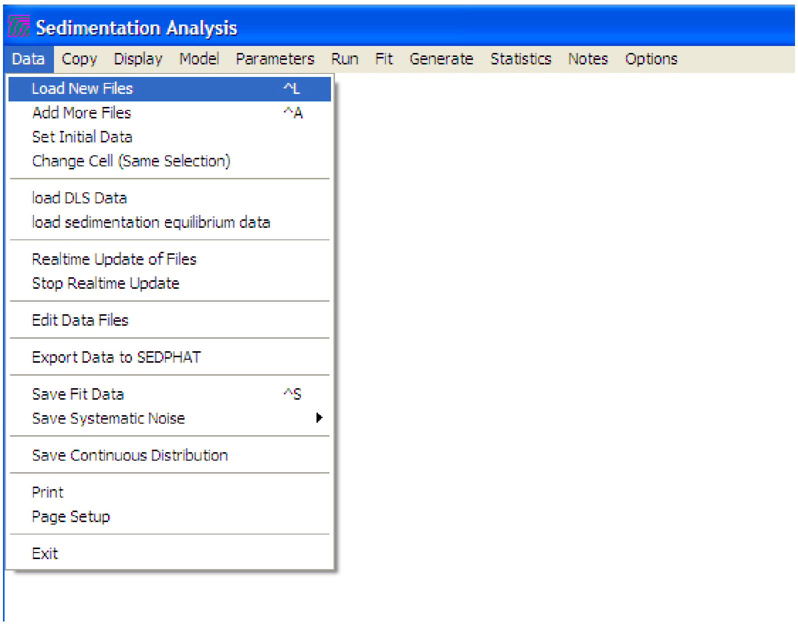
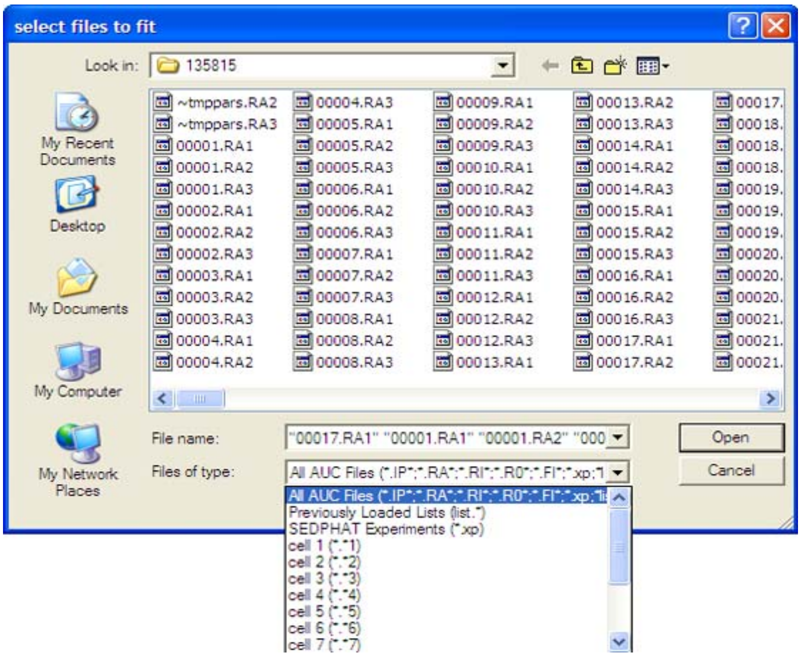
Basic AUC SV data analysis with Sedfit

- Sedfit could be downloaded at http://www.analyticalultracentrifugation.com/download.htm,
- Sample file for this tutorial can be downloaded in BCF AUC page. http://140.109.66.224/AUC.htm
- It is recommended to run Sedfit with multi-core CPU to shorten the fitting time. Please verify the CPU of your computer before installing Sedfit.

