

## DEFit Quick Start Guide

This guide is in development stage. There are too many features to list anyway. I recommend users to simply play around and see what happens.

1. Select Input File Type
  - a. If the input file is ASCII format (\*.txt,\*.dat,\*.asc, etc.) type in the correct column numbers for the data in the “Data Columns”. If your data doesn’t have an imaginary component, you can ignore the third column, or -to be safe- type in “0” in “Im(y)” box.
2. Load the Data: Click “Browse”
  - a. Input data must have time units in nano seconds. Check the unprocessed data plot for correct time axis. Make sure there are no multipliers in the axis (displayed as  $10^{-3}$  at bottom right corner of the plot). If your input data’s time axis is in micro seconds, type in 1000 in the “Time Scale” box to convert to nano seconds.
  - b.  $t=0$  must correspond to primary Hahn echo ( $2*\tau$  after the first pulse). If your acquisition software has some other definition for the time axis, correct this by setting “Time offset (ns)”. As a result, the Processed Data plot will have the highest echo modulation intensity at  $t=0$ .
3. If phase correction is not desired, uncheck “Phase”.
  - a. Uncheck “Plot imaginary” for zoomed in display of real data.
4. Cutoff data points to be discluded in the analysis from left and right if desired.
5. Adjust the interval where background will be fit.
  - a. Uncheck “Subtract” if background subtraction is not desired.
  - b. “Bckg rms” is root mean square deviation between data points and the background curve *within the interval* where background is fit.
  - c. Mod Depth is the ratio of pure echo modulation after background subtraction to entire echo intensity, i.e.  $(y(t=0)-y_{\text{bckg}}(t=0))/y(t=0)$
6. Noise
  - a. The green circles in “Data” plot are what DEFit assumes as baseline in your data. For the statistics in the analysis to be reliable (f-test, contour plots), the baseline must have only random noise with as many points as possible. The number of points is calculated automatically, however, you can change it at the “Advanced Settings” dialog.
7. Hit “Run”.
  - a. Initialization may take a few seconds, please be patient.
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8. When MC minimization is complete, hit “Contour”. Play with contour settings.
9. Check out the command window output for error information from contour runs.

## Reference:

Sen KI, Logan TM, Fajer PG. "Protein dynamics and monomer-monomer interactions in AntR activation by electron paramagnetic resonance and double electron-electron resonance." *Biochemistry*. 2007 Oct 16;46(41):11639-49.

