Instructions for Fast acquisition and data processing using Nanalysis (1.4 T) NMR spectrometer and MatLab

by Dr. Nuwandi Ariyasingha and Dr. Jonathan Birchall

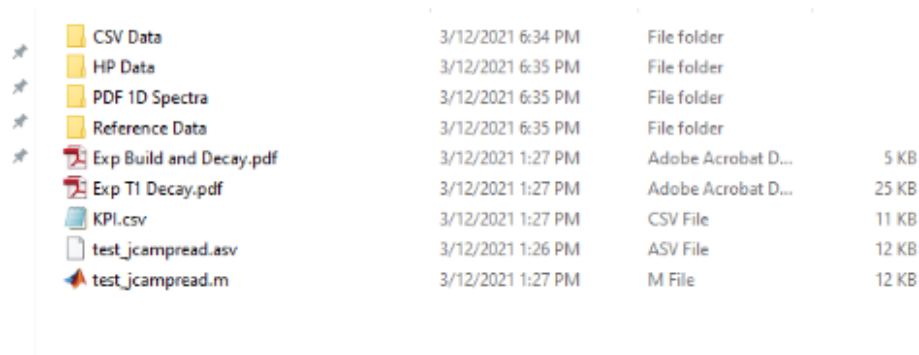**A) *Data Acquisition Procedure (Turning on the individual scan capability) on 1.4T Nanalysis:***
Turning Pable mode on and running the spectral acquisition:

1. Remove any old files saved under "Results" in the spectrometer (this will keep the spectrometer performance better, please make sure to copy all the files to an external drive before deleting them from Nanalysis)

2. Enable debug keys: by doing the following:
   (i) press F12 on USB keyboard connected to the device
   (ii) then type "debug", an icon will appear that the debug has been toggled ON, Hit "Ok"

3. Turn on individual-scan save capability ("Pable flag") by pressing "Ctrl+P" – this will enable the Pable mode; Hit "Alt+Tab" to double check that the Pable mode is truly ON.

4. Select Lock Nucleus "1H", Solvent "Generic 1 Peak".

5. Select Experiment "Designer", Under 1D change the Receiver Gain to "Fixed, 10db", the pulse duration should be set to what is equal approximately 15 degrees (2.6 us).

6. Select the script by hitting "Manager", selecting "FastCapture", then press "Edit", then "Use", finally hit "Done"

7. Set up acquisition parameters, including the number of pseudo 2D scans desired, Good parameters: 20 ppm width, 512 points, 64 scans, 0 dummy scans, spectral center 5 ppm, pulse width of 2.6 us.

8. Prepare/insert the sample into the spectrometer, Hit "GO" to initiate spectral acquisition.

9. When the scans are complete, leave the acquisition screen (by pressing "done". A window will appear asking whether to save the files hit "no" because the individual scans are saved automatically). Important: make sure to note down the file numbers in the book !!

10. Use the same protocol (8-9) to acquire thermally polarized spectra of the desired sample.

Note: you will have 64 files for each experimental acquisition run (this will be the same for both HP data and for the thermal data, with 64 files for each data set. We are only interested in the "Signal.dx" files and not the "lock.dx" files.)

B) Data Processing Procedure (MatLab):

1. First go to the "Results" folder on the spectrometer and copy all the files ("signal.dx) that needs processing.
2. Copy the files in to "Gen 3" computer (connect via TeamViewer, please check with Ed or Radwan for the availability of "Gen 3" computer). (Path- Desktop=> Pablo=> "desired folder". Copy a previous "pable folder" and rename it with a new name , *eg*. "ddmmyyX" (see below).

📁 CSV Data	3/12/2021 6:34 PM	File folder		
📁 HP Data	3/12/2021 6:35 PM	File folder		
📁 PDF 1D Spectra	3/12/2021 6:35 PM	File folder		
📁 Reference Data	3/12/2021 6:35 PM	File folder		
📄 Exp Build and Decay.pdf	3/12/2021 1:27 PM	Adobe Acrobat D…	5 KB	
📄 Exp T1 Decay.pdf	3/12/2021 1:27 PM	Adobe Acrobat D…	25 KB	
📄 KPI.csv	3/12/2021 1:27 PM	CSV File	11 KB	
📄 test_jcampread.asv	3/12/2021 1:26 PM	ASV File	12 KB	
📄 test_jcampread.m	3/12/2021 1:27 PM	M File	12 KB	

"ddmmyyX" folder will look like above with sub-folders in it.
HP data folder will have all the HP files (signal.dx) that is copied from the spectrometer.
Reference Data folder will have all the thermal spectral files (signal.dx)
CSV Data folder will have the data files created as .csv files.
PDF 1D Spectra folder will have all the spectral files created after data processing, saved in the pdf format.
"Exp Build and Decay" shows the generated build up and decay curves (will be explained later)
"Exp T1 Decay" shows the generated T1 decay curve (will be explained later)
"test_jcampread.m" is the Matlab master file to run the code.
"KPI.csv", key performance indicator file shows all the output values such as Ha, Hb integrals, enhancement values etc in an excel file.

3. Make sure to copy your HP and thermal experiment data in JCAMP-DX format to the appropriate folders before running the MATLAB script. This done by opening the folