2D-PASS processing in ssNake

8th November 2018

1 Introduction

A 2D-PASS (Phase Adjusted Spinning Sidebands) experiment attempts to separate isotropic lines from spinning sidebands. This is especially useful in cases were there are a lot of different sites, each with a considerable chemical shift anisotropy (CSA). If the MAS spinning speed can be increased to a value higher than the strongest CSA, an isotropic spectrum can be obtained in a normal 1D NMR experiment. In the intermediate cases, severe overlap of sidebands can make it difficult to recover isotropic information. In a 2D pass experiment, after proper processing, each order of sideband is contained in a spectre spectrum. The centre spectrum of this series will be the isotropic spectrum.

The following will describe how to process such 2D-PASS data in ssNake.

2 Data

Delivered with this tutorial is a ^{13}C data set of alanine. `13C_Alanine_1250Hz.fid` is a 2D-PASS spectrum of alanine measured at 300 MHz and 1250 Hz MAS. The spectrum was obtained on a Varian system.

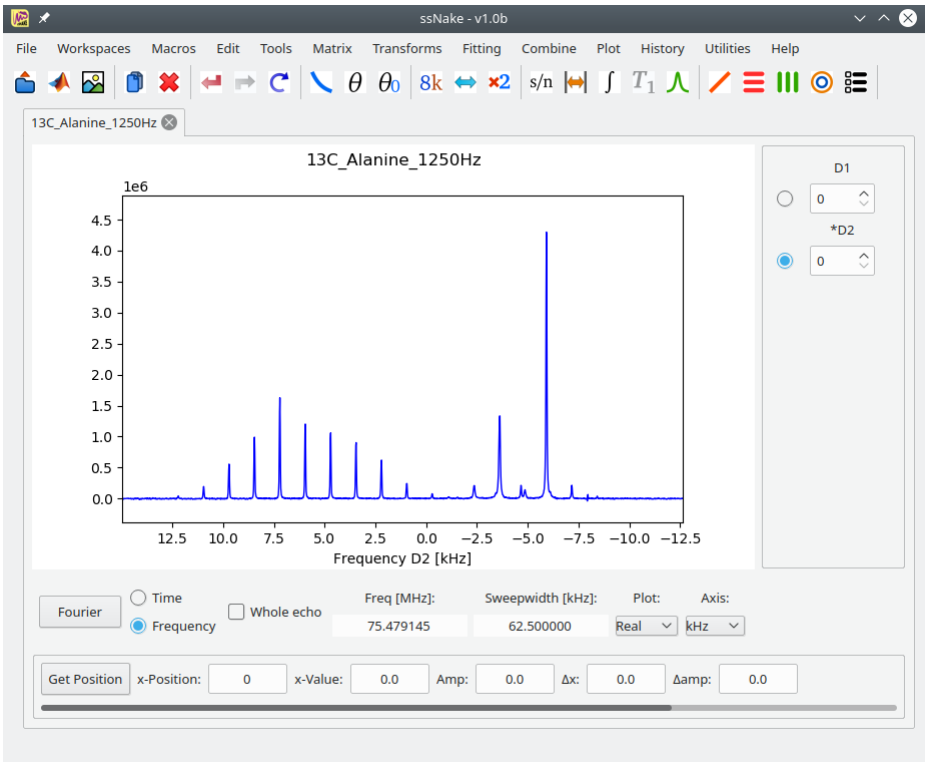
3 Processing the data

To start, we will process the more accessible spectrum of alanine.

- Open the Varian file `13C_Alanine_1250Hz.fid` using `File` ⟶ `Open`
- Fourier transform via the 'Fourier' button
- Clear the current ppm reference via `Tools` ⟶ `Reference` ⟶ `Clear Current Reference`[1]
- Phase the spectrum using `Tools` ⟶ `Phasing` and use 8.1° 0th order phasing
- Set the size to 4096 using `Matrix` ⟶ `Sizing`

[1] For some reason the reference of this data set is very wrong. I do not know why.

