

DOSY

Diffusion Ordered Spectroscopy

- a manual for AVneo400 and AVneo800 with Topspin 4.3 -
March 2024



Written by Isabelle Gerz and Knut T. Hylland in 2022 and 2024,
modified for AVneo400 and AVneo800 by Knut T. Hylland and
Frode Rise in 2024

Theory

The DOSY experiment allows to see how fast molecules diffuse in your NMR sample. Diffusion only creates a net change if there is a concentration difference. Therefore, you would expect a diffusion measurement to require a concentration gradient. As you have learned, NMR samples must be homogeneous, and we cannot employ a real concentration gradient. Instead, NMR uses a trick, by having a gradient pulse (for which a special gradient coil is needed, not all instruments have one). A gradient pulse allows to give the magnetisation vector a spatial 'label'. Two such pulses are employed, and the time span in between the two gradient pulses is called Δ (not to be mistaken for δ , the duration of the gradient pulse). The farther the molecule has travelled during Δ and the stronger the gradient (δ), the bigger the mismatch between the defocussing and refocussing pulse. This mismatch causes an attenuation (becoming less) of the peaks. For the DOSY spectrum, we need to collect a series of 1D proton spectra with increasing gradient strength (so-called transients). The DOSY spectrum itself is created from these spectra by fitting either the intensities or the integrals of the peaks. Thereby, the diffusion constant can be determined and you obtain a pseudo 2D spectrum which, instead of cross peaks like you have encountered them in 2D NMR, shows the measured diffusion coefficient of each peak. If you have a mixture of molecules, they are separated by diffusion coefficient (all peaks of one molecule should have the same), earning DOSY the nickname 'NMR chromatography'. A word of caution in the end: This technique, while powerful, is easily disturbed by other phenomena that make the observed proton travel: chemical exchange and convection, for example due to a temperature gradient in your solvent, create false results. The more viscous your solvent is, the lesser the convection issue. Therefore, DMSO and water are the most popular DOSY solvent.

The DOSY experiment is used in all kind of subfields of chemistry:

- Aggregation
- Hydrogen bonding, Ion Pairing, etc.
- Host-guest complexes and supramolecular assemblies
- Identification of mixtures, especially in food chemistry
- Estimate the molecular weight of polymers

For the theory behind it, consult the respective chapter in 'High Resolution NMR Techniques in Organic Chemistry' by Claridge.

A shorter summary can be found on

<http://chem.ch.huji.ac.il/nmr/techniques/other/diff/diff.html>

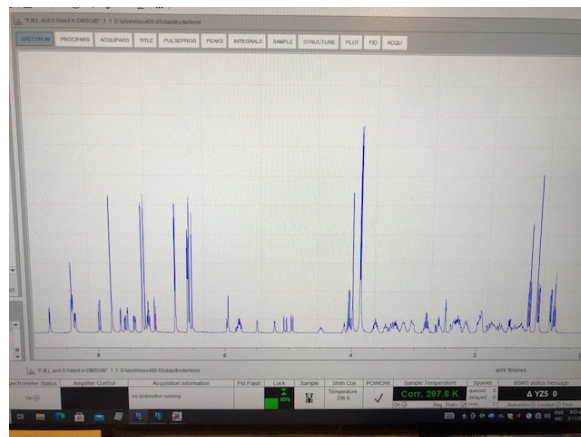
A more pharmaceutical perspective on

<https://www.sciencedirect.com/science/article/pii/S0079656599000035>

Experiment

A. Acquisition

Start by running a normal ^1H experiment (**Exp. 1**) the usual way. Make sure the spin is off. Phase and calibrate the spectrum.



Find the **rg** value by typing “**rga**”, this is needed for the next experiments. Use **wrpa** to make a second experiment in **Exp. 2**.