1. Click on “Data” and choose “Load New Files” to open your data.



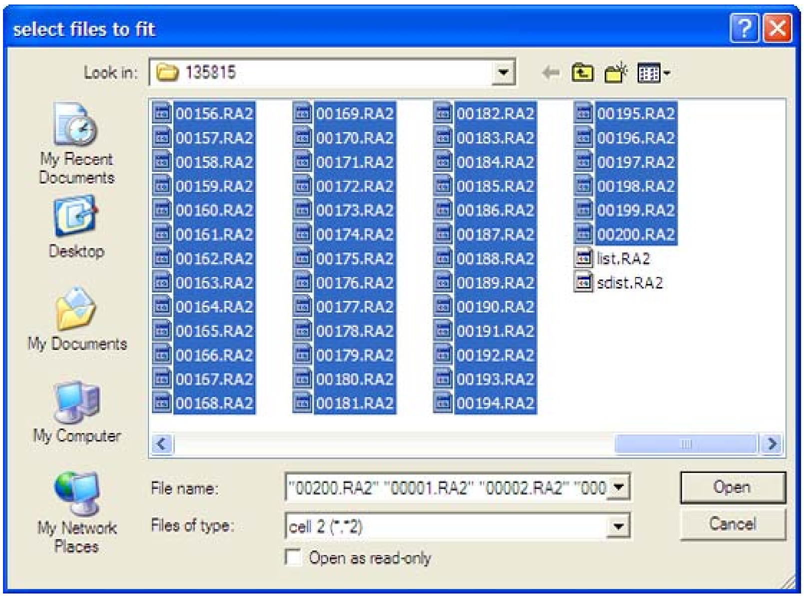
2. Choose the desired data type, namely, cell 2 (*.*2) in this tutorial.



File name: "00017.RA1" "00001.RA1" "00001.RA2" "000

Files of type: All AUC Files (*.IP*;*.RA*;*.RI*;*.R0*;*.FI*;*.xp;*l

3. Click on the 1st scan file, hold on “Shift” key and click on the last scan file to select every scan. Click “Open” to load the files. It is recommended to include all files if possible.



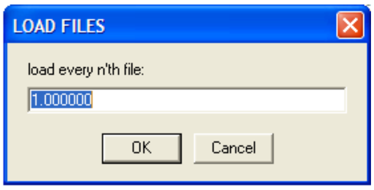
Look in: 135815

File name: "00200.RA2" "00001.RA2" "00002.RA2" "000

Files of type: cell 2 (*.*2)

☐ Open as read-only

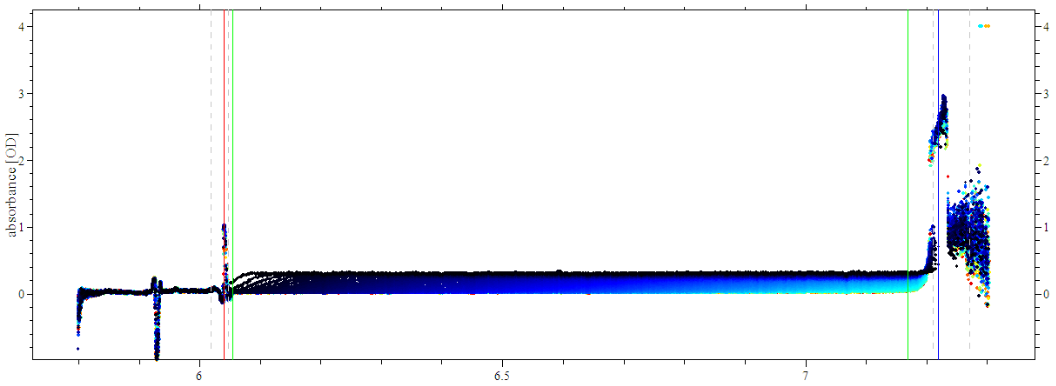
4. In the pop-up dialogue, enter the desired file loading interval; recommended value is “1”, which means loading every files in the selected range.