This should show:

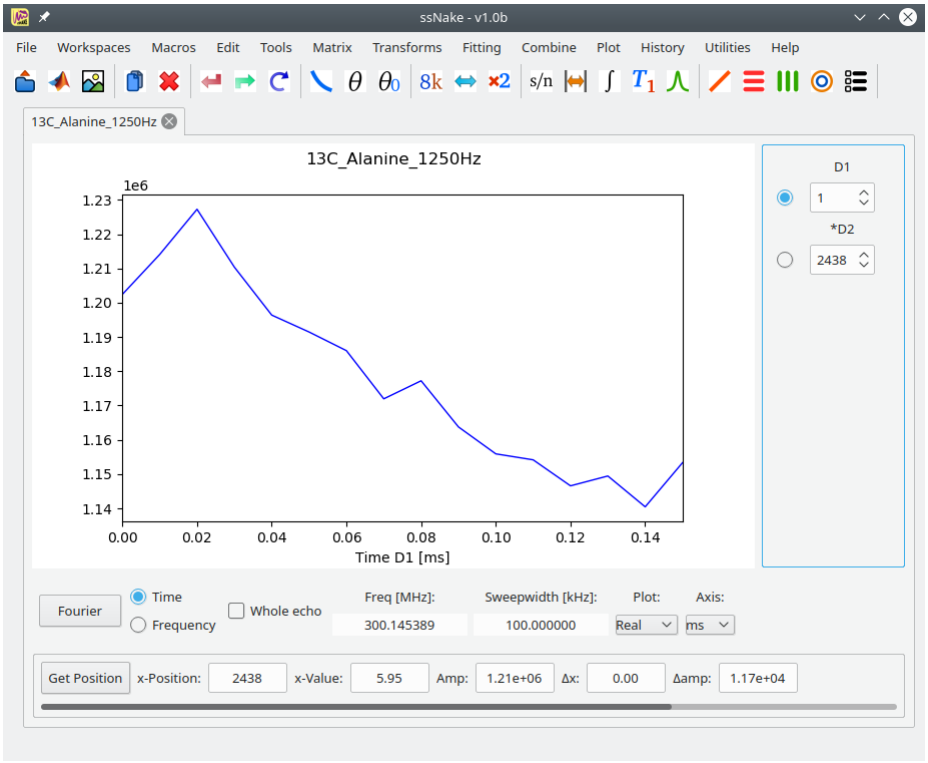


Scrolling though D1, we can clearly see that all the sidebands change their phase, while the centrebands of each site stay in-phase. This is the way this experiment works, and allows to distinguish sidebands from centrebands.

We will now change the view to D1, and process this dimension.

- Fill in '2438' at the D2 box in the sideframe, and press enter

This now shows the change of the centreband of this carboxyl group over the separate PASS spectra:

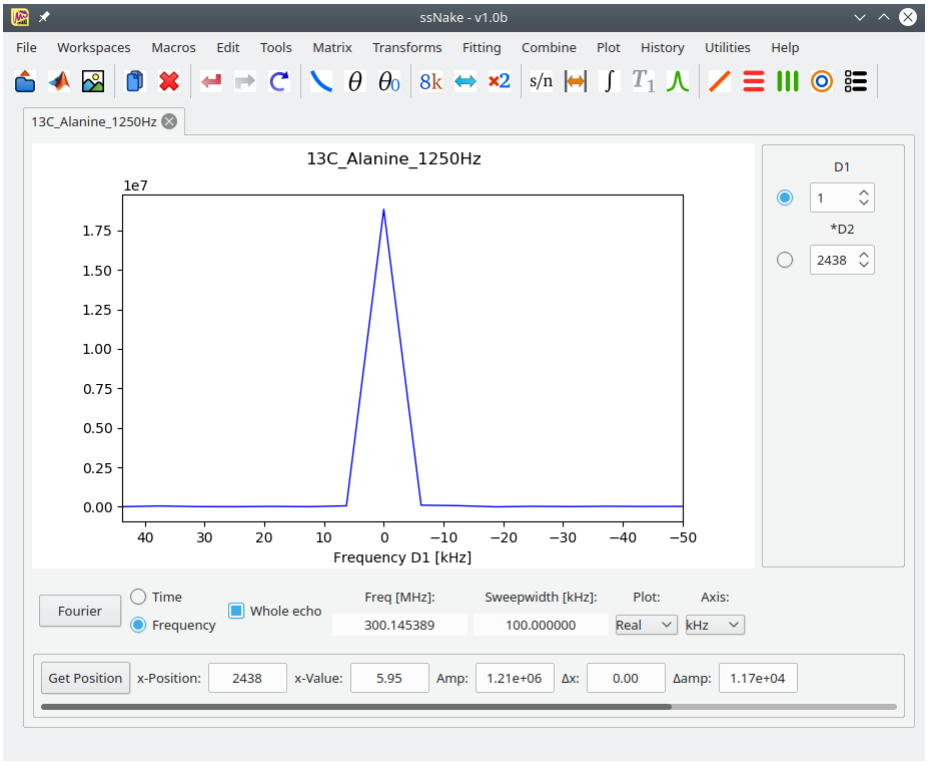


We now want to Fourier transform also along this dimension. However, we need an extra trick. In a regular Fourier transform, the first point of the FID is always multiplied by 0.5, to get a flat baseline spectrum (i.e. to get rid of the frequency-independent Fourier term). This works well only if our signal decays to 0. In the current case, we do not really have an FID, and no decay to 0 is present. We must therefore tell ssNake to not multiply the first point by 0.5. To do this, we enable the 'Whole echo' mode in the bottom frame (tick the box).

After this, we can do transform:

- Fourier transform via the 'Fourier' button
- Clear the current ppm reference via `Tools` ⟶ `Reference` ⟶ `Clear Current Reference`

This now shows:



What we see here, is that one one point has intensity: the centre point. This is exactly what we expect here. Every point stands for a specific order of a sideband. The centrepoint is the isotropic (order is 0) value. As our position 2438 in D2 stands for an isotropic peak, it makes sense that only the 'order is 0' point has any intensity: the sidebands are spectate.

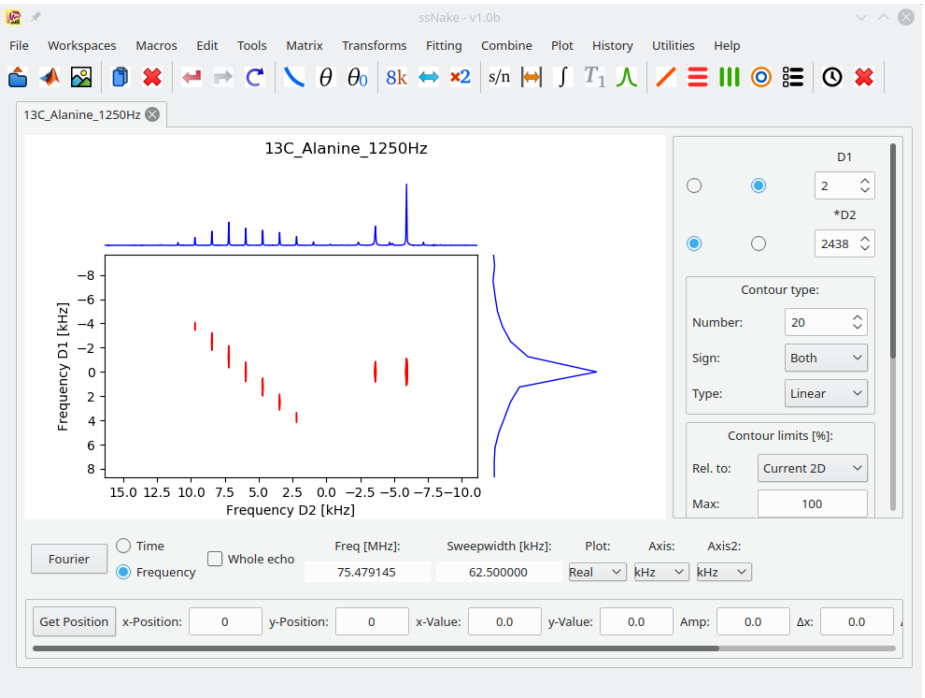
To aid in the view of the data, we must correct the spectral width. Each point corresponds to a specific order of sideband, so between neighboring points, there should be 1250 Hz difference in frequency (i.e. the spinner frequency). In this case we have 16 points, so we must correct the spectral width accordingly:

- In the bottomframe, set the Sweepwidth to 20 kHz (16 * 1.250)