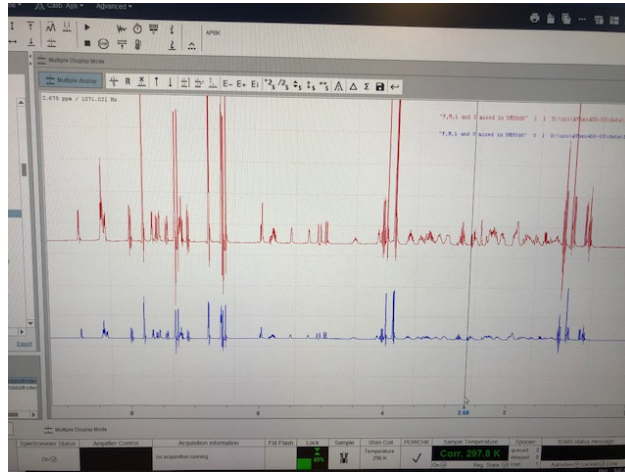
- re 2
- Change the pulse program in the acquisition parameters to **stebppg1s1d**
- Type **d20** (this is Δ) and set it to a value between 50 and 100 ms (beware, the entry is in seconds, so you should write something between 0.05 and 0.1)
- Type **p30** (this is $\frac{1}{2} \delta$) and set it to a value between 1 and 3 ms (beware, it is given in μs , so you should write something between 1000 and 3000)
- Set **gpz6** (this is the gradient strength) to 5% (can also use 2%)
- Set **gpz7** to -17.13%
- Set **gpz8** to -13.17%
- Type **gpnam**.
- Set **GPNAM** for **6,7**, and **8** to **SINE.32** (other options might work as well).
- **getprosol**
- set **ds** to 8 or another value $n*2$ as desired
- set **ns** to 16 or another value $n*16$ as desired (32 give better “chromatographic” separation)
- Set **d1** to 1 or another value as desired. Higher **d1** values gives better data.
- Set **RG** to the same value as for the initial ^1H experiment (**no rga, important**)
- **zg, efp** and phase correction as needed

Look at your spectrum. Ideally, it looks similar to your previously collected ‘normal’ proton.

Use **wrpa 3** to copy everything to **expno 3**.

Go to **expno 3** and change **gpz6** to 95% (can also use 98%). Use the same **RG** value as above in **expno 2** (**no rga, important**). Use **getprosol** and **zg** to obtain a second spectrum that you **wrpa 4** to have a safety copy in **expno 4**.

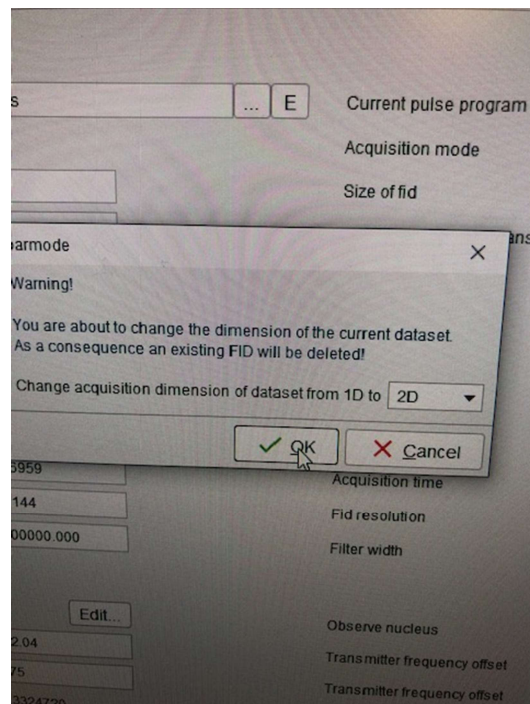
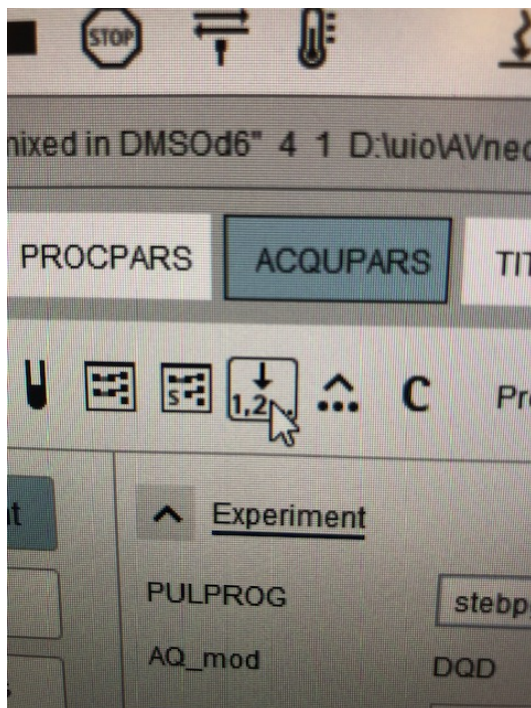
Use the overlay function to compare the spectra in **expno 2 and 3** with 5 and 95% gradient strength. No signals should have disappeared completely, but the ones that are most affected should only have 5% of their initial intensity in the 95% spectrum



