Dynamics Center Tutorial:
General Dynamics

This tutorial contains a selection of spectra to be used in the exercises.

These data are confidential and must not be further distributed.

To run the tutorial, just copy the folder tutorial_dc to your disk, e.g. into c:\

The following files then become available:

c:\tutorial_dc

Diffusion	pseudo 2D diffusion spectrum
REDOR	pseudo 2D REDOR spectrum
T1	pseudo 2D T1 spectrum
tutorial_dc.pdf	this document

Contact:

Dr. Klaus-Peter Neidig +49 721 5161 6447 Peter.Neidig@bruker-biospin.de
Dr. Klaus Zick +49 721 5161 6135 Klaus.Zick@bruker-biospin.de

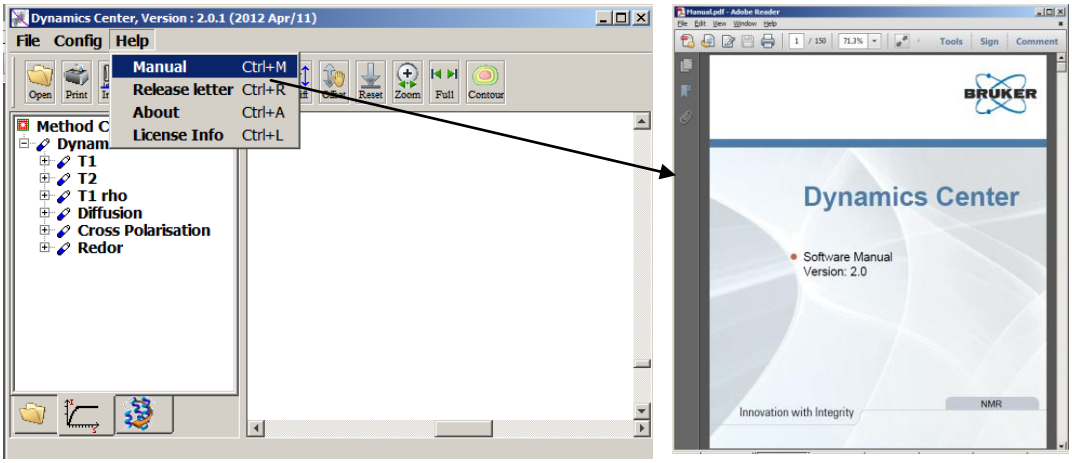
The following assumes that the Dynamics Center software is installed and can be started, i.e. at least a FlexLM TOPSPIN_1D license is contained in the license file.

Last updated:

April 30, 2012

Exercise 1: General Features

- Start DC. Note that the manual is available under **Help/Manual.**



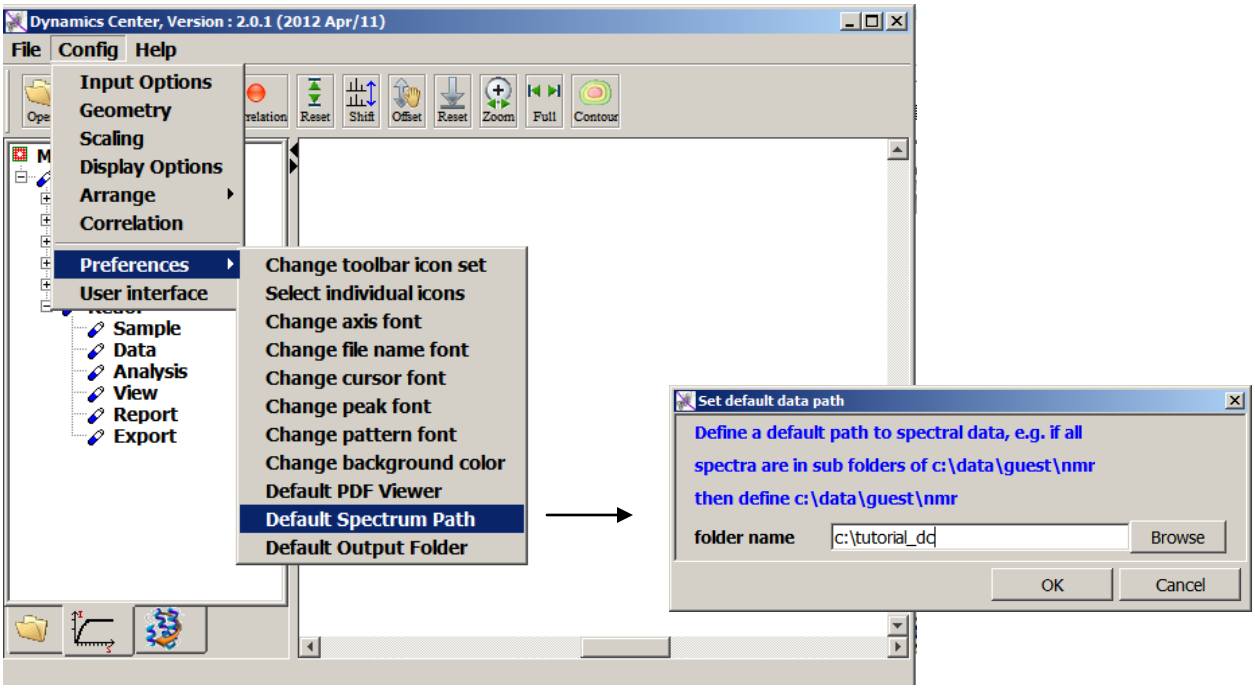
The Dynamics Center window contains three tabs in the lower left corner. The first from the left gives access to a file system explorer, the second is used for general dynamics and the rightmost is used for protein dynamics.

This tutorial is concerned only with general dynamics, so only the general dynamics tab will be used. Select this tab. A method tree containing **T_1**, **T_2**, **T_{1rho}**, **Diffusion**, **Cross Polarization** and **Redor** will appear.

- Note that the main menu bar is used for global purposes such as settings.

Data analysis is performed entirely by activating methods on the method tree. The main menu bar only serves for some global purposes, especially setting preferences.

- Use **Config/Preferences** to customize the DC.

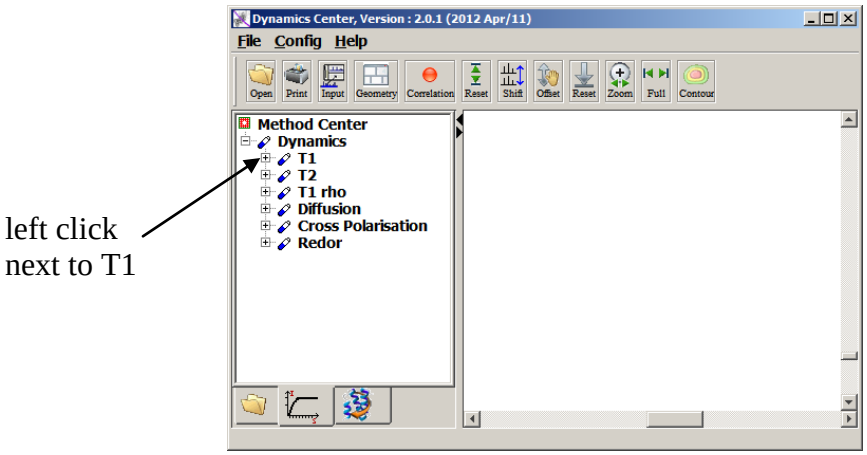


The options available under Config make it possible to customize the appearance of the Dynamics Center and to choose the starting directory for spectra searches. Use **Config/Preferences/Default Spectrum Path** to choose the directory that will serve as the root when choosing spectra to analyze in the DC. Set the directory to the directory containing the spectra used in the tutorial. For example, since the data were unpacked to c:\tutorial_dc here, the data path is set here to c:\tutorial_dc.

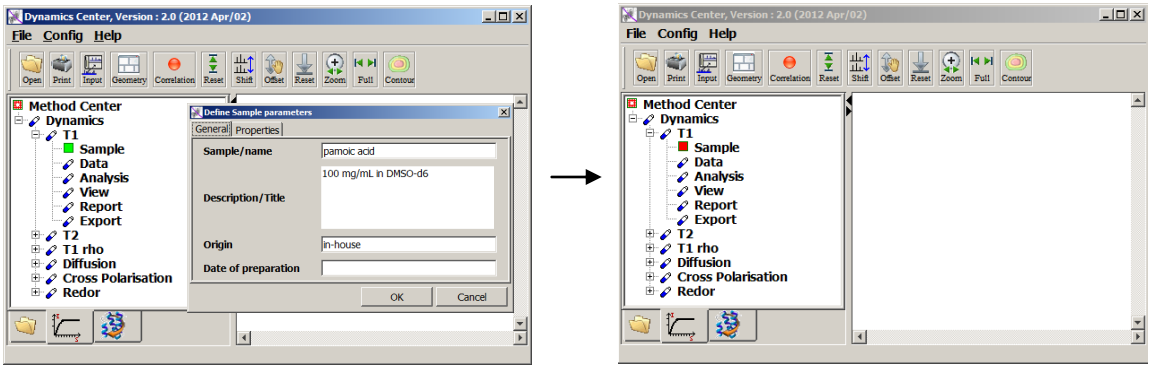
Exercise 2: T1 Method

In this example, we will analyze a ^{13}C inversion-recovery T_1 experiment of pamoic acid using the standard Bruker pulse sequence t1ir.

- Expand the method tree for T_1 analysis by clicking with the left mouse button on the + box next to T1 on the method tree (or double-click on T1).

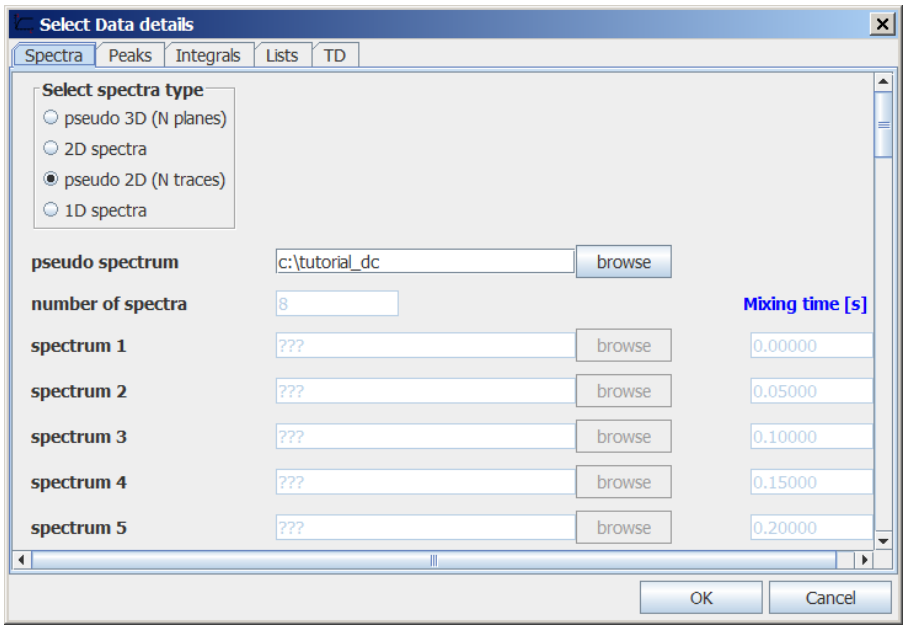


This expands the tree and shows components named **Sample**, **Data**, **Analysis**, **View**, **Report** and **Export**. To do a complete T_1 analysis, execute the components in order from top to bottom.



After clicking on **Sample**, the box next to Sample turns red and the dialog box "Define Sample parameters" appears. Information entered here is printed on the final report generated after analysis. After entering an appropriate name and description (the sample in this example is the Bruker standard 100 mg/mL pamoic acid in DMSO) or accepting the defaults and then clicking OK, the box next to Sample turns **green**.

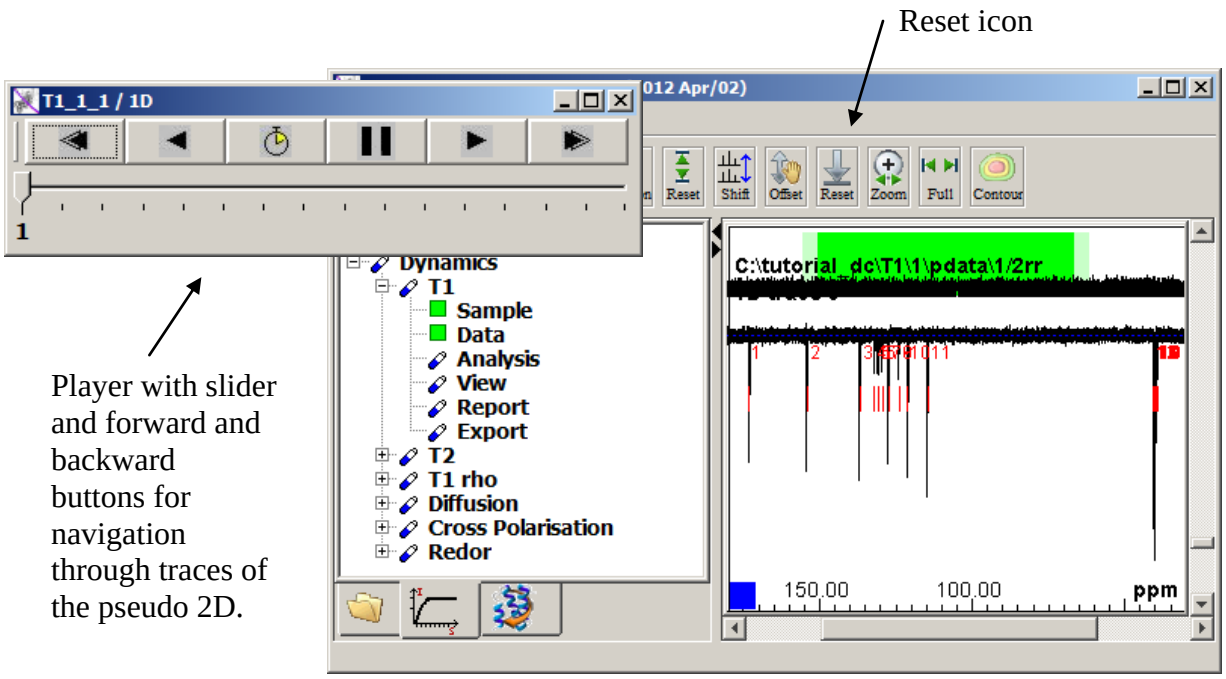
- Left click on **Data** to choose the dataset and verify related parameters.



After clicking on Data, the box next to Data turns **red** and a dialog window with several tabs shows up. Be sure that **pseudo 2D** is selected in the Spectra tab and use the **browse** button right of the field containing a directory to navigate to the directory containing the processed data, **c:\tutorial_dc\T1\1\pdata\1**. Note that the dialog field

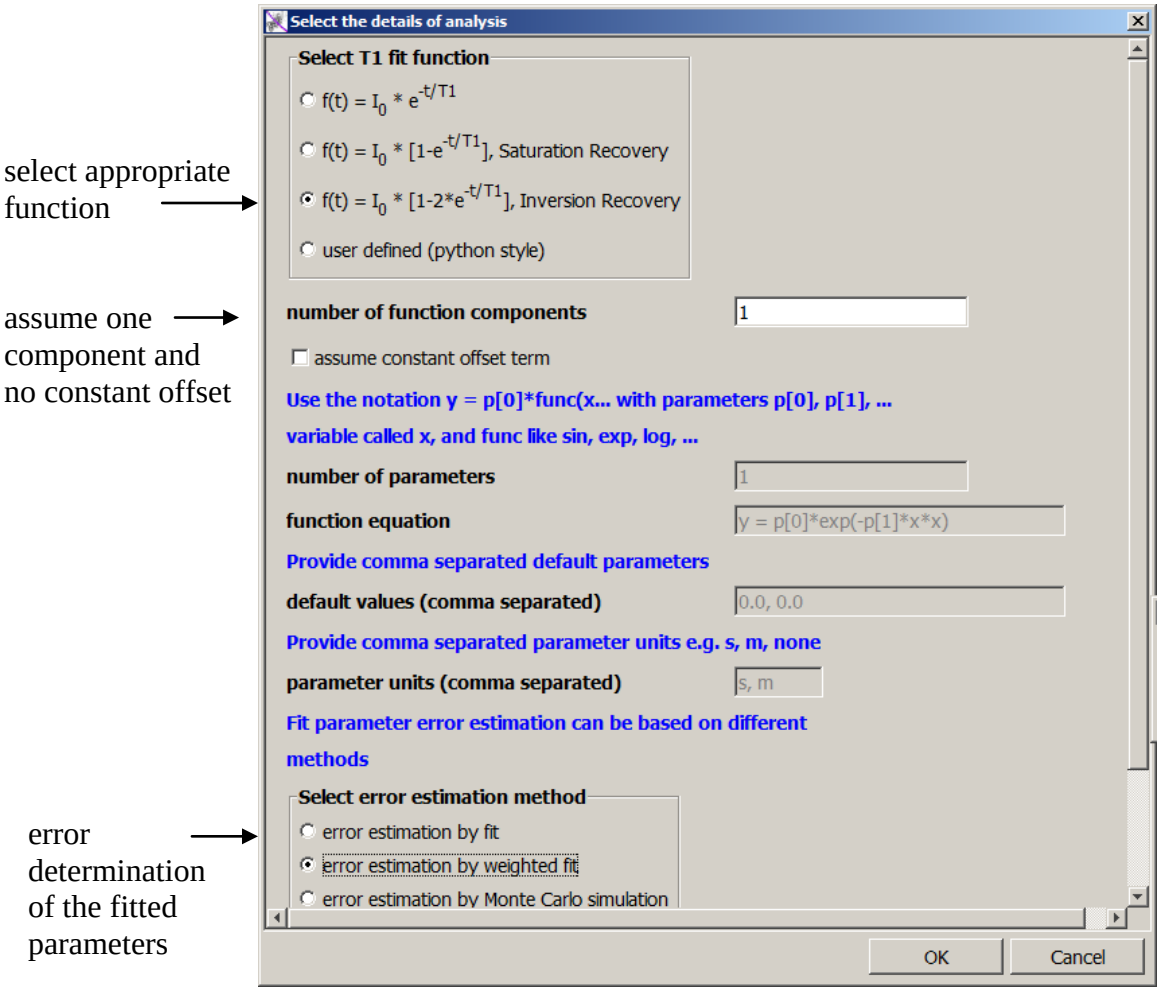
already contained the directory chosen under **Config/Preferences/Default Spectrum Path**.