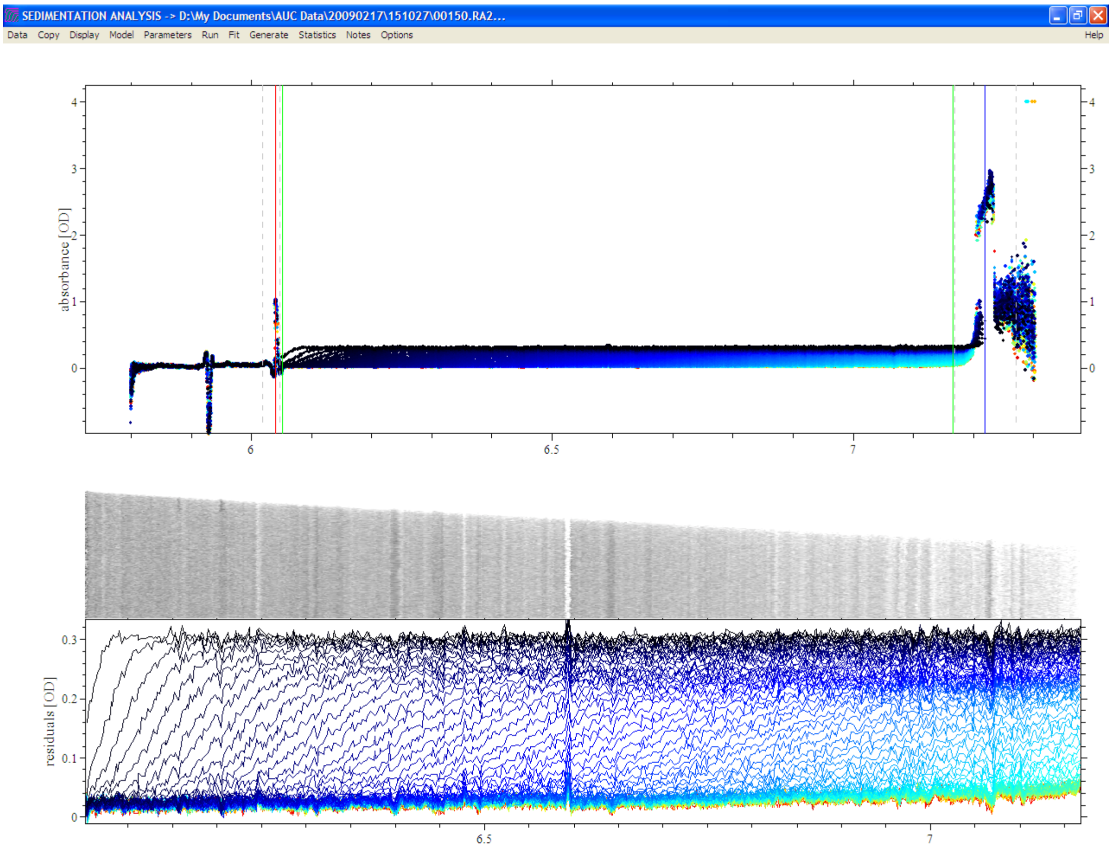
load every n'th file: 1.000000

5. If the “Load Previous Analysis?” dialogue pops-up, choose “No” to start a new analysis.

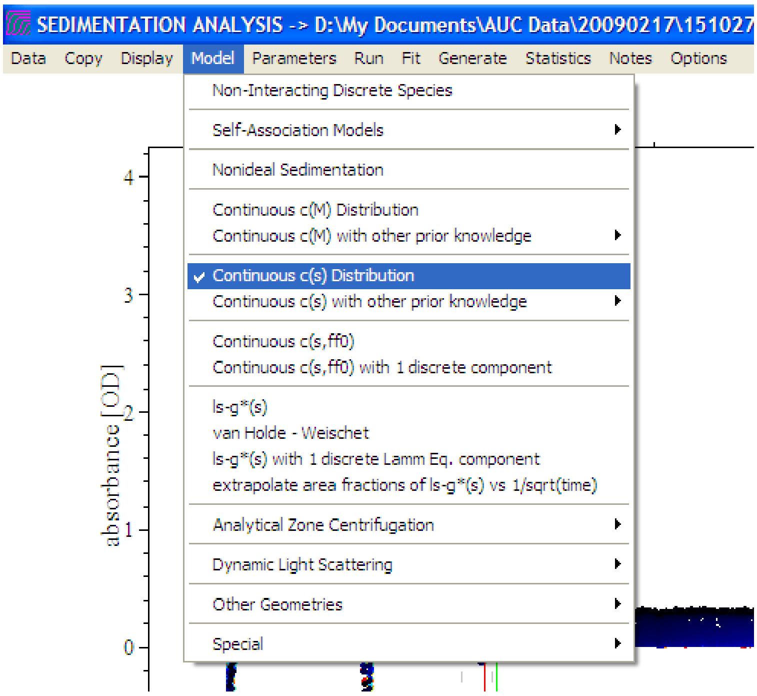
6. Drag and drop the red line to indicate cell top, then “confirm” to move fit limit (green line); move the blue line to indicate the cell bottom, then “confirm” to move fit limit (green line). You may zoom in an area by drawing a rectangular when right mouse button is pressed down, and may back to full scale by double right-click.