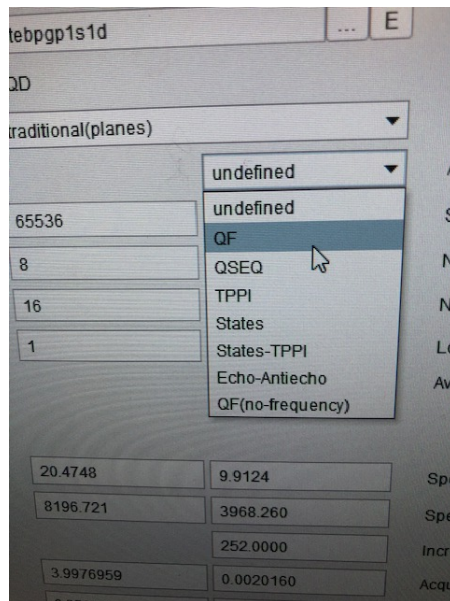
If the spectra do not fulfil the requirements, you need to change **p30** and **d20** and rerun both spectra (one with 2 % and one with 95 % gradient strength). Increasing either will increase the attenuation, while decreasing them will reduce the attenuation (If any signals were gone, you need to decrease them, while if you want a stronger effect, you need to increase them). Changing **p30** has a more pronounced effect than changing **d20**. **NEVER** set them outside of the range given above, you can irreversibly damage the instrument.

When you have found a satisfactory setting, go to **expno 3**, copy the experiment by **wrpa** to **expno 5**. This creates a new experiment **Exp. 5**. This experiment will be the one running the series of 1D experiments. Open the Acquisition Parameters tab in **expno 5**.

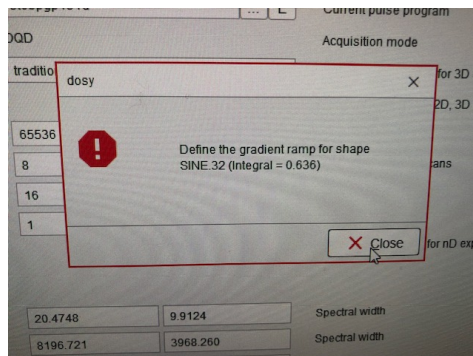
- Change the pulse program to **stebppg1s**
- Change the acquisition dimension to 2D:



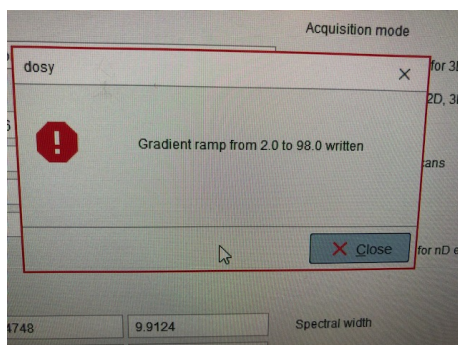
Set **FnMODE** to **QF**



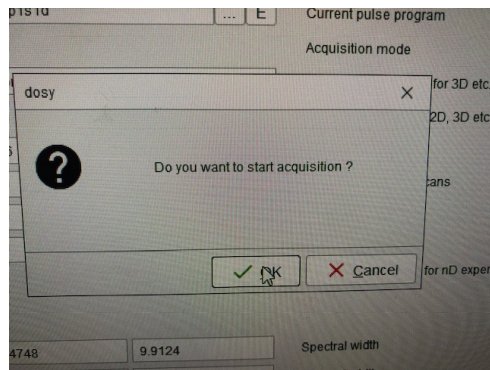
Set the **TD** in **F1** to 16. Check if **d20** and **p30** are set correctly (the values you used before on the 1D).
getprosol, **rga. dosy** (this opens a command window. The red cross doesn't mean you created an error. Just press close and the settings window will open.).



The ramp is set for you to 2-98 %,

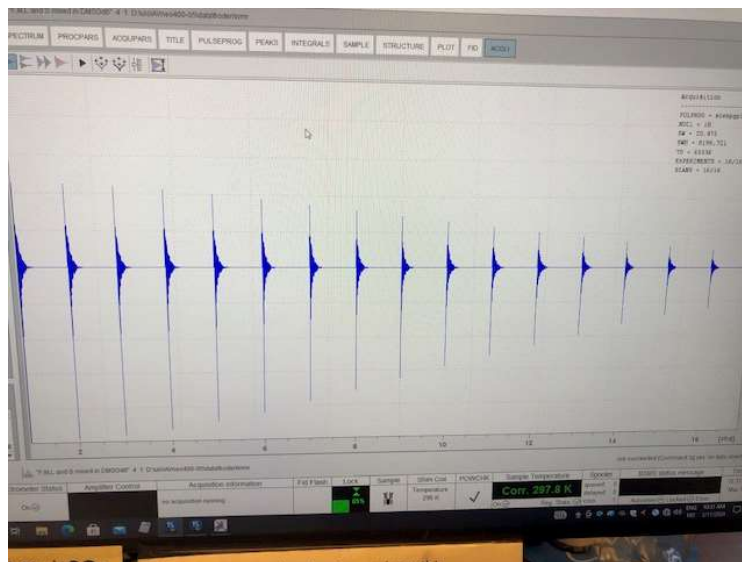


choose number of points (16) and choose a linear ramp (l). Agree to start the experiment.



