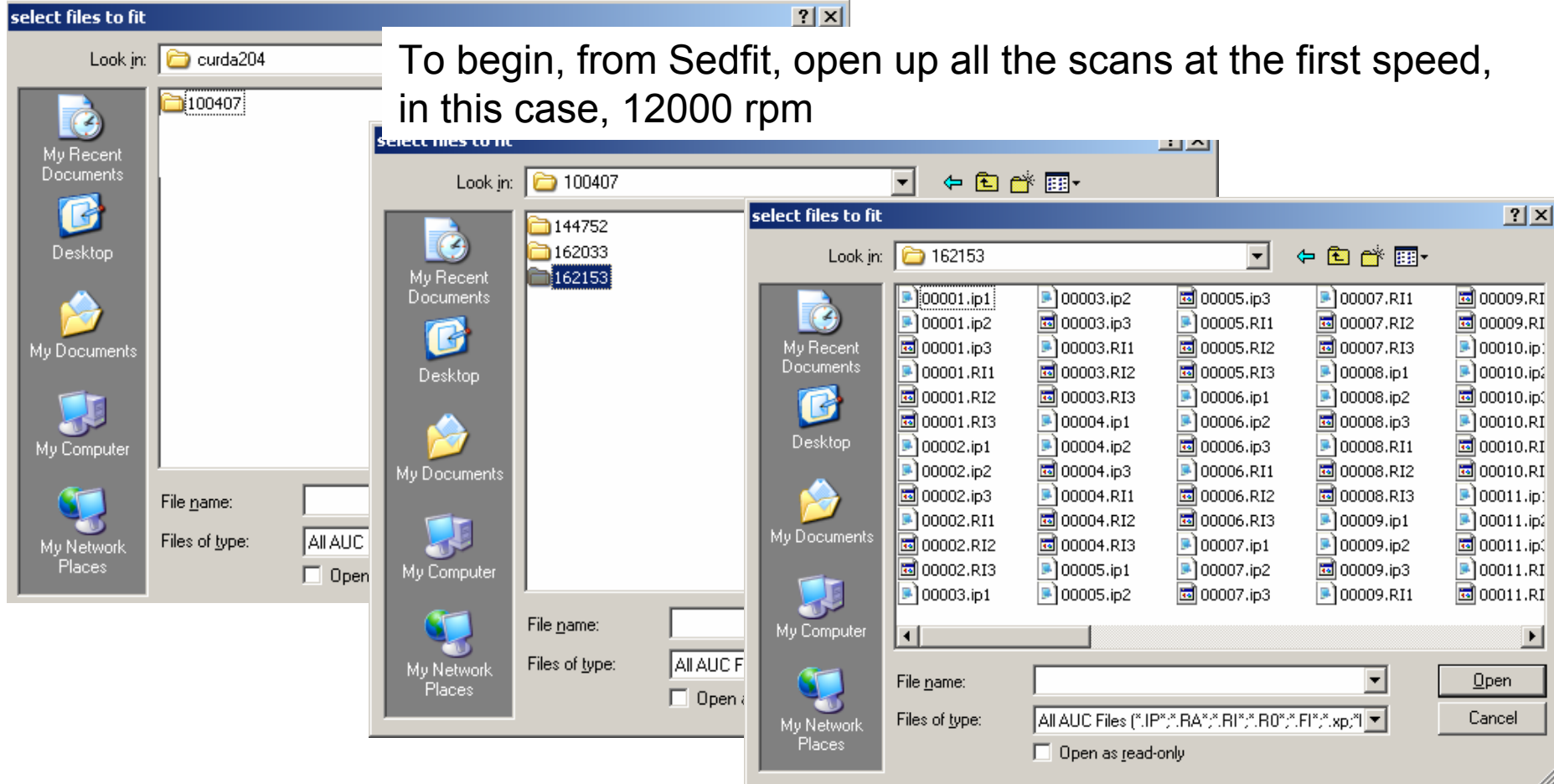


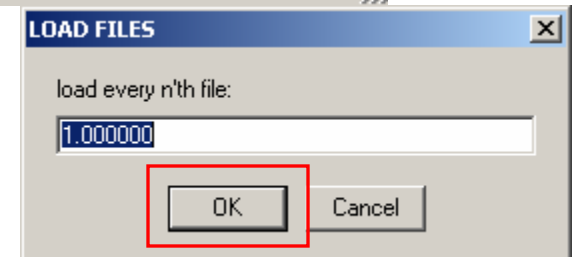
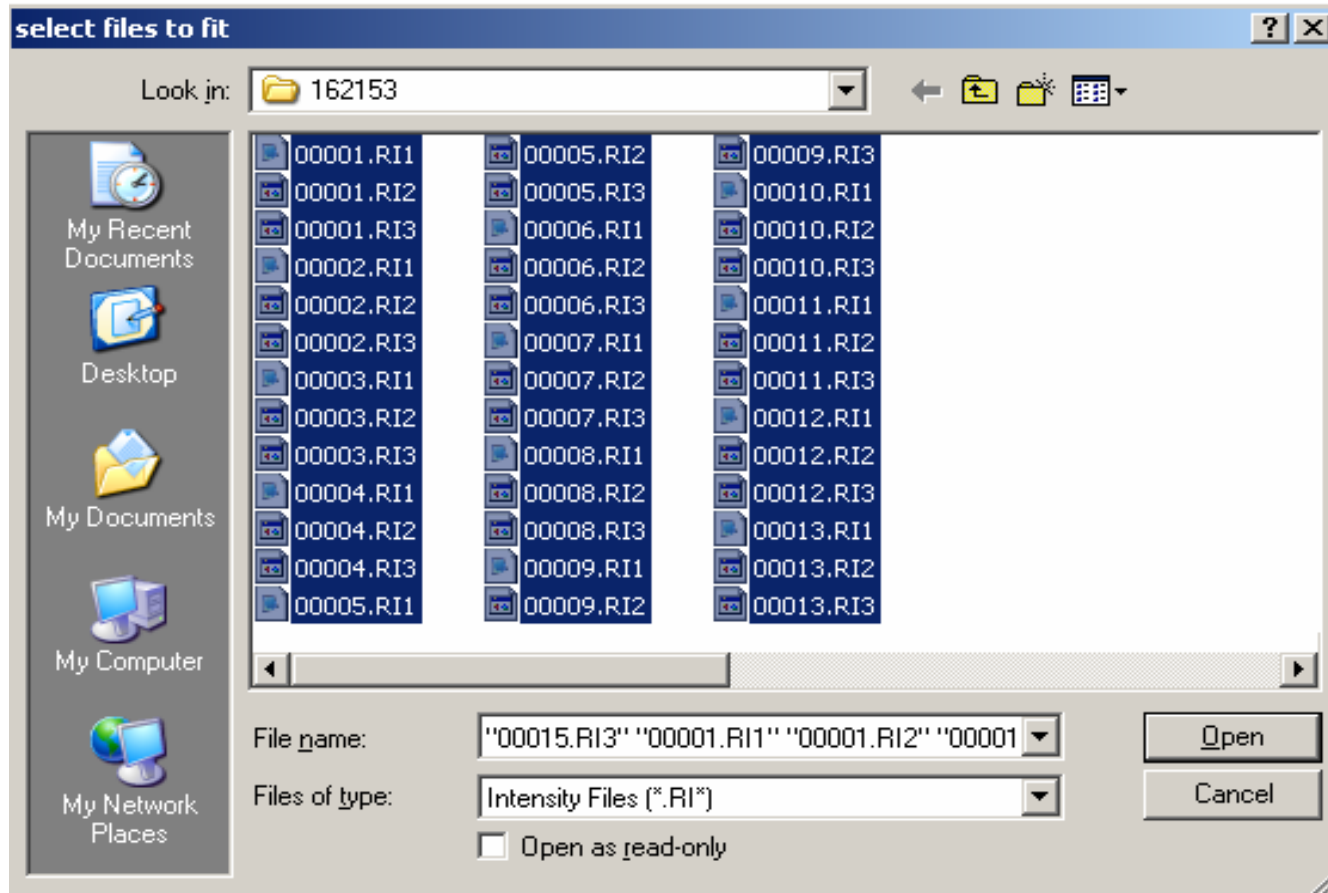
This tutorial will demonstrate how to use SEDFIT to sort interference and absorbance data from a Sedimentation Equilibrium experiment to determine if scans are in equilibrium.

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.

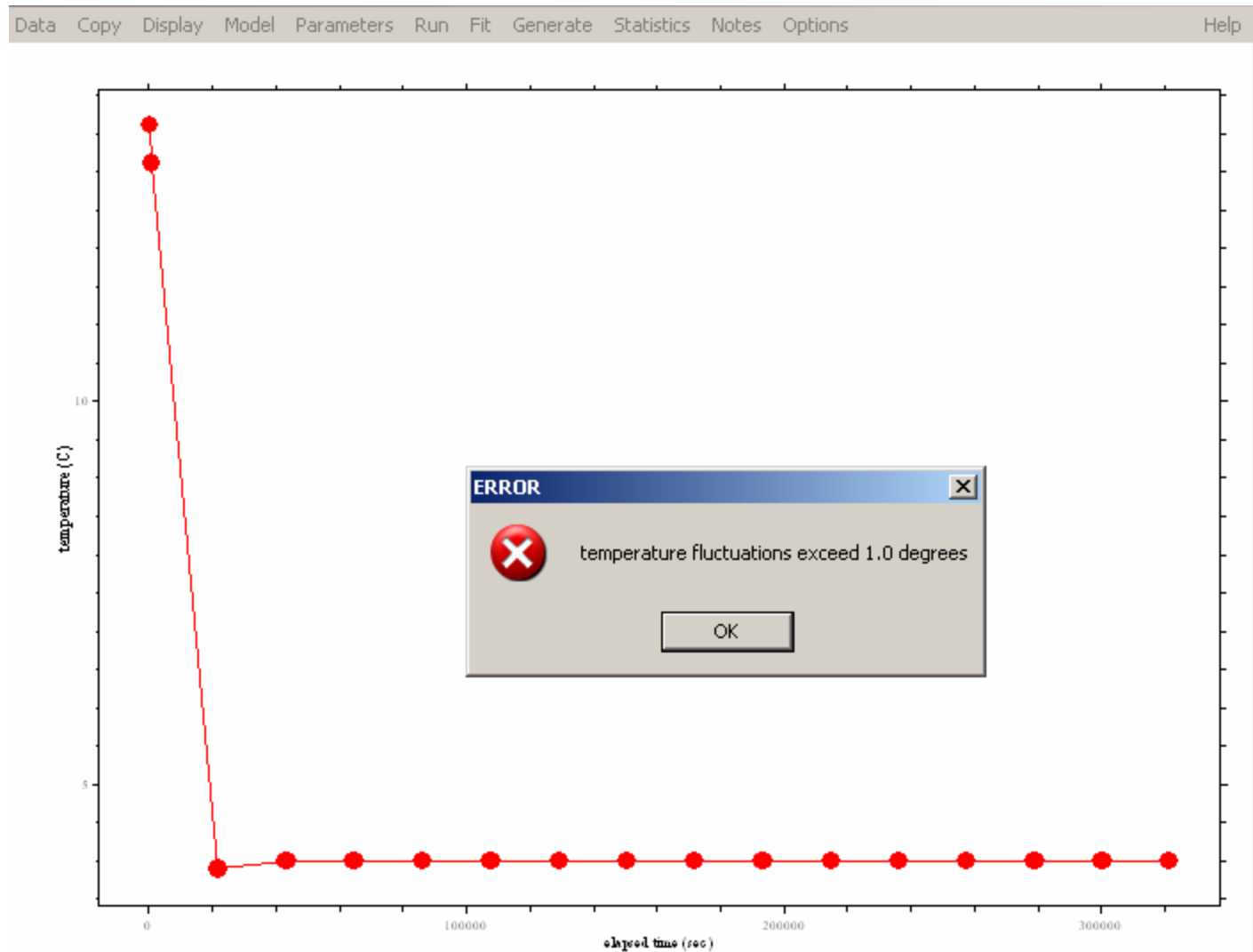
To begin, from Sedfit, open up all the scans at the first speed, in this case, 12000 rpm



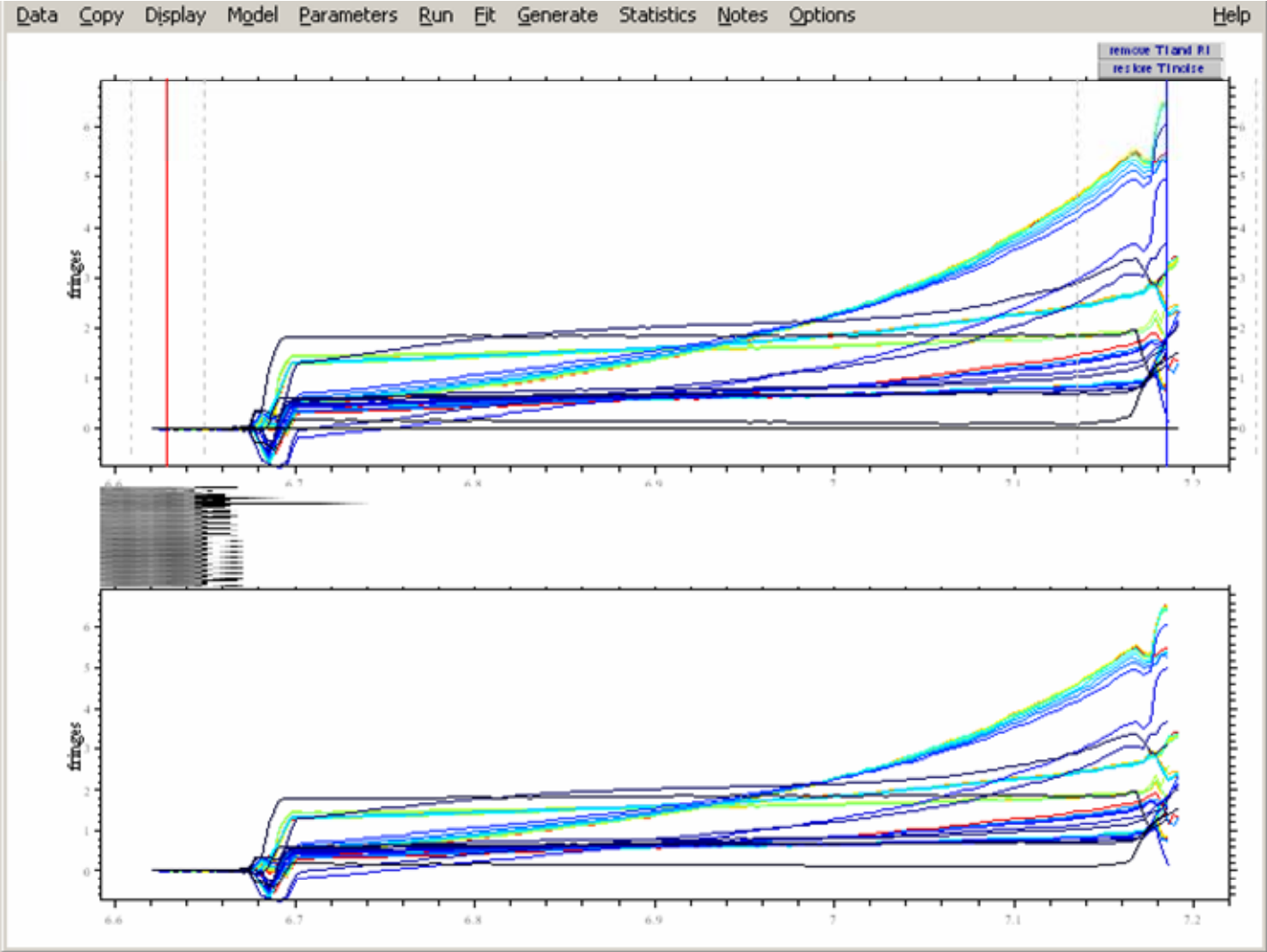
Then, open up all the Interference scans for all the cells in the run.