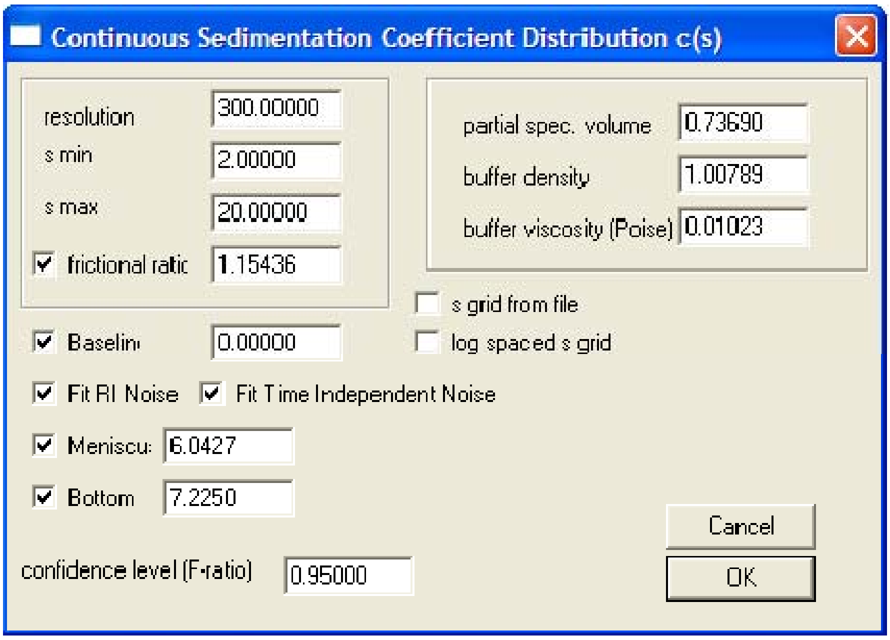
7. The data range is shown at the bottom row of the screen.



8. Under "Model" pulldown, choose "Continuous c(s) Distribution" for Sedimentation coefficient (S).



9. Click on “Parameters”, enter the “partial spec. volume”, “buffer viscosity” and “buffer density” for your sample (buffer). These parameters can be calculated in Sedtrep software. In this tutorial, partial spec. vol. of BSA=0.7369, the density and viscosity of 1.05X PBS=1.00789, 0.010229, respectively.



Continuous Sedimentation Coefficient Distribution c(s)