B. Data Treatment

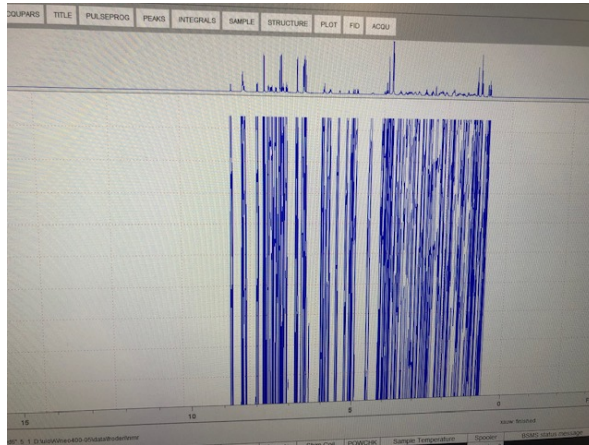
Once the experiment is completed, use **wrpa 6** to copy the data into a new experiment (**Exp. 6**). Do all the processing in the copied version.



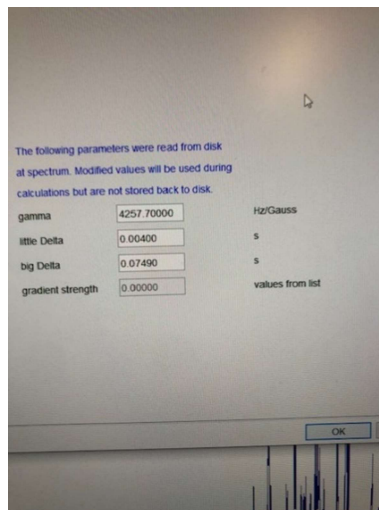
- Go into the processing parameters.
- In **Window Function**, Set **LB** of F2 to 2
- In **Baseline Correction**
 - Set **ABSG** for F2 to 0
 - Set **ABSF1** for both dimensions to 1000
 - Set **ABSF2** for both dimensions to -1000

Execute **xf2**.

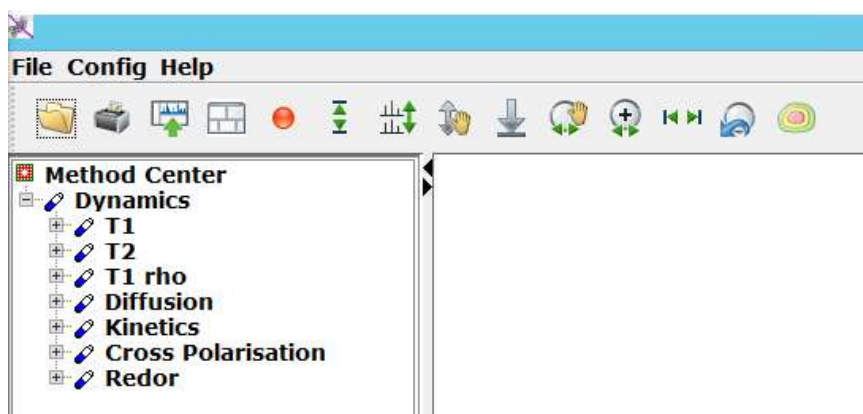
Xf2 shows you the following pictures if TD(F1) is set to either 16 or 32. Phase the spectrum manually, **but only the rows**. You do this by **rser 1 efp apk** and maybe manual phasing. Look in edp for the two phasing constants and write them down. Close the temp window and open edp and change if necessary the two phasing constants. Do **xf2** again and execute **abs2**



Execute **setdiffparm**. If you forget setdiffparm all entries in the following window will be zero and all fails later on.

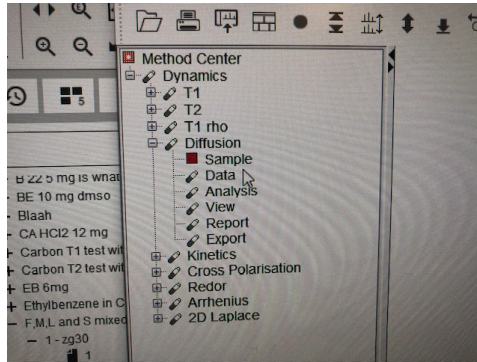


Open the **'Dynamics Centre'** by typing **dyn** into the command line in toprosin, or you find it on your desktop.



Double click the Diffusion option. This opens six subchapters. You will go through them in the order they are displayed.