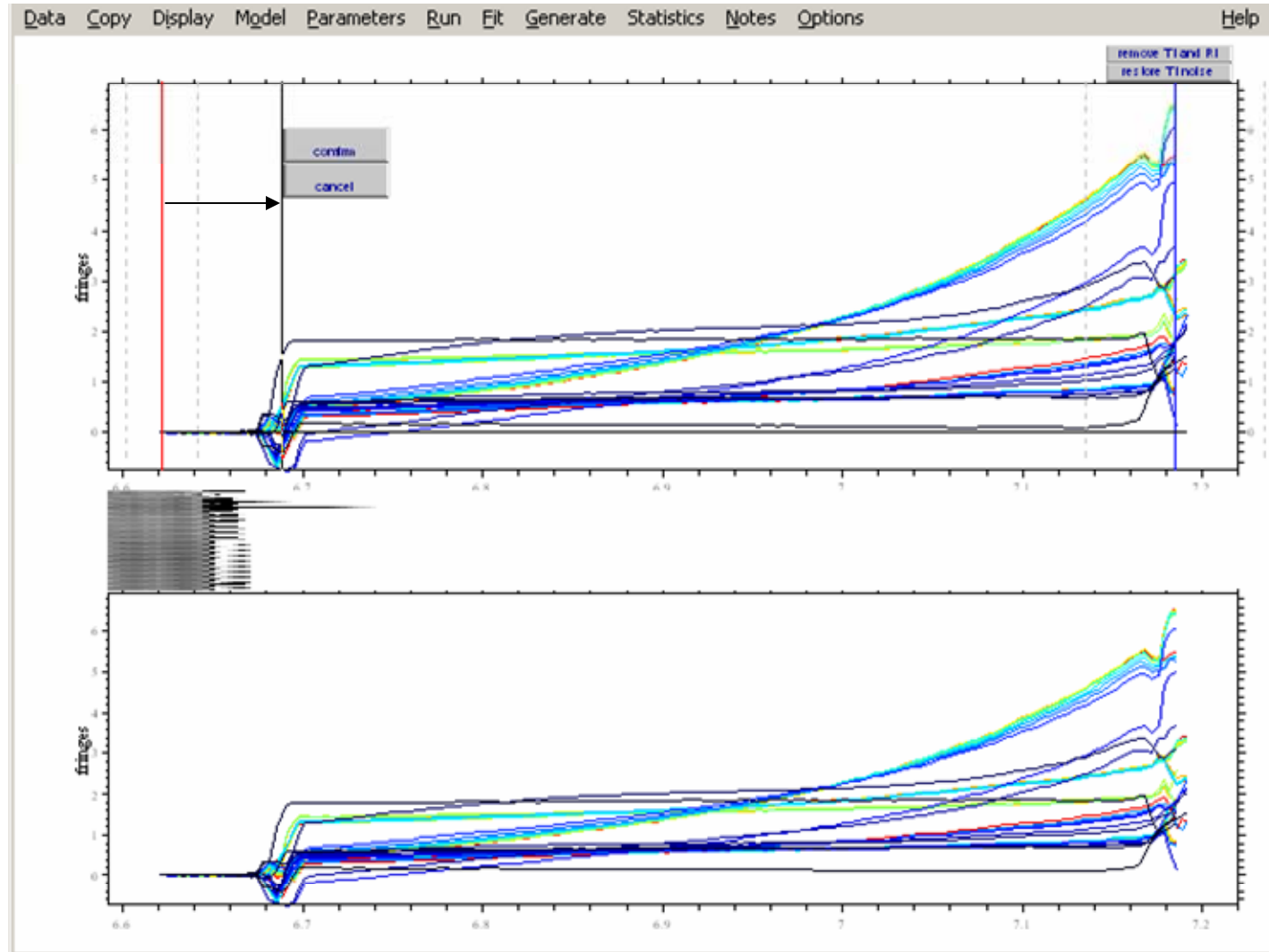
Since it isn't necessary to begin SE runs at thermal equilibrium, the temperature vs time plot is shown. These initial temperature fluctuations are irrelevant here, so, just hit OK.



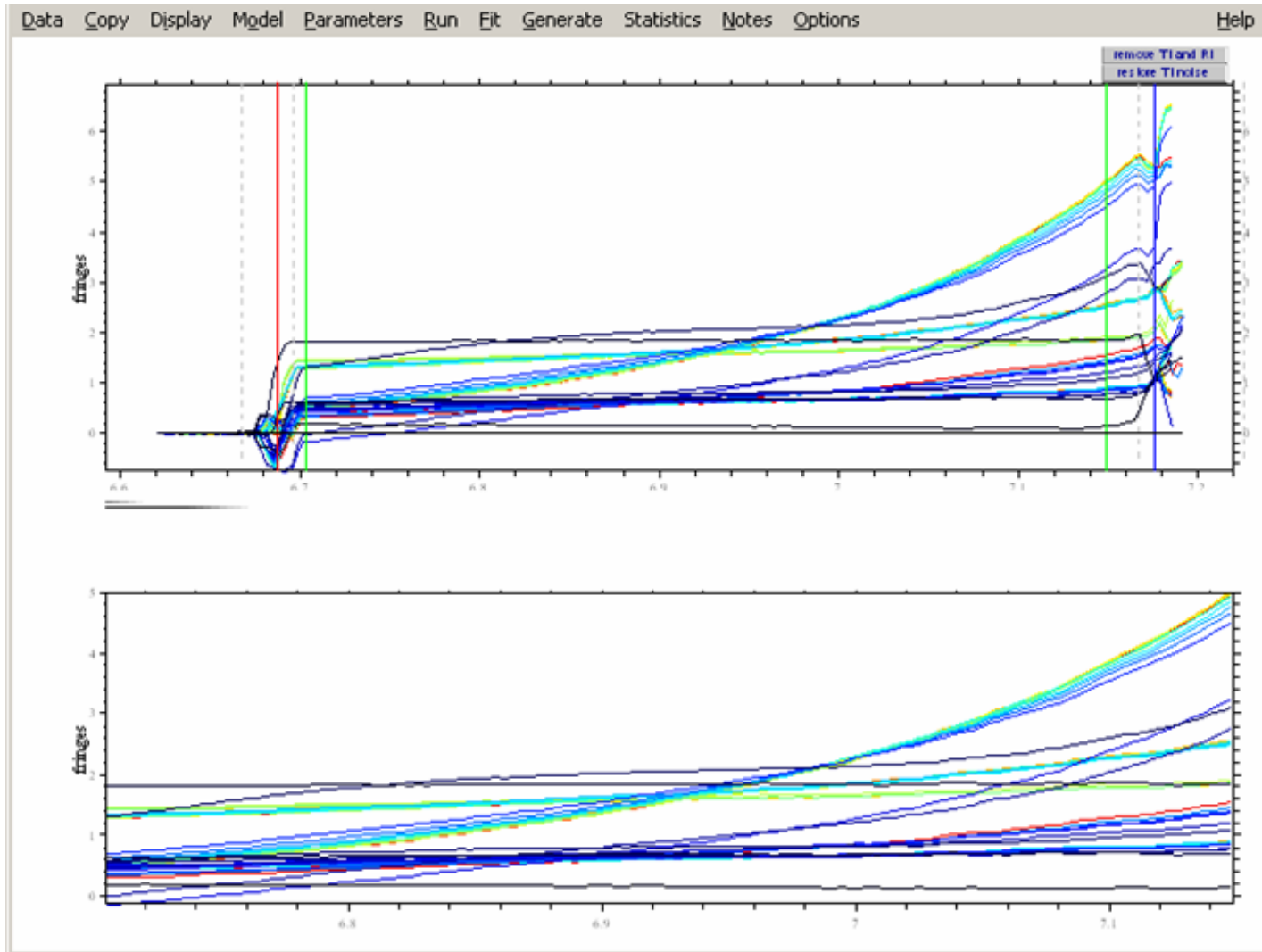
Interference scans from all 3 cells at 12000 rpm are shown. Next, set the meniscus, the bottom and the data limits.



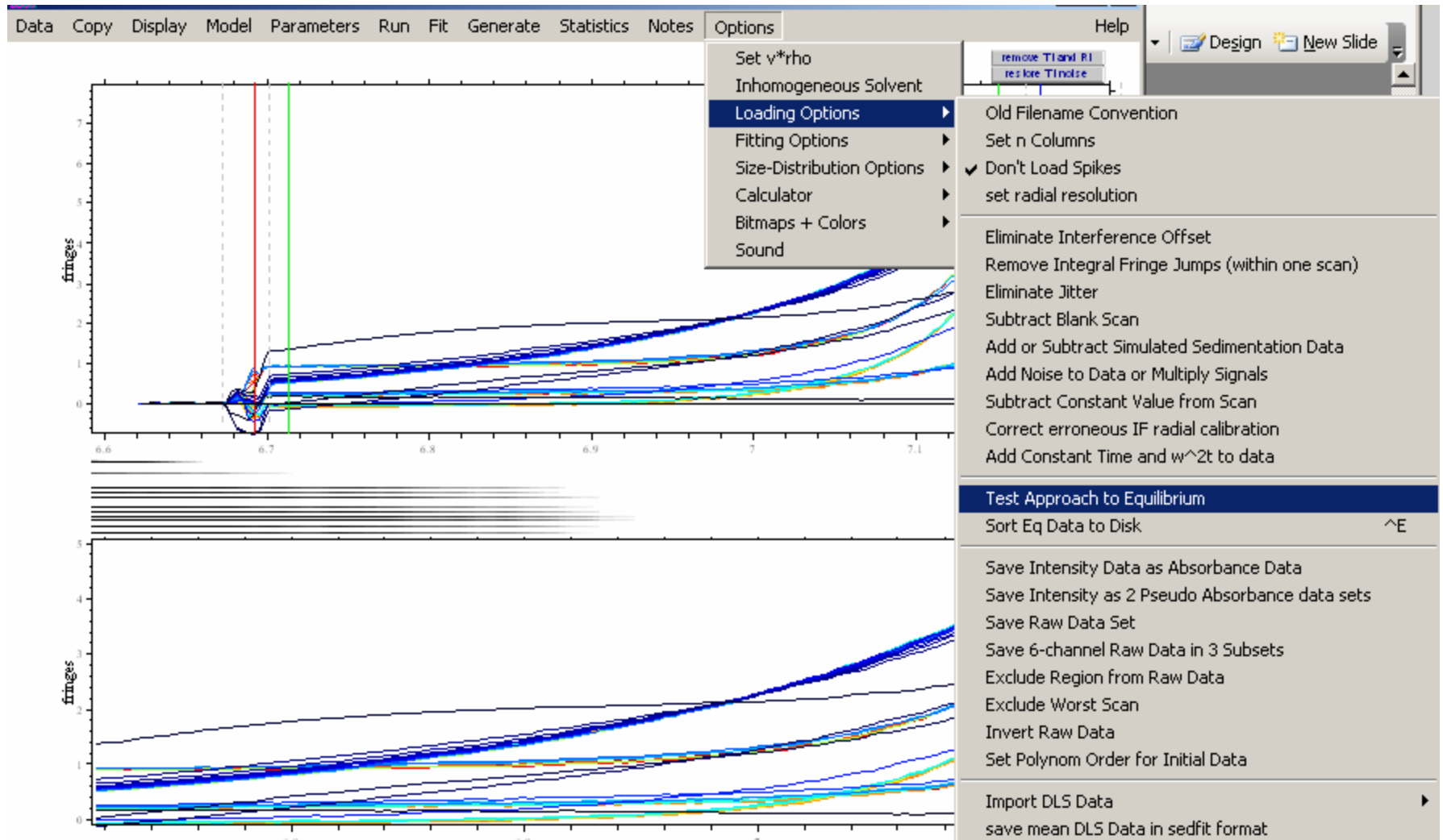
Set the meniscus by dragging the red line to the proper position and hit confirm.
Do the same for the bottom and the data limits.