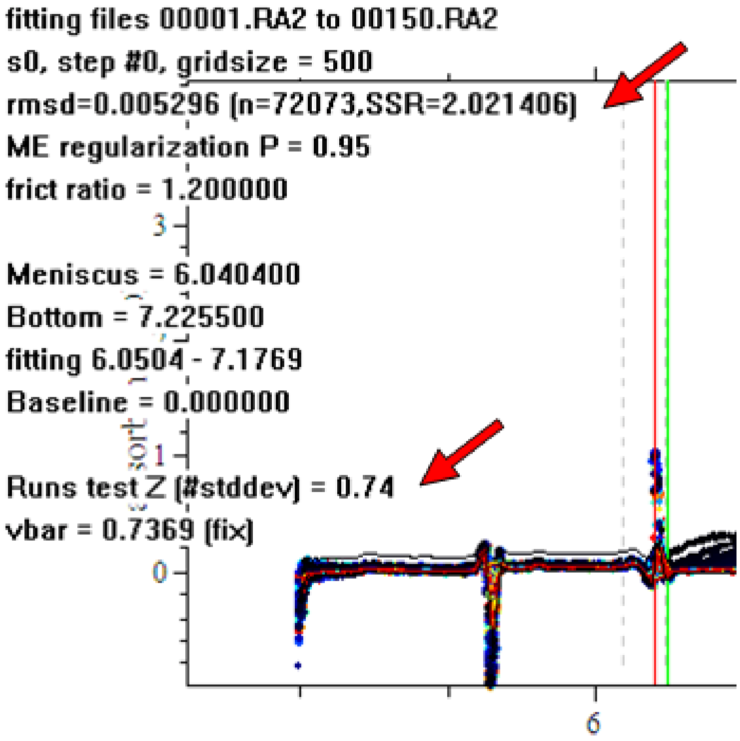
resolution 300.00000
s min 2.00000
s max 20.00000
☑ frictional ratic 1.15436
partial spec. volume 0.73690
buffer density 1.00789
buffer viscosity (Poise) 0.01023
☐ s grid from file
☐ log spaced s grid
☑ Baselin 0.00000
☑ Fit RI Noise ☑ Fit Time Independent Noise
☑ Meniscu 6.0427
☑ Bottom 7.2250
confidence level (F-ratio) 0.95000

Check the parameters that are to be optimized by Sedfit, such as frictional ratio, Baseline, etc.

The resolution and distribution range(S min and S max) can be changed according to your sample and the first test run. The default value for “resolution” is 50, which means to divide the distribution range in 50 points (The practical range of resolution is 50-300, note that higher resolution takes longer fitting time).

The frictional ratio is influenced by the shape of sample particle, for globular protein, the ratio is near 1.2; for rod-like particles, near 1.8. It is impossible to have the frictional ration less than 1.0.

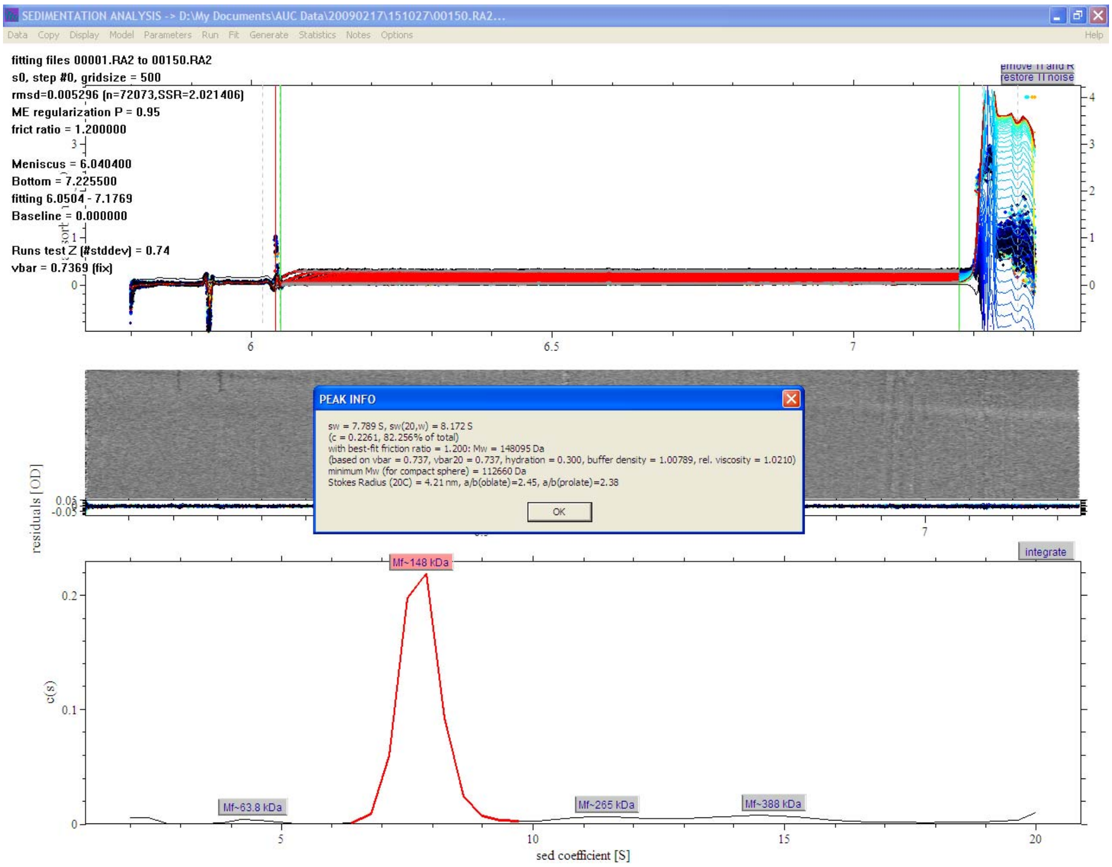
10. Click on “Run” to fit the data without using nonlinear regression method. The result is shown on the upper-left part of the screen after fitting procedure has finished. You may judge the quality of fitting by the values of “rmsd” and “Runs test Z”. Lower “Runs test Z” means better fit, and the recommended upper limit of “Runs test Z” is 30.