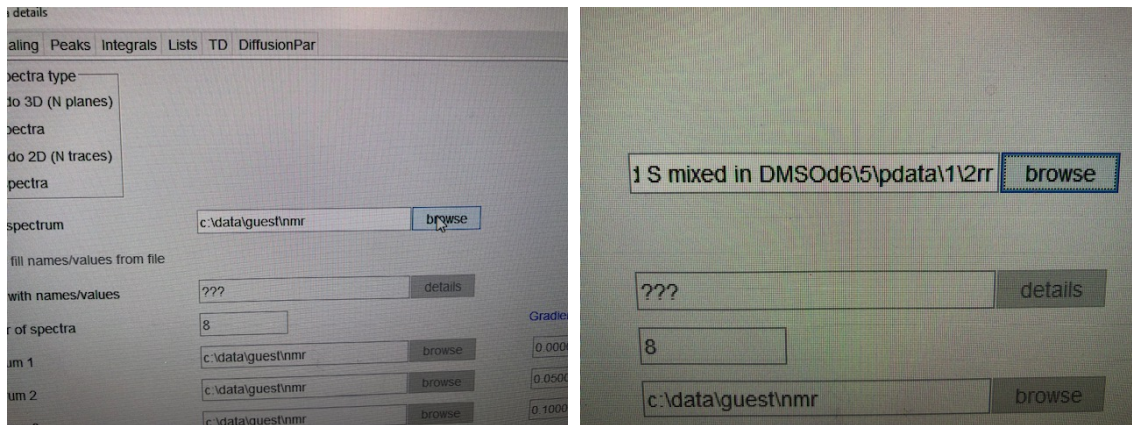
Sample



Double click 'sample'. Give it an appropriate name.

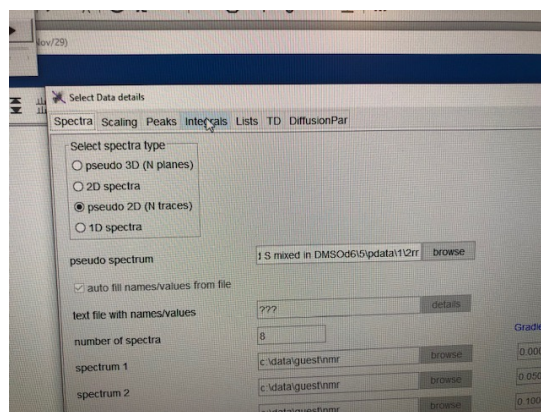
Data

A window pops up with multiple tabs. In the 'spectra' tab, browse for your experiment in the **pseudo spectrum** line. >This PC >NMR (D:) > uio > the instrument you took the experiment on > data > your user > nmr > experiment name > experiment number > pdata > proc number > 2rr



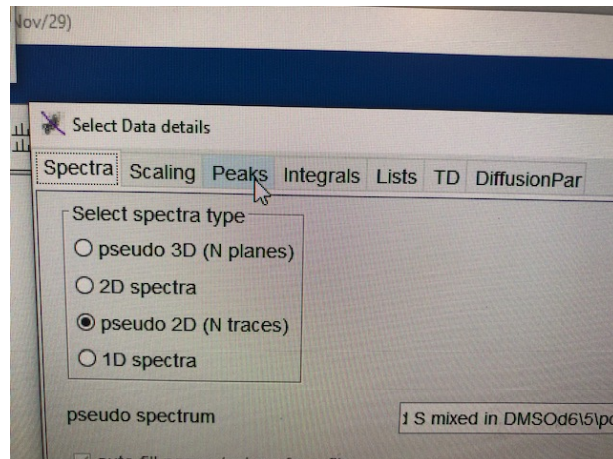
The path can differ between computers, essentially you need to find your spectrum.

Then go to the **integrals** tab in the Data window.

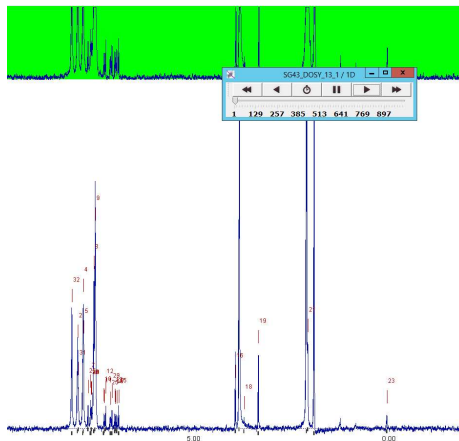


Choose 'use peak area (user defined) integrals).

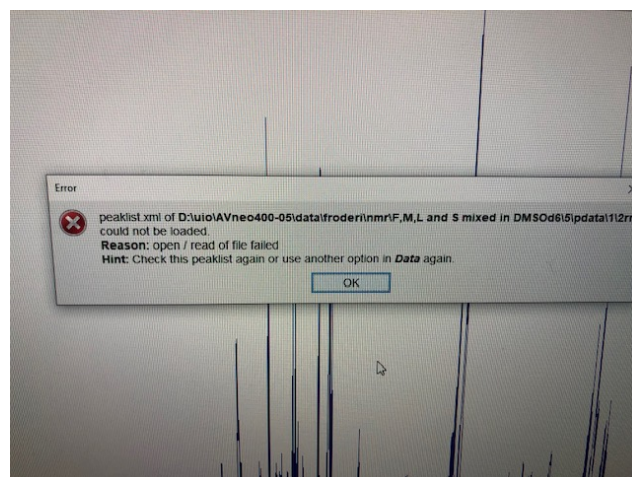
Then go to the **peaks** tab. Under 'select spectra types', several options can be used (if using the default pseudo 2D an error message might occur, but this can be ignored).



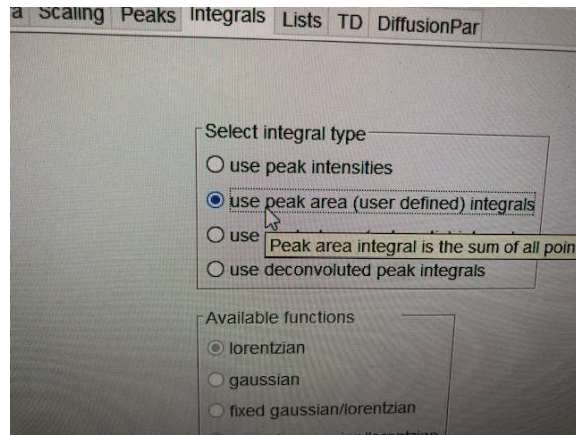
Press OK. Wait until the red square next to **Data** becomes green. The screen now shows something like below:



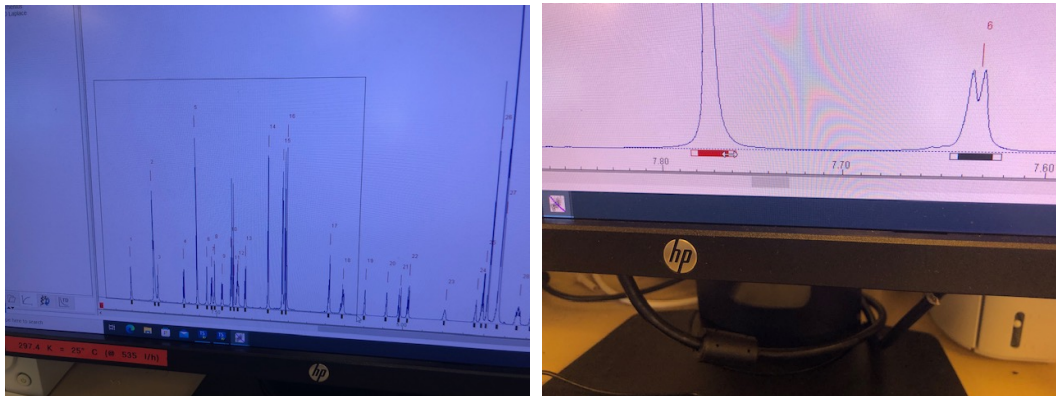
You can press the play button in the pop-up window to see the signal attenuation progress, but this is not necessary.



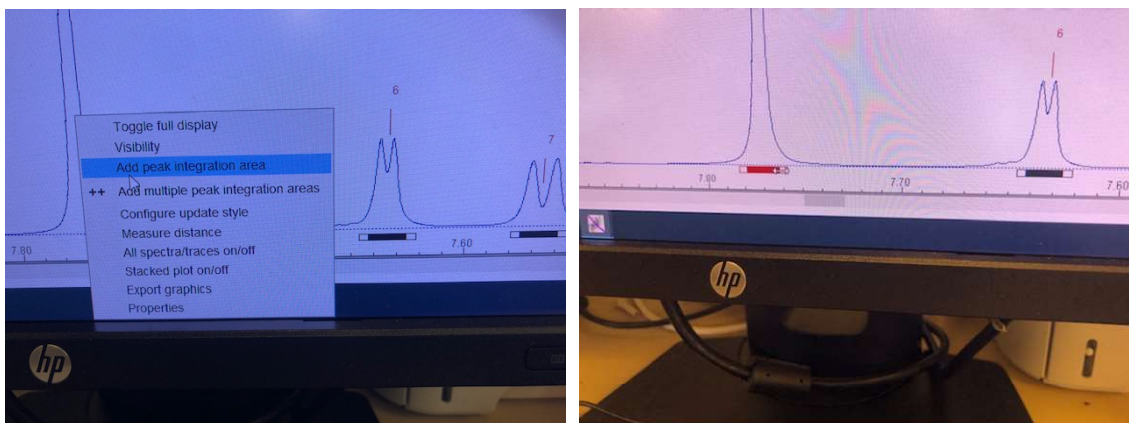
Somewhere you get the error message above. You have to select Integrals in the data tab and select use peak area (user defined) integrals for the dynamic center program before you define the integrals.



