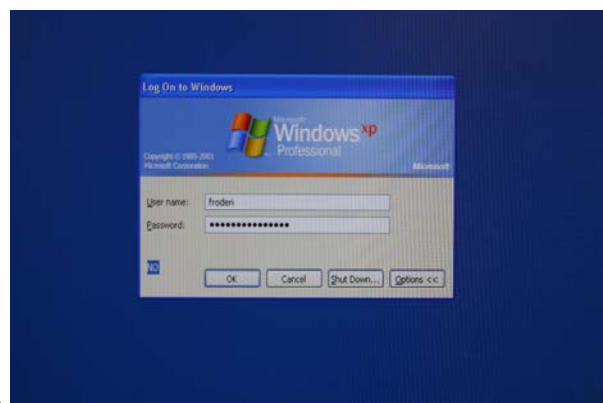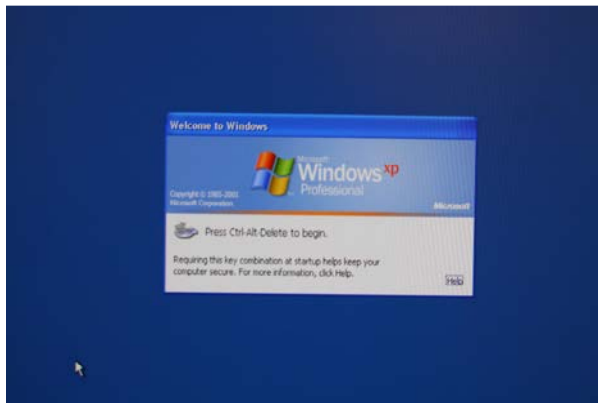KJM 5280

III

Processing of nmr spectra with emphasis on TOCSY, NOESY and HSQC

Version 1.1

TopSpin 1.3 patch level 10

Windows XP

© F. Rise May 2018

Find the Windows XP PC that is booked for you kjemi-nmr11 (DPX200 instrument), kjemi-nmr09 (DPX300 instrument), kjemi-nmr07 (DRX500 instrument), kjemi-nmr12 (in room Ø 319), kjemi-nmr10 (Ø 319), kjemi-nmr08 (Ø 332), kjemi-nmr02 (Ø 332) or kjemi-nmr13 (Ø 319). This manual is assuming that you already have set your password on the computer. See the KJM 5280 ^{1}H nmr introductory manual if you do not remember how to set your password. Sit down and log in.
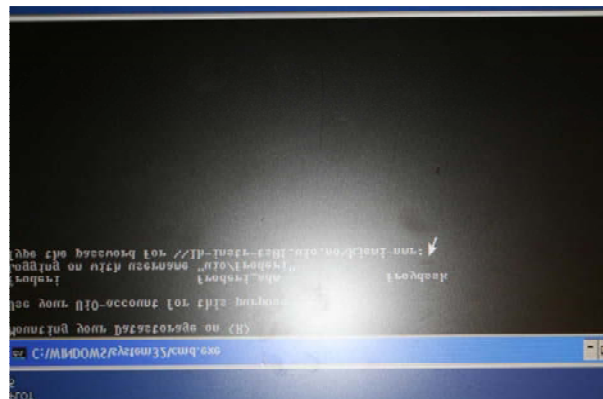

Locate the data storage Icon (S not R as in the pictures) on the desktop and double click on it. Type your UiO password in the black window that pops up and press enter/return. There is no response on the screen when typing. If you have done it correctly the black window goes away and you have connected the central storage location for nmr data at UiO to the computer you are working at.
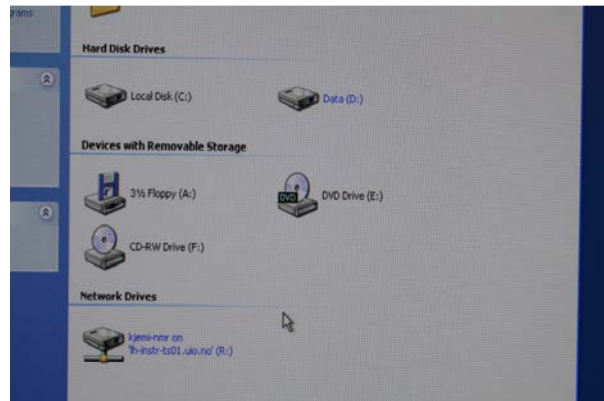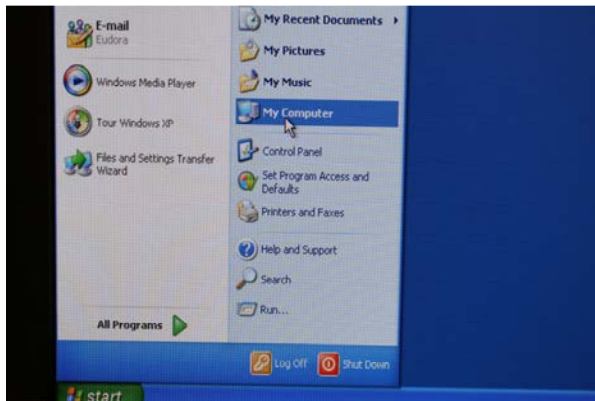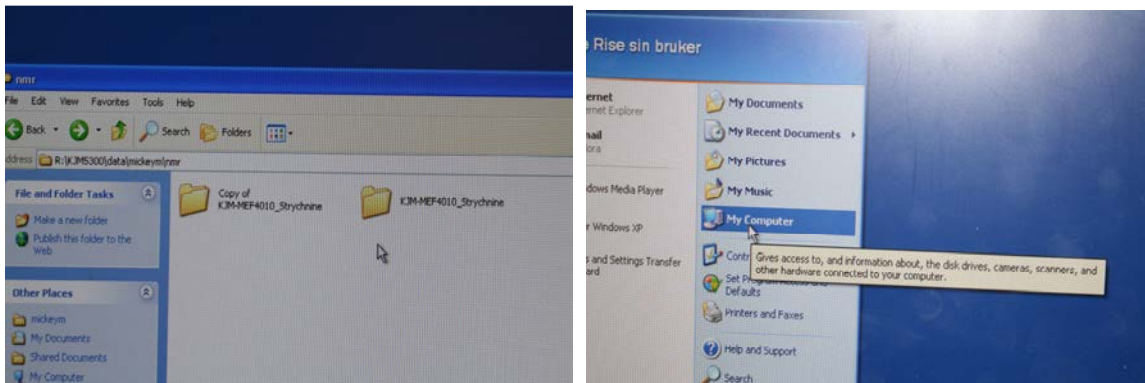
Click on Start and Locate My computer. Open it and see the C disk, the D disk and the S disk you just connected to.
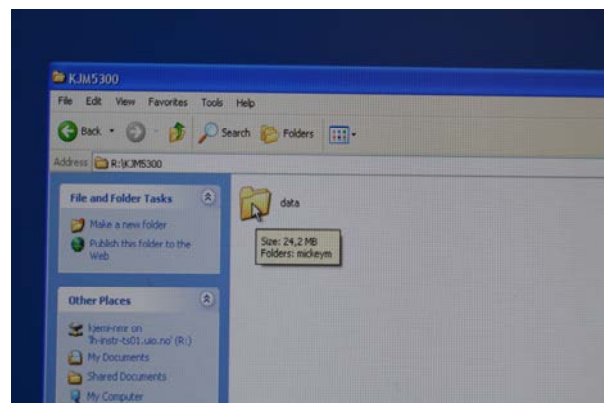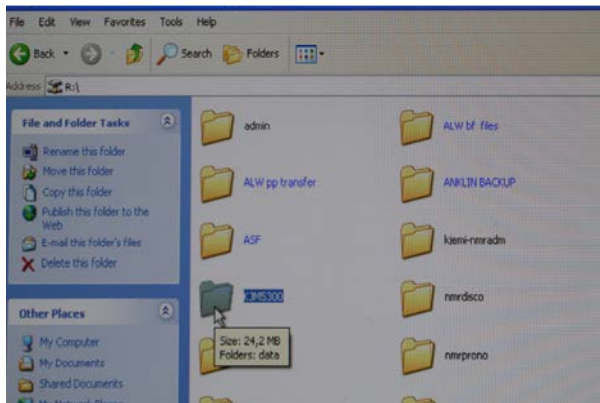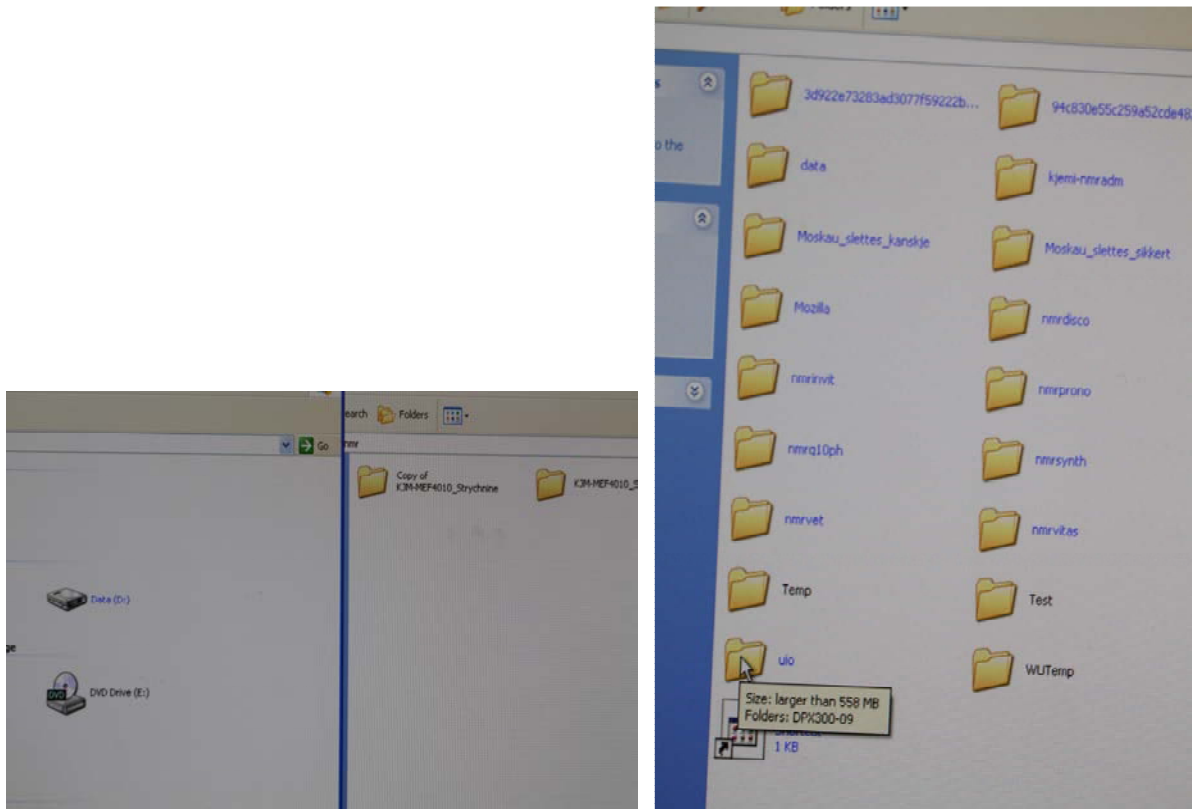

The task now is to locate the data you are going to work with. Click on the S disk then on the KJM 5300/5280 folder followed by the data folder.
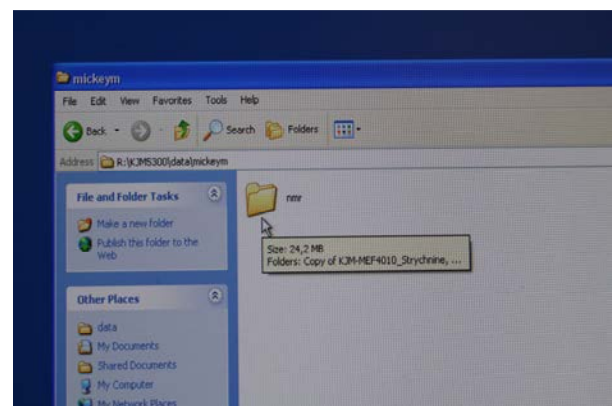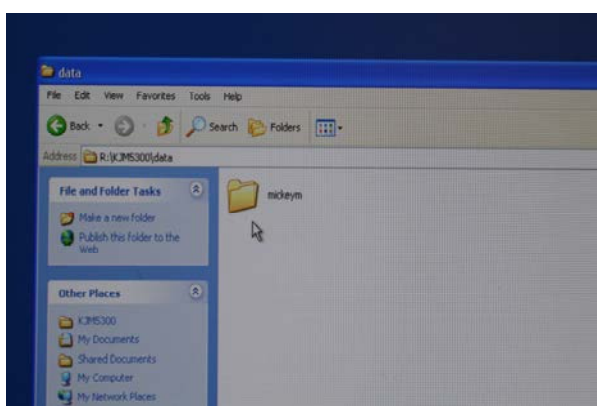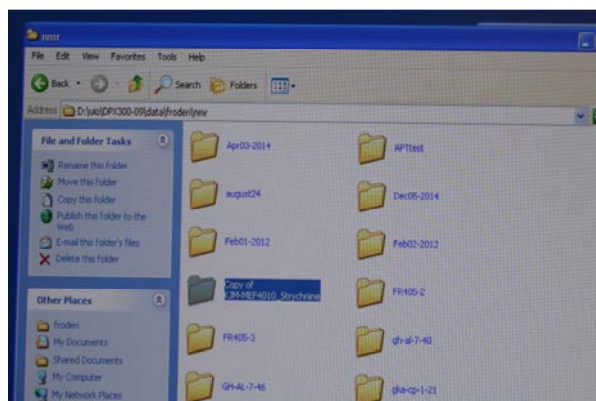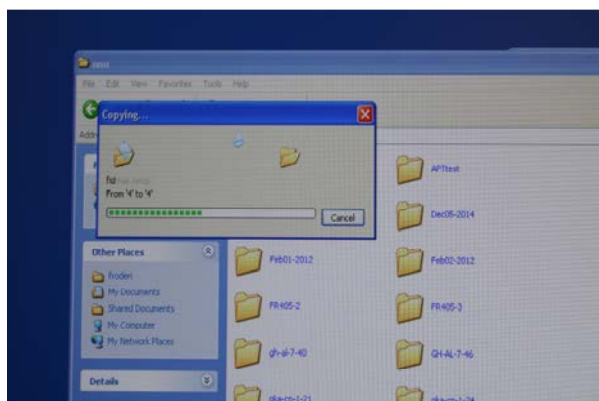

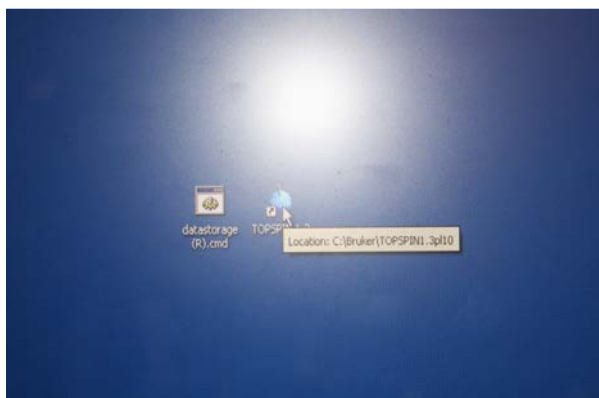Inside data you discover Mickey Mouse which contain nmr.

Mickey holds two folders. Your task is now to copy one of these to your nmr folder in the D drive on the PC.
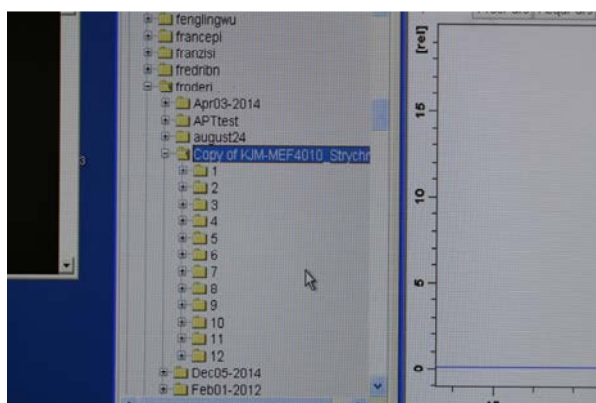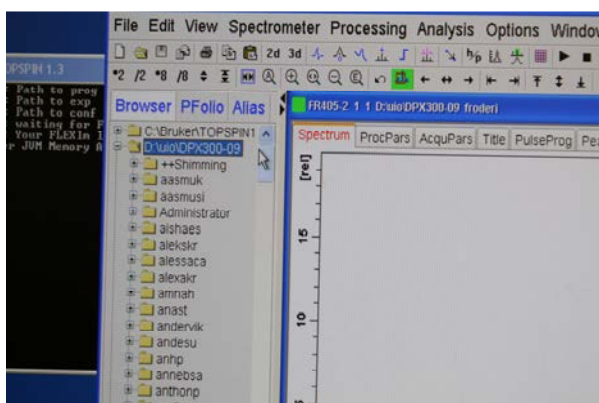

Double click on the D drive and locate the uio folder and click on it.

The DPX300-09 appears. Open it and open the next level as well (Data).

Inside Data all users who have used the TopSpin program appears. If you have no nmr experiments on this PC you are in serious trouble and you have to create yourself, but not at the Windows level but rather by using TopSpin. (see another page ??) to create yourself and your file structure. See pages 3 through 6 in the KJM 5280 ^{1}H nmr introductory manual. Continue as described here if you your username appears. Click on your folder, nmr appears.



Click and drag one of the two Mickey Mouse folders to your nmr folder.

Wait until completion.

Start TopSpin.

