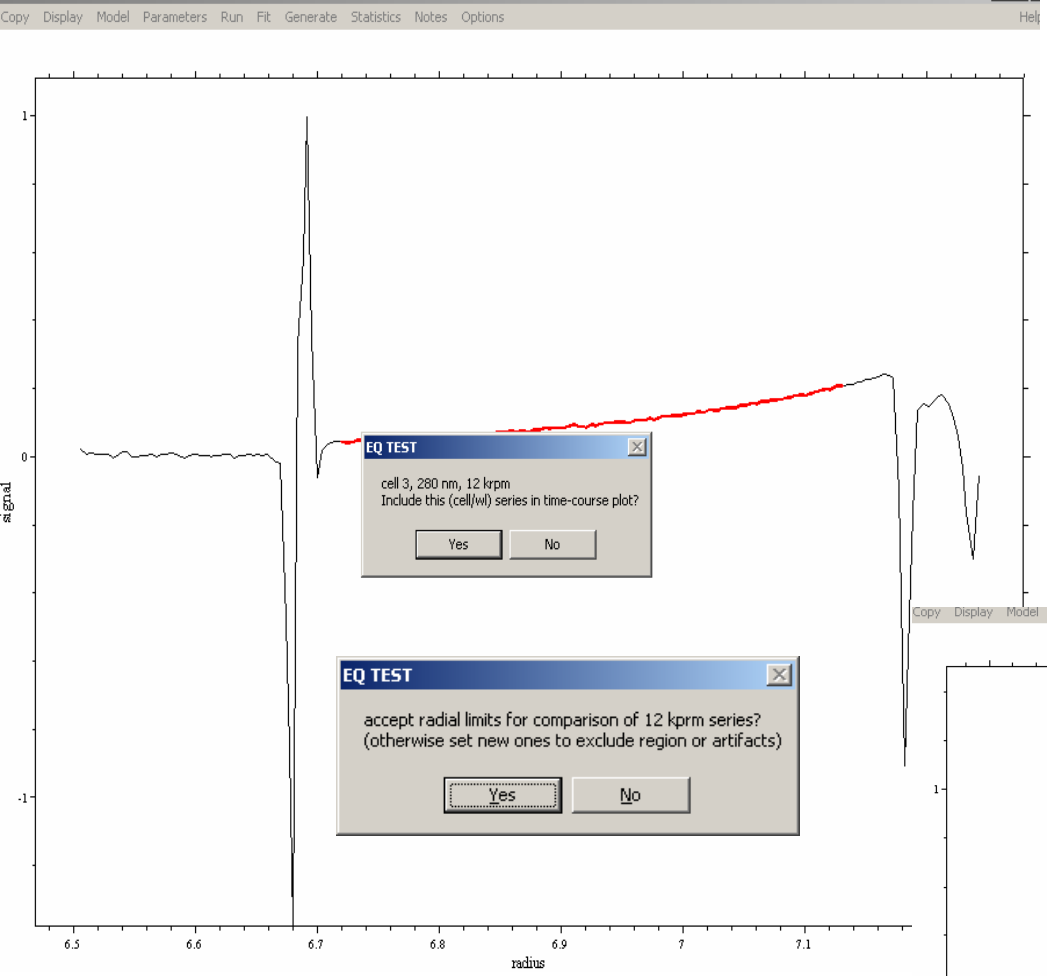
Then, answer Sedfit's prompts for each cell at each wave length.



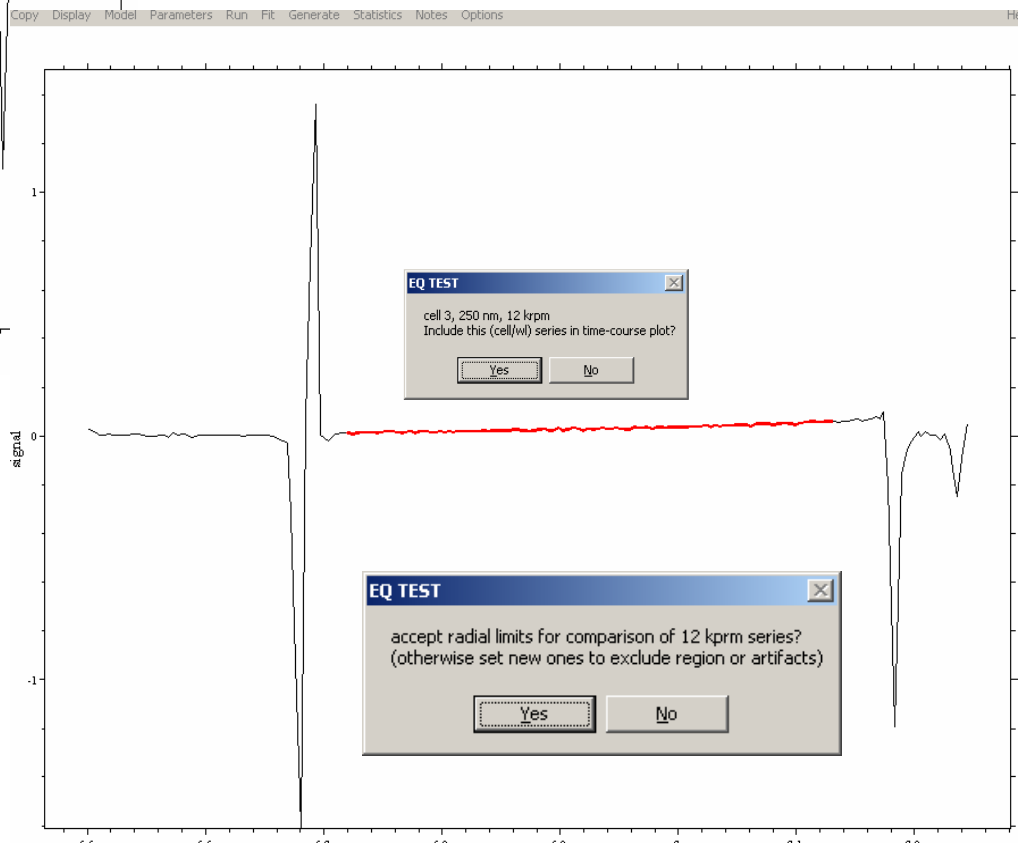

Copy Display Model Parameters Run Fit Generate Statistics Notes Options
signal
EQ TEST
cell 2, 280 nm, 12 krpm
Include this (cell/wl) series in time-course plot?
Yes
No
EQ TEST
accept radial limits for comparison of 12 kprm series?
(otherwise set new ones to exclude region or artifacts)
Yes
No

Cell 2.