In the main Dynamic Center window (green above) you zoom in on the signals, pick peaks and integrate the signals of interest. When picking peaks and integrating, do not choose overlapping signals, their analysis will be misleading.



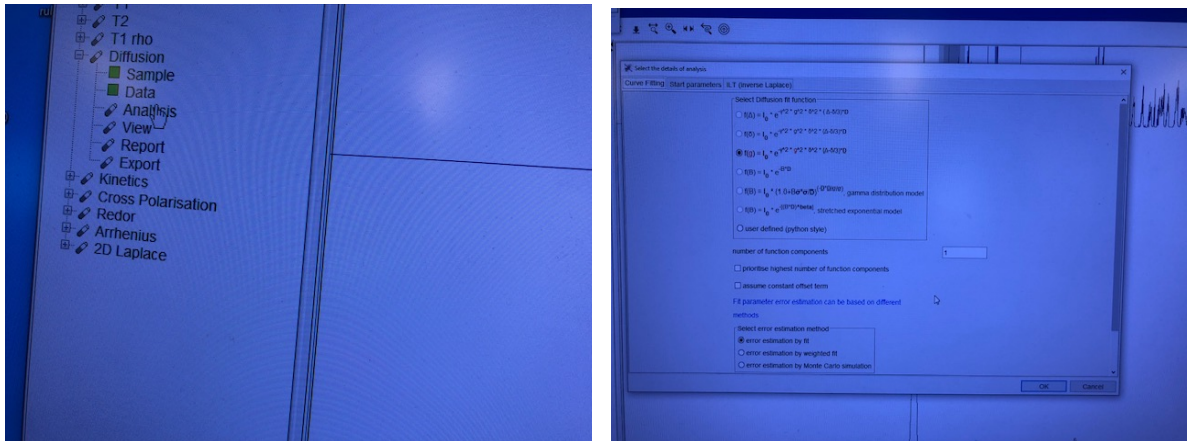
You zoom by clicking with the mouse either left or right button (figure it out yourself) and hold the button while dragging. Right-click on the signal of interest and choose "Add peak integration area" from the list that appears.



A small box will appear under the signal (it will not cover the entire range of the signal). Adjust the integral by dragging the black part, you affect the placement and you can stretch them by dragging the white borders. When done, repeat the procedure for the next signal.