Open the D:\uio\DPX300-09 (or similar) folder in the left pane of the TopSpin program interface. Open yourself and confirm that Mickey Mouse is transferred correctly. You will have 12 different nmr-experiments to play with. If the D:\uio\DPX300-09 is not visible you have to add it as described in the "KJM 5280 ^{1}H nmr introductory manual" pages 4 and 5. Click on experiment 1.
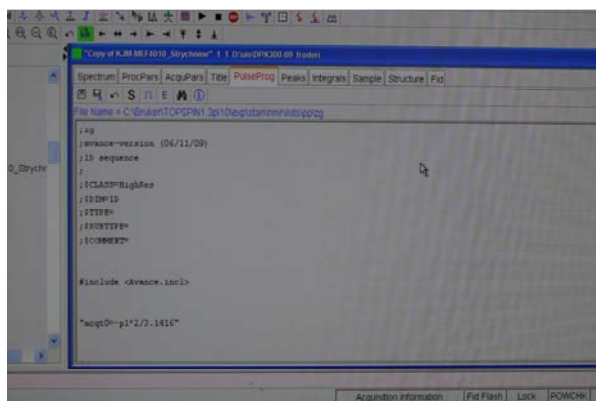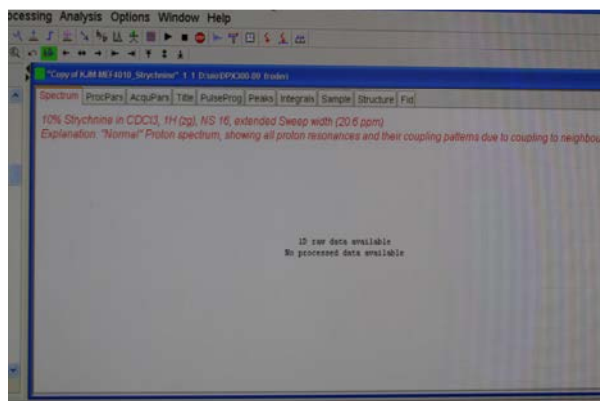
You shall now process this 1H nmr experiment as you learned by using the: "KJM 5280 ^{1}H nmr introductory manual. How to do this is not shown here ☺ Read in the other manual if you do not remember it yet. If you the reader is participating in KJM 5280 your goal is to learn this to a high level so you seldom have to look in the manuals later. Click on the PulsProg button.
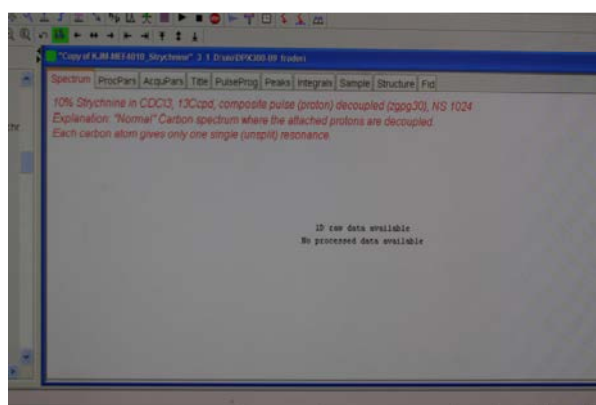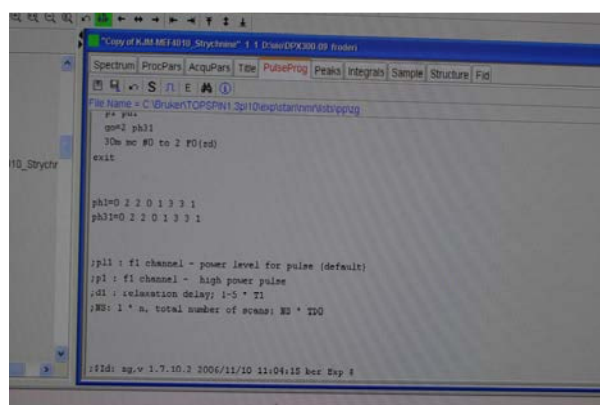

The simple 1H nmr pulse program pops up. This is written in a proprietary software only used by Bruker Biospin. Get used to look at the bottom part of the pulse program. Important information about possible restrictions and limitations concerning numbers to be put into the Parameter Set/Experiment is shown here. Although not much here for this simple experiment.
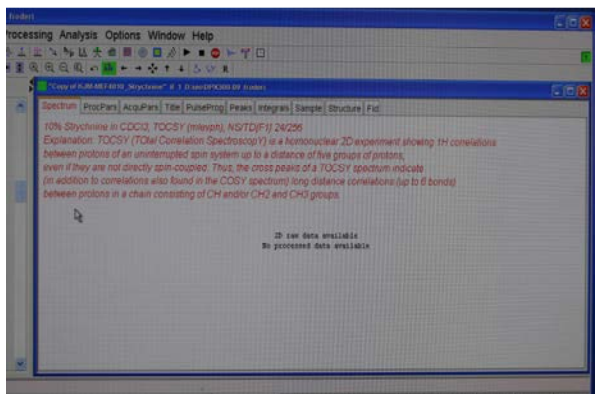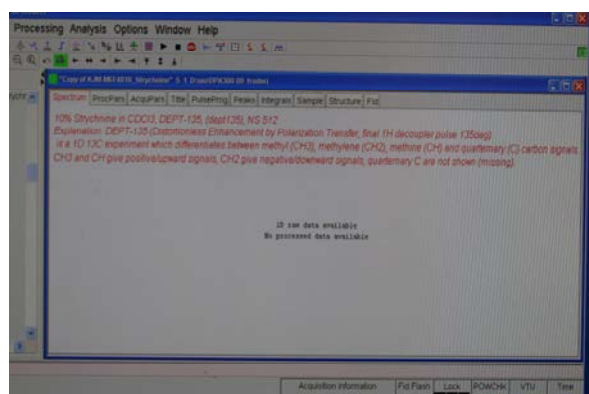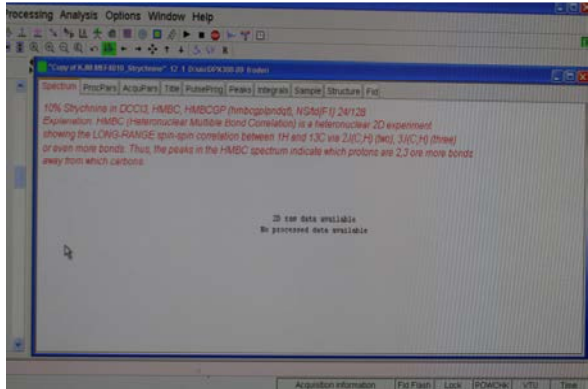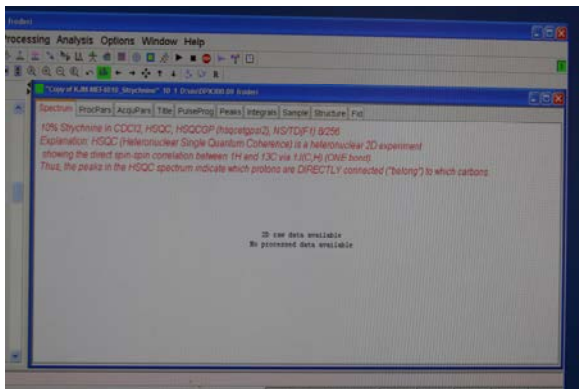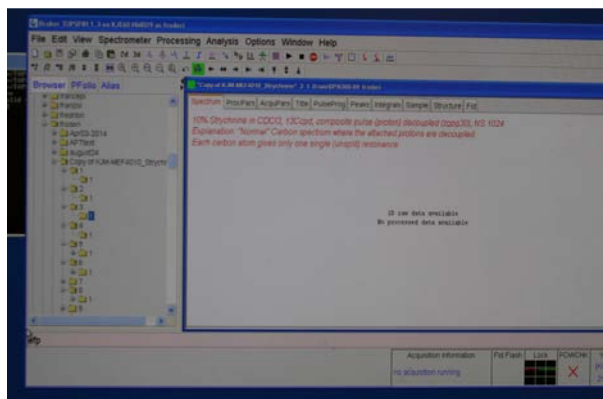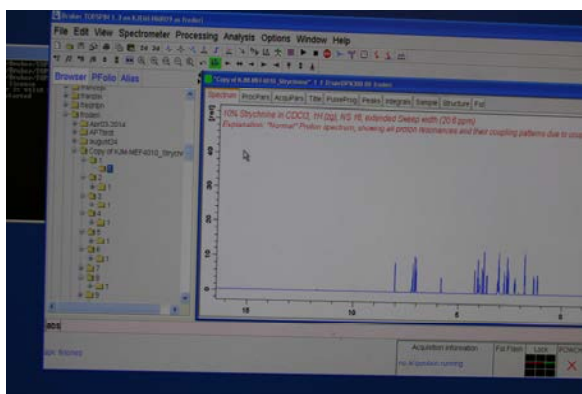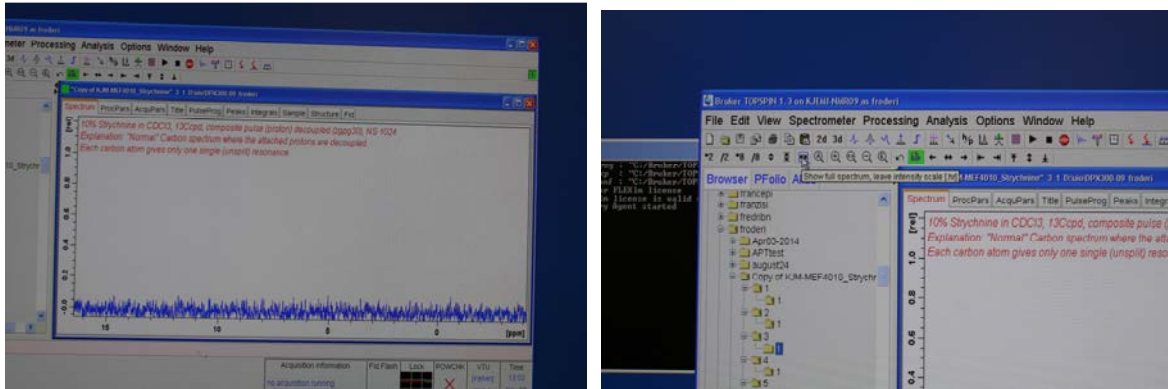
Open them all and read the red information text at the top. Process all one-dimensional experiments as you learned in: "KJM 5280 ^{1}H nmr introductory manual."
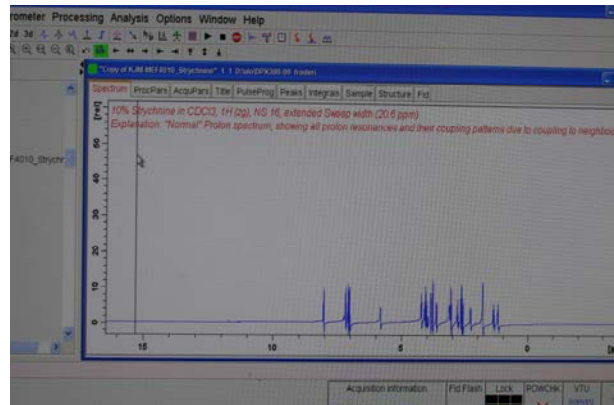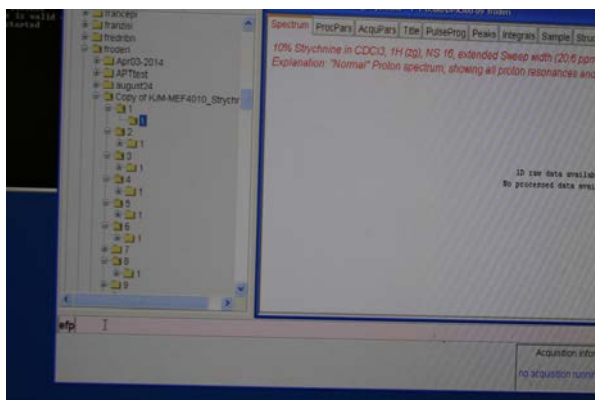

efp, apk, manual phasing, referencing the ppm scale, integrating and all that stuff and much more ☺ is your responsibility now.


When done with the ^{1}H experiment open a ^{13}C one dimensional experiment.

When done efp-ing the FID you are really surprised. There are no resonances in the spectrum??? The program is stupid – it opens the spectrum with the ppm scale of the previous spectrum that was open – that is the ^1H spectrum and the ppm scale of proton spectra are much narrower than ^{13}C. Locate the button that brings up the whole spectrum.
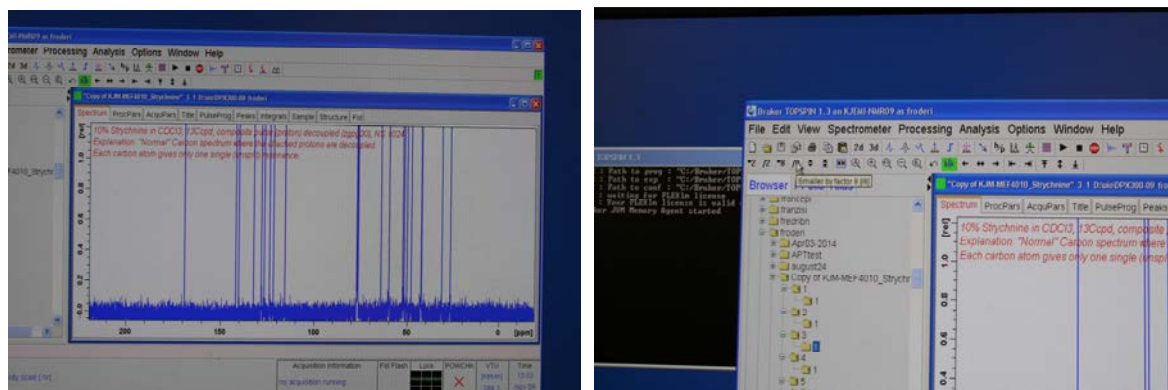

The whole spectrum shows up, but in this case with a too high intensity. Locate the /2 and /8 buttons and button for continuous change of intensity. Familiarize yourself with the functionalities.
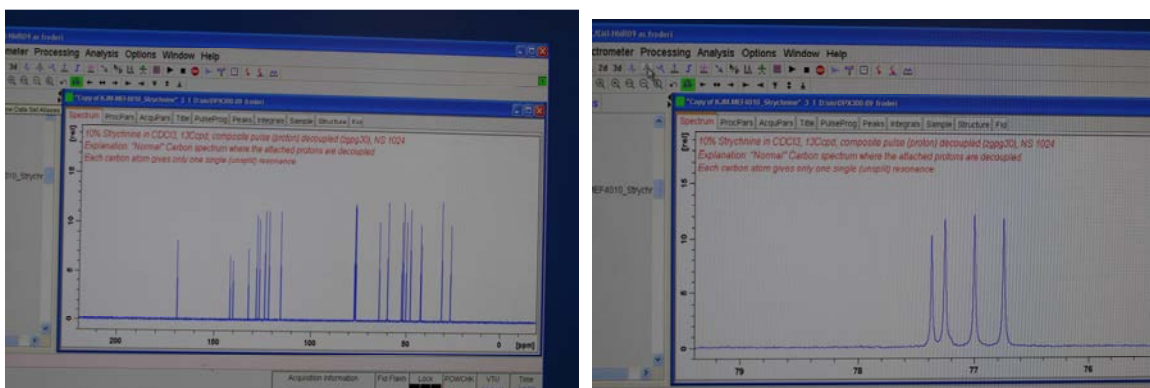

Locate the peaks to be used for referencing. Here the triplet peak for $CDCl_3$ carbon atom.
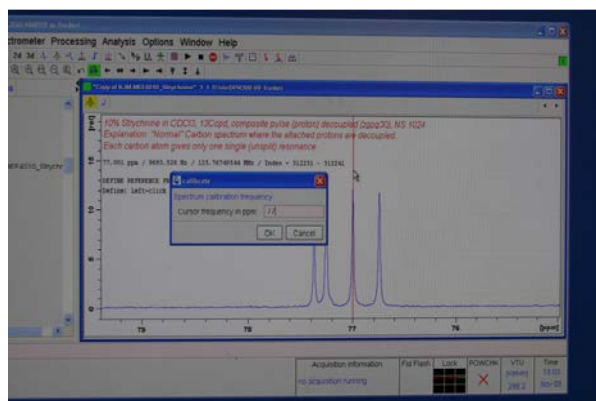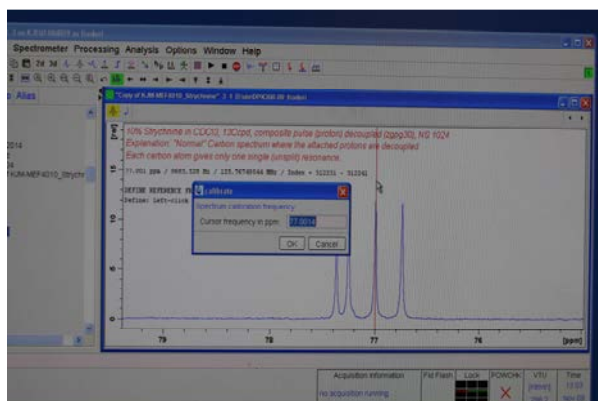
Do the calibration of the central peak. Make peak numbering visible on the spectrum and print the spectrum. See other manual if not remembering how to do this yet.
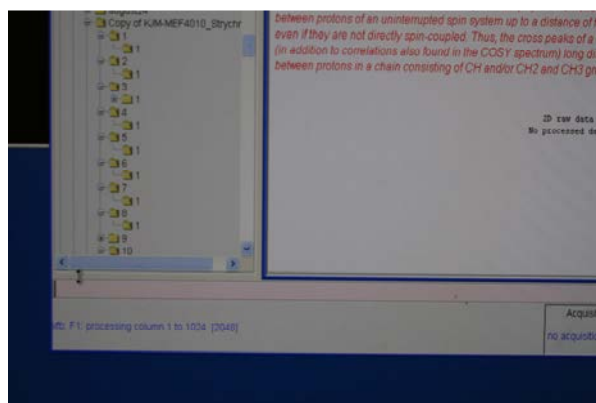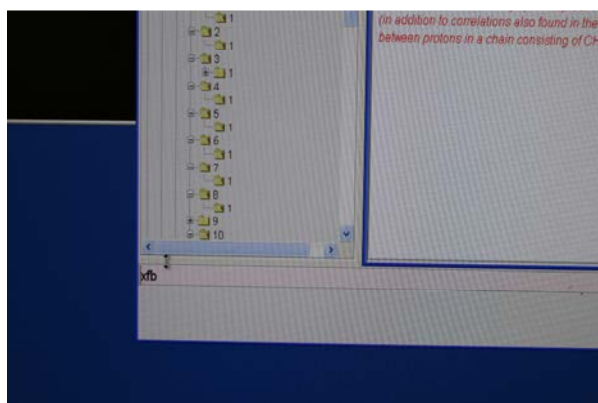