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.



The screenshot displays the Sedfit software interface. The top menu bar includes: Data, Copy, Display, Model, Parameters, Run, Fit, Generate, Statistics, Notes, Options, and Help. The 'Options' menu is open, showing a list of settings. The 'Test Approach to Equilibrium' option is highlighted in blue. Below the menu, two plots are visible. The top plot shows 'fringes' on the y-axis (ranging from 0 to 7) and a time axis (ranging from 6.6 to 7.1). It features several data series in various colors (black, blue, green, red) and two vertical dashed lines, one red and one green, at approximately 6.7. The bottom plot shows 'fringes' on the y-axis (ranging from 0 to 5) and a time axis (ranging from 6.6 to 7.1), displaying multiple data series in various colors.

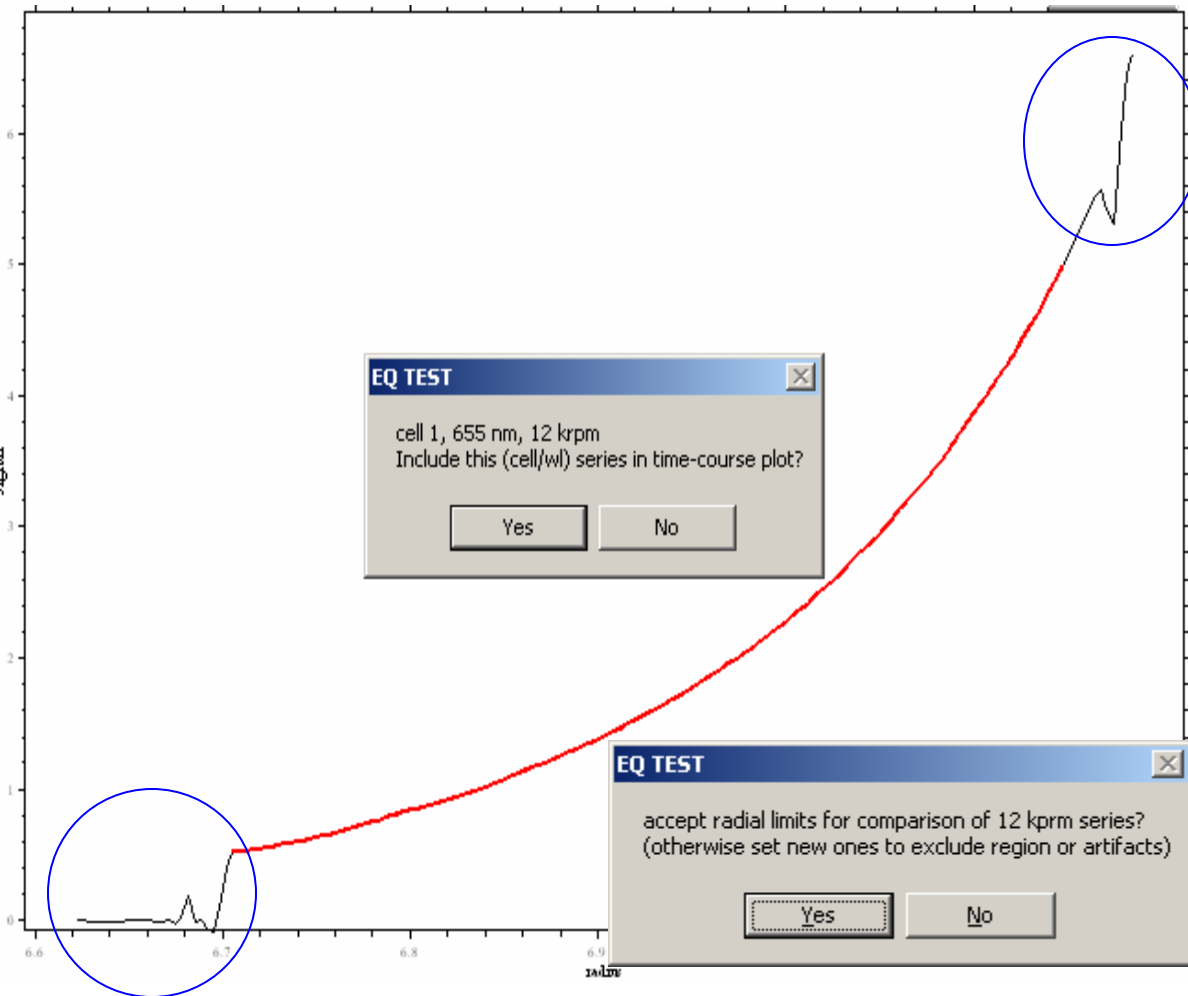
Options menu items:

- Set v^*rho
- Inhomogeneous Solvent
- Loading Options**
- Fitting Options
- Size-Distribution Options
- Calculator
- Bitmaps + Colors
- Sound

Options menu sub-items:

- Old Filename Convention
- Set n Columns
- Don't Load Spikes
- set radial resolution
- Eliminate Interference Offset
- Remove Integral Fringe Jumps (within one scan)
- Eliminate Jitter
- Subtract Blank Scan
- Add or Subtract Simulated Sedimentation Data
- Add Noise to Data or Multiply Signals
- Subtract Constant Value from Scan
- Correct erroneous IF radial calibration
- Add Constant Time and w^2t to data
- Test Approach to Equilibrium**
- Sort Eq Data to Disk ^E
- Save Intensity Data as Absorbance Data
- Save Intensity as 2 Pseudo Absorbance data sets
- Save Raw Data Set
- Save 6-channel Raw Data in 3 Subsets
- Exclude Region from Raw Data
- Exclude Worst Scan
- Invert Raw Data
- Set Polynom Order for Initial Data
- Import DLS Data ▶
- save mean DLS Data in sedfit format

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.

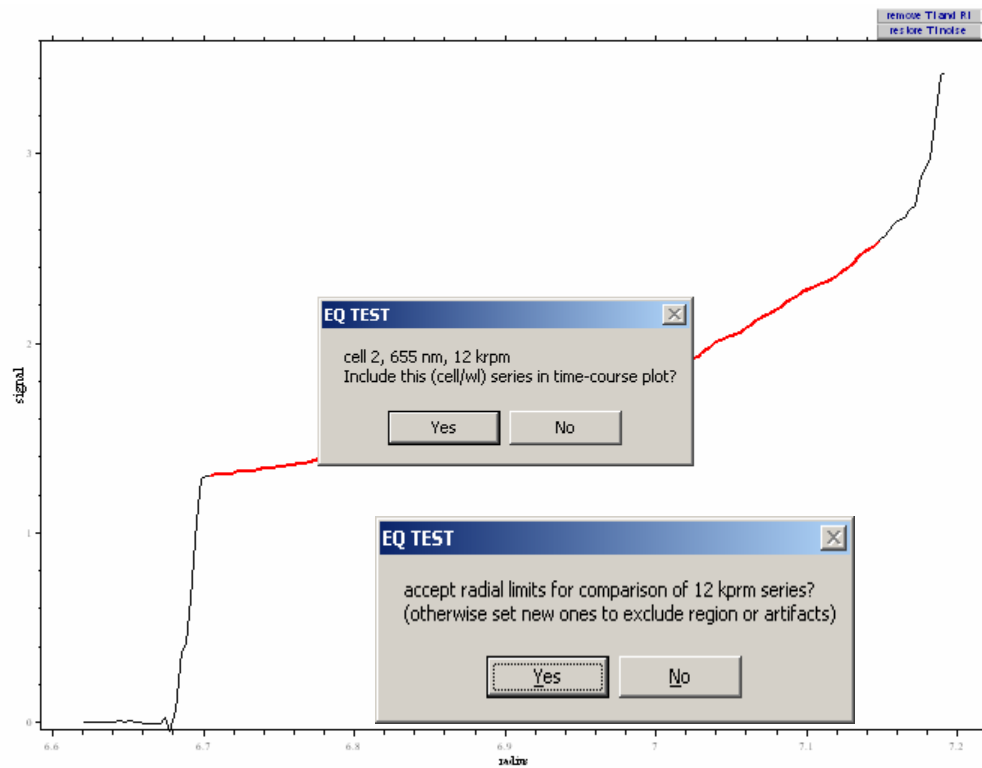


Re-set radial limits with these 2 windows, then again include the cell in the plot and accept the new limits.

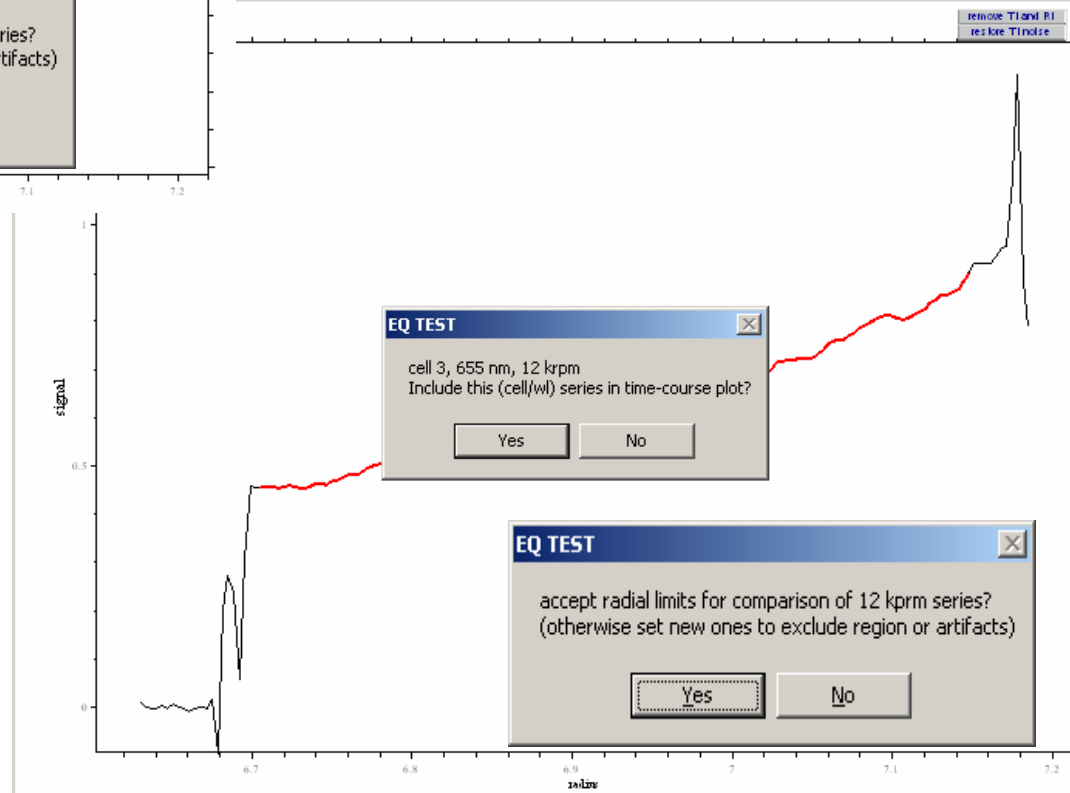
EQ TEST (top):
r-min:
6.708980
Buttons: OK, Cancel

EQ TEST (bottom):
r-max:
7.134380
Buttons: OK, Cancel

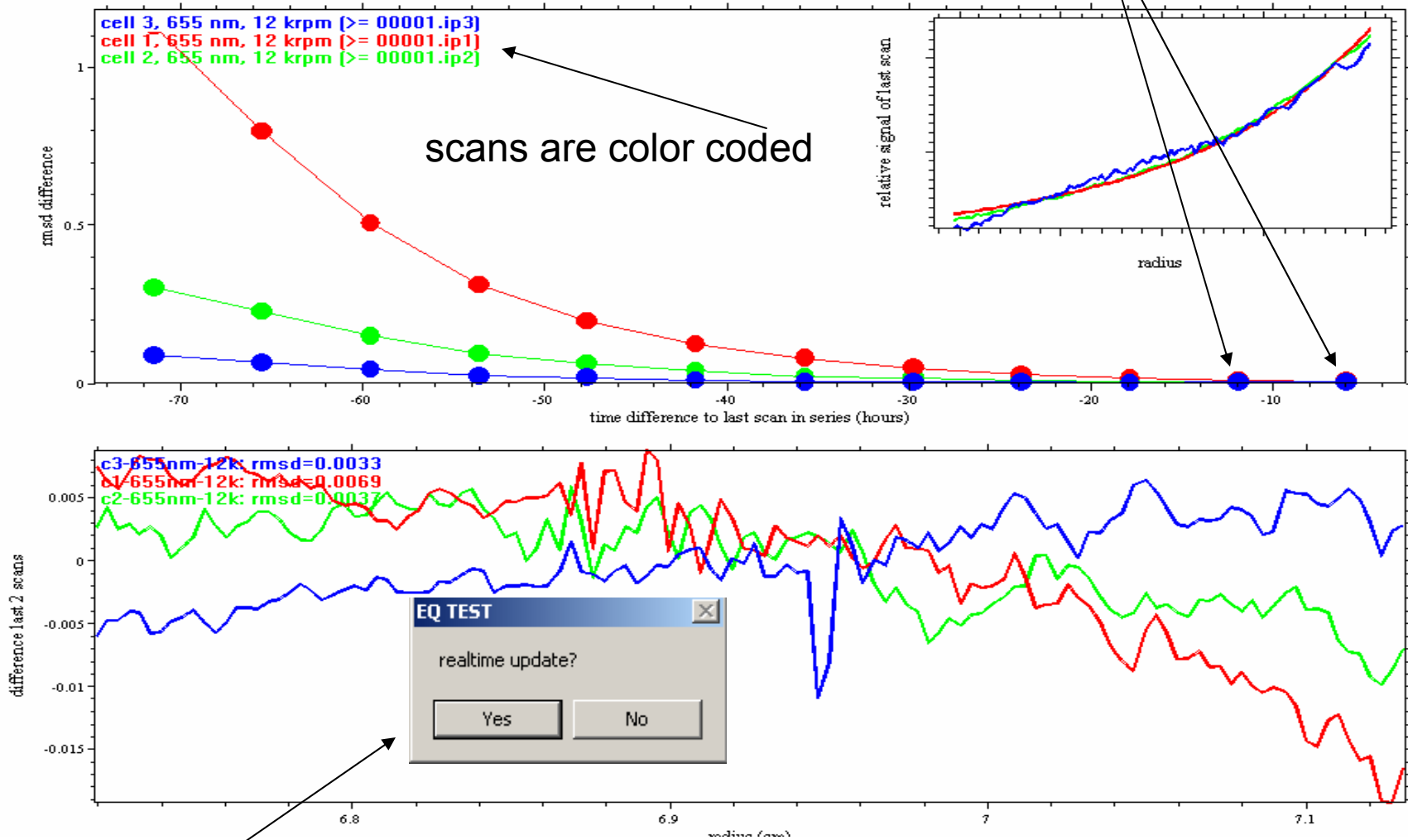
As a alternative, you can reload all the scans and reset the data limits taking care not to drag the lines too close to the meniscus or the bottom.