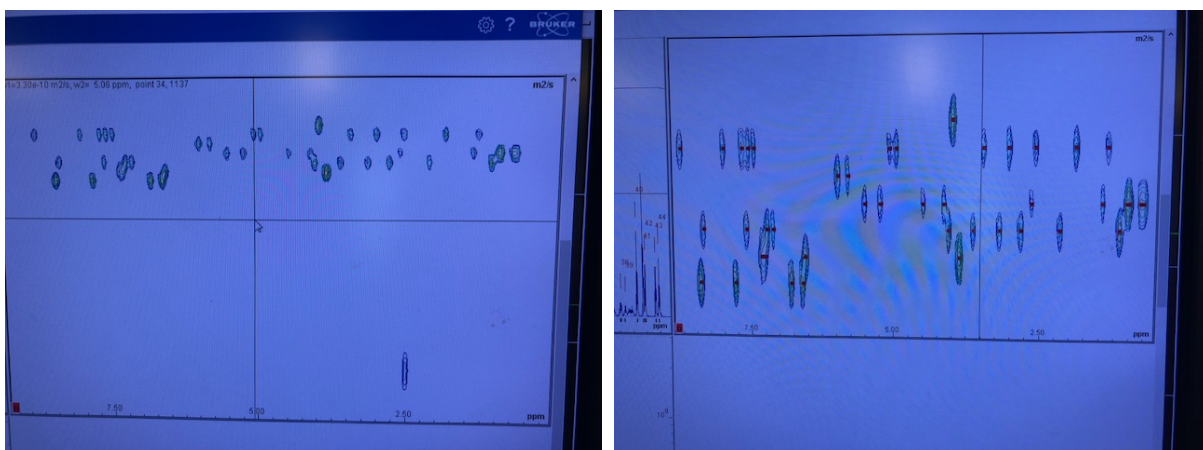
Analysis

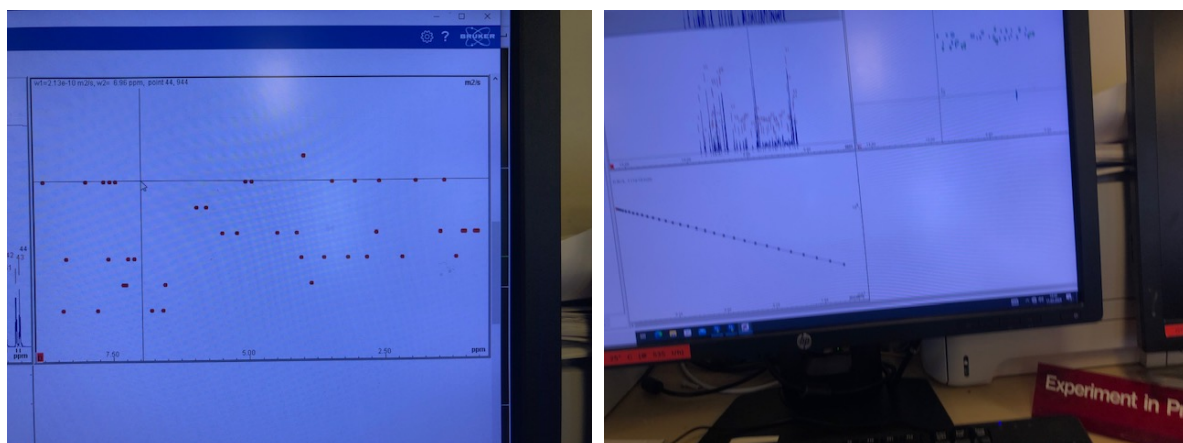
Double click the 'analysis' button. Again, a pop-up window is opened. Several parameters that can be adjusted.



- **Number of function components** (if you have a good fit with one component, only use one).
- **Error estimation method** (both 'error estimation by fit' and 'error estimation by weighted fit' are reasonable choices. It really depends on the measurement.)
- **Integral areas** (this is not in the pop-up, but done as in **data** you will have to re-click **analysis** after adjustment). If you have followed this manual the integrals are already done

Press ok. A window stating that the fit curves have been calculated. If there are failed fits, you should change parameters mentioned above.





View

Double click **view** and press ok. (If issues are encountered, you can try to change the **display x variable** to the second option. Furthermore you can adjust the DOSY plot range in the DOSY plot tab, if you want to. For that, it is important to know where your diffusion coefficients are, so we only recommend doing that on later iterations.)

Wait until the square is green. By hovering over the peaks, their respective fits are shown. If the fits are not good, we recommend going back to the above steps and adjusting the bullet points addressed in **analysis**. If only some fits are off, you should try to change your peak picking and integrals.

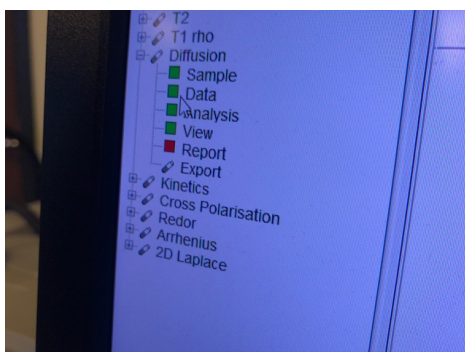
The fits are displayed in a pseudo 2D spectrum – your DOSY! If you were successful with the fitting, you should see that each component's peaks (this can be species originating from different compounds or

from the same, e.g. aggregates) have 'cross peaks' in a horizontal line (more or less). This line corresponds to the diffusion coefficient for your component.

Once you are satisfied with your DOSY spectrum, save it as 2rr spectrum (option when you right-click on the pseudo-2D spectrum). A window where that asks you to save the data under a new procno. This will transfer the spectrum back to topspin.

Report

Double click 'report'. Press the 'browse' button and find your user folder for the instrument (the same as the one where we got the data from earlier). Save the pdf under an appropriate name.



(Other processing methods that don't use the dynamics centre can be found under: <http://nmrweb.chem.ox.ac.uk/Data/Sites/70/userfiles/pdfs/diffusion-nmr.pdf>)

Note down the diffusion coefficients of your sample. How many components are there?

If the 2rr spectrum you transferred into TopSpin is functional (sometimes the file is corrupted for reasons unknown), make a DOSY plot in the plot editor. If the file does not work, use the plot in your pdf. Specify the parameters you used to acquire the DOSY (δ , Δ , number of transients, NS, pulse sequence).

Calculate the Mw of the components in your sample using the SEGWE calculator from the Manchester NMR Methodology Group. There you can also find their scientific publication on the calculator.

<https://nmr.chemistry.manchester.ac.uk/?q=node/432>