Since the spectrum was acquired with a Bruker standard pulse program, the DC knows to look at the files in the dataset and sets all parameters, including the mixing times, correctly. Glance through the other tabs to see the options, but accept the standard settings (including automatic peak picking) and click OK to continue. The Data component in the method tree turns **green**, the pseudo spectrum chosen is loaded, the first trace is shown and a player appears which enables navigation through the different traces of the spectrum.



The peaks used for further analysis are those shown as numbered in the visible trace. It may be helpful to zoom in on certain peaks by drawing a box around them with the left mouse cursor (click and drag to draw the box) and then releasing the mouse cursor. Double clicking shows the whole spectrum. You can adjust the baseline by moving the mouse pointer to the baseline, then clicking and holding with the left mouse button and moving the mouse forward and backward. The Reset icon returns the baseline to its default position.

- Execute curve-fitting by clicking on **Analysis**.



The relaxation curves will be fit for each of the peaks currently numbered on the screen. To add another peak, hover over it with the mouse cursor, right click, and select "add peak"; to remove a peak, hover over it, right click, and select "delete peak". The appropriate fit function for the T_1 experiment performed must be selected in the dialog window, but when a Bruker standard pulse sequence is used, the correct fitting function is chosen automatically. This is the case here, with pulse program t1ir. We will assume that only one component is present for each peak and that no constant offset term needs to be added to the fit equation, so the **number of terms** is set to **1** and the tick box for **offset** is **switched off**. The user may specify a fit function in Python style, but this is only needed in rare cases. In the bottom area, details about error determination of the parameter fit can be given. Error estimation by **weighted fit** is a good choice. It takes into account the experimentally determined errors of the peak integrals/intensities involved.

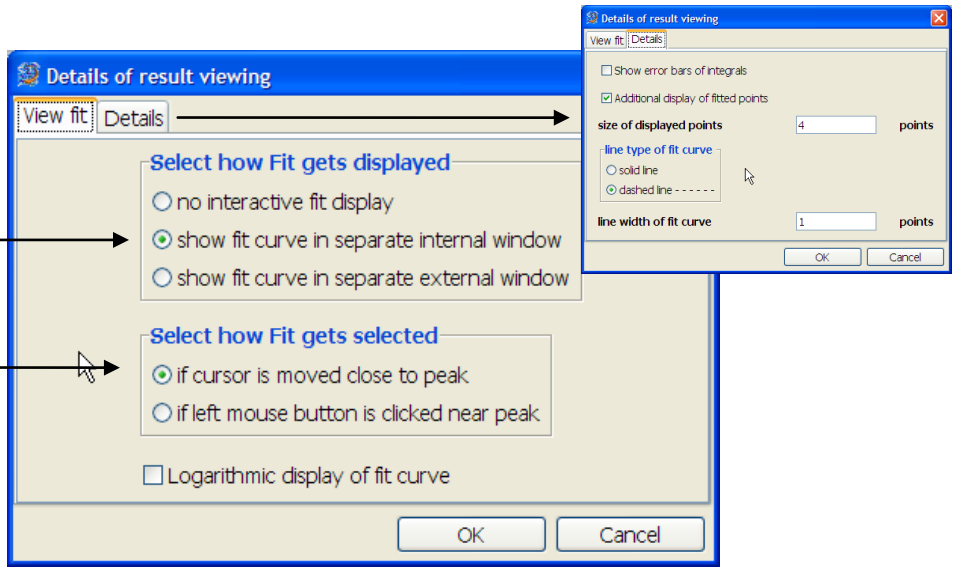
The curve fitting is finished within moments.

- Click on **View** to see the fit curves just calculated.

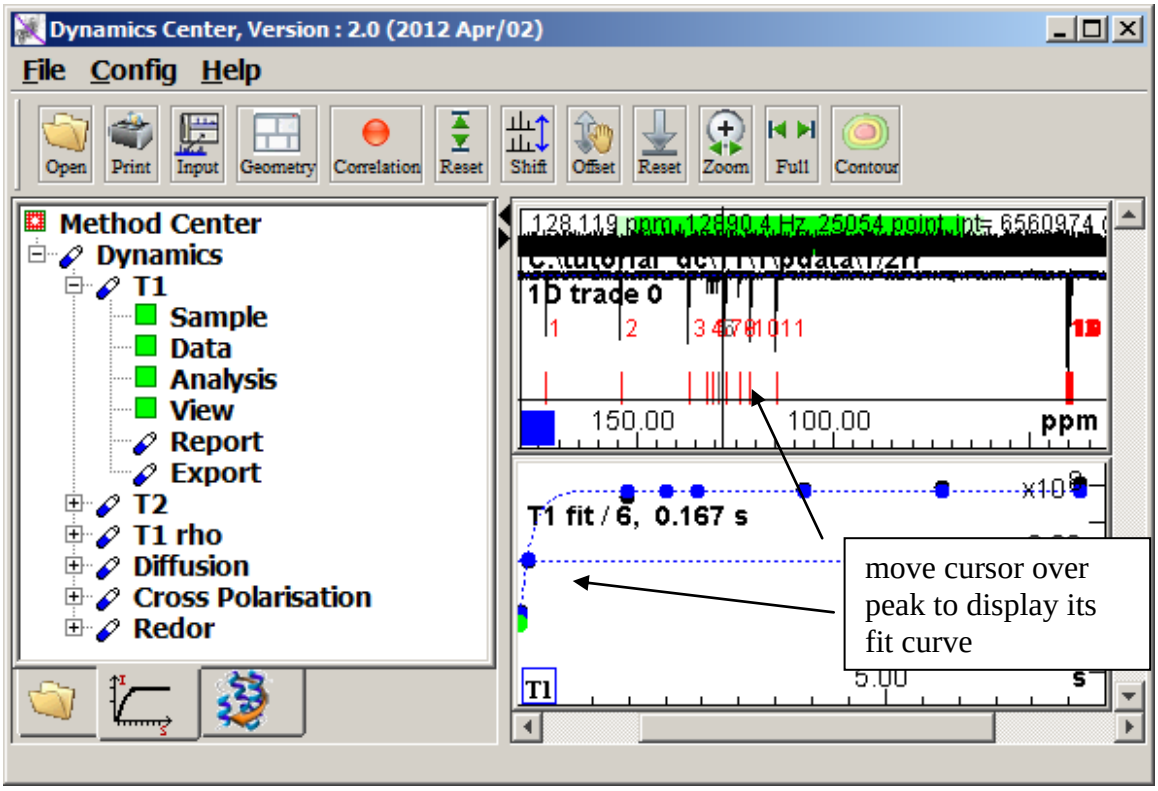
To display the fit curves of peaks by hovering over the corresponding peaks in the spectrum and make the fit curve display inside the Dynamics Center window, use the settings shown:

show fit curve display inside the Dynamics Center Window

display each fit curve by hovering with the mouse cursor over the corresponding peak