TOtal Correlation SpectroscopY is a homonuclear 2D technique which is used to identify ^1H spin systems. In principle, all members of a J coupled spin system couple with all the other members regardless of how many bonds there are between the protons, as long as there are no breaks in the chain of CH, CH_2 and CH_3 groups. In practice, you will only see coupling over a distance of five groups of protons. The longer the so-called spin-lock period (d9) the more couplings are seen. Locate the TOCSY spectrum – open it and read the text to get more information about what TOCSY stands for.
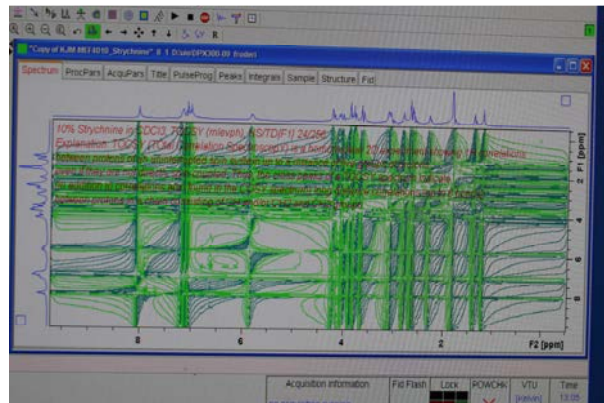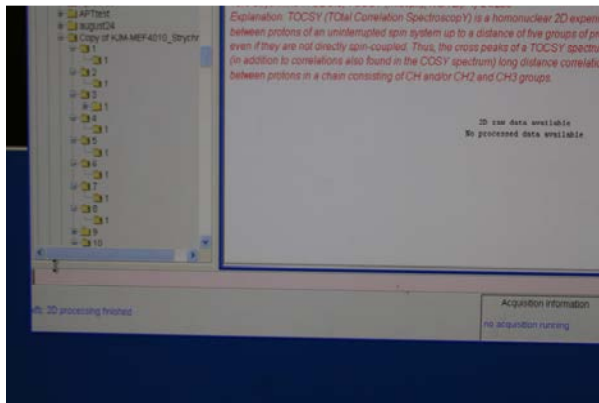
Then you Fourier transform the dataset with the command xfb and not with the command efp. A really ugly spectrum shows up. Lots and lots of challenges here ☺
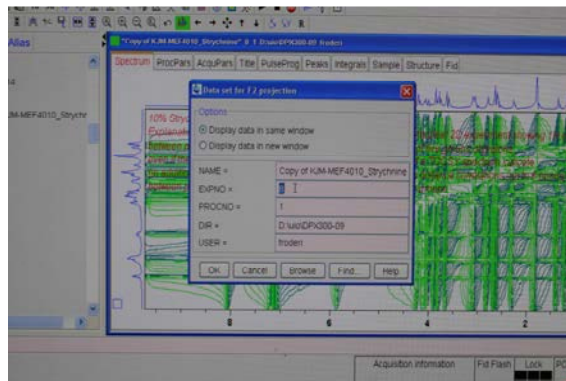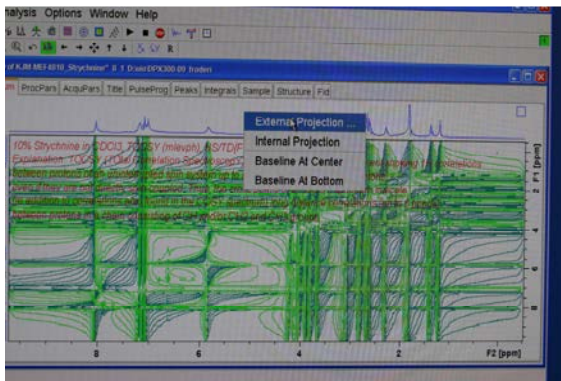

Before phasing the 2D spectrum the "ugly" internal 1D projections is in need of being exchanged with External projections. Right click on a projection and left click on External projection. Ensure that all information for the F2 (horizontal) project is correct, in this example the EXPNO 8 is changed to EXPNO 1 where a 1D ^{1}H spectrum is residing - hopefully you have processed it already.
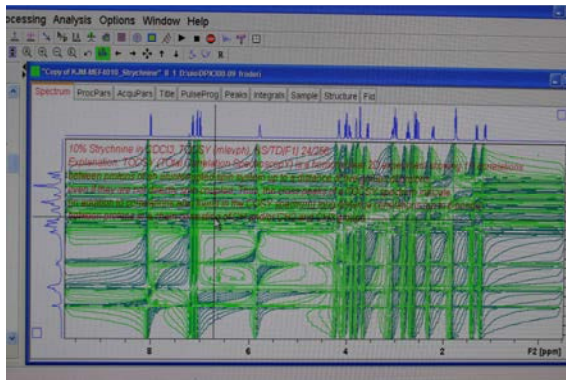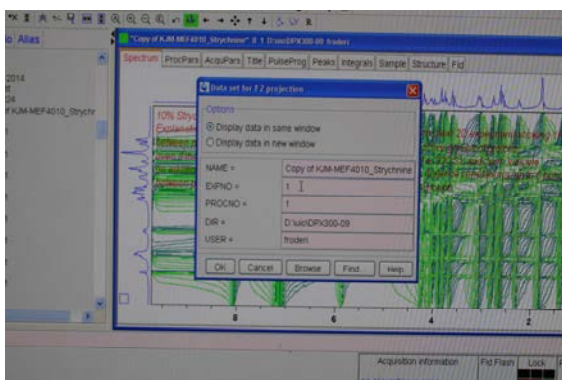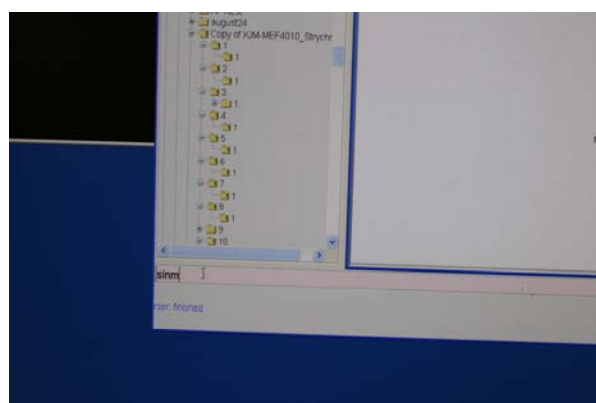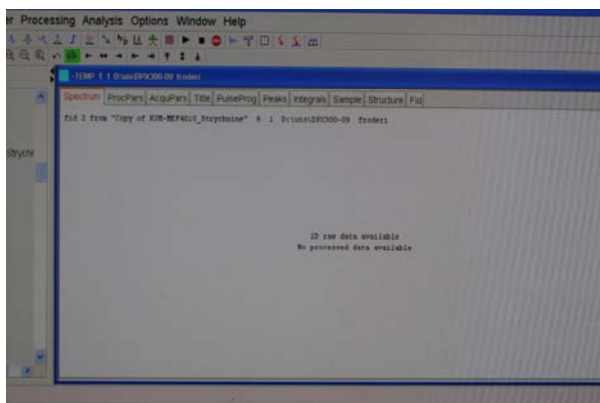

Click on OK and observe the much nicer 1D ^{1}H spectrum on the horizontal side of the 2D.
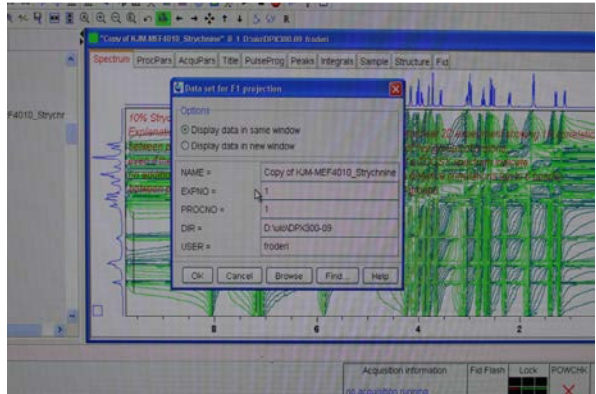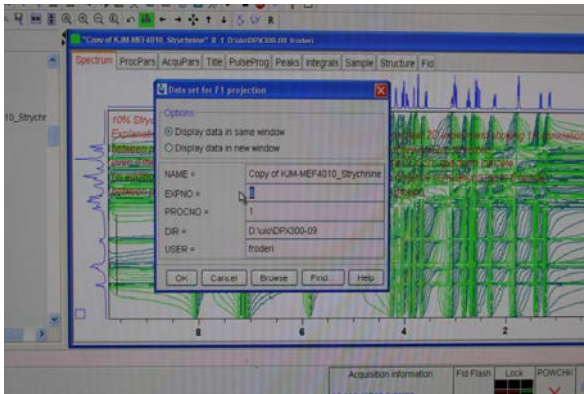
Do the same for the vertical (F1) projection.
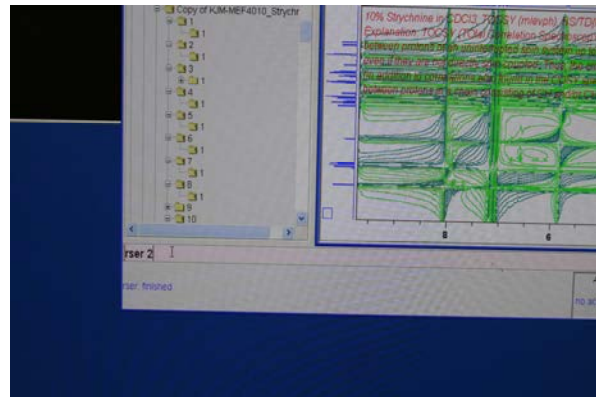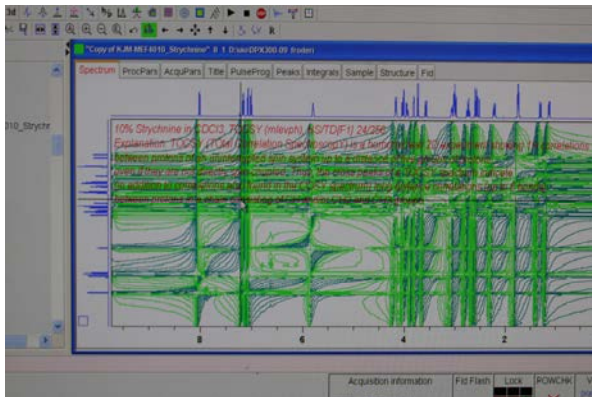
Now the task of fixing up the 2D spectrum starts. A part of the 2D dataset, the so called serial file 2 is used. Type the command rser 2 (read serial row 2) (in some versions of TopSpin the command is rsr 2) in the command line.


Observe that this extracted part of the 2D dataset ends up in a temporary file. ~TEMP 1 1. In TOCSY and ROESY spectra the serial file/row 2 is used. When this procedure is used for other 2D experiments rser 1 or rsr 1 is used. Type the command sinm. Sinm implements a sinusoidal multiplication of the real FID – giving the intermediate section of the FID higher value relative to

the start and end. You are now maybe for the first time encountering the use of so called window functions to change the appearance of the FID before Fourier transforming it.
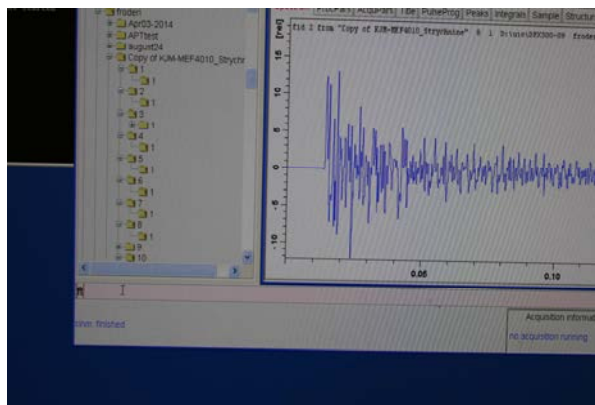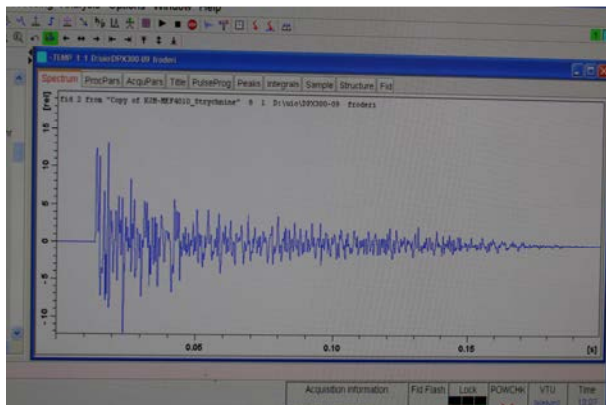

The FID appears and is processed with the command ft. Usually we process 1D FIDs with efp and not with ft. The e in efp means exponential (function) treatment of the raw data - before Fourier processing (fp). Another example of application of a window function.
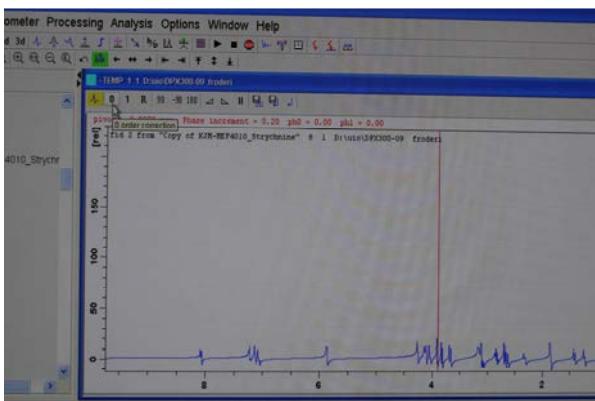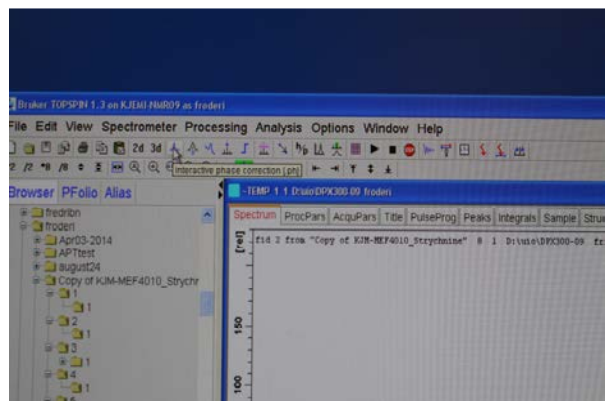

The spectrum is ugly with peaks up and down. Locate the Interactive phase correction button and use it, observe the chosen location of the red line. Phase the spectrum around the red line with the zero-order correction button in combination with the mouse.
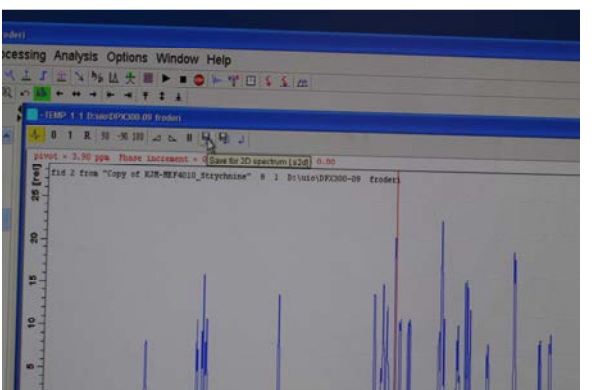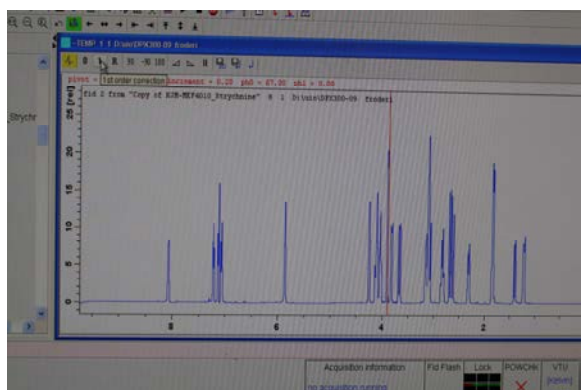
Optionally you continue with the first order correction button and the mouse. In the current case this was not needed. Locate the 2D saving button, use it and use it twice.
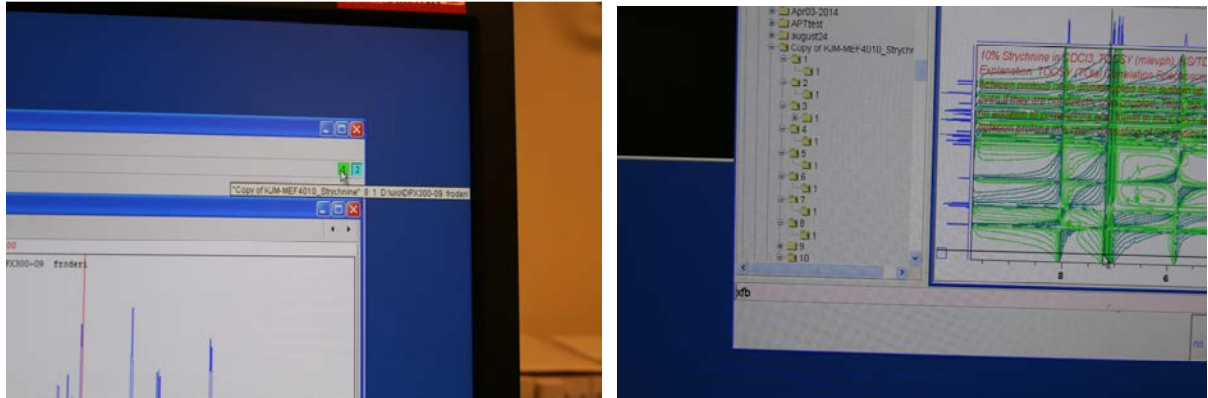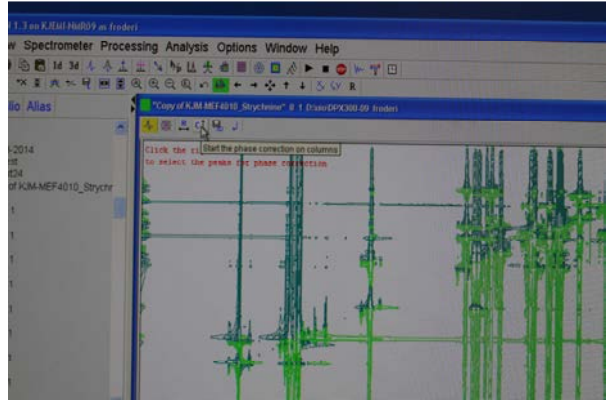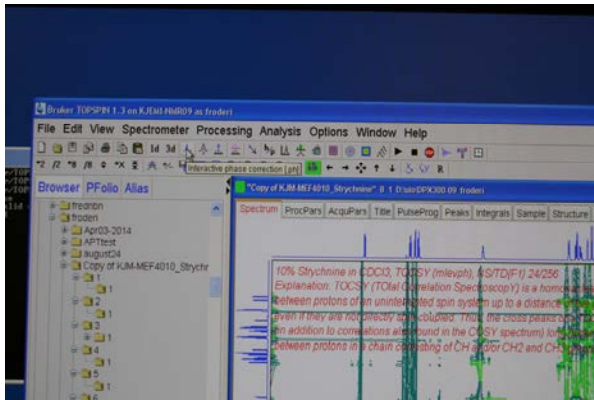