To have a more proper view of the data, lets view it as a contour plot:

- Move to D2 by clicking its radiobutton in the sideframe
- Set the plot to contour via `Plot` ⟶ `Contour`

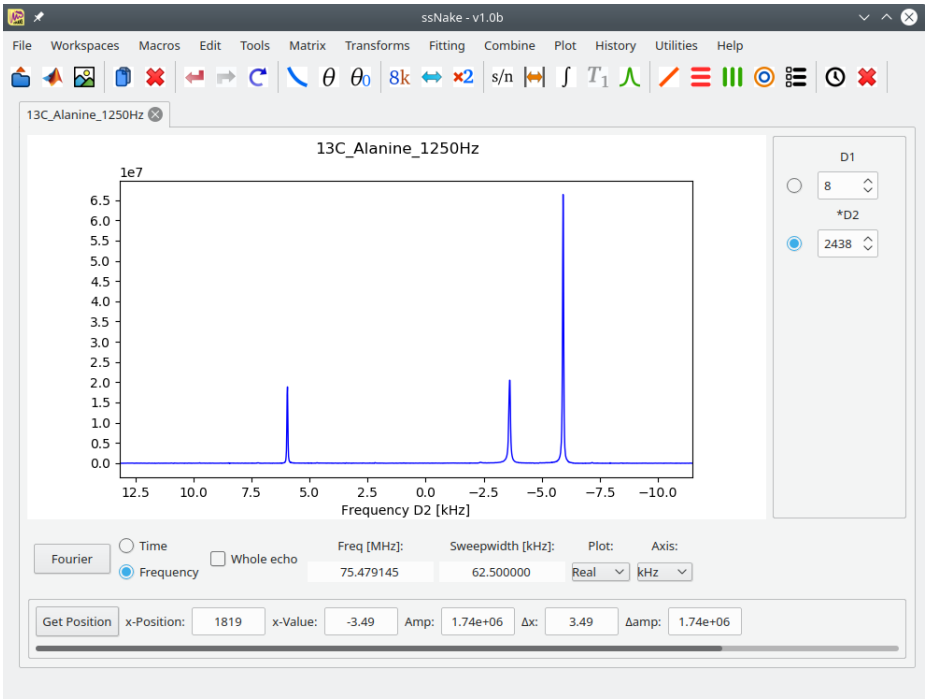
This should show (zoomed):



Here we can see that the two lines on the right only have a centreband (hardly any CSA). However, for the carboxyl group on the left, a clear pattern emerges. Every sideband is contained in a specific trace along D1, and are therefore separated. When viewing this centreband (i.e. position 8 along D1) we will see an isotropic spectrum:

- Set the plot to 1D via `Plot` ⟶ `1D Plot`
- Fill in '8' at the box of D1 in the side frame, and press 'enter'

This should show (zoomed in):



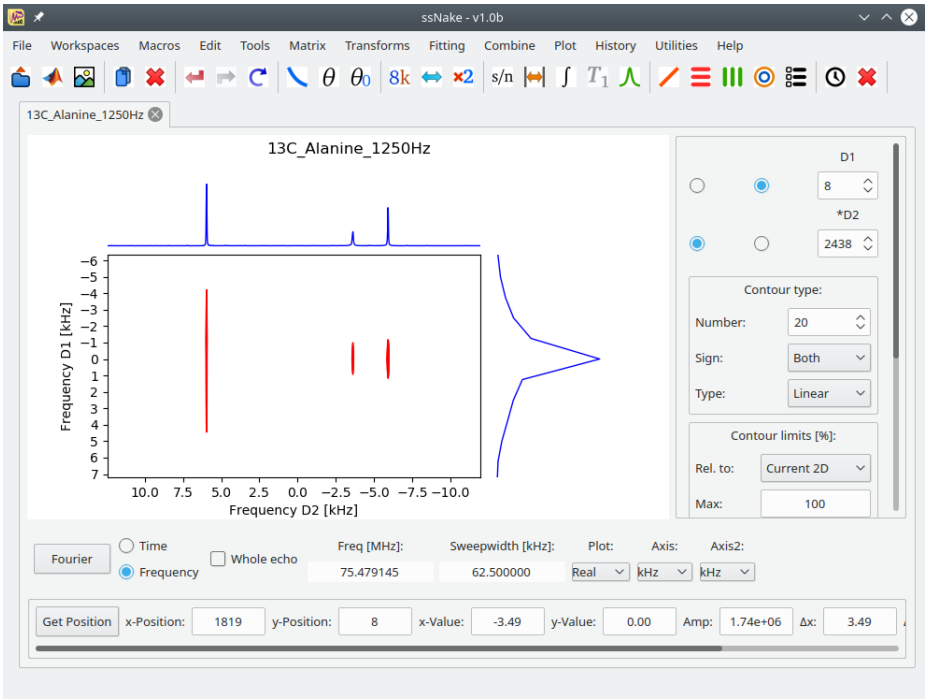
Which is a very nice isotropic spectrum. However, by only looking at this single trace, we are losing a lot of signal! It is therefor better to sum all the sidebands for each site. This is actually very easy for 2D-PASS data.