fitting files 00001.RA2 to 00150.RA2
s0, step #0, gridsize = 500
rmsd=0.005296 (n=72073,SSR=2.021406)
ME regularization P = 0.95
frict ratio = 1.200000
Meniscus = 6.040400
Bottom = 7.225500
fitting 6.0504 - 7.1769
Baseline = 0.000000
Runs test Z (#stddev) = 0.74
vbar = 0.7369 (fix)

11. You may change the distribution range and resolution by going back to “Parameters”, and run the fitting again. Once you are satisfied with the fitting result by “Run”, you may “Fit” your data using nonlinear regression algorisms by choosing “Fit” command on the top panel. “Fit” command is used only to optimize the nonlinear parameters, such as sedimentation coefficient, diffusion coefficient, fractional ratio, molar mass, meniscus, bottom, binding constant, etc. (Note: “Fit” will take much longer time than “Run”)

12. After the fitting process is done, the result is automatically displayed. For example, the distribution of S in this tutorial is shown in the bottom graph. In "Continuous c(s) Distribution" model, the calculated Mw of each peak can be displayed by pressing "Ctrl+M" or by choosing "Show Peak Mw in c(s)" in "Display" menu. You may click on the Mw box to show other analysis results (i.e. S, Sw, diameter, shape……etc).



```
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fitting 6.0504 - 7.1769
Baseline = 0.000000

Runs test Z (#stddev) = 0.74
vbar = 0.7369 (fix)
```

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sw = 7.789 S, sw(20,w) = 8.172 S
(c = 0.2261, 82.256% of total)
with best-fit friction ratio = 1.200: Mw = 148095 Da
(based on vbar = 0.737, vbar20 = 0.737, hydration = 0.300, buffer density = 1.00789, rel. viscosity = 1.0210)
minimum Mw (for compact sphere) = 112660 Da
Stokes Radius (20C) = 4.21 nm, a/b(oblate)=2.45, a/b(prolate)=2.38
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13. If the fitting parameters disappear due to screen refresh, it could be recalled by "Ctrl+o" or "Show Last Fit Info Again" in "Display" pulldown..

14. You may analyze scans of other cells using the same selection range and parameters by choosing "Data" - "change cell (same selection)".

∗ If you encounter any problems when using Sedfit, you are welcome to discuss with BCF staff; or you may access the Sedfit on-line help at: http://www.analyticalultracentrifugation.com/sedfit_help.htm