Go back to where the 2D TOCSY spectrum is located. Type the 2D Fourier transformation command xfb. If you did not click twice on the save 2D button above the result will most likely be much uglier.
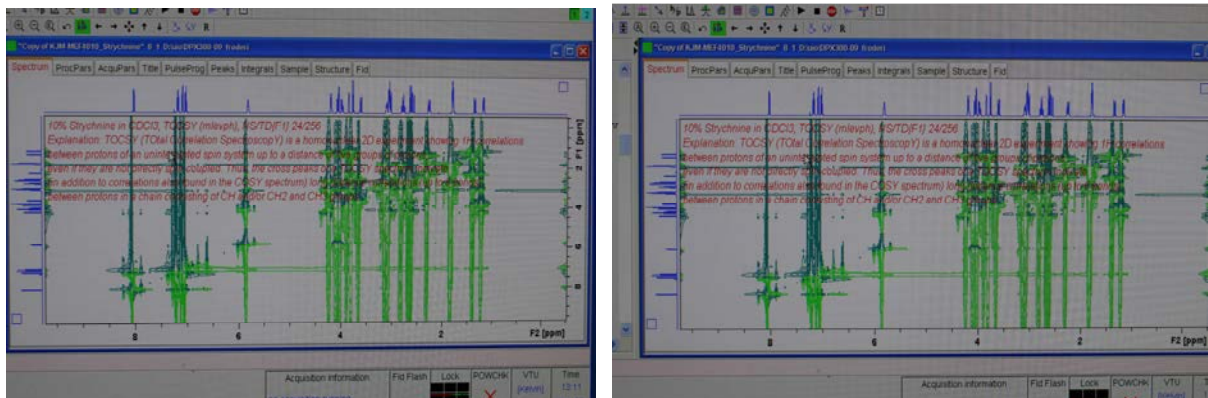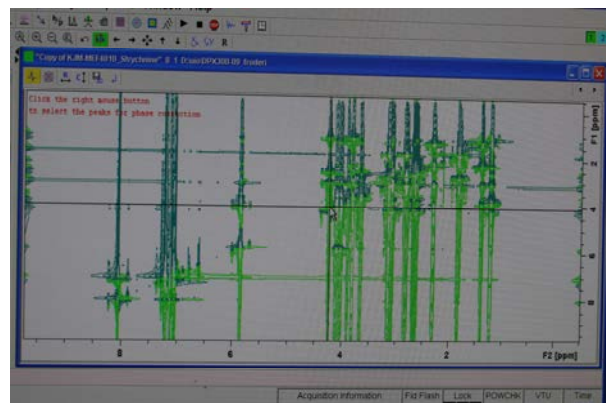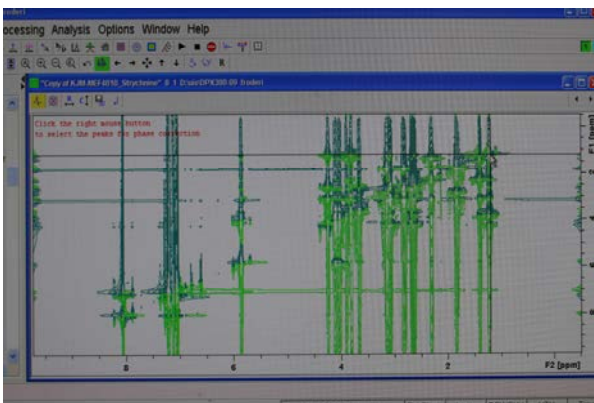

The spectrum is much nicer horizontally, e. g. in the F2 dimension.
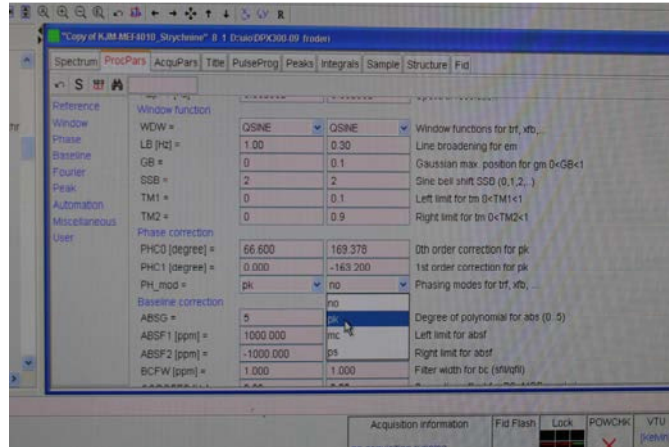
Either type edp or click on ProcPars and change PH_mod to pk.

Time to phase the indirect F1 dimension. Locate the Interactive phase correction button and use it. Find the F1 phasing button = start the phase correction on the columns and click on it.
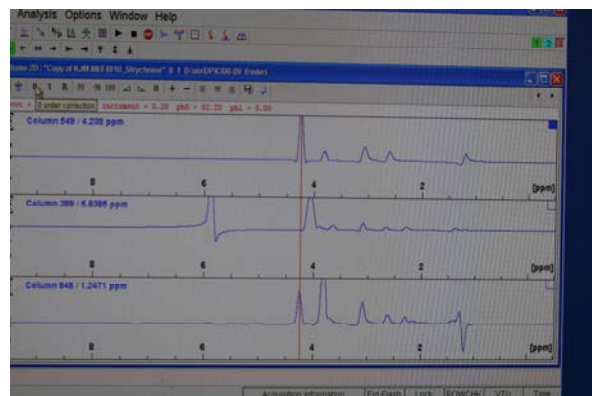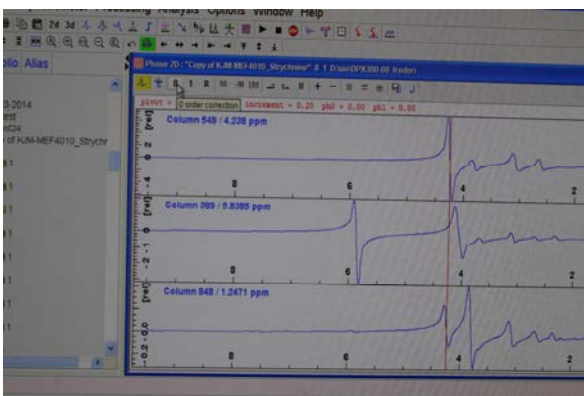

Find points on the diagonal to use, 3-4 of them. It has to be signals at your chosen points. On the right/up one or two in the middle and one to the left/lower part of the spectrum.
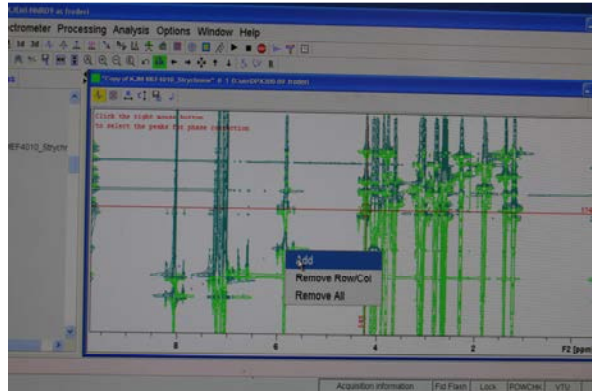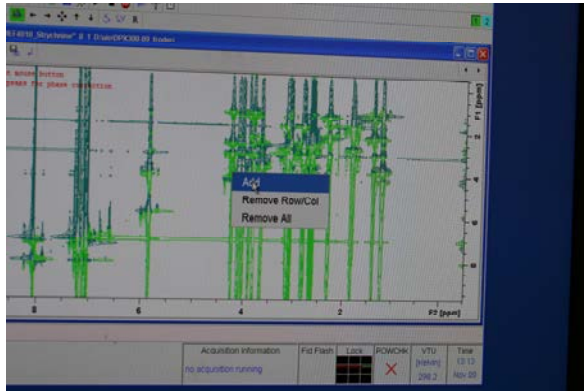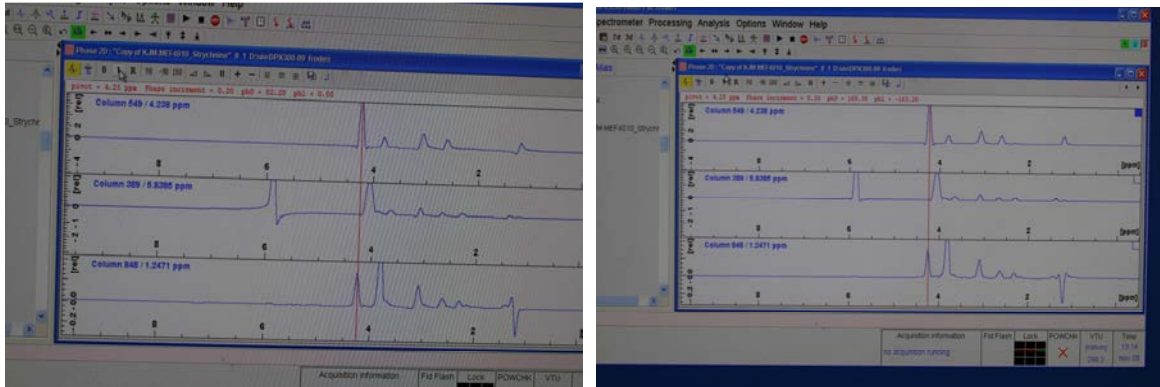
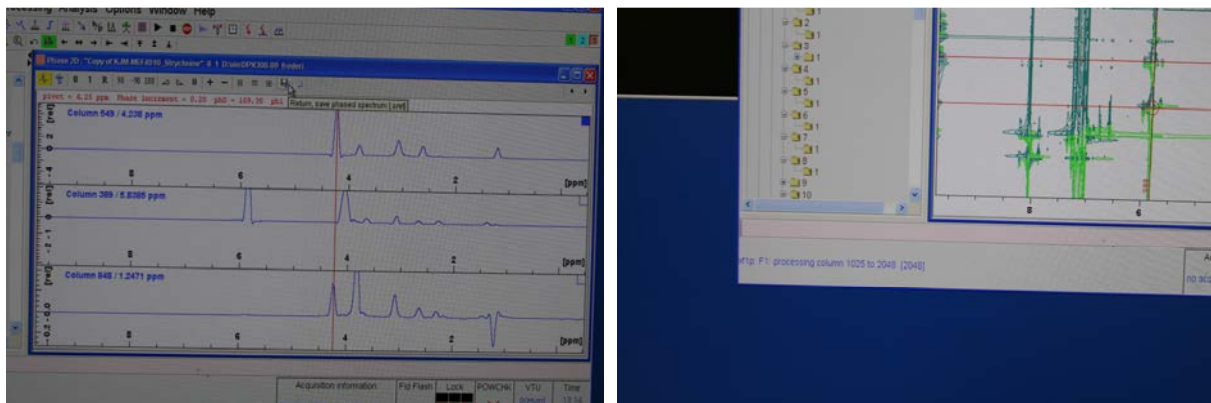Successively use left and right mouse buttons for the addition purpose.
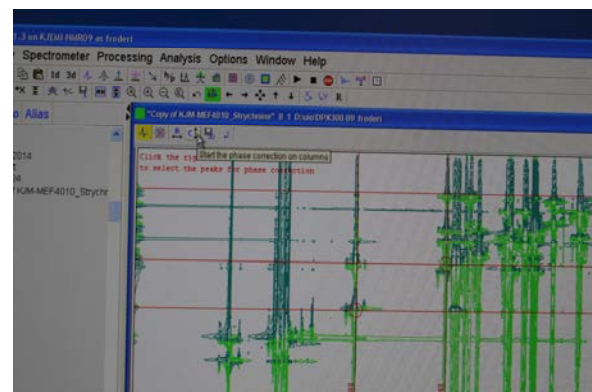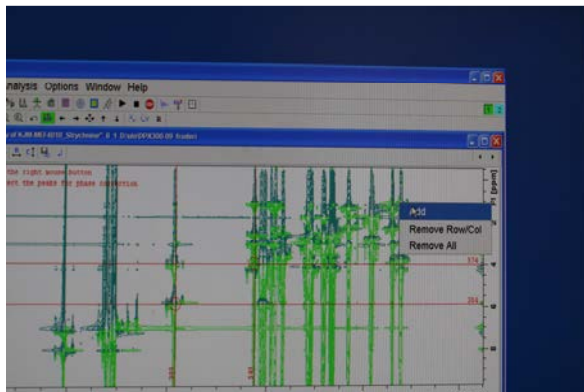
When the selection process is over please locate the Start the phase correction on column button and click on it.
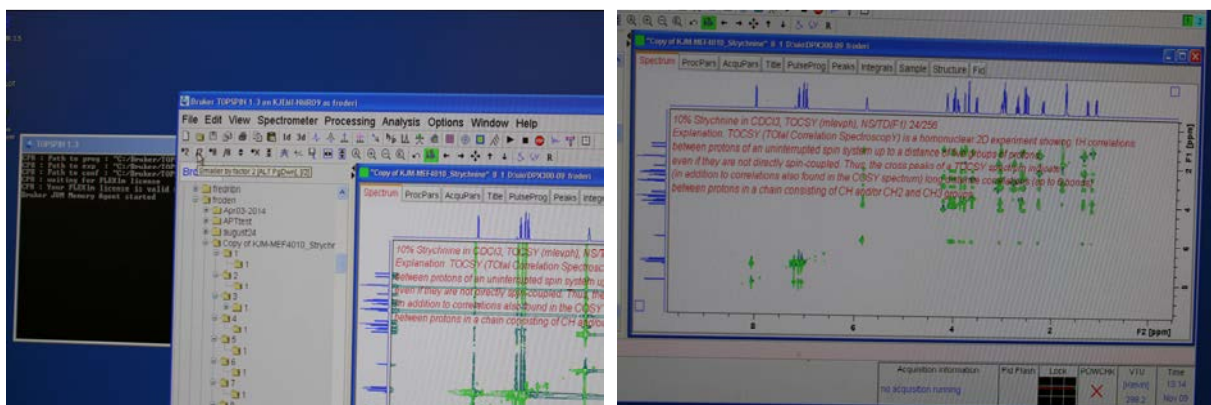

Use the zero-order phase correction button together with the mouse to fix the phasing around the red cursor.
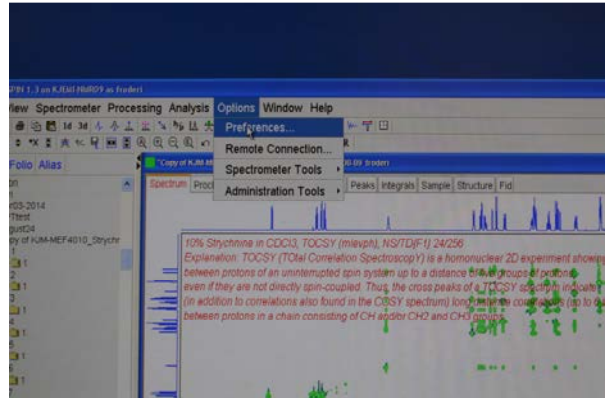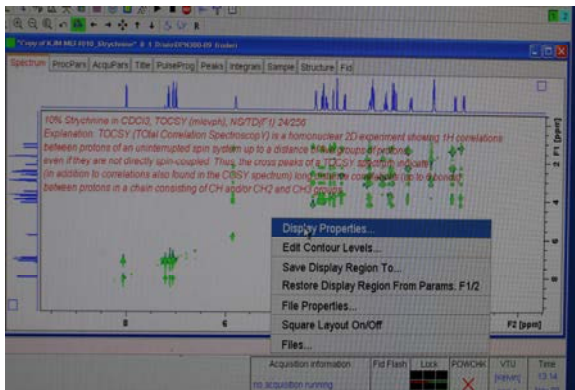
Complete the phasing process with 1ˢᵗ order corrections.

Save the result. Click on the button twice. The computer Fourier transform the data along the F1 axis.


Reduce the intensity if needed. The 2D spectrum looks much nicer. But the ugly green and blue colors chosen as default is maybe in need of being changed to something nicer?
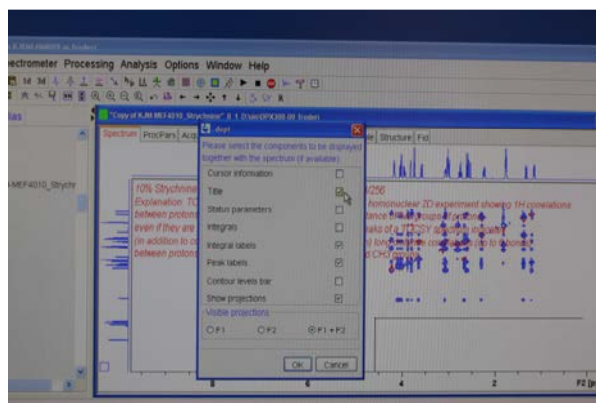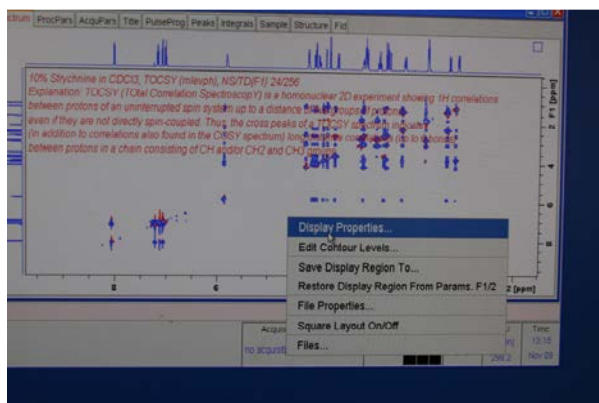
Right click on the spectrum and select Display properties and realize this was the wrong choice. Find Options and Preferences.
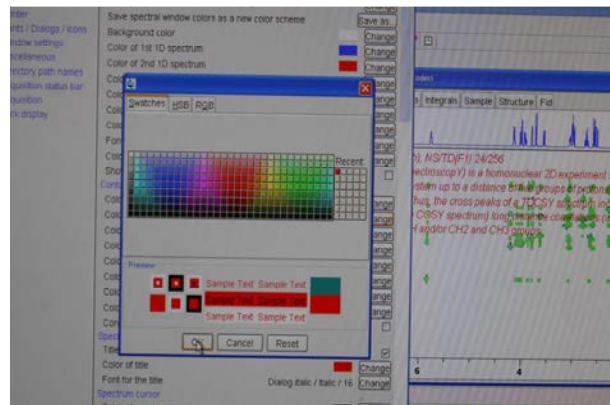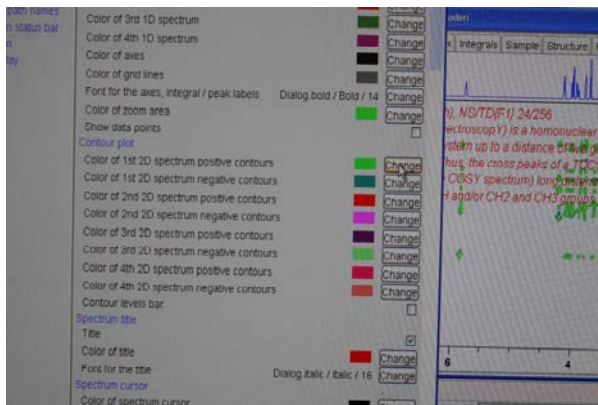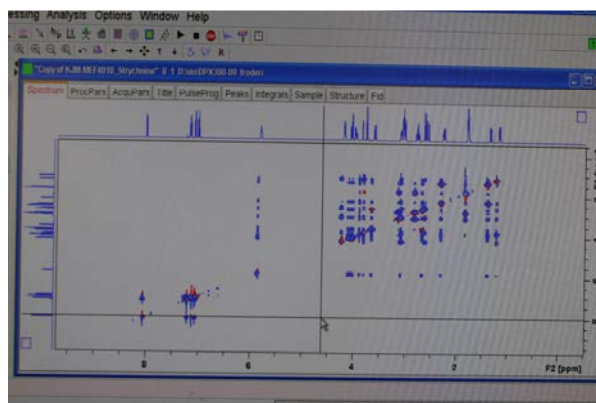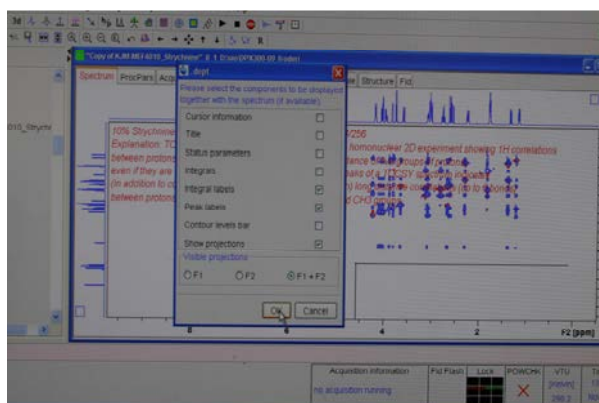

Change the 2D colors as shown above. Select for instance Bright red and Bright blue for positive and negative contours.
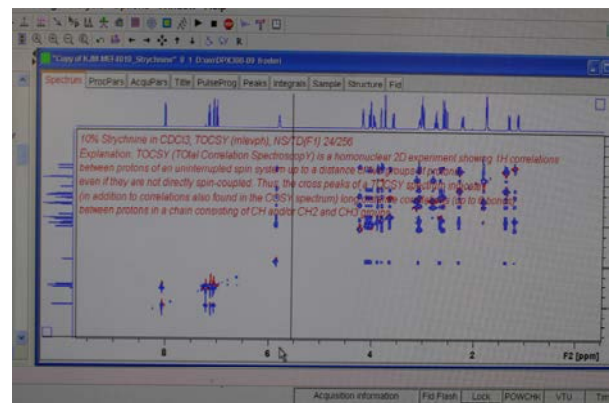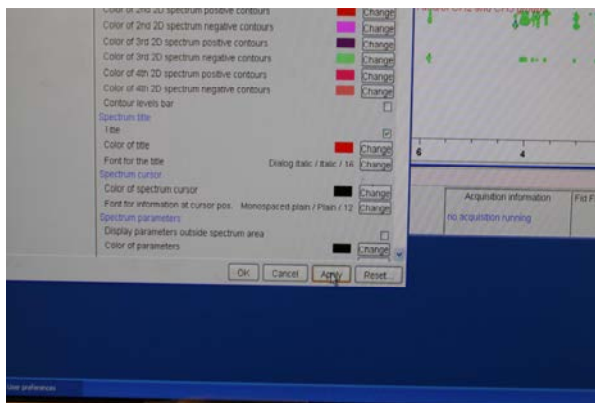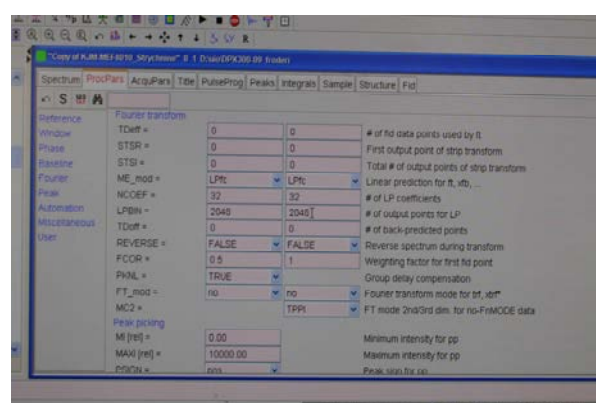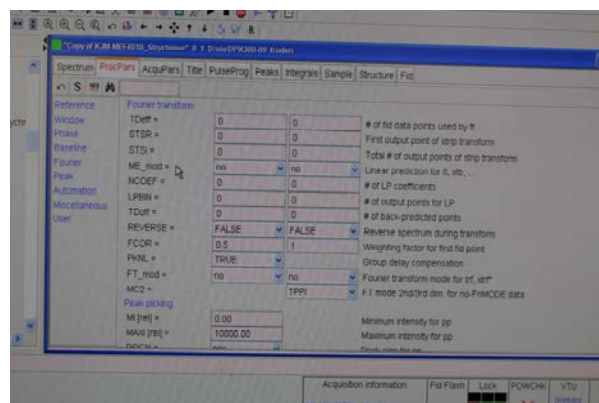

Apply!

What about all the red text that we have read many times? Is there a way of removing it? Right click on the spectrum and select Display Properties. Remove Title.


Click on OK.

You will now get the first introduction to linear prediction. Linear prediction is an artificial way of improving the nmr data by making the data set larger based on the existing data. The computer is asked to calculate the most likely FIDs that would have been obtained if the experiment had been going on for longer time. Click on Acqupars tab and look for the TD (time

domain) for F1 and F2 (no picture of this!!). Change the parameters around ME_mod (ME-Mod = maximum entropy method) in the ProcPars (edp) section as shown here. TD and SI are somehow connected for a non-Linear predicted nmr data set. Try to figure out how.
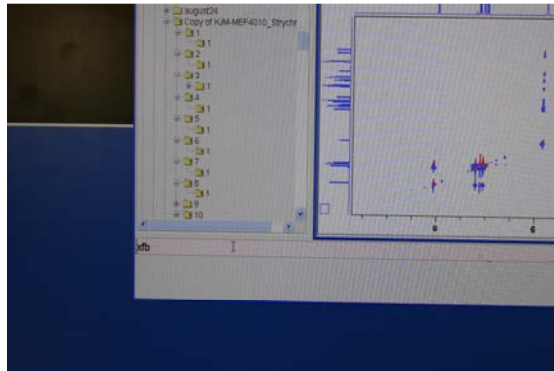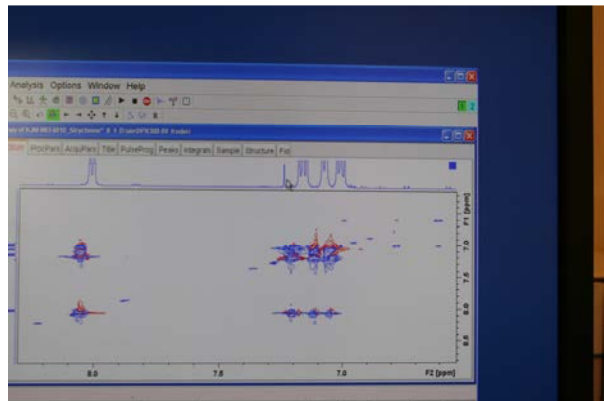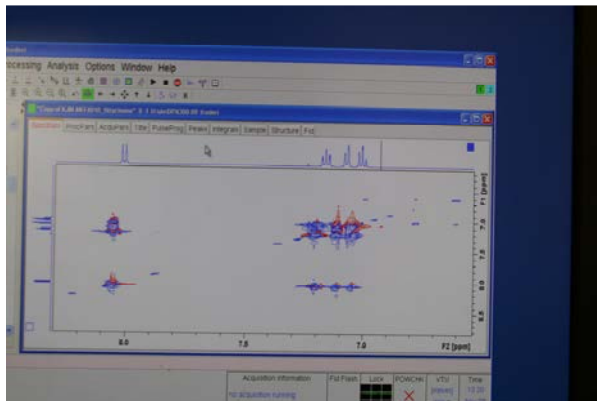

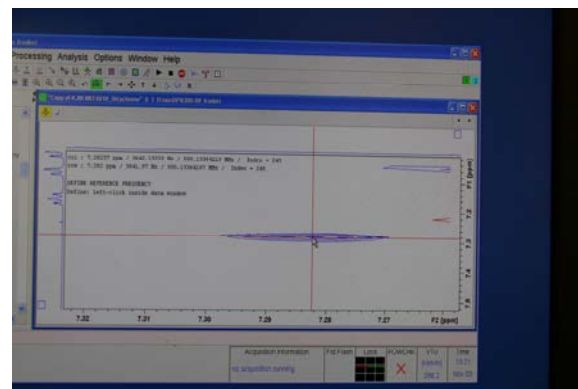Perform a new two-dimensional Fourier transformation e.g. xfb.
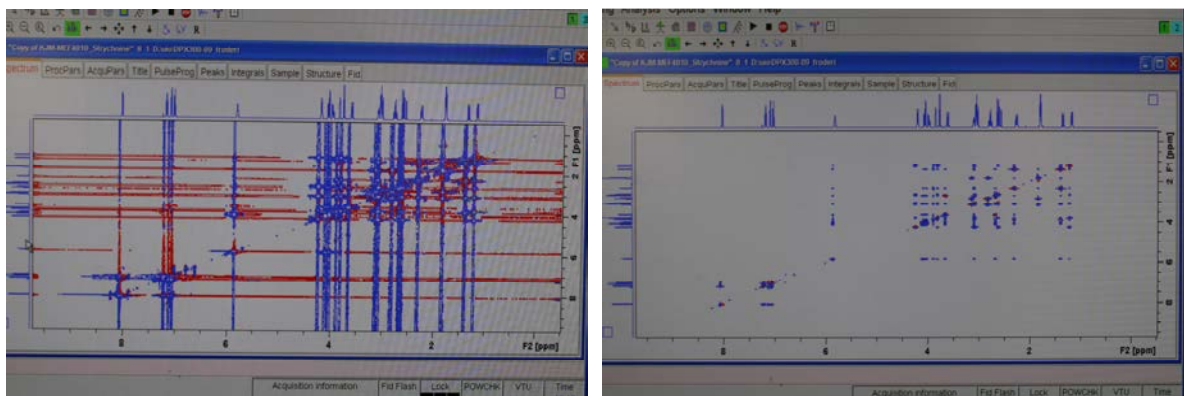
Reduce the intensity and (hopefully) observe that the peaks have become sharper/narrower in both dimensions.
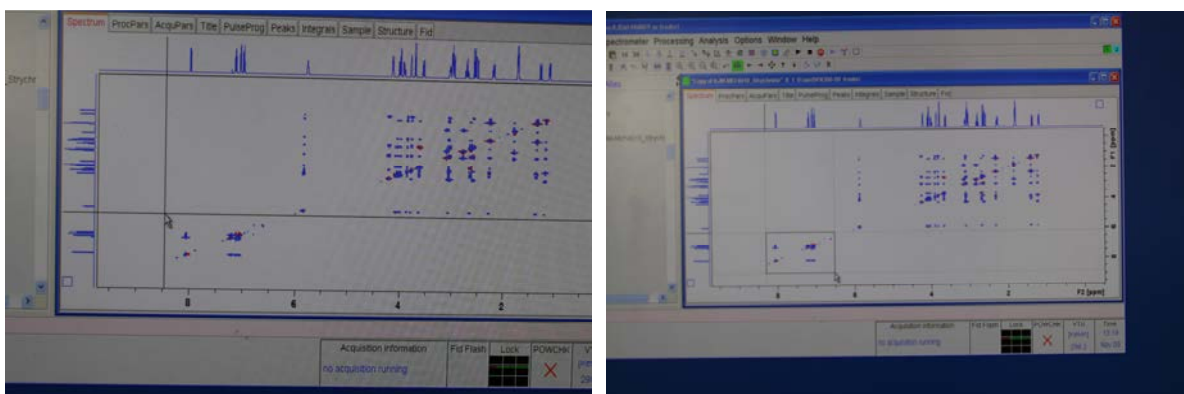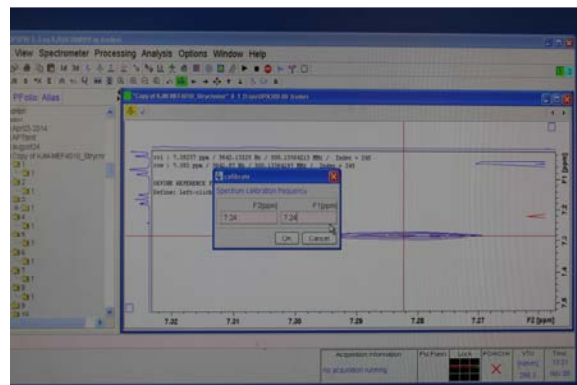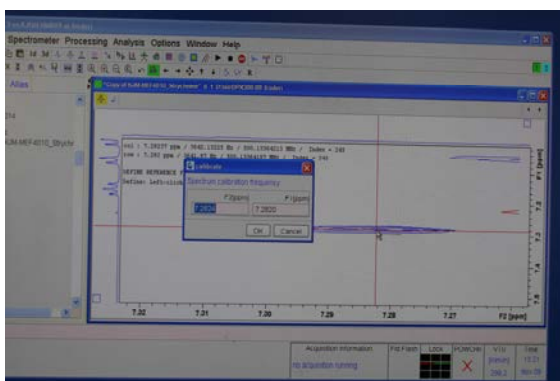

Use the mouse to select the area to be used for referencing the spectrum. Click and drag.
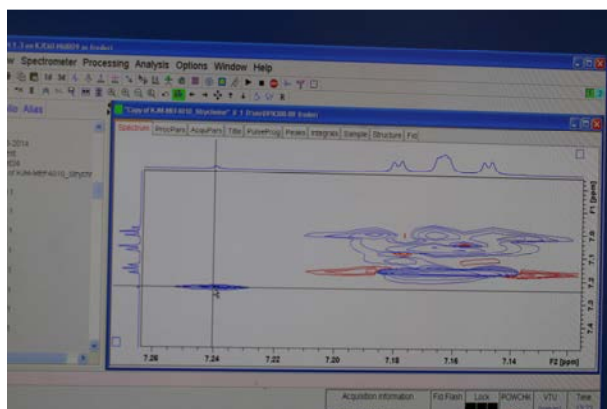
In this case the residual $CHCl_3$ in the solvent $CDCl_3$ is used for referencing. Find it.
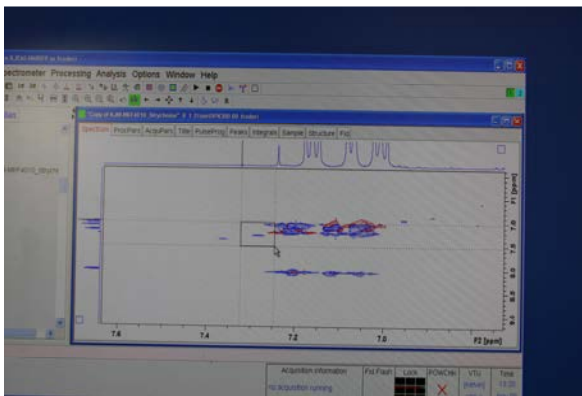
Expand it. Use the referencing button (not shown here). Mark the correct position with the cursor.

Change the numbers to correct values and click OK.

It seems like the chloroform peak is aligned correctly between the 1D spectra on the side and the 2D peak. Plot the expansions as you wish. If you are not remembering how to do this please use another manual as help.

Load the TOCSY pulse program by clicking on PulseProg button. There are two major variations of the Total Correlation Spectroscopy nmr experiment. TOCSY and DIPSI. Those interested in Metabolomics nmr should note that the standard TOCSY experiment on biological samples used in the UiO labs on the 800 MHz instrument is a DIPSI experiment. To complicate the situation even more; the pulse program used in the regular organic chemistry TOCSY experiments is called mlevph since the inventor Malcolm Levitt called the pulse program after himself. The name of the pulse program in any Parameter set is seen as shown above. Scrolling down shows you all parameters with numerical values you ought to check if an experiment does not work out of the box. Especially d9 the TOCSY mixing time must be handled carefully. Read in a text book about the TOCSY mixing time.

We are now moving from TOCSY to NOESY nmr ☺

NEW

Nuclear Overhauser Effect SpectroscopY identifies spin pairs undergoing cross-relaxation. The most common cross-relaxation "method" is called magnetic dipolar coupling. The NOE "goes" through space and is extremely dependent upon distance. NOESY experiments gives information about which nuclei are close in space. The same information is obtainable from the 1D experiment NOEDIFF. NOEDIFF is faster for small molecules. NOESY is used for large molecules with hundreds and thousands of correlations. For the regular user NOESY is simpler to set up and run since the BRUKER NOEDIFF experiment is not easy to perform. Open the NOESY experiment and then you take a look at the pulse program. Look for the delay d8 (the so called mixing time) and the minimum number of NS (number of scans) for each increment along the F1 axis.
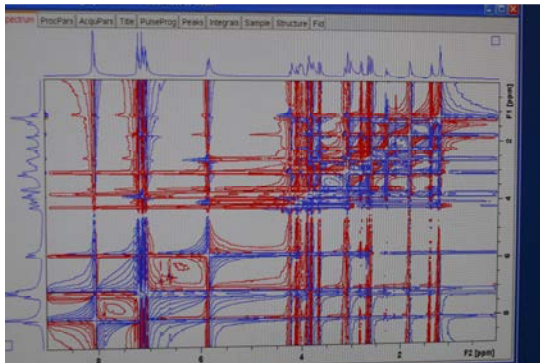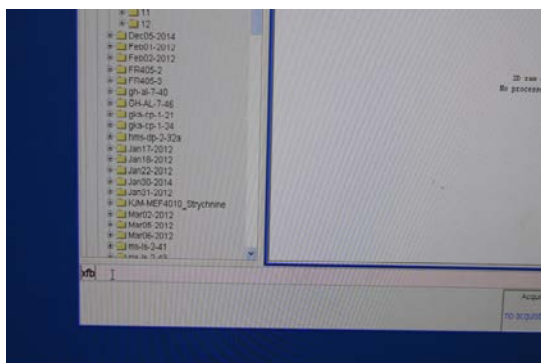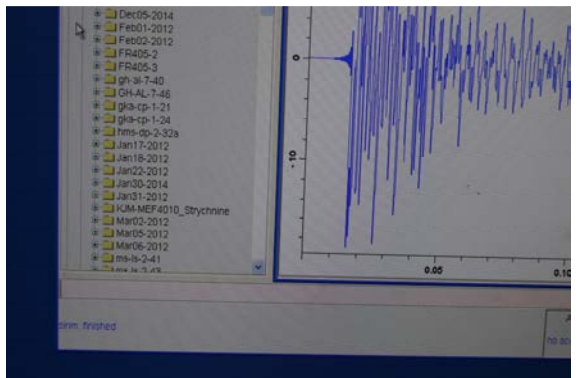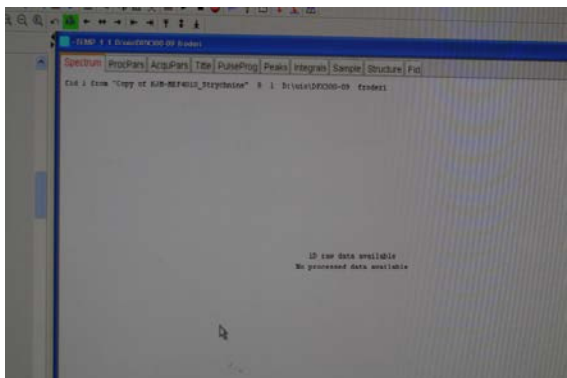

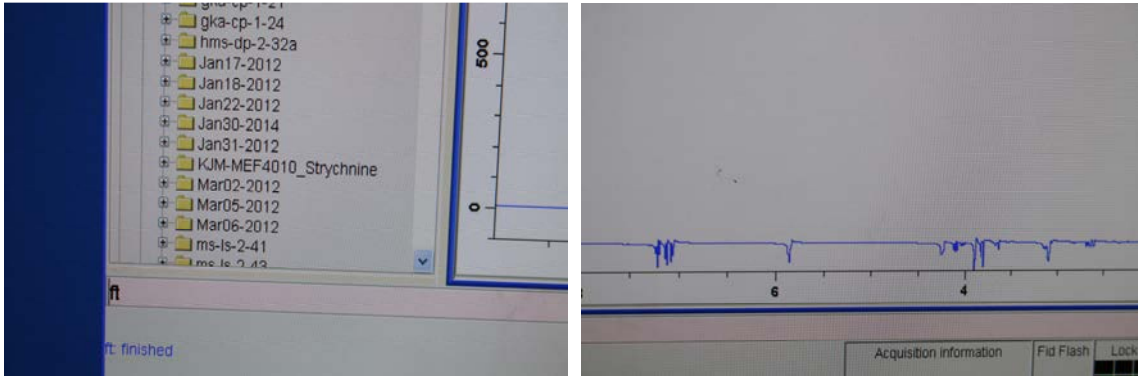Fourier transform the NOESY experiment and observe how ugly it is.
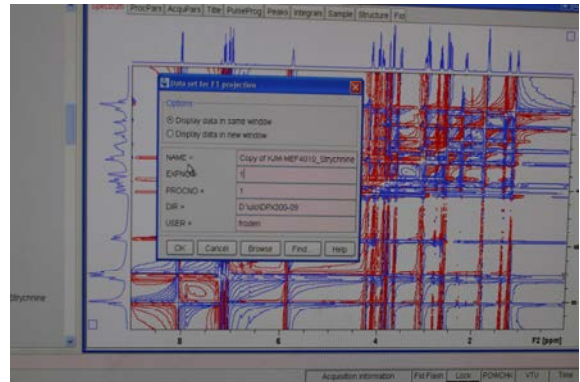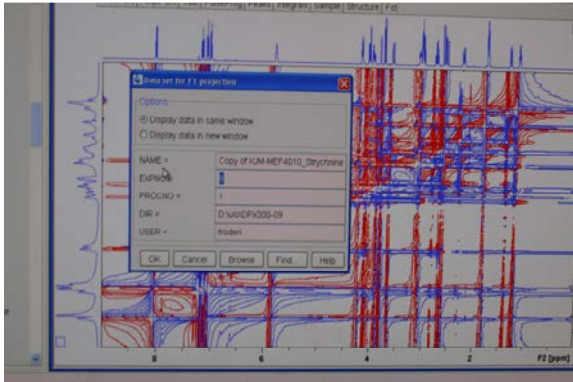
Put in external projections on both sides. You ought to know how this is done now. If not remembering look back in this manual.
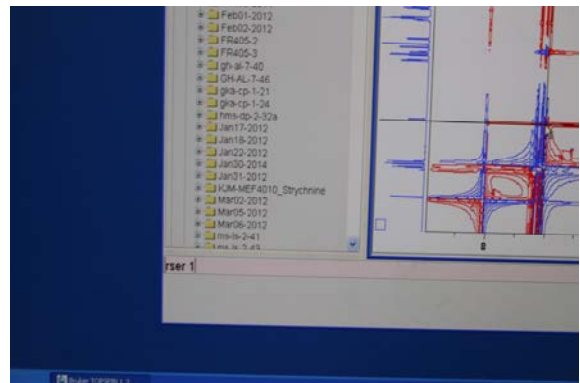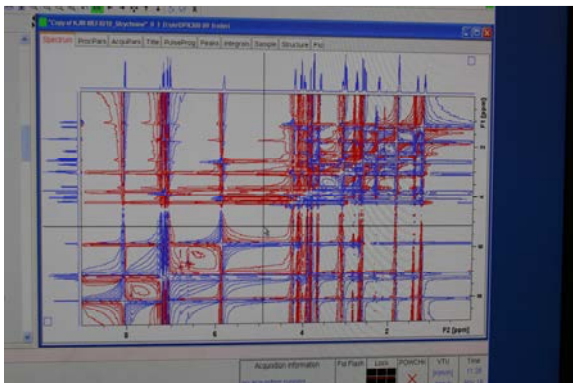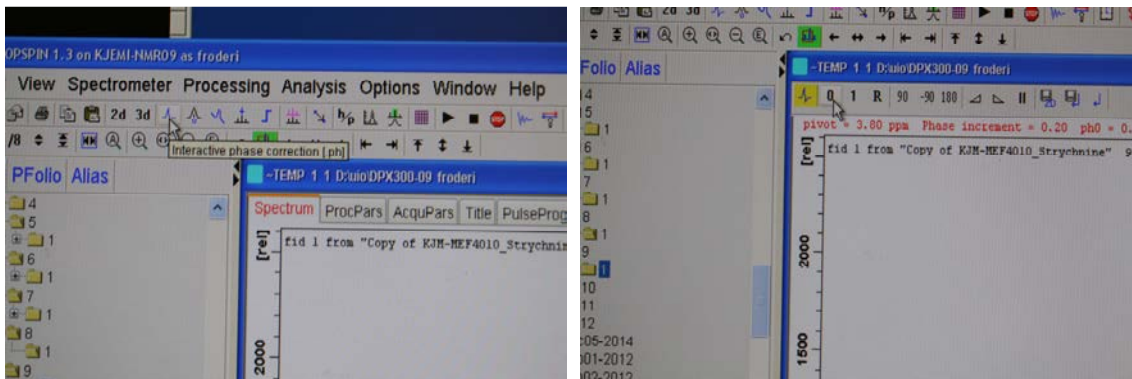

Read serial file 1. rser 1 or rsr 1.

The single FID jumps as usual into the temporary experiment. sinm changes the FID and shows the modified FID to you.
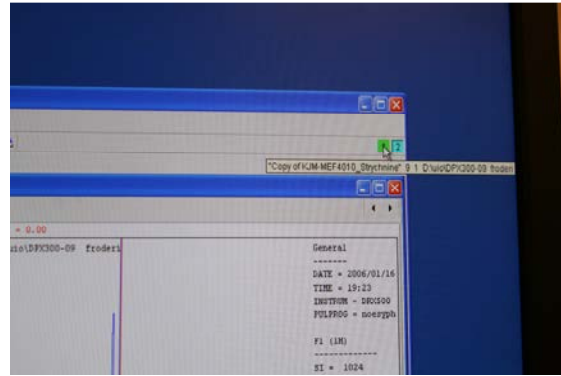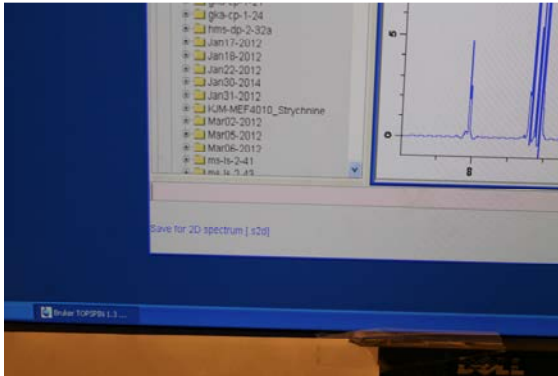
ft and the spectrum is here, although upside down and phased badly. Fix it ☺

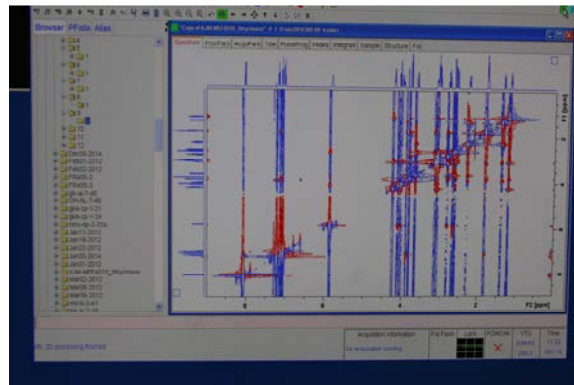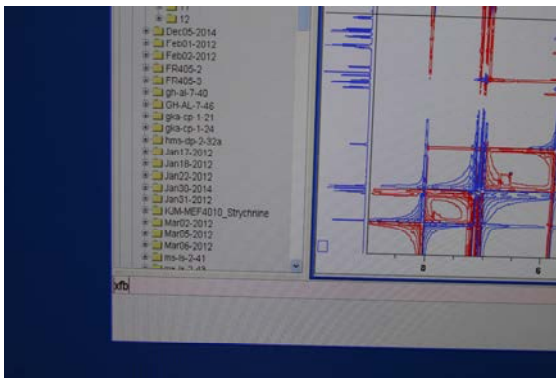And here we go again with zero and first order phasing. You now ought to remember which buttons to click and how to drag the mouse up and down while holding in the left mouse button?
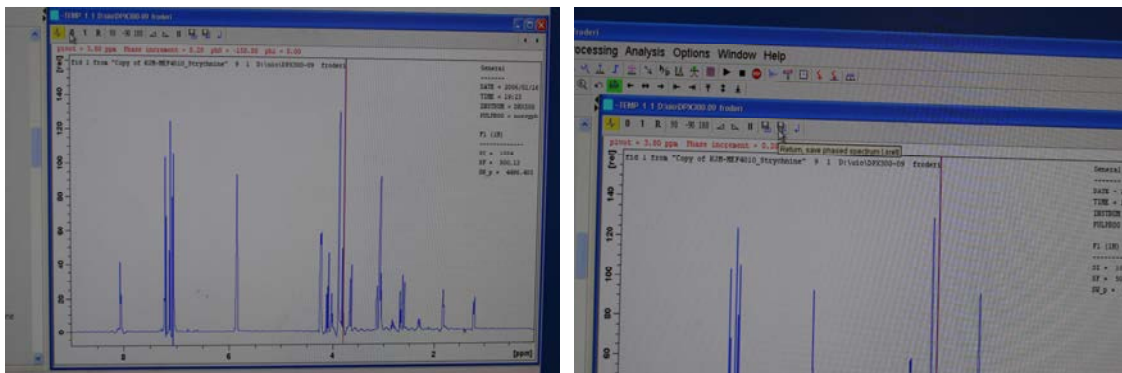
Save as 1D or 2D? Sometimes saving must be done twice.

Go back to window 1 (not expno 1).