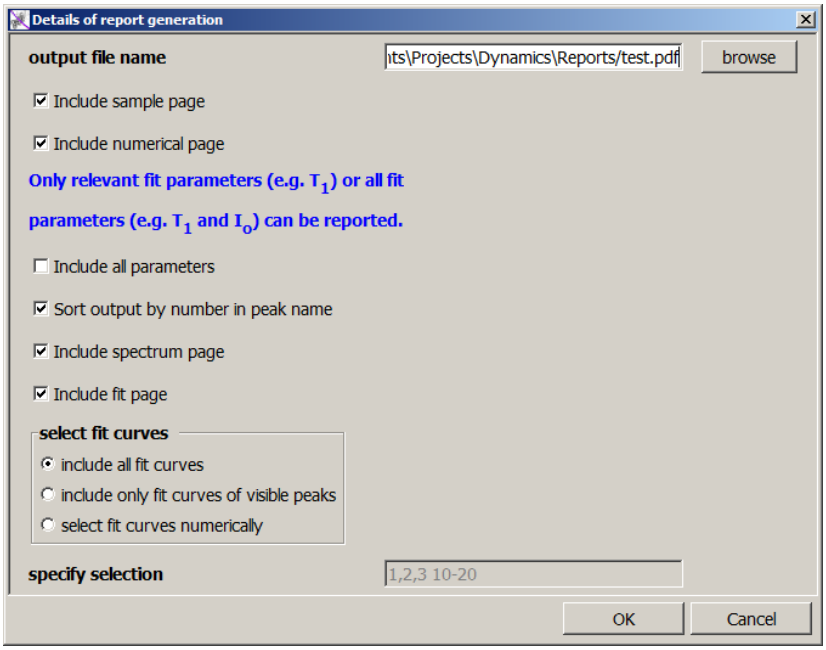
Some further details, e.g. logarithmic displays or display details, can be set under the **Details** tab. After clicking **OK** and moving the mouse to a peak, the display should look as follows:



A right mouse button click in the fit curve display window brings up a context sensitive popup menu. The menu depends on whether you are close to an experimental point (**black** filled circle which then turns **red**) or not. If the cursor is not near an experimental point the popup menu offers, among other options, "**Properties,**" which gives the details of the curve fitting.

- Click on **Report** to generate a report containing the fit curves.

This feature is fairly self-explanatory. A window entitled "Details of report generation" pops up (see below), allowing the report to be customized. After clicking OK in that window, the report is generated, and Acrobat Reader will be launched to show the report, if Acrobat Reader is available. To choose a different PDF viewer, use **Config/Preferences/Default PDF Viewer**. Similarly, to choose a different directory for saving reports to, use **Config/Preferences/Default Output Folder**. Part of the report showing the fitted relaxation parameters is shown below.

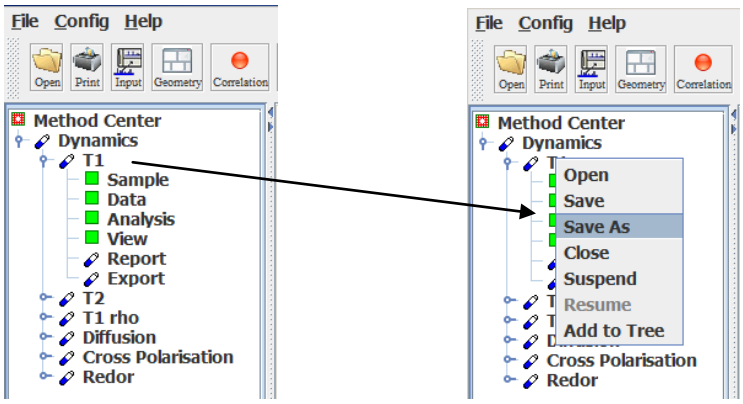


T1 Analysis
C:\tutorial_dc\T1\1\pdata\1\2rr

Fitted function:	f(t) = Io * [1 - 2*exp (-t/T1)]
Random error estimation of data:	RMS per spectrum (or trace/plane)
Systematic error estimation of data:	worst case per peak scenario
Fit parameter Error estimation method:	from fit using calculated y uncertainties
Confidence level:	95%
Used peaks:	automatically picked peaks
Used integrals:	peak intensities
Used Mixing time:	all values (including replicates) used

Peak name	F2 [ppm]	T1 [s]	error
1	173.078	1.22	0.04500
2	153.869	1.57	0.05136
3	136.669	1.04	0.04243
4	131.999	0.176	0.02991
5	130.591	0.208	0.03953
6	129.312	0.167	0.03139
7	127.190	1.03	0.04806
8	123.810	0.188	0.02830
9	123.770	0.188	0.03261
10	121.080	1.59	0.05235
11	114.456	1.49	0.04437
12	40.551	8.83	1.399
13	40.540	9.46	1.541
14	40.336	8.98	0.5845
15	40.125	9.26	0.2519
16	39.921	8.96	0.2048
17	39.713	8.99	0.2408
18	39.501	9.07	0.5998
19	39.297	9.42	1.697
20	20.380	0.0925	0.03714

- Click on **Export** to export fit parameters to a text or Excel file. To save all work to a project file, **right click** on T1 on the method tree and select **Save As** from the popup menu that appears.



You can specify an arbitrary name for project files, for example **c:\tutorial_dc\T1.project**. The extension **.project** is advised. The next time you want to repeat the T1 analysis of the same spectrum, you can select **Open** from the popup menu and load the project file again.

We can directly continue with the next exercise (the Dynamics Center can handle multiple analyses in parallel), or we can clean up the display by selecting **Close** or **Suspend** from the T1 popup menu.

The other relaxation analyses follow the same basic pattern as the T1 analysis: describe the sample, choose the data, analyze it (paying attention to the options), view it, and generate a report or export a file.

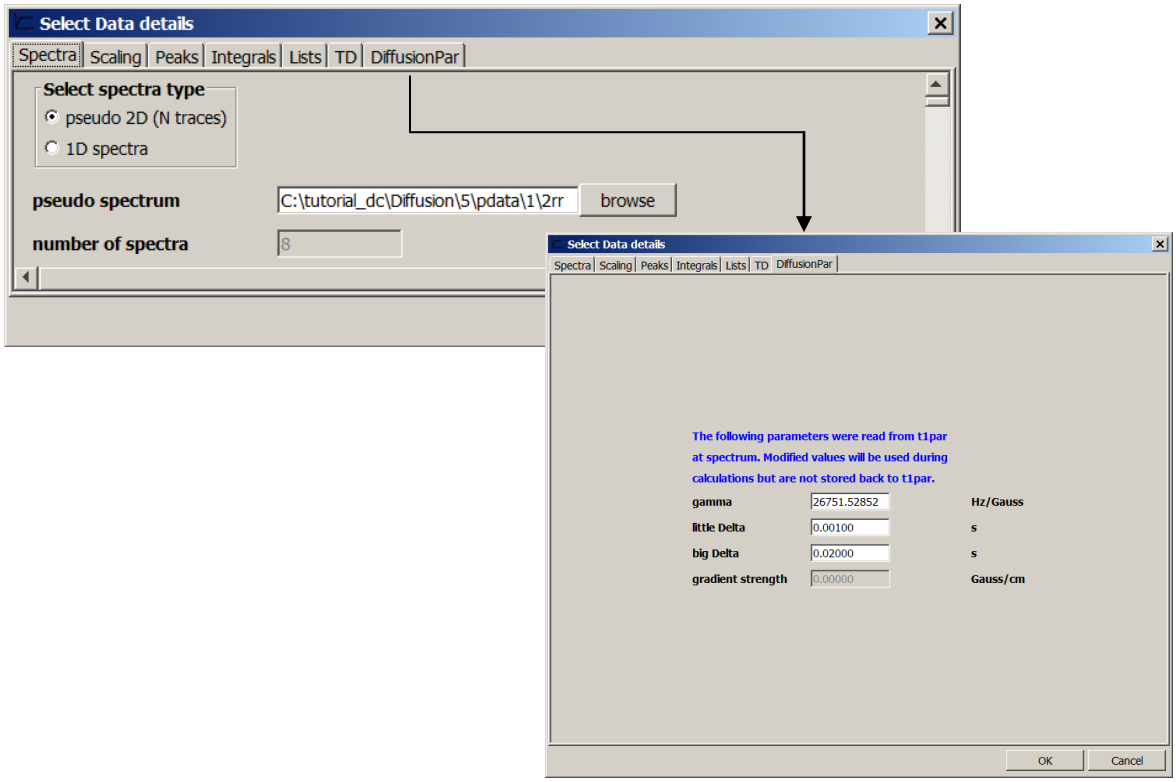
Exercise 3: Diffusion Method

A diffusion analysis follows the same basic scheme as other relaxation analyses such as the T1 analysis done in the previous exercise. There are, however, some important differences. Here, we will analyze a DOSY experiment run on "doped water": 1% H_2O in D_2O doped with 0.1 mg/mL of $GdCl_3$ and containing 0.1% $^{13}CH_3OH$ acquired on a diffusion probe using the stimulated-echo sequence diffSte.

- To start with, **right click** on the diffusion method, select **open** from the popup menu and specify the project file stored in the previous exercise. Even though that project file belonged to a different method, the project can be opened and any relevant information (such as sample information) is applied to the new project. Quantities that need to be different or new parameters not used in the first method must, however, be set.

After loading the project file, the **Sample** component turns green on the method tree. We can directly proceed with **Data**.