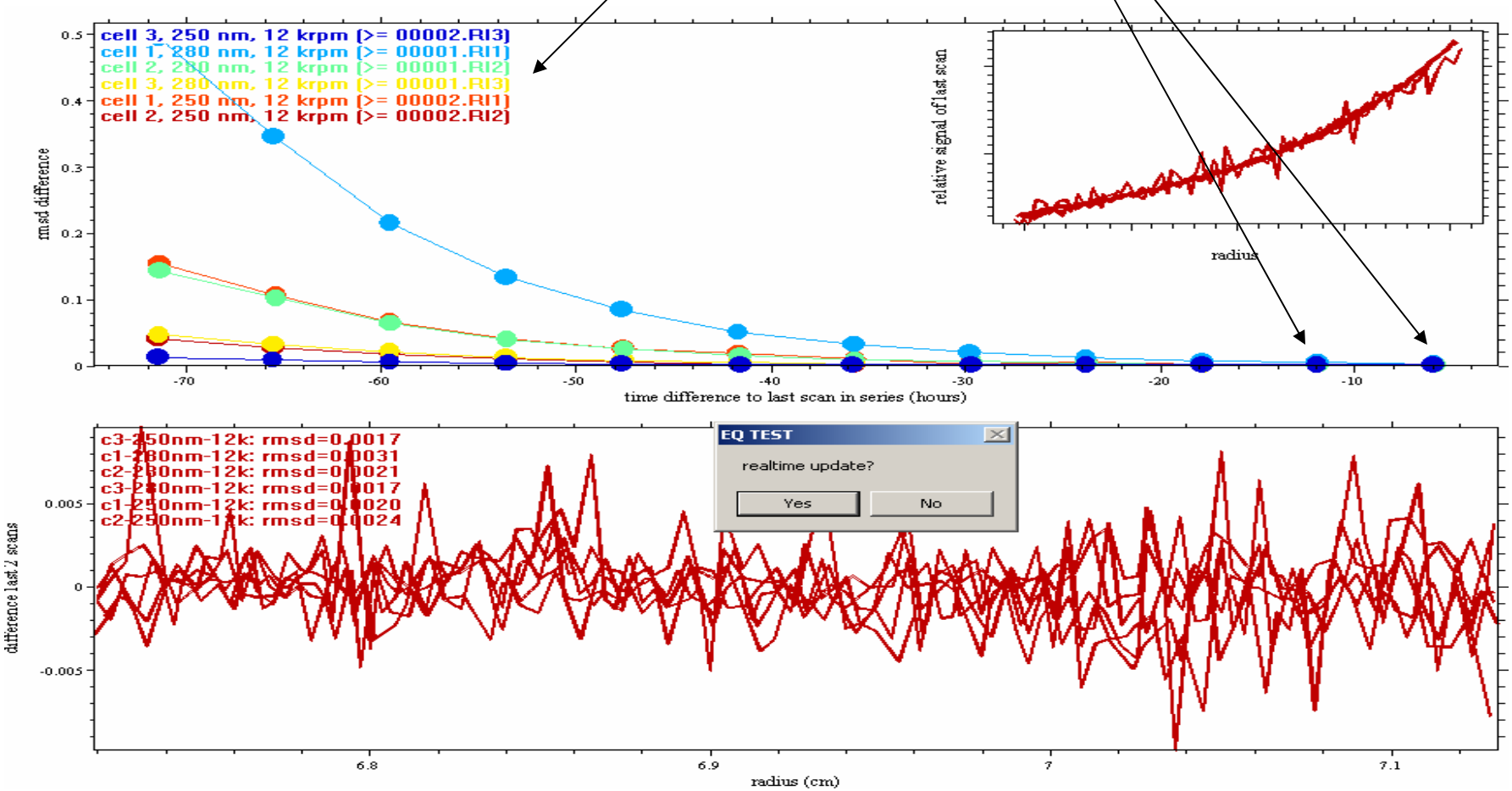

Copy Display Model Parameters Run Fit Generate Statistics Notes Options
signal
EQ TEST
cell 2, 250 nm, 12 krpm
Include this (cell/wl) series in time-course plot?
Yes
No
EQ TEST
accept radial limits for comparison of 12 kprm series?
(otherwise set new ones to exclude region or artifacts)
Yes
No


Copy Display Model Parameters Run Fit Generate Statistics Notes Options
EQ TEST
cell 3, 280 nm, 12 krpm
Include this (cell/wl) series in time-course plot?
Yes
No
EQ TEST
accept radial limits for comparison of 12 kprm series?
(otherwise set new ones to exclude region or artifacts)
Yes
No
signal
radius

Cell 3.


Copy Display Model Parameters Run Fit Generate Statistics Notes Options
EQ TEST
cell 3, 250 nm, 12 krpm
Include this (cell/wl) series in time-course plot?
Yes
No
EQ TEST
accept radial limits for comparison of 12 kprm series?
(otherwise set new ones to exclude region or artifacts)
Yes
No
signal

A plot of rms difference over time will be displayed comparing each scan with the previous scan. Here also, the rms difference is unchanged between the last 2 time points. The scans are in equilibrium.
Also, notice that the scans are color coded which allows us to see each individual scan as it approaches equilibrium.



At this point, we would stop the run, change the Methods to reflect the next speed, save the new parameters and start the new method.