2D-PASS processing in ssNake

15th January 2019

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 single spectrum. The centre spectrum of this series will be the isotropic spectrum.

This tutorial will describe how to process such 2D-PASS data in ssNake.

2 Data

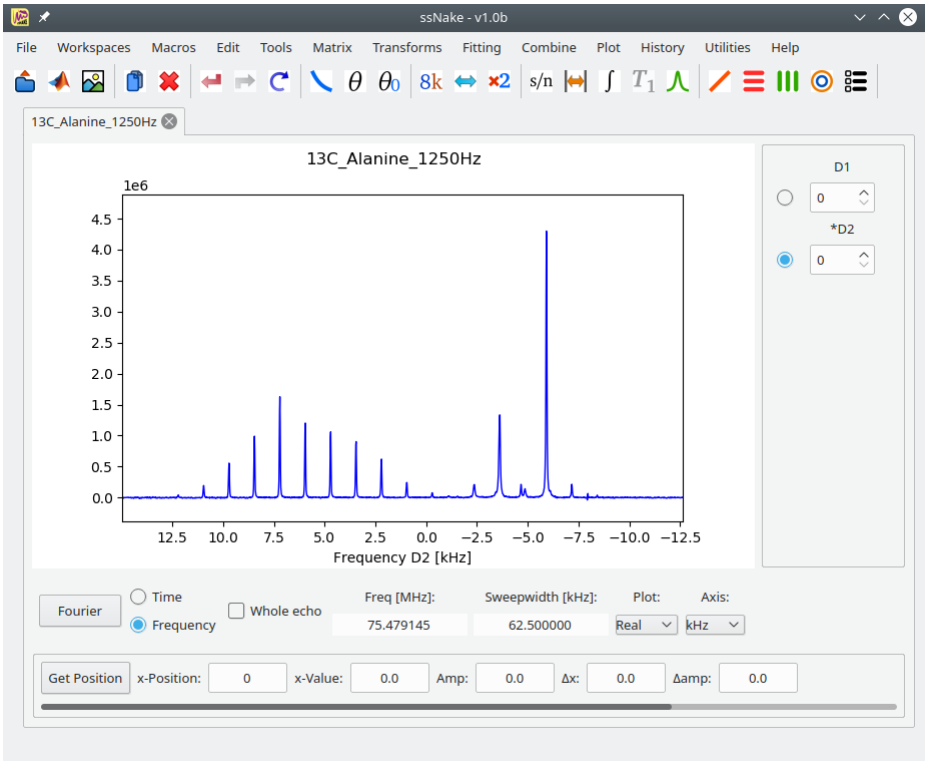
Delivered with this tutorial is a ^{13}C data set of alanine. `13C_Alanine_1250Hz.fid` is a 2D-PASS spectrum of alanine measured at 300 MHz and 1250 Hz MAS. The spectrum was obtained using a Varian spectrometer.

3 Processing the data

- 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`

This should show:

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

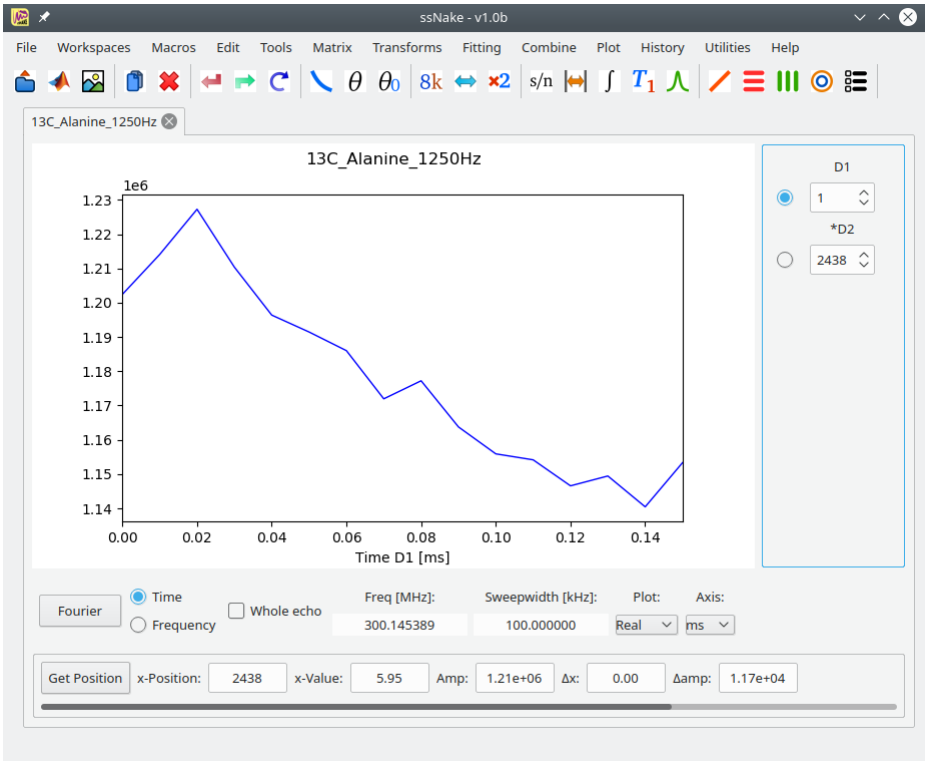


Scrolling though D1, we can clearly see that all the sidebands change their phase, while the centrebands of each site stay in-phase. This is the way this experiment works, and allows to distinguish sidebands from centrebands.

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

- Using the 'Get Position' button, the trace of the carboxyl centreband can be determined to be 2348
- 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:

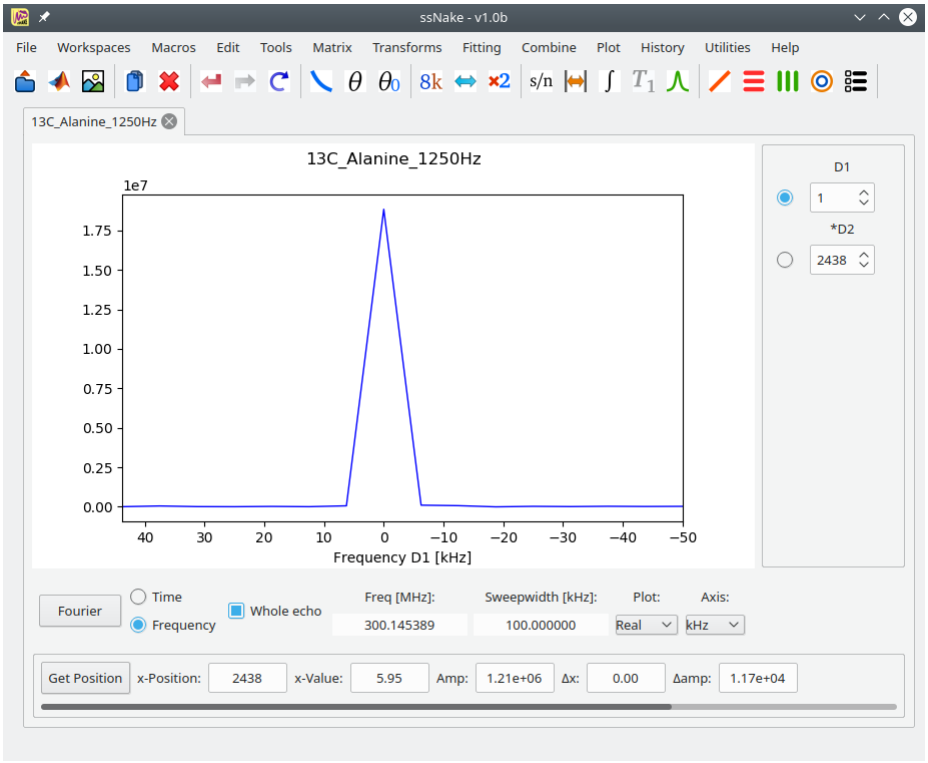


We now want to Fourier transform also along this dimension. However, we need an extra trick. In a regular Fourier transform, the first point of the FID is always multiplied by 0.5, to get rid of the baseline offset. This works well only if our signal decays to 0. In the current case, we do not really have an FID, and no decay to 0 is present. To correctly process this dimension, the 'Whole echo' mode should be enabled. To do this, tick the 'Whole echo' box in the bottom frame.

After this, we transform this dimension:

- 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 data point has intensity: the centre point. This is exactly what we expect here. Every data point represents a specific order of a sideband. The centrepoint is the isotropic (order is 0) value. As our current trace (position 2438 in D2) is along the isotropic peak, only the central point should have intensity.

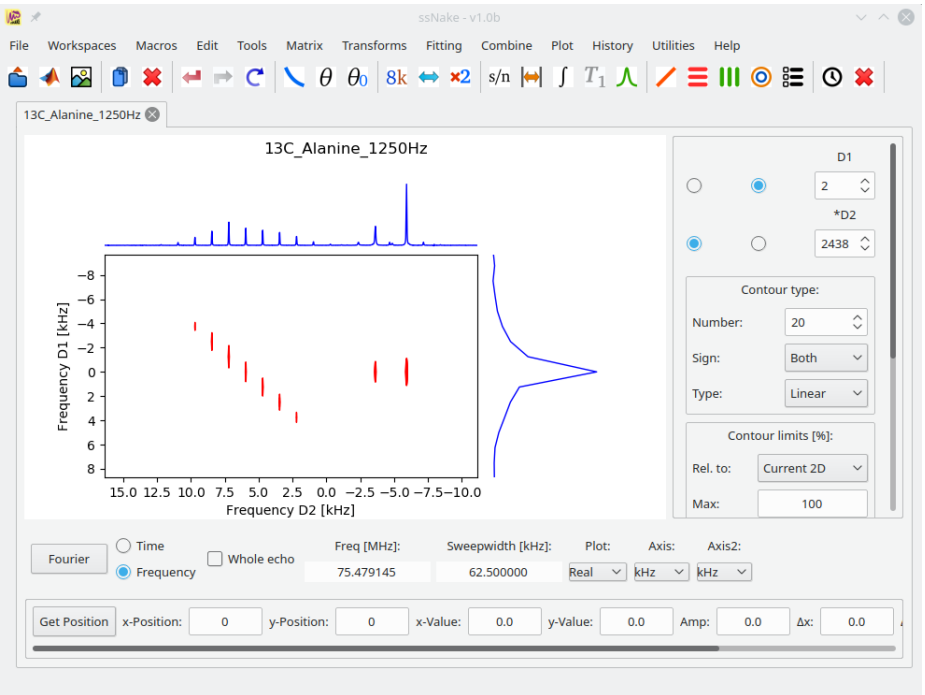
To aid in the view of the data, we must correct the spectral width. Each data point corresponds to a specific sideband order, so between neighboring data points there should be a frequency difference of 1250 Hz (i.e. the spinner frequency). There are 16 data points in dimension 1, so we must correct the spectral width accordingly:

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

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

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

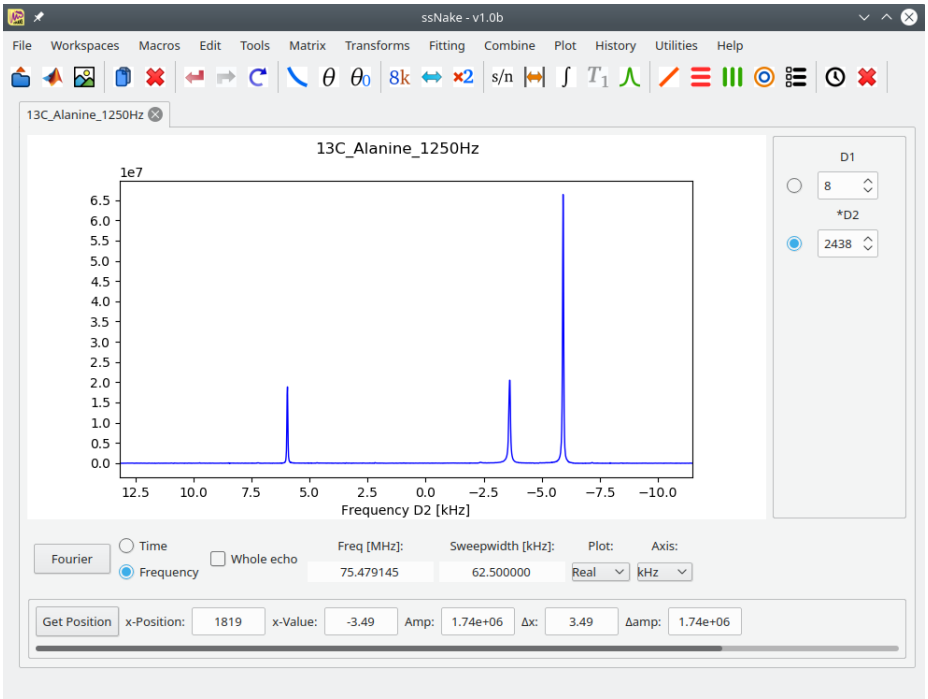
The spectrum should look similar (zoomed) to:



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. When viewing the centrebands (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):



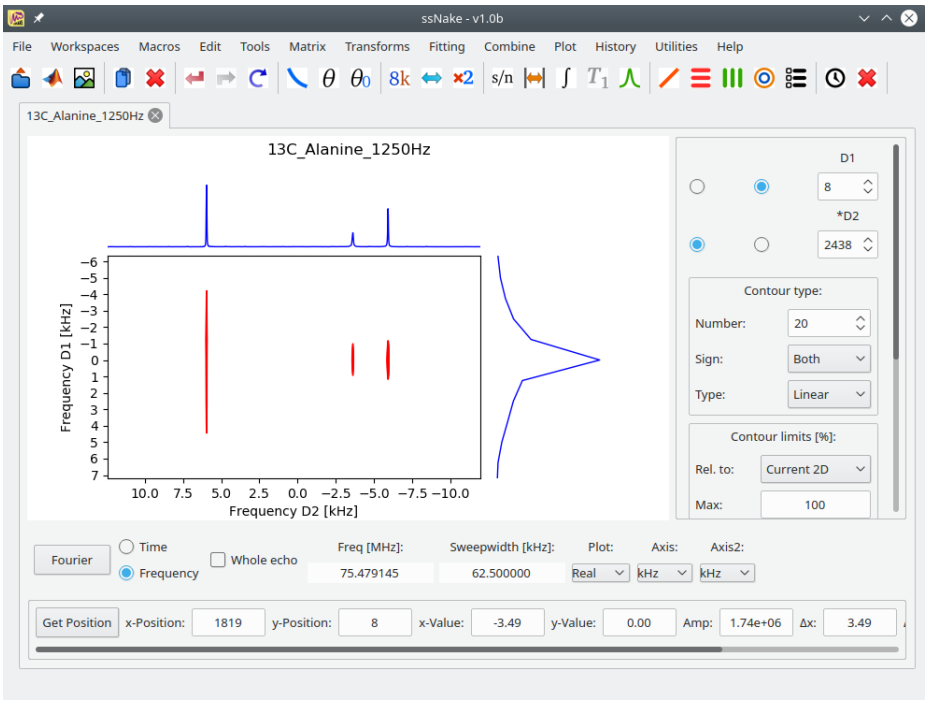
Which is a very nice isotropic spectrum. However, by only looking at this single trace, we are losing a lot of signal! It is therefor better to sum all the sidebands for each site. This is actually very easy for 2D-PASS data.

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

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 for 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.

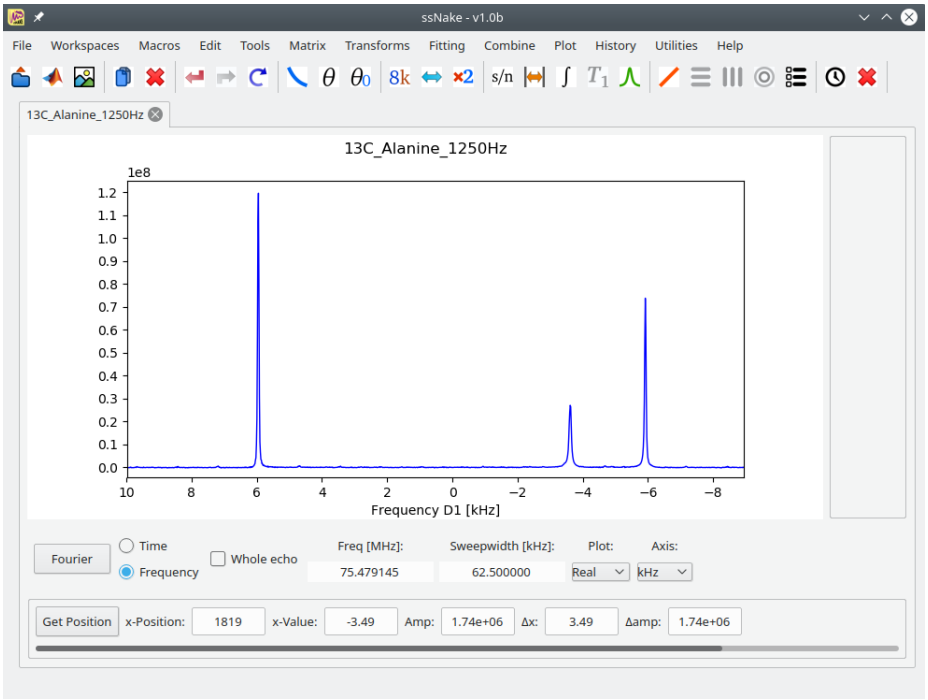
This should result in:



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'

The spectrum should now look like:



Note that, the carbonyl resonance is much more intense, compared to the centreband spectrum above. Comparing the intensity of the lines between the centreband spectrum and the summed spectrum tells us something about the amount of anisotropy that is present.