- Set the plot to contour via `Plot` ⟶ `Contour`

Now we again have the contour plot shown above. What we will now do is shear the data in such a way that all the sidebands of the carboxyl group end up along a vertical line along D1. As we have 1250 Hz as a spinning speed, the spectrum that contains the first order sidebands much be shifted by −1250 Hz for it to align with the centreband. The same holds fro the second order sideband (shift by −2500), etc. As we have corrected the spectral width of D1, we can do this via a shearing transform.

- Shear via `Matrix` ⟶ `Shear` with '1' as a constant, '2' for direction, and '1' for axis.

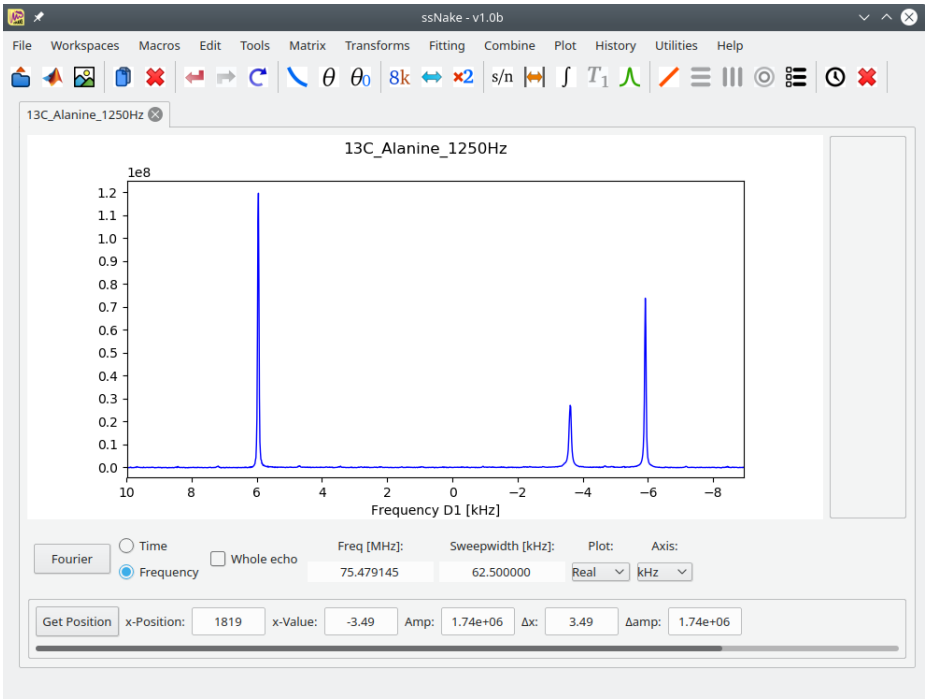
This should give:



which shows a proper alignment of the sidebands. As a final step, we will sum this data along D1:

- Set the plot to 1D via `Plot` ⟶ `1D Plot`
- Switch to D1 using the radiobutton in the side frame
- Sum via `Matrix` ⟶ `Region` ⟶ `Sum` and push 'Ok'

This shows:



Note that, when compared to the single trace spectrum above, the carbonyl has much more intensity in this case (relative to the other lines). Comparing the intensities of the lines for the single trace versus the sum tell us something about the amount of anisotropy that is present for each line.