New 2D Fourier transformation. How is your spectrum in the vertical dimension?
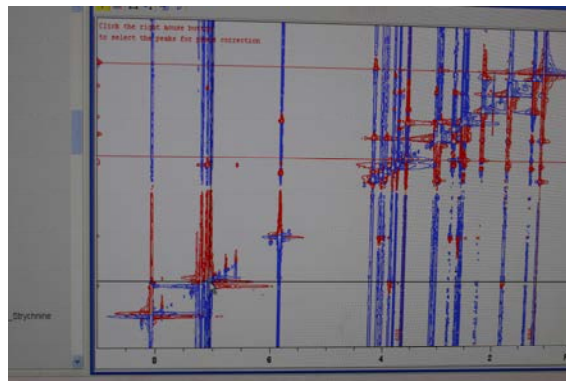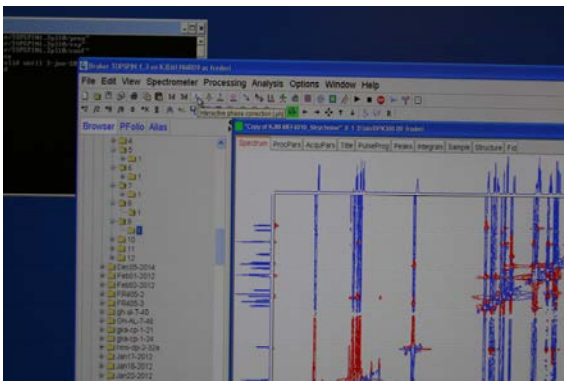
Manual phasing. Select 3-4 points.
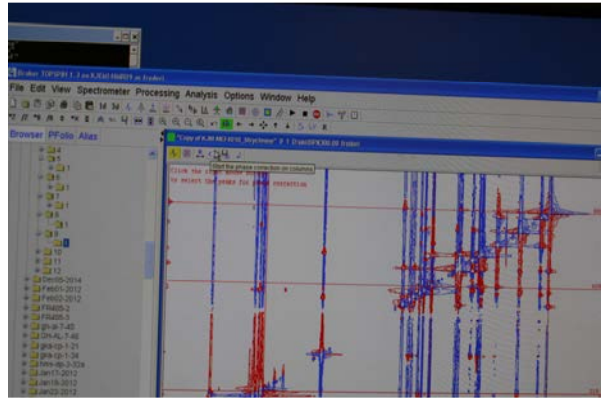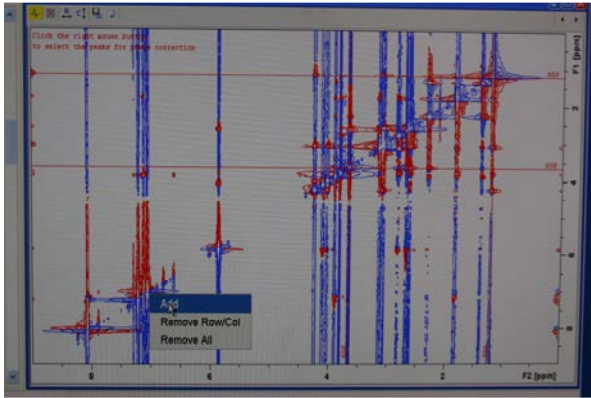
Add the points. Select columns.
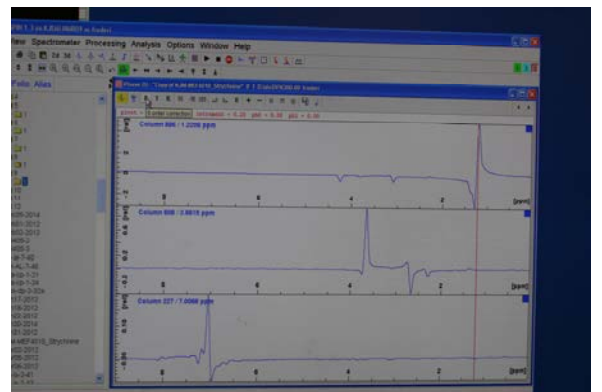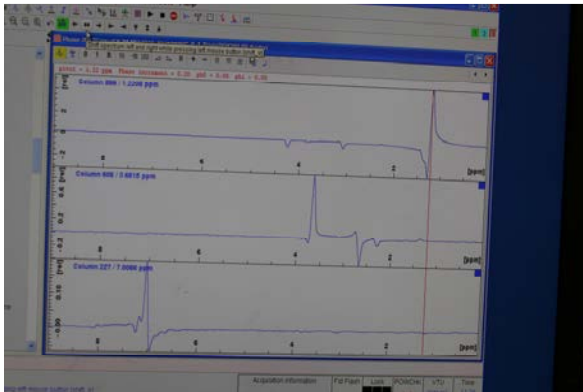
Use Zero and First order phasing. Seeing the three blue squares to the right? Ideally only one of them shall be colored, as shown in the small window.
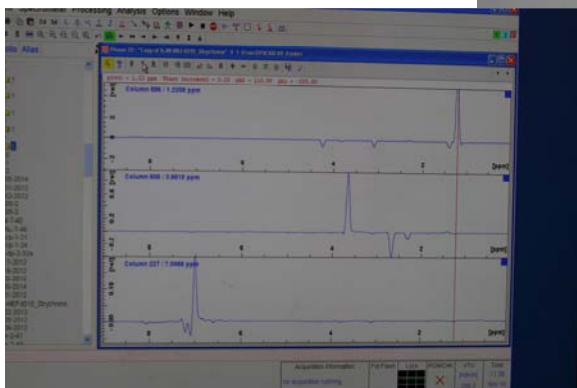
Click on save (right picture).
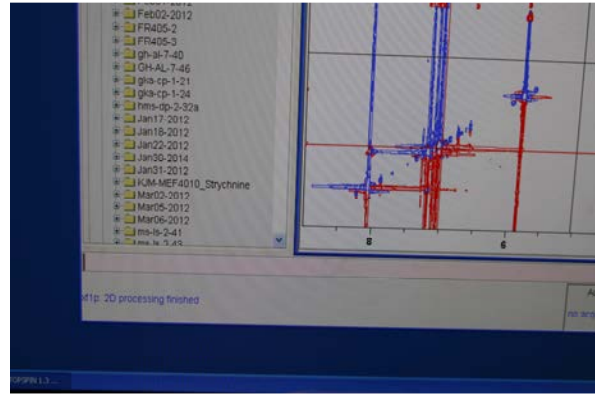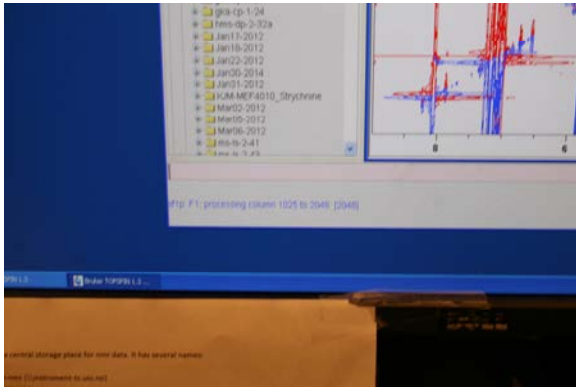
When until the new 1D Fourier transformation is completed.
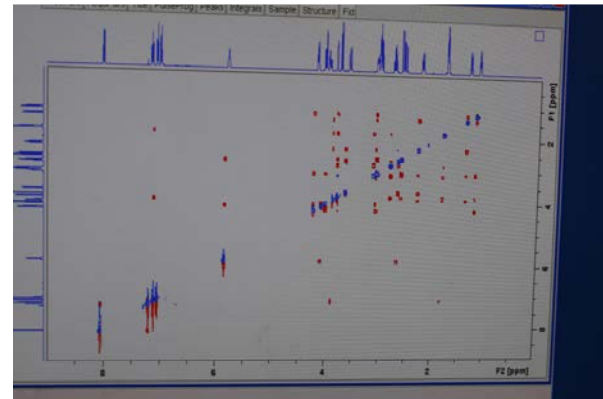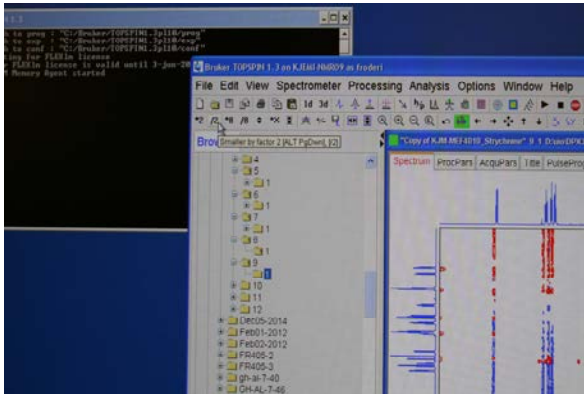
Reduce the intensity and realize that the 2D phasing is still a bit ugly.
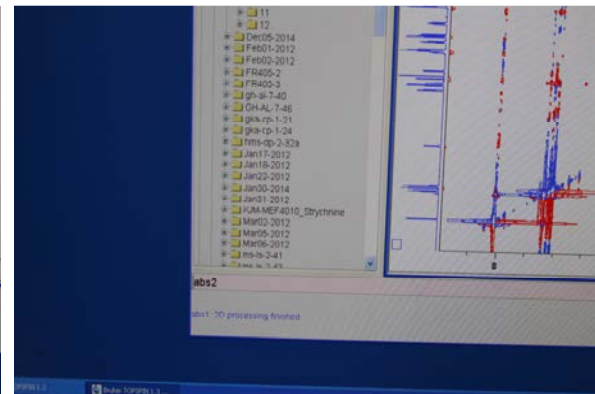
You can try abs1 and abs2 to flatten the spectrum in a similar way that abs does for a 1D spectrum.
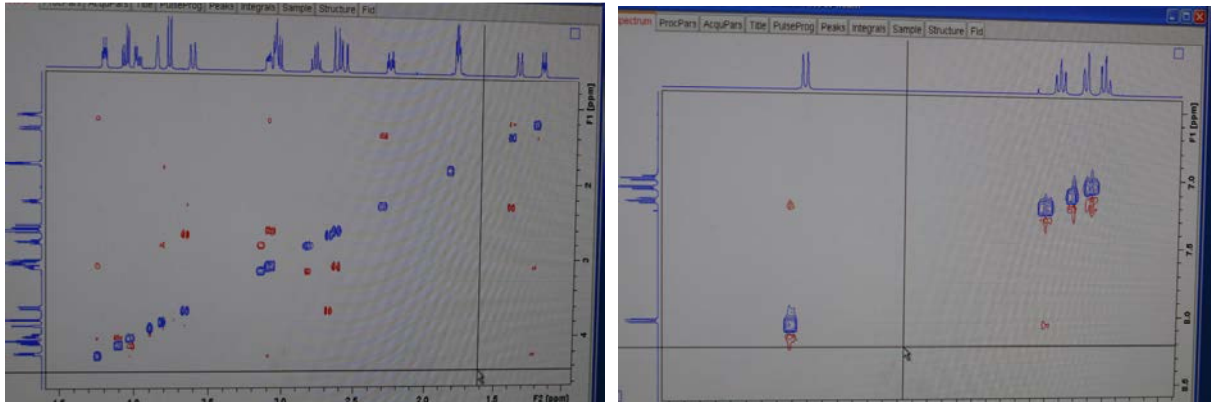
A bit better. But the spectrum is not aligned with the 1D external projection spectra. If you at this stage still have an extremely ugly spectrum you have to start the phasing process once more. It is generally hard to clean up 2D nmr spectra and sometimes you fail whatever you are doing. Go in to the edp menu and set all phasing correction numbers PHC0 and PHC1 for both F2 and F1 to zero and do a new 2D Fourier transformation and start again with the phasing. This procedure is not shown with pictures since you are now becoming half way an expert and ought to do this on yourself.
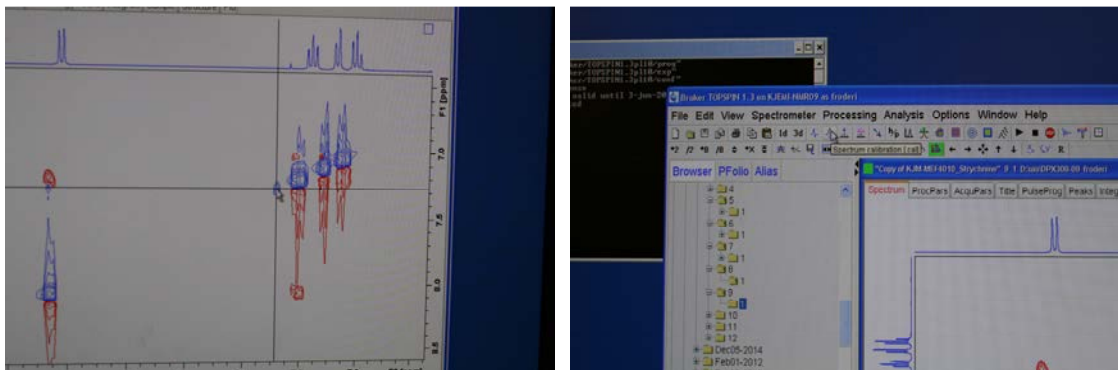
Locate the solvent peak and then locate the referencing button – and use them!
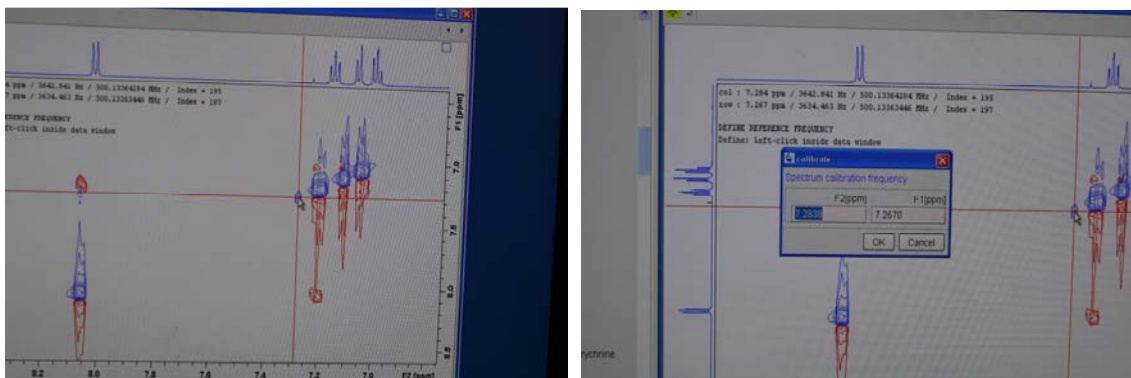
It sure is aligned wrongly. You are seeing the wrong numbers?
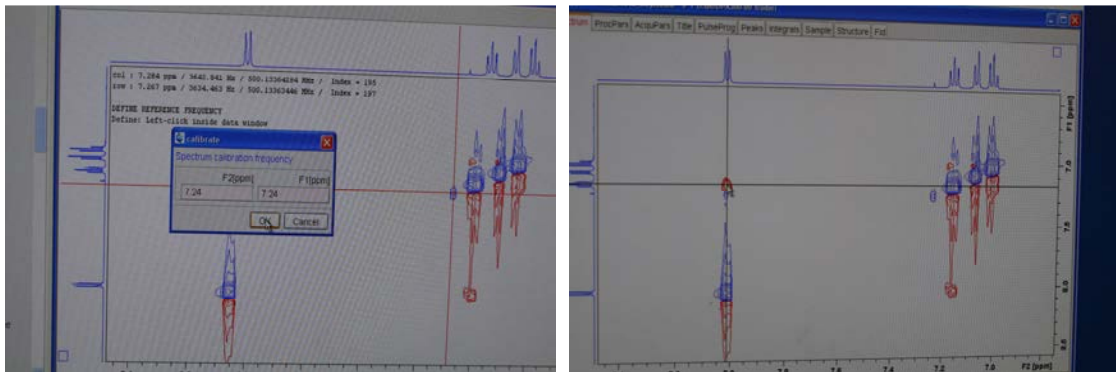
Much better. But we are still not satisfied when it comes to the T1 noise/F1 streaking. Read about T1 noise in a text book.

We can cheat a bit and use a trick. The command syma looks in both directions and remove whatever is missing in one of the dimensions. And since T1 noise is along the F1 axis it often works. Similarly, the command sym is used for 2D spectra that is phased only one way. Peaks in one way only (up from the screen/paper or into the screen/paper). Print/plot your spectrum and expansions of it.
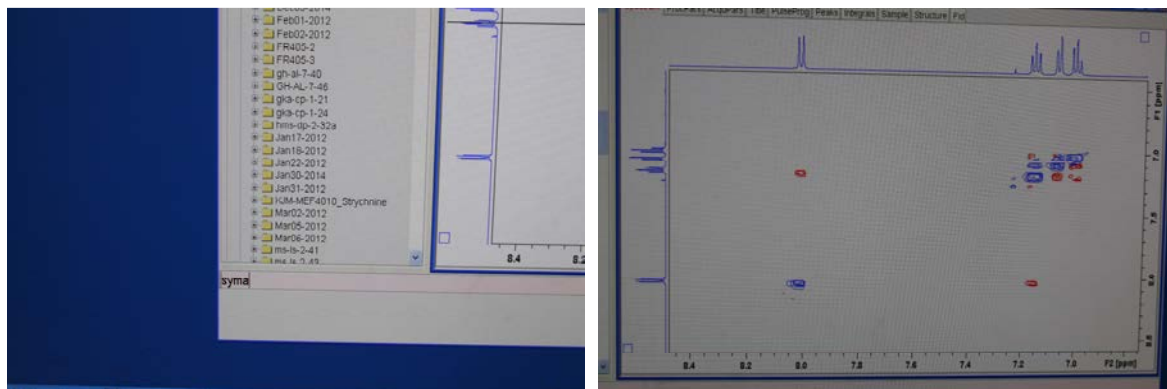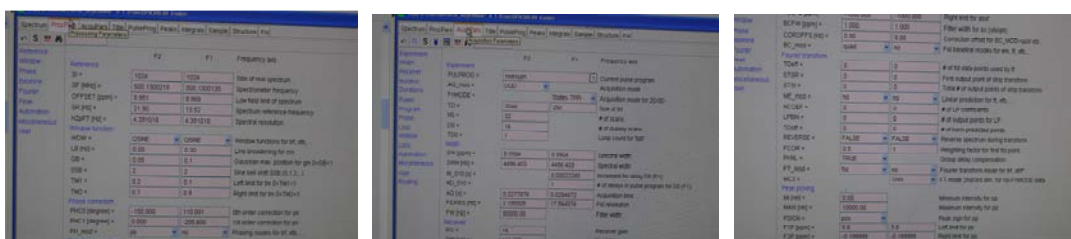

Finally, you ought to look at the SI numbers for F2 and F1 in the Processing parameters section (edp command) and the two corresponding numbers TD in the Acquisition parameters section (eda command). For those of us planning to use nmr heavily in their science ME_mod, NCOF and LPBIN is of interest as well.
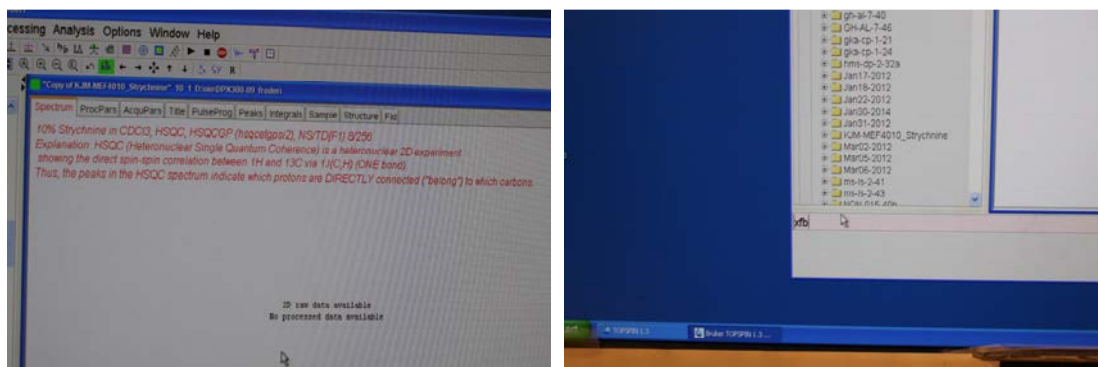
Open the HSQC data set and xfb it to get the spectrum. You are now entering the great world of naming confusion in nmr. HSQC means heteronuclear single quantum correlation – the name stems from the Physics behind the experiment. The sister experiment HMBC means heteronuclear multiple bond correlation – the name originates from the chemical information what we see in the experiment. This is the freedom the inventors of new experiments have. Heteronuclear Single Quantum Coherence nmr spectra observe the ^1H-^{13}C correlations in molecules. via the 13C chemical shift evolution of a single quantum coherence. In KJM 5280 you will run your own HSQC experiment with the help of the program ICONNMR on one or more of the the AVII400, AVIIIHD400, AVI600 and AVII600 nmr instruments. If you later move on to the next more advanced nmr course; KJM 9250 you will obtain the spectra manually.
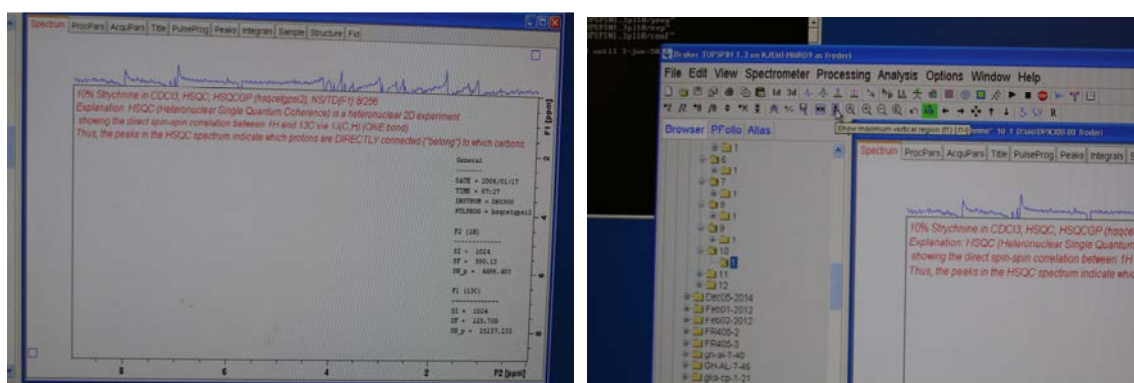

Often total confusion happens at this stage. There are no peaks/correlations in my spectrum! The TopSpin program is to blame. Look closely at the ppm scales on F1 and F2. They are very limited. Why? The program remembers the ppm settings from the previous experiment. Look for the two buttons that brings in the maximum ppm width. They are close to each other.
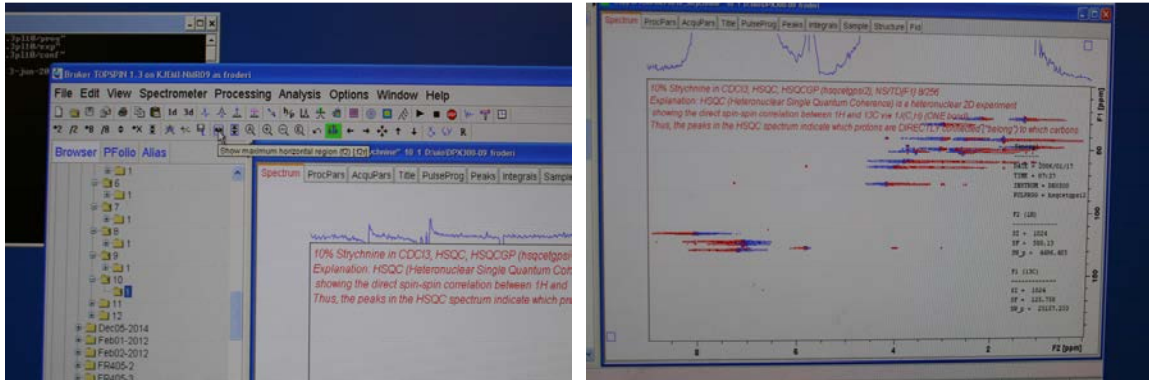
Phasing in F1 (horizontal) seems accidentally to be fairly OK here.
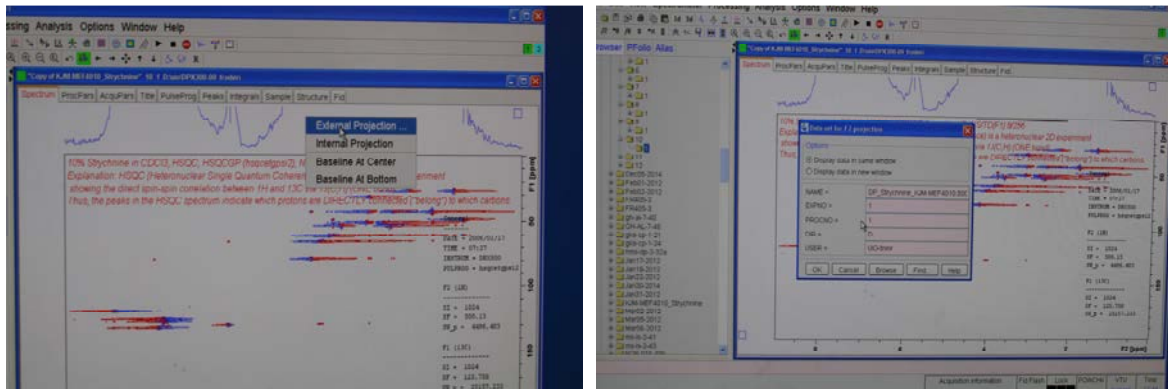
Change the ugly 1H internal projection with an external one. And realize it is now much more difficult than before. Many more of the entries in the pop up window are now wrong. NAME, EXPNO, DIR and USER must all be corrected. If you make 3 out of 4 correct and click OK it will not work and those that were made right are back to wrong the next time your try to do this.
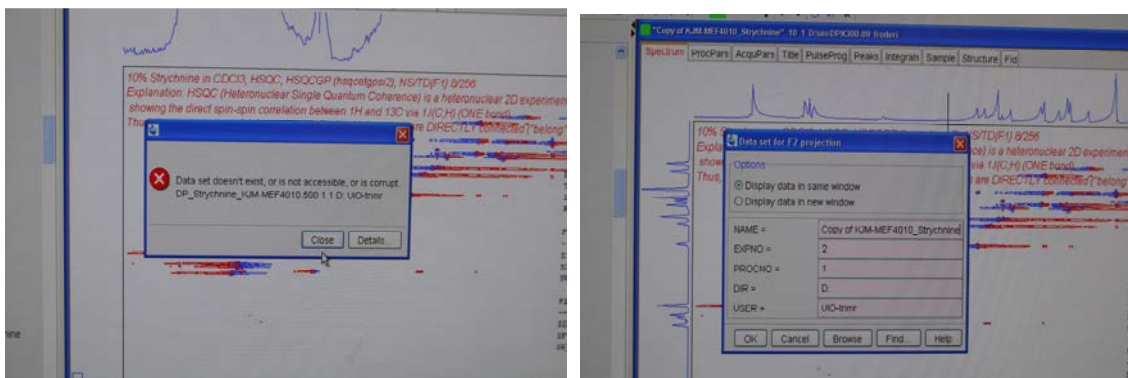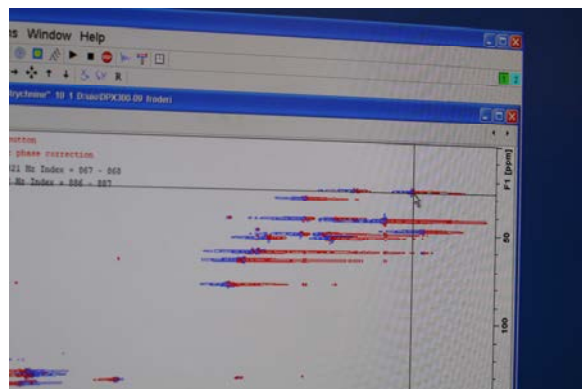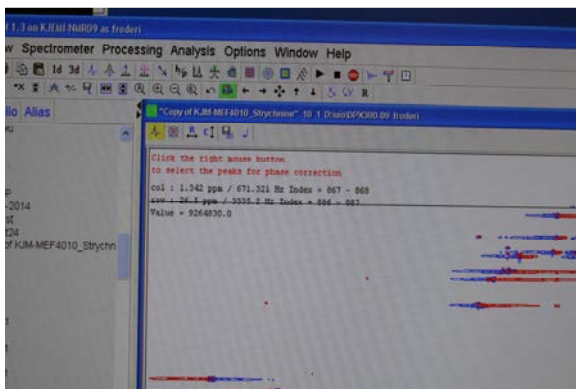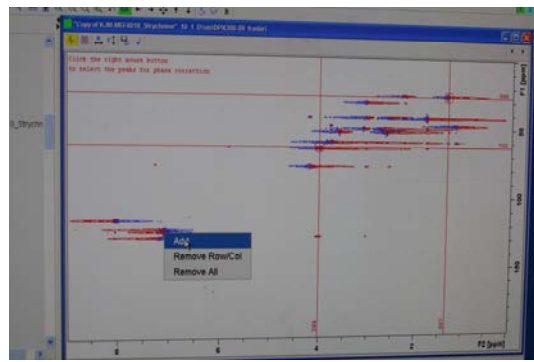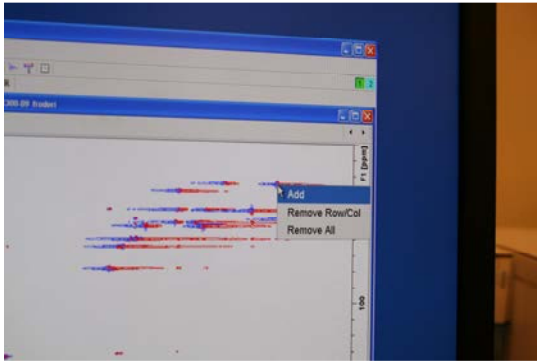

Here we go again ☺
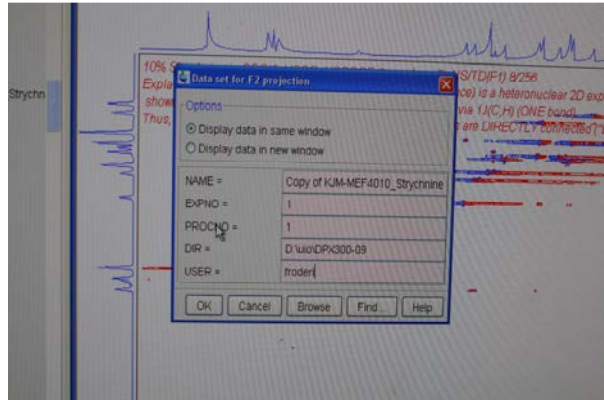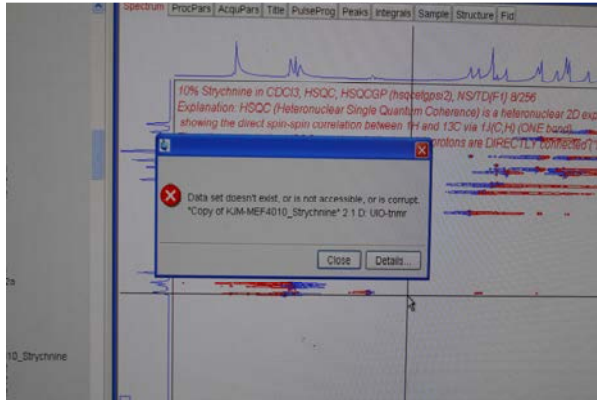
Still wrong! And maybe right in the right picture?
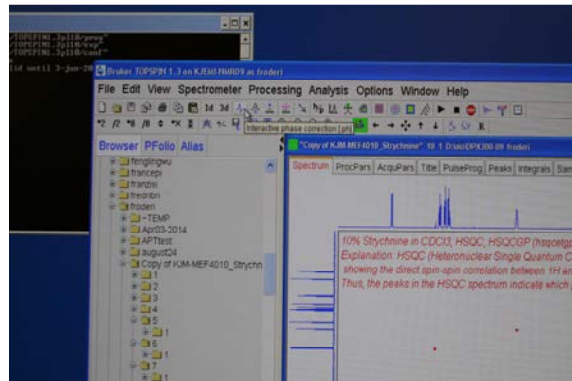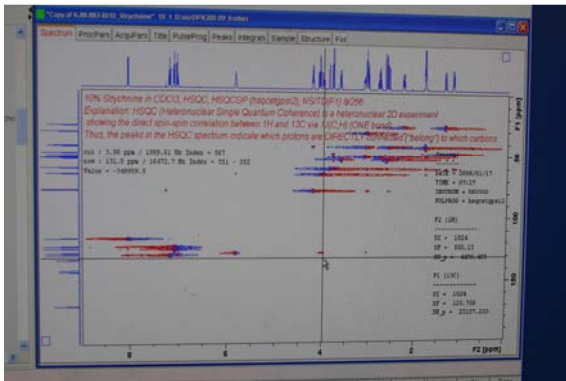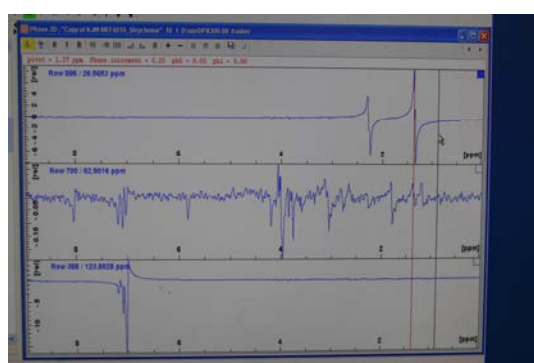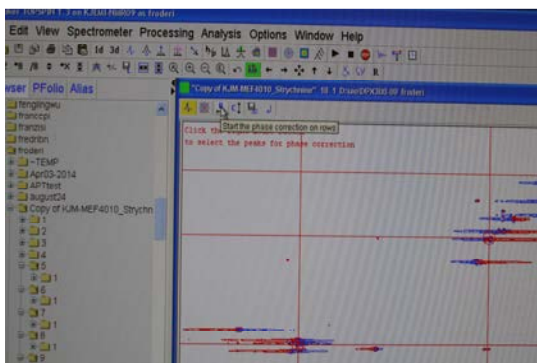

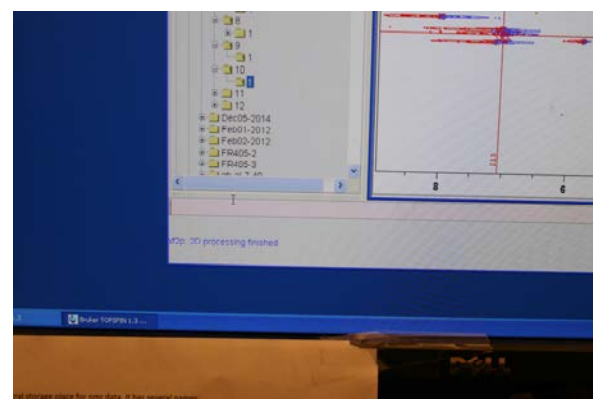Finally, it is time to start the phasing process of this spectrum. Choose the interactive phase correction button.
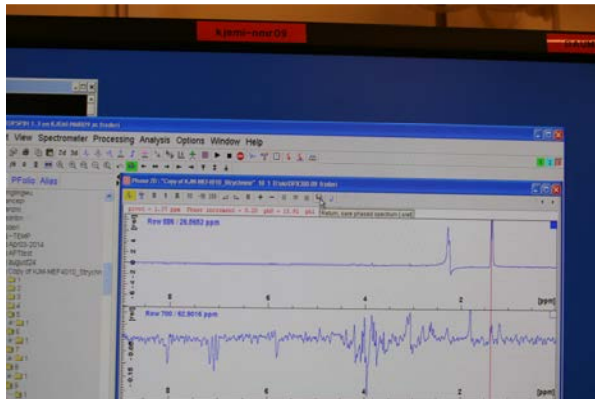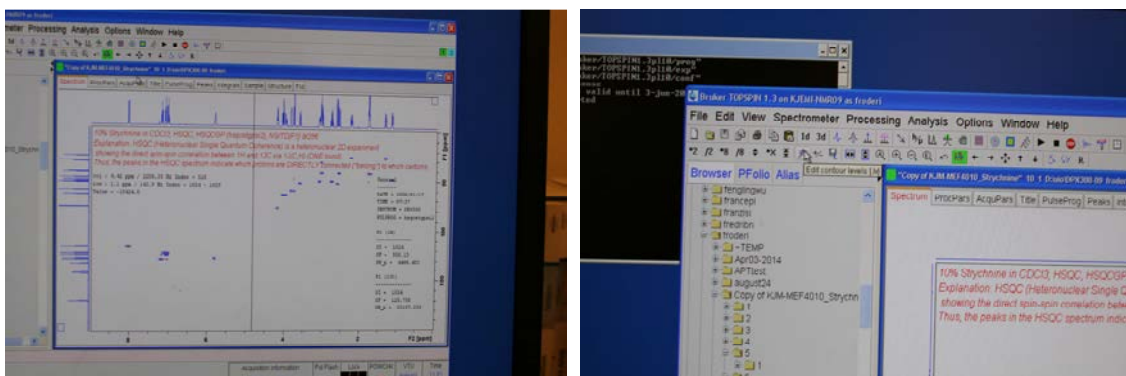

Select your three/four phasing points.

Add, add and add.

Do the work.

Again and again. Go in to the edp menu and set all phasing correction numbers PHC0 and PHC1 for both F2 and F1 to zero and do a new 2D Fourier transformation and start again with the phasing.

Finally after a series of rounds the spectrum looks almost OK. We are about to embark upon referencing the ppm values for both the ^{13}C and the ^{1}H axes now. But first we will learn to edit the contour levels which might be helpful in finding the mid-point of the referencing 2D peak.
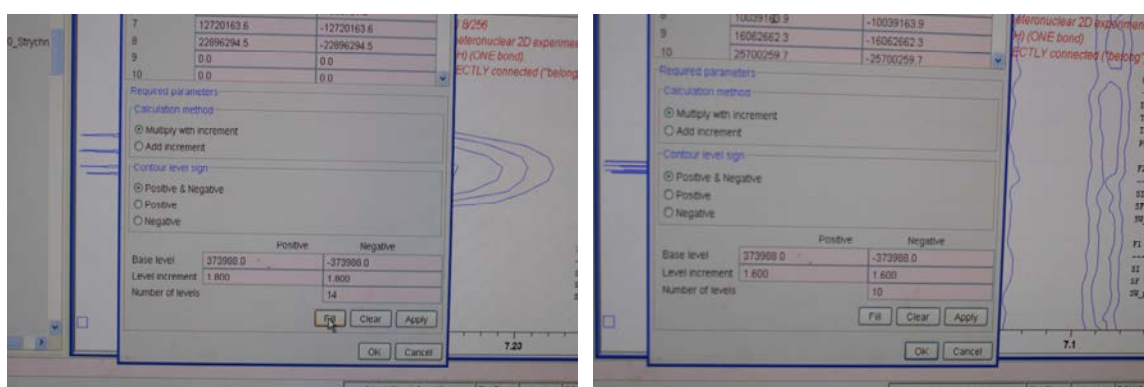
First find the referencing peak. In this document the 2D peak at 77.0/7.24 ppm from the residual ^{13}C^{1}HCl$_3$ in the solvent CDCl$_3$ is used. Fool around with the Level increments and Number of levels. Click on Fill, Apply and OK and observe the result. Do it again and again until you have a good result.
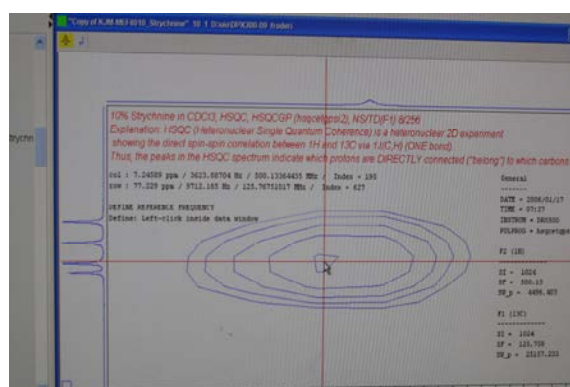
Select the correct button for opening the referencing sub menu. (You shall remember how to do this now). Find the center and select it.
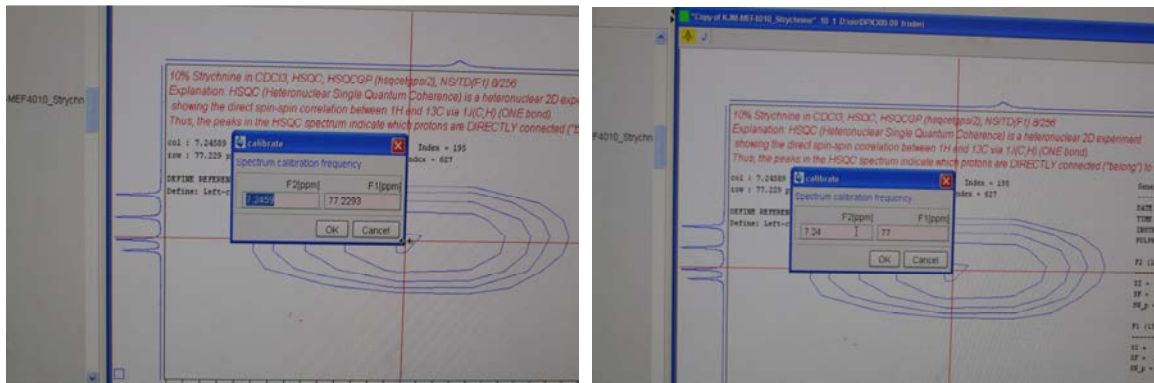
Exchange the wrong numbers with correct numbers. Select between OK or Cancel.
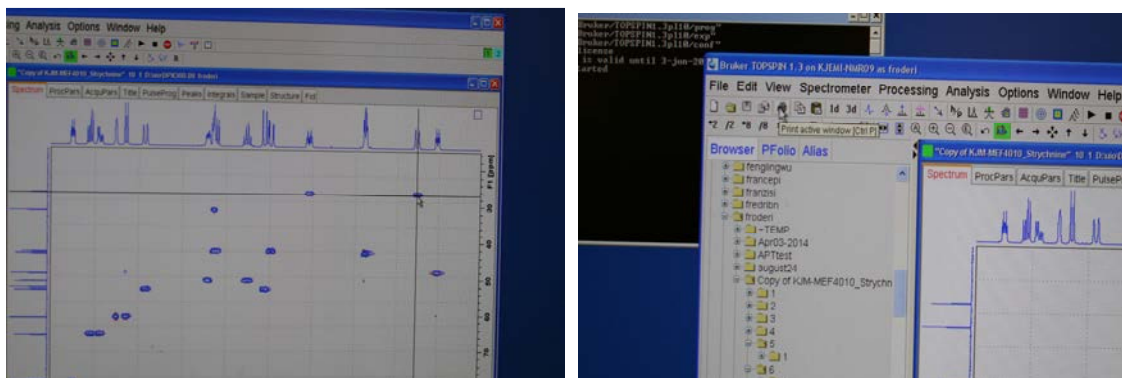
The 2D correlation peaks are now aligned nicely.   You are observing that two protons are sitting on the same carbon? The Hs are in dif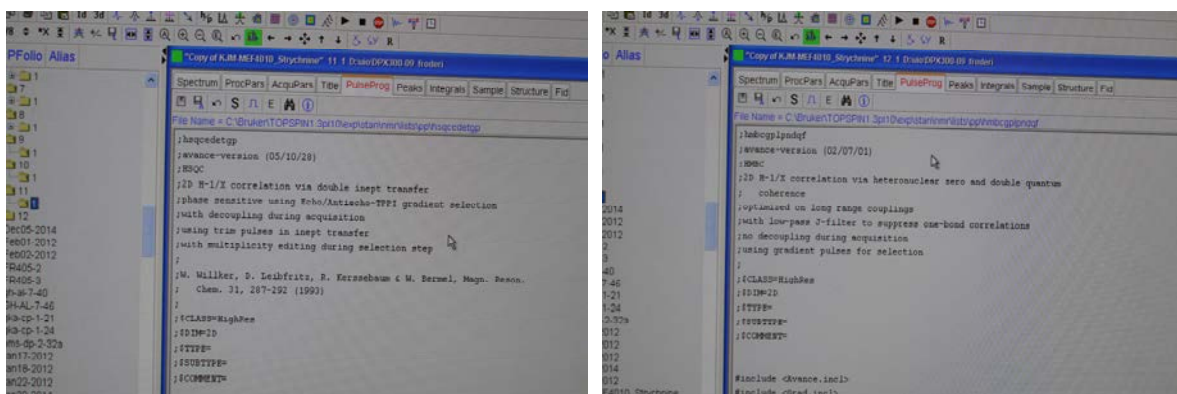ferent magnetic environments and are getting different chemical shifts due to the stereo chemical properties of the molecule. Print/plot your spectrum and expansions.

The experiment HSQCEDETGP (pulse program hsqcedetgp) sorts CH_2s from CH and CH_3 phase wise (up/down). The higher the magnetic field the worse the phasing challenge is. The electronics on the 600s and the 800 are not able to send uniform radio signals over the wider Hz

range in the higher magnetic field instruments. On the 400s this is usually not a problem. In the course KJM 9250 so called shaped pulse versions that alleviate these problems in HSQCEDETGP and other experiments are introduced.

Process the rest of the unprocessed Mickey Mouse experiments.