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

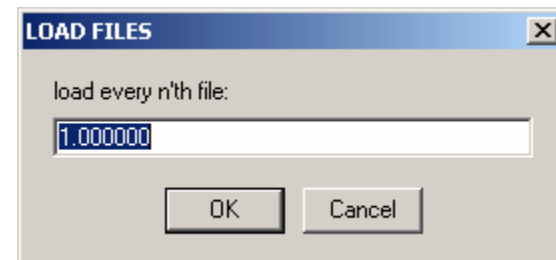
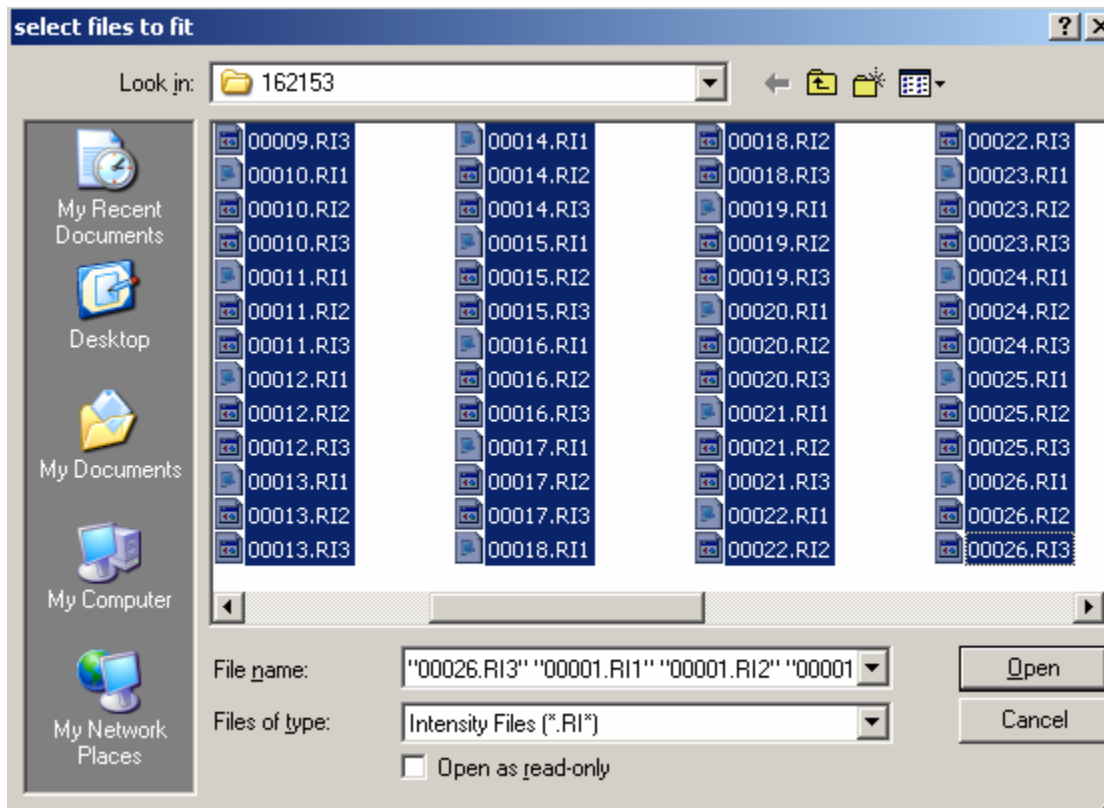


A plot of rms difference over time will be displayed comparing each scan with the previous scan. As you can see here, the rms difference is unchanged between the last 2 time points. The scans are in equilibrium.

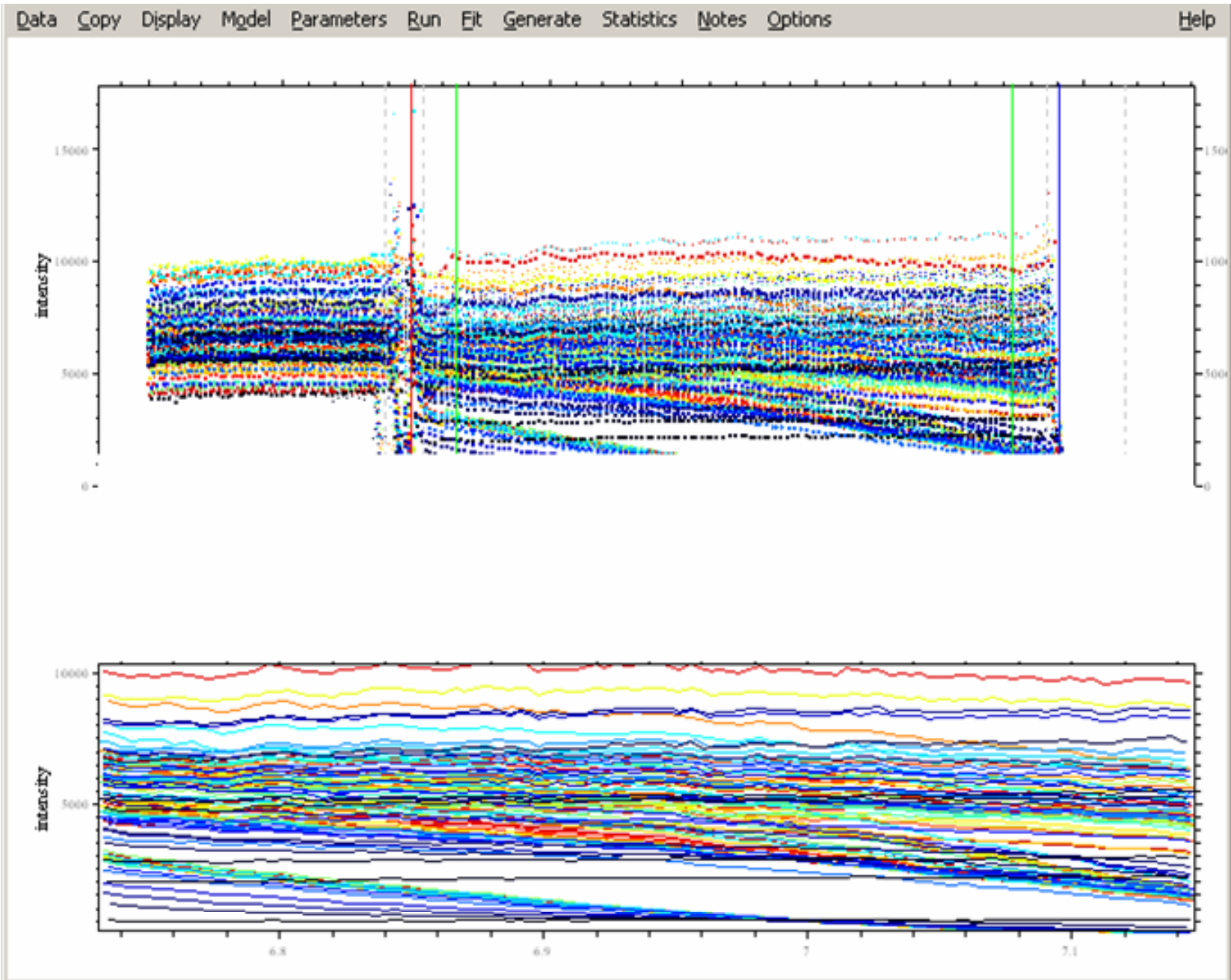


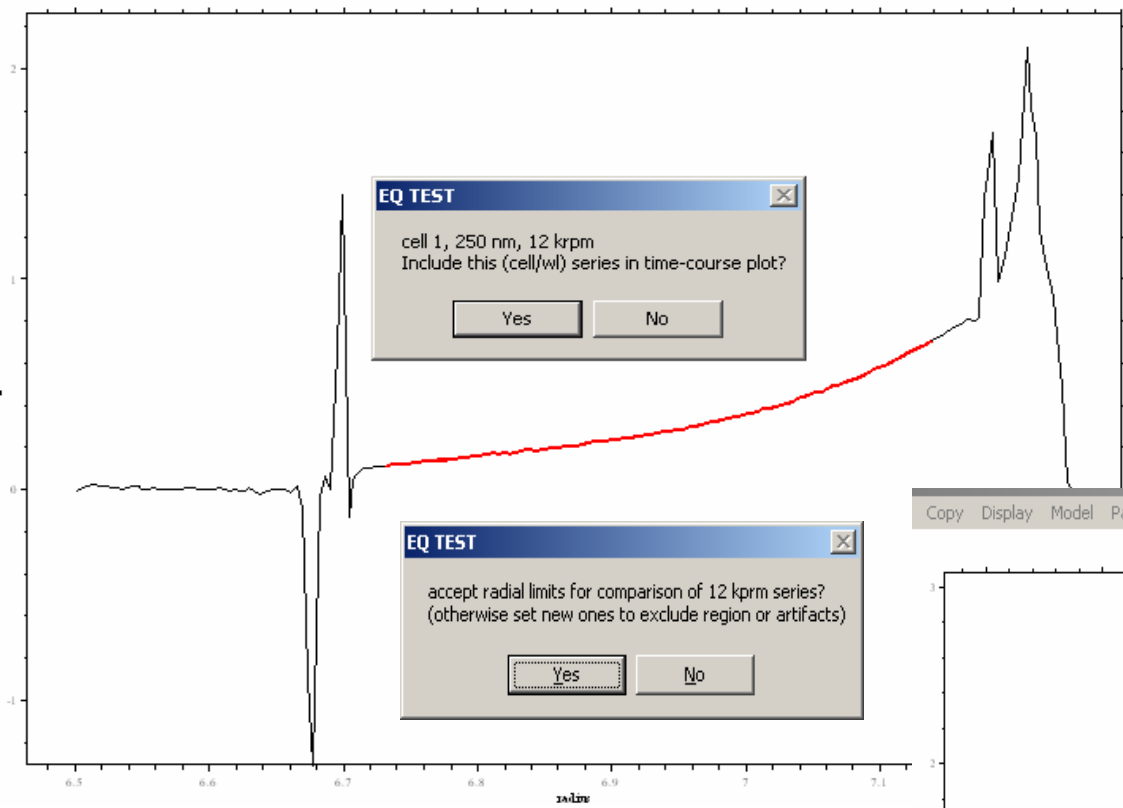
Sedfit gives us the option to view this time plot in real time as the SE run is progressing

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.

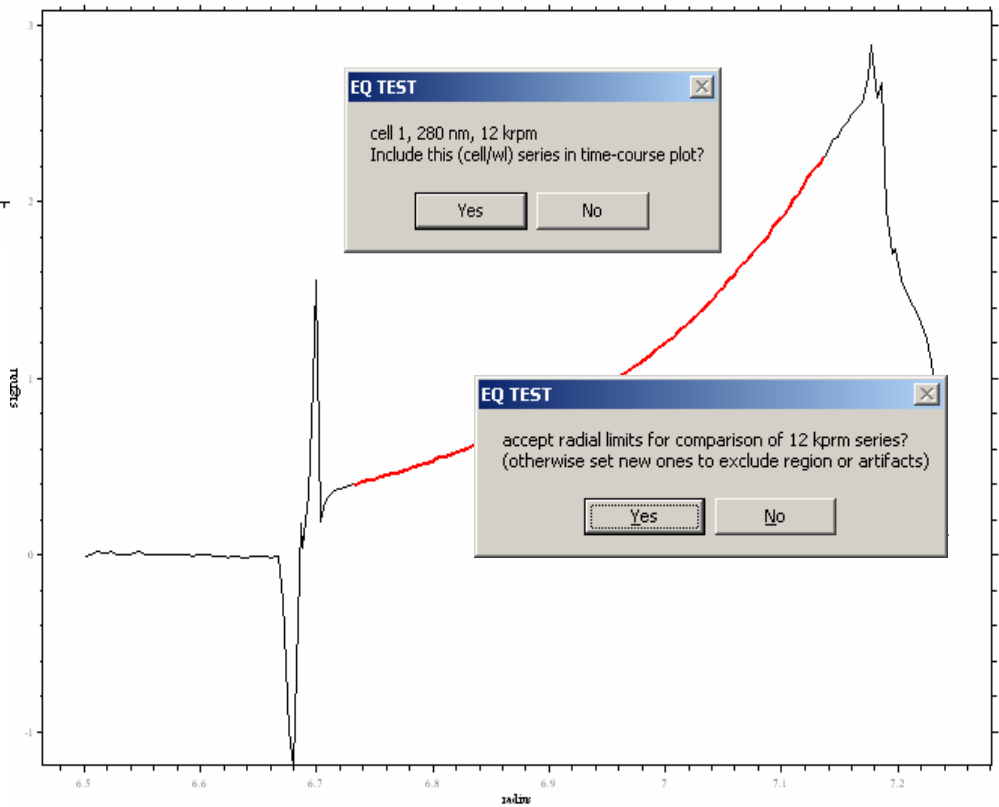


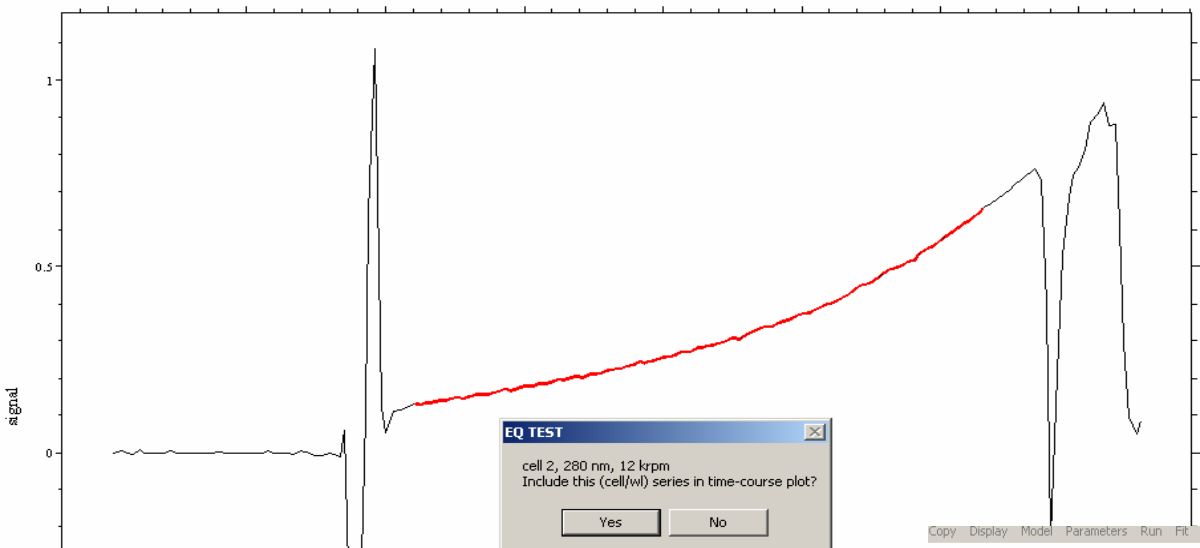
Set the meniscus and bottom. Then set the data limits between them.



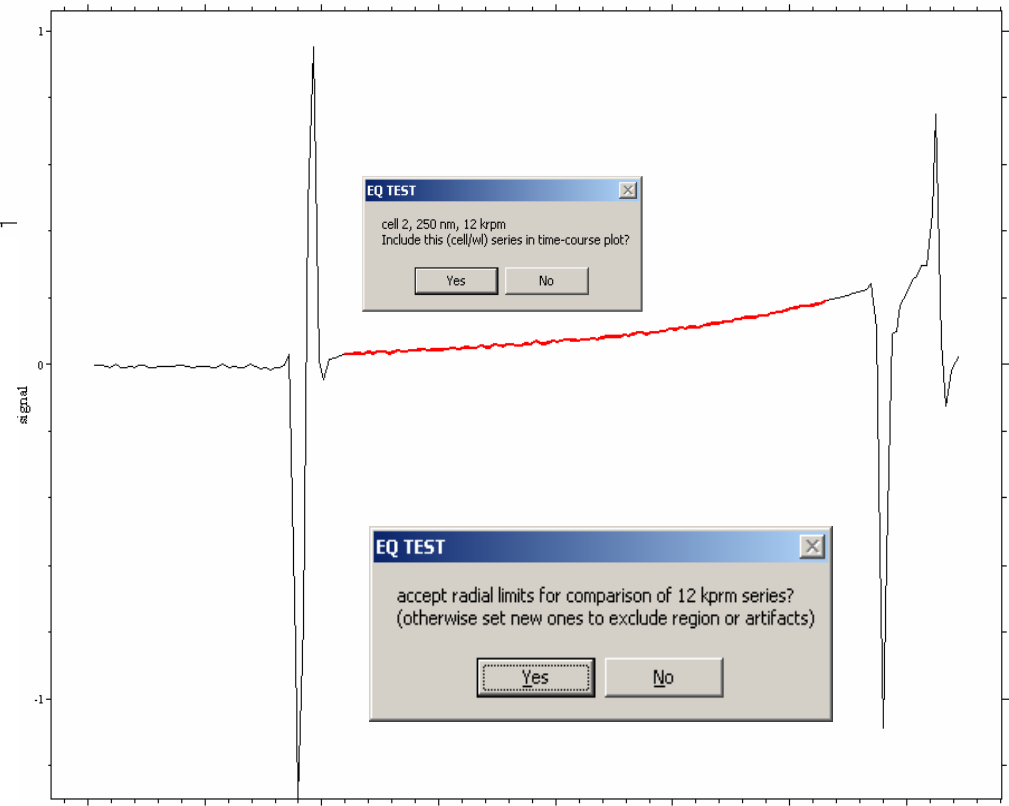


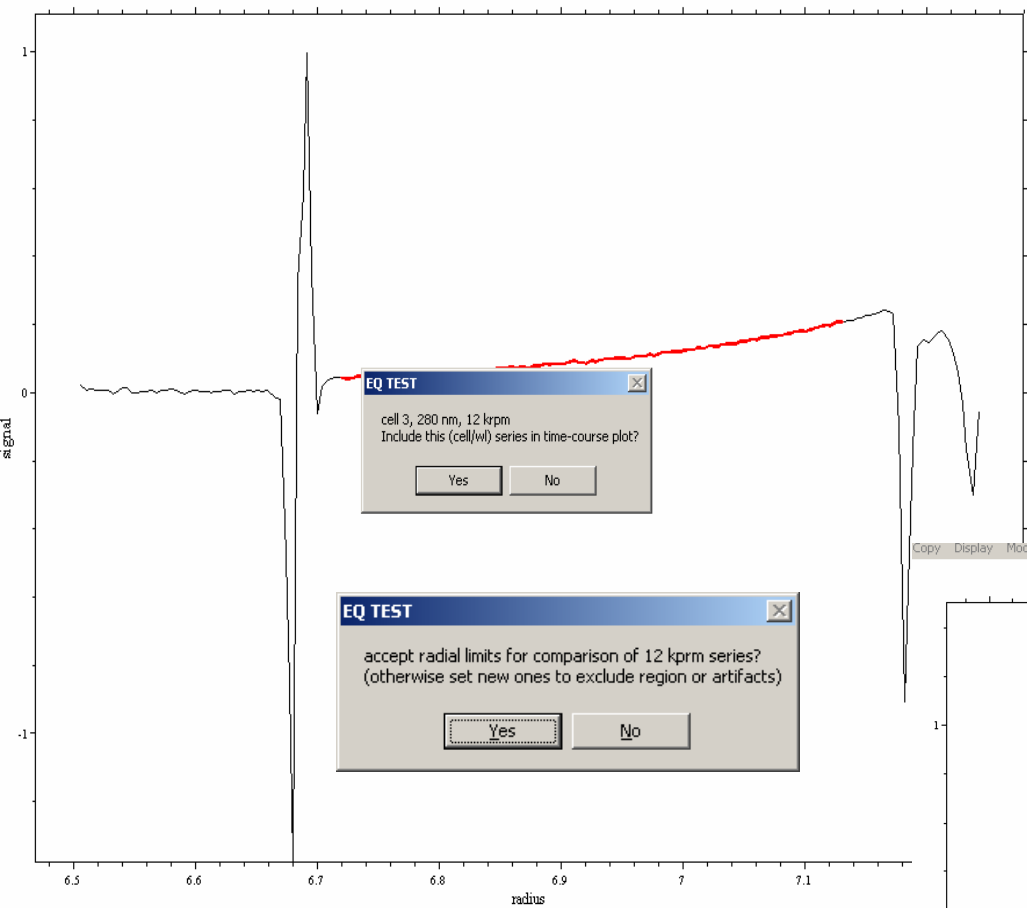
Then, answer Sedfit's prompts for each cell at each wave length.



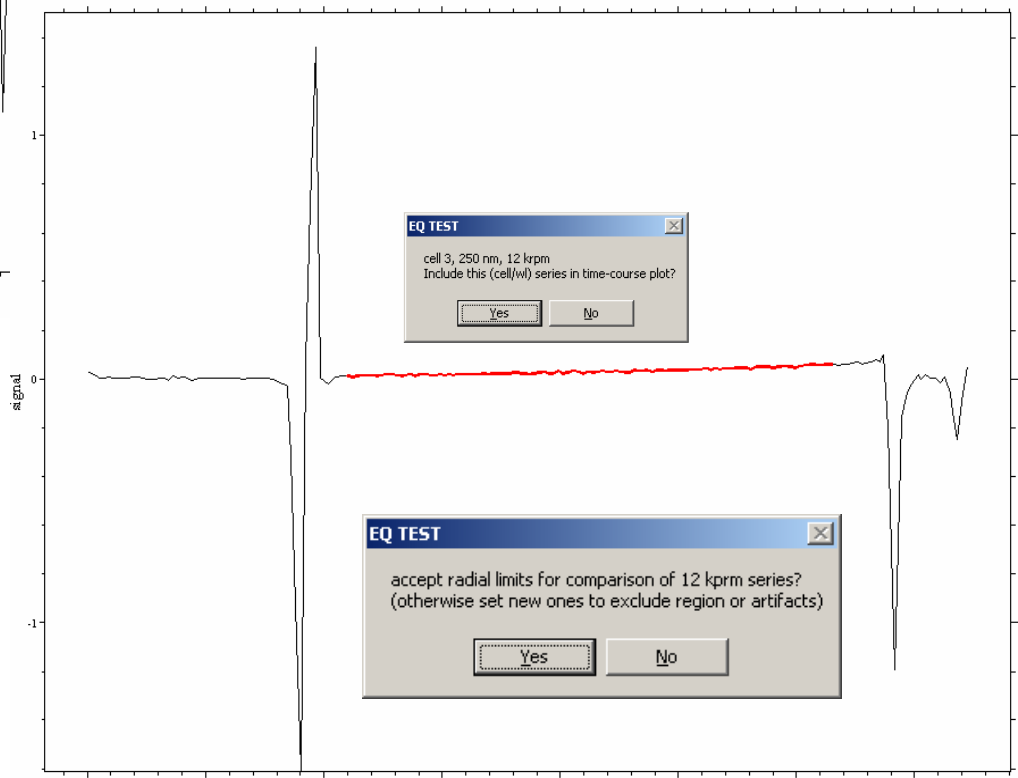


Cell 2.



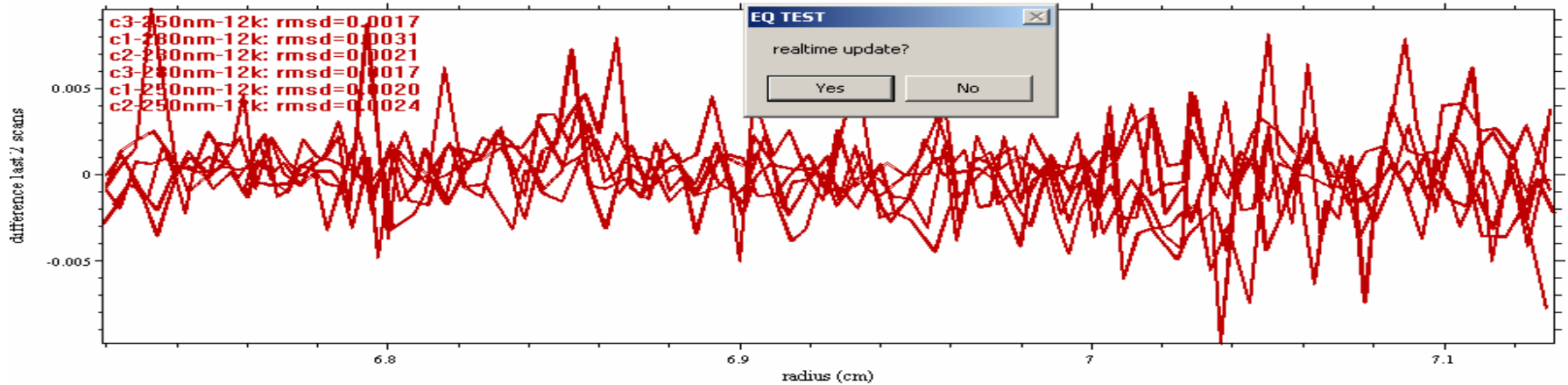
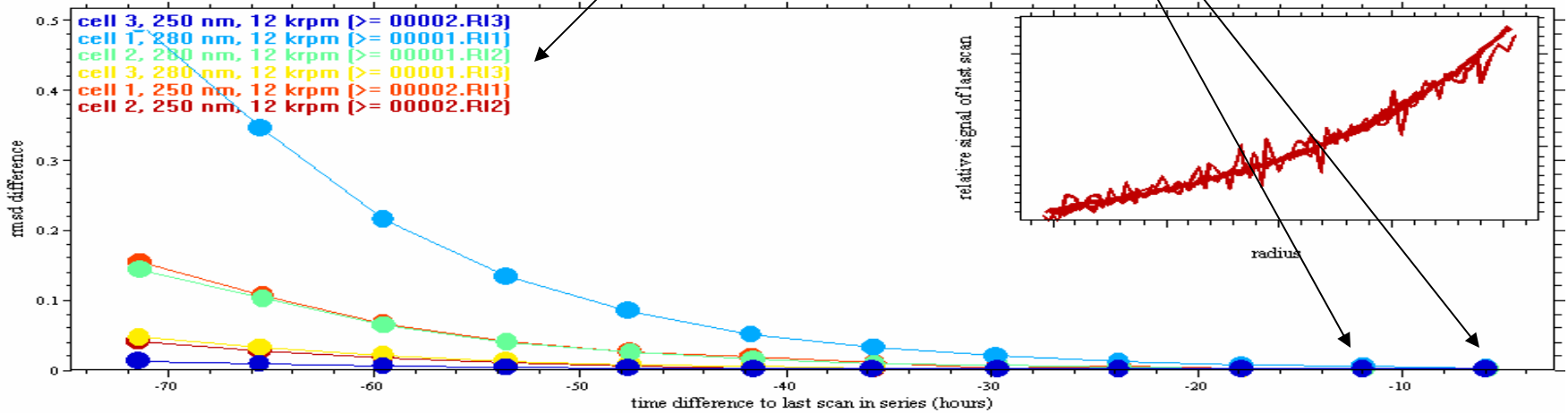


Cell 3.



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.