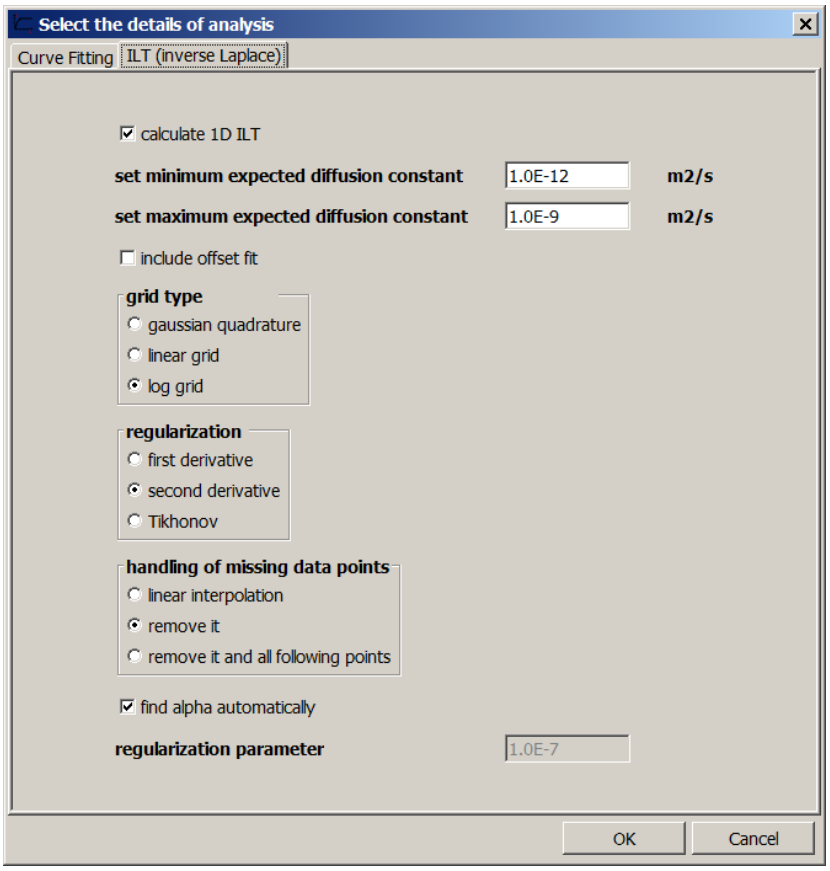
- **Data** opens the usual dialog window for selecting the spectrum type and the location of the data. Select pseudo 2D, and c:\tutorial_dc\Diffusion\5\pdata\1\2rr.
  An additional tab named **DiffusionPar** is now available. Parameters needed for the analysis are loaded from the **t1par** parameter file saved with the dataset. Usually, the values are correct, but they should be checked over.



The parameter that was varied during the experiment (here the gradient strength) is greyed out, but it is possible to modify the other parameters. Changing them here will cause new values of parameters to be used in subsequent calculations, but the **t1par** parameter file will not be updated from the Dynamics Center. After clicking OK, the spectrum is loaded and the first trace is shown along with a data slider.

- **Analysis** is the next step to perform. There are two differences as compared to some other methods. One is that, depending on the experiment, different fit functions have to be used for the curve fitting. An automatic pre-selection is done, but do check that the correct function is really selected. The other major difference is that besides curve fitting, there is an alternative **Inverse Laplace Transform** (**ILT**) technique available. Mathematically it is completely different compared to fitting; it requires more computer resources and the careful set-up of

several parameters. The selection of these parameters is critical and may lead to very different possible solutions of the underlying equations. ILT is particularly relevant if the data contain distributions of diffusion constants but the number of individual constants is not known.

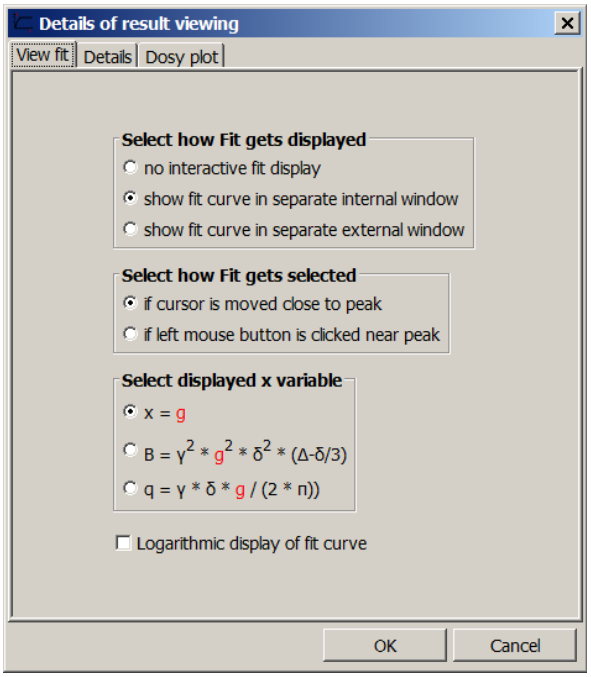


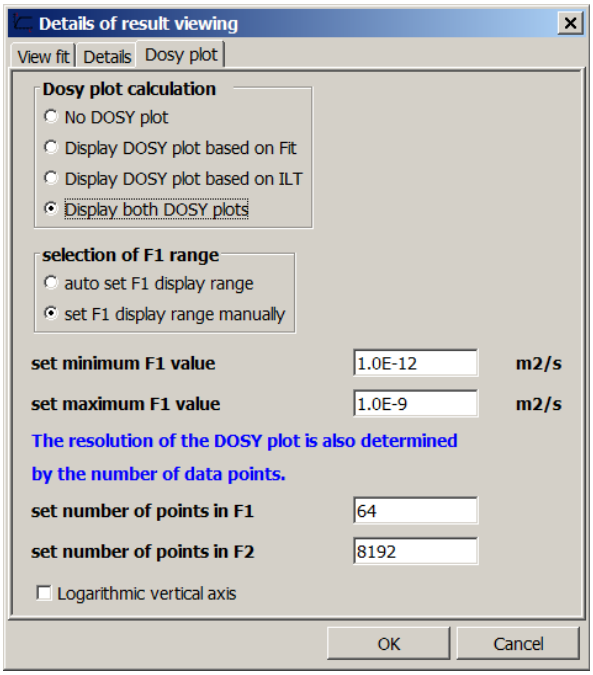
The settings shown here are default values. They are reasonable, except that the expected range of diffusion constant from 1.0×10^{-9} to 1.0×10^{-12} m^2/s seems to be questionable. After clicking OK, both the fits and ILT are calculated.

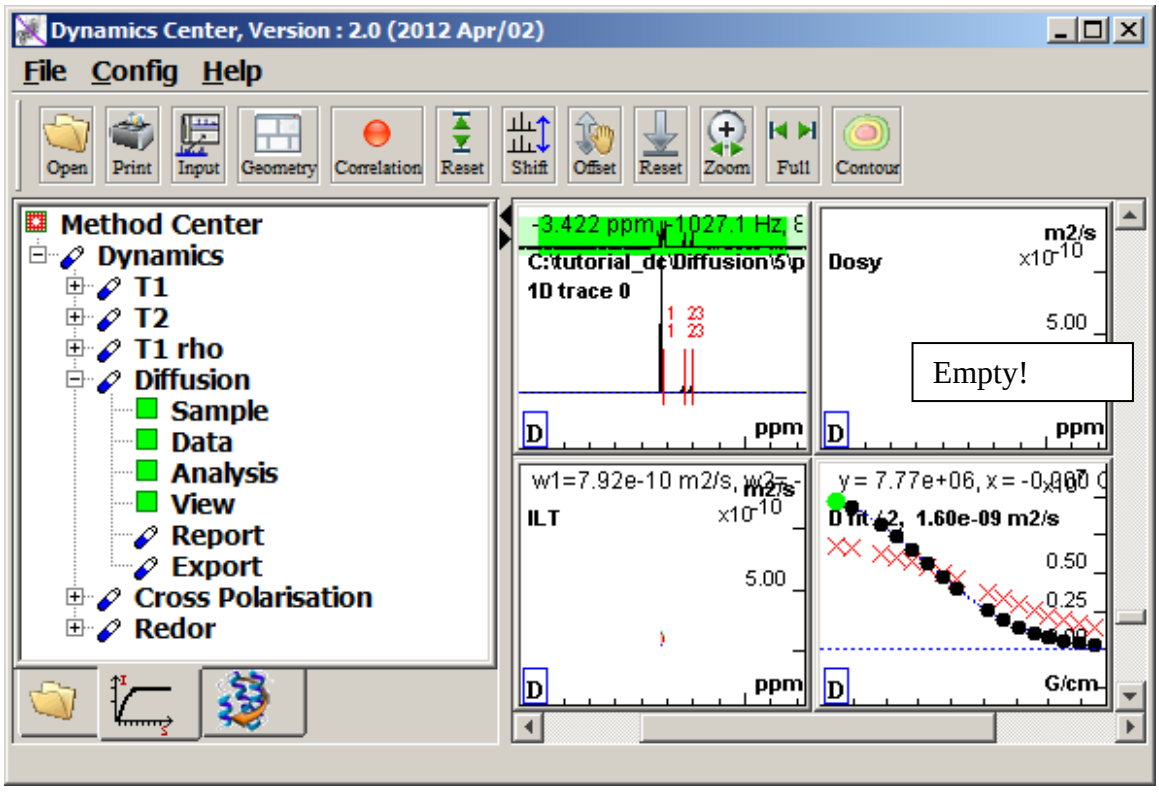
- **View** is again used to define details of the display and then to cause the DOSY curves and ILT to be displayed. Some more settings are required here than for relaxation measurements. One reason for this is that, depending on the selected fit function, different types of x variables can be used for the fit curve display. In the current example, the gradient strength was varied; this can be used for the fit curve display, but the decay can also be visualized as a function of so-called **B** or **q** values.

  There is also a tab to set the details for the 2D **Dosy** displays which show diffusion constants on the vertical and ppm on the horizontal axis. The F1 display range should be set according to the values already used for ILT, here 1.0×10^{-9}

and 1.0×10^{-12} m^2/s. It makes sense to display and compare both Dosy plots, one based on fit, one based on ILT.

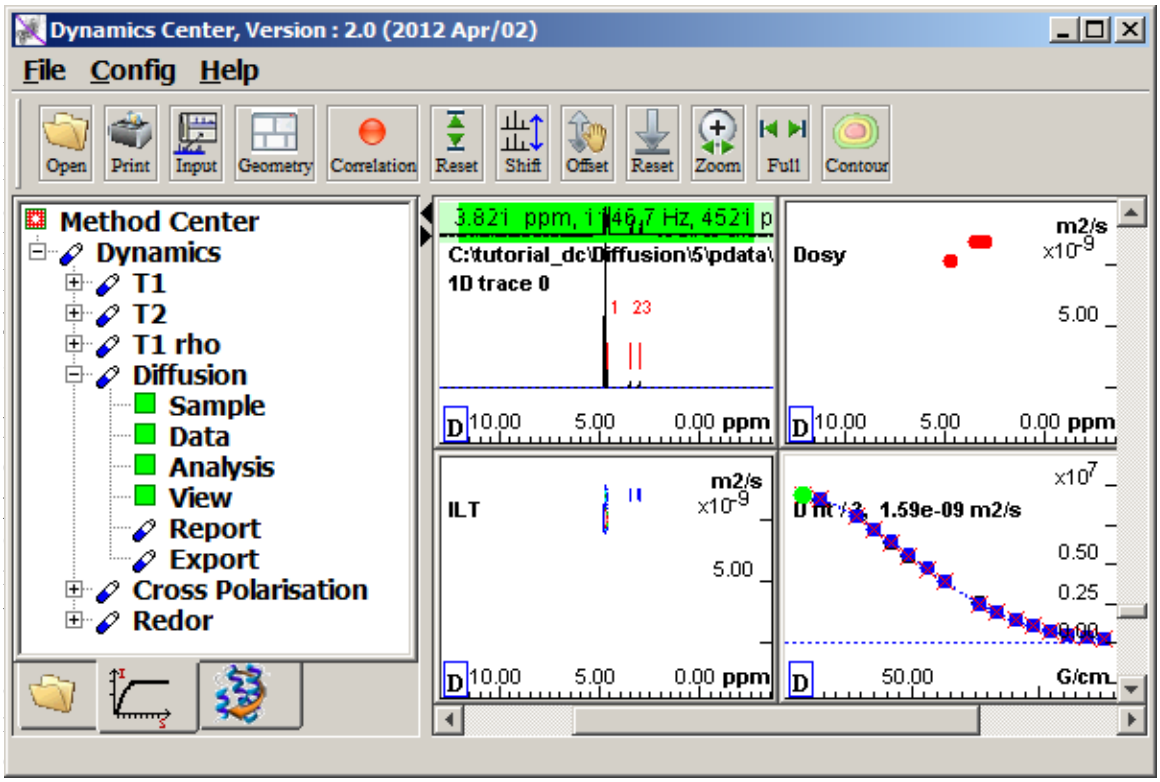






The result looks bad! Not only is the Dosy plot based on fit empty but the Dosy plot based on ILT only contains a single peak which is at the wrong F1 position. It should have occurred at about 1.8×10^{-9} m^2/s. The fit curve display shows **black** experimental, **blue** fitted and **red** ILT backcalculated points. While black and blue points are in good agreement, the red crosses deviate a lot. From this we can draw the following conclusions: (1) The selected Dosy display range of 1.0×10^{-9} to 1.0×10^{-12} m^2/s is too small, otherwise we should have seen a good Dosy plot based on the curve fitting. (2) The expected range of diffusion values used for ILT was also too small and gave poor ILT results. Setting the range of diffusion constants to 1.0×10^{-8} to 1.0×10^{-12} m^2/s in all cases (under Analysis / ILT and View / Dosy plot) yields a much better result.

Now the Dosy plots based on fit and ILT are very similar: the calculated diffusion constants are as expected and experimental, fitted and ILT backcalculated points are in good agreement on the fit curve display. It should be noted that the actual numerical results of this experiment depend strongly on the exact temperature and calibration of the gradient unit used.



Export can be used to generate a report of the DOSY plot and a text or Excel file containing the fit parameters. **Right click** on the name of the method (Diffusion) and select Save as… to save the project as, for example, DOSYpamoic.project.

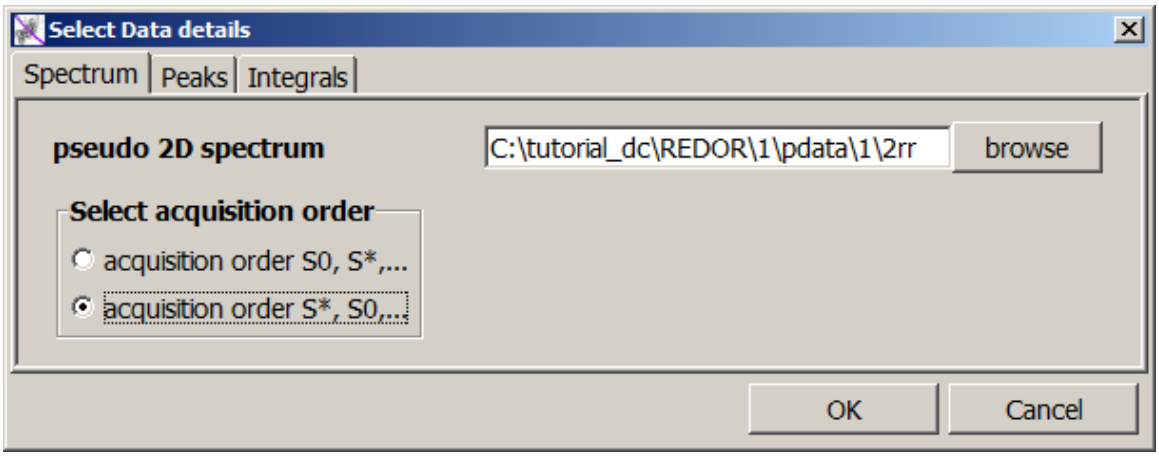
Exercise 4: Redor Method

In this example, we will analyze a $^{13}C\{^{15}N\}$ REDOR curve on ^{15}N-labelled glycine acquired using the standard Bruker pulse sequence cpredori. Again, we will follow the steps of the appropriate method.

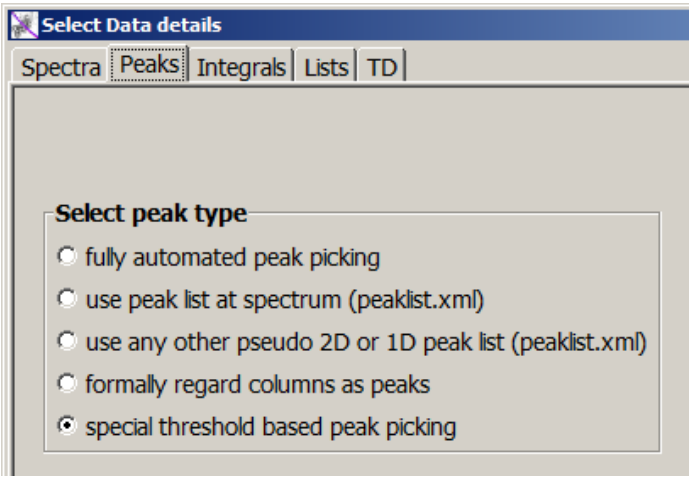
- Either **right-click** on Redor, click on Open and select some project file to begin with, or **double-click** on Redor, then **left-click** on **Sample** to enter sample information.

In either case, the box next to Sample will turn green.

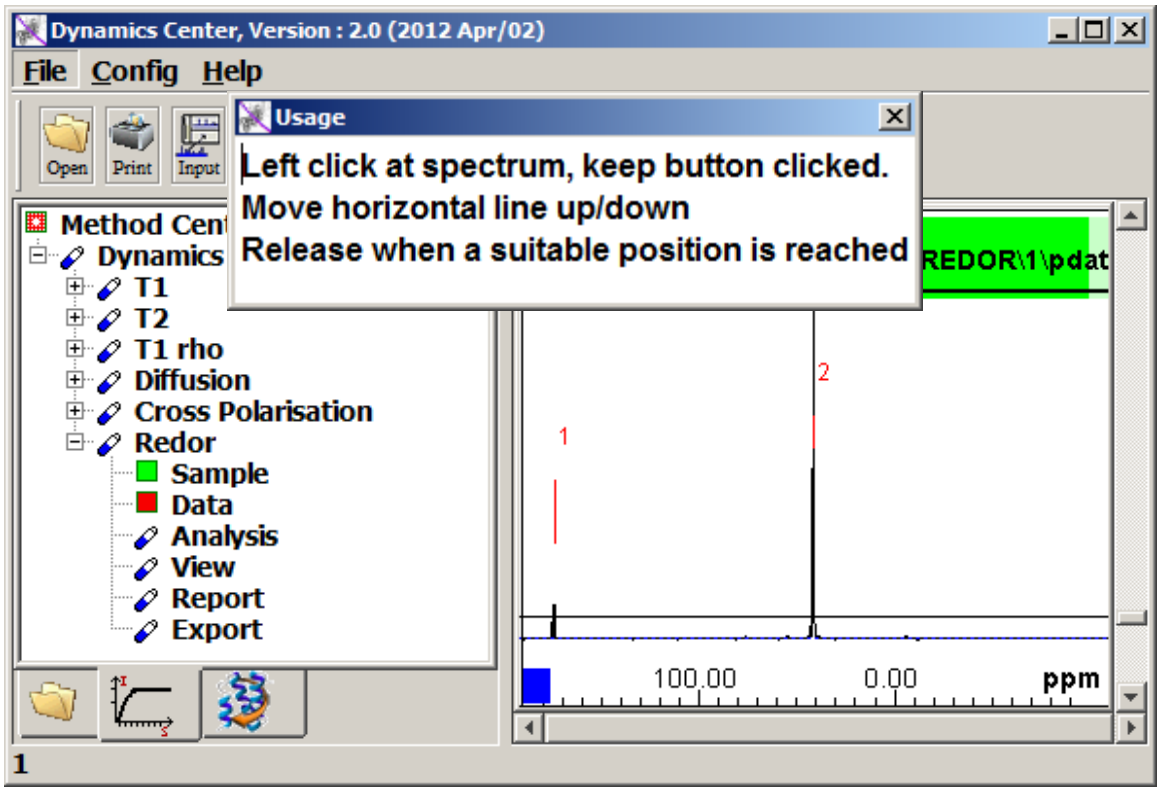
- **Left-click** on **Data** to set the data path (c:\tutorial_dc\REDOR\1\pdata\1; DC adds the \2rr automatically if pseudo-2D is selected) and the acquisition order (S*, S0 with this pulse sequence).



Also, under the Peaks tab, under "Select peak type", choose "special threshold based peak picking.

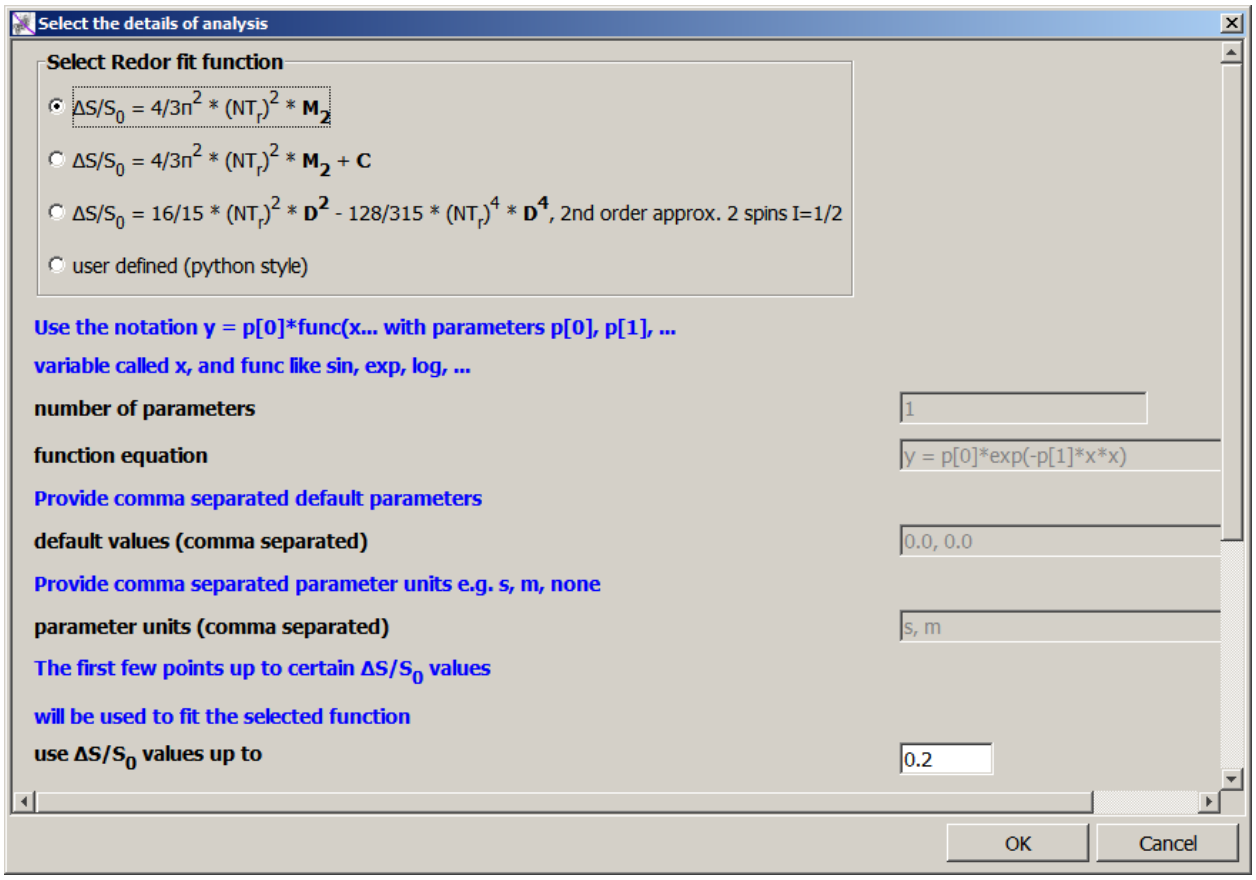


After clicking OK, a message will pop up stating to **click and hold** with the left mouse button while dragging the mouse to set the threshold. Set the threshold so that the two tallest peaks are selected. This option is not necessary here (fully automated peak picking would have been successful), but if we had had spinning sidebands that we wanted to avoid picking, this option would have been useful.

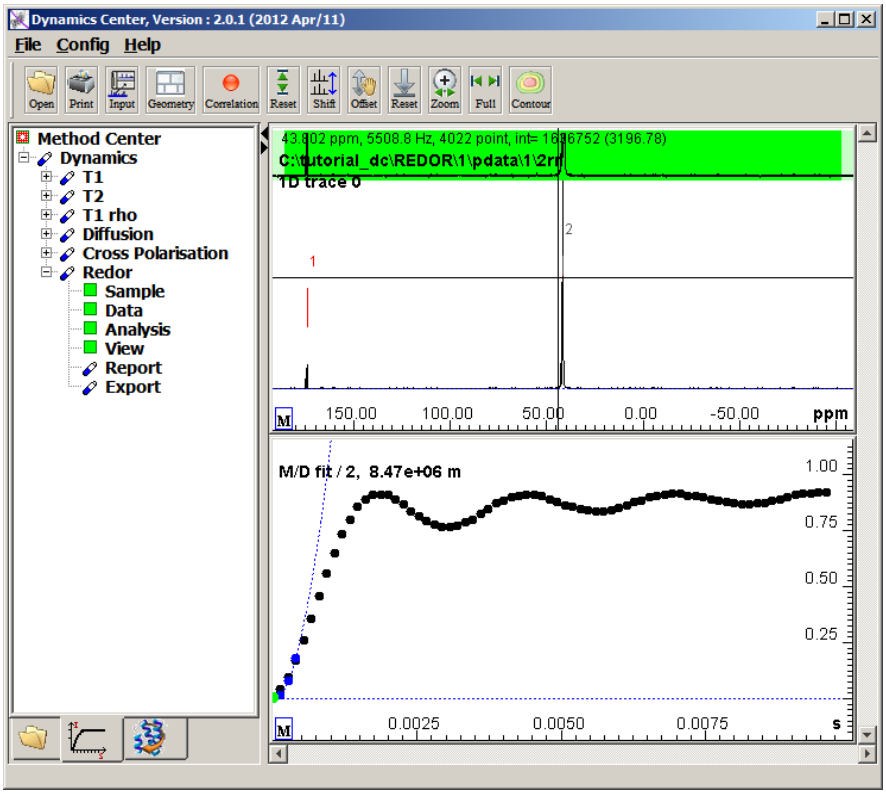


After doing this, the spectrum appears with a data slider for navigation through the traces of the pseudo-2D spectrum.

- **Left-click** on **Analysis** to open the analysis dialog box. There are two main options for fitting REDOR curves. The first is to do a second-moment analysis and the second is to solve for the dipolar coupling directly. We will first fit the curve to obtain a second moment by using the first available REDOR fit function. Accept the default maximum $\Delta S/S_0$ value of 0.2. This constrains the program to fit only the beginning of the REDOR curve. Click OK.



- **Left-click View** to show the spectrum in an internal window.

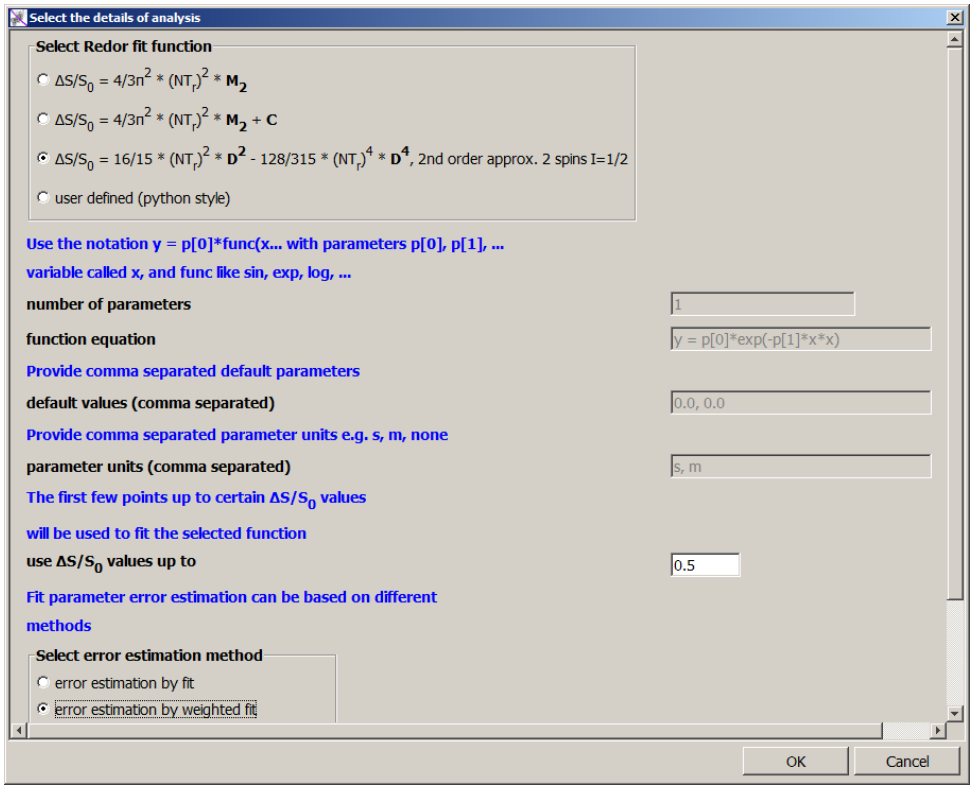


Fitting for the second moment for the alpha carbon at 41.1 ppm yields a second moment of M_2=8.47×10^6 Hz^2. Using the two-spin approximation

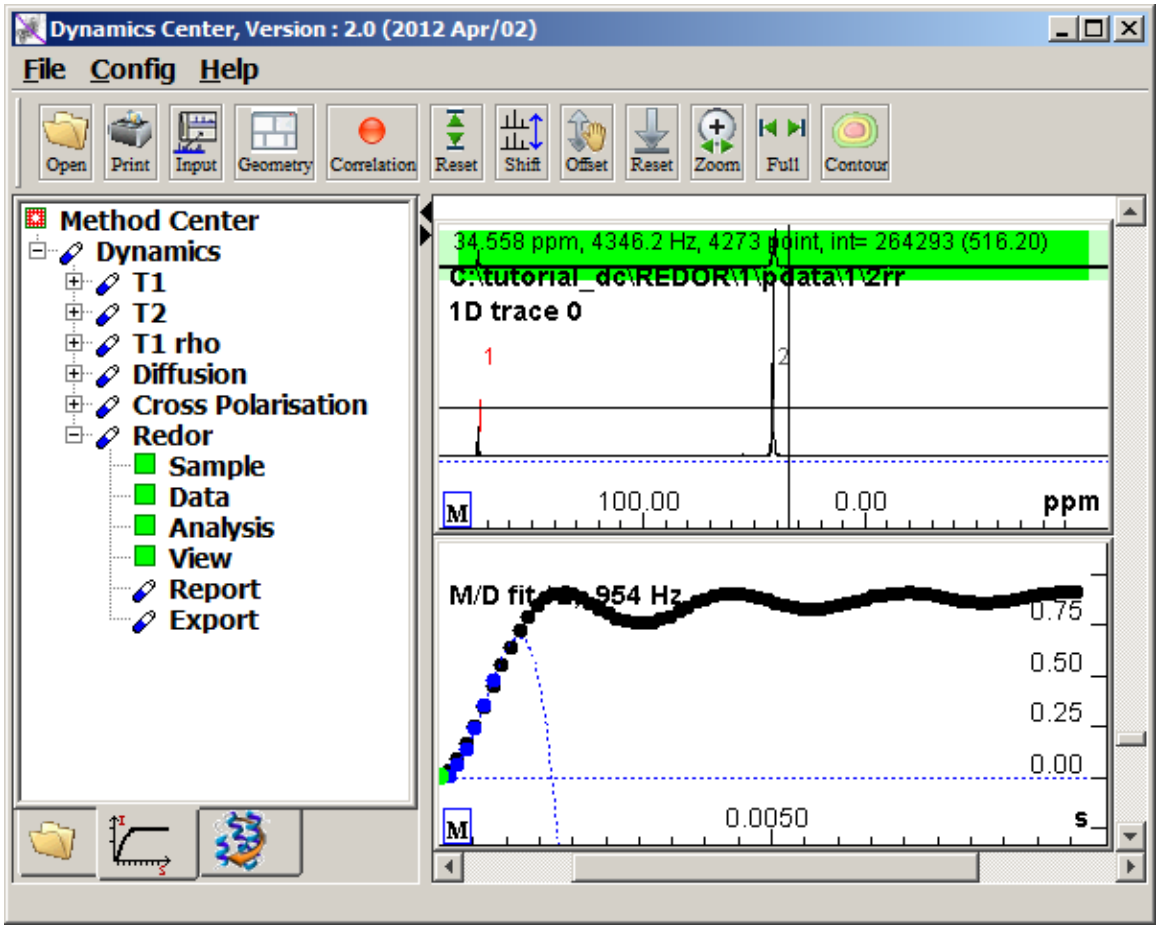
$$D = \sqrt{\frac{60}{48\pi^2} M_2}$$

yields a dipolar coupling D=1036 Hz.

- Now, **left-click** on **Analysis** again to refit the curve to solve directly for the dipolar coupling between the ^{13}C and the ^{15}N. Because the equation contains fourth-order terms, it can accurately describe more of the REDOR curve than the second moment equation, so allow $\Delta S/S_0$ values of up to 0.5.



- **Left-click** on **View** to update the curves displayed (the curves are not updated automatically).



Here the dipolar coupling is reported directly to be *D*=954 Hz. This is much closer to the true value than the value generated with second moment analysis.

- Finally, generate a **Report** or **Export** the fit parameters by **left-clicking** on the appropriate entry in the method tree. There is an extra option under the Simpson tab of the Report window for generating an output file for use with the SIMPSON solid-state NMR simulation program.[1] Save the project by **right-clicking** on Redor and choosing "Save As…".

[1] Bak M., J.T. Rasmussen & N. C. Nielsen, JMR, Vol 147, 2, 296-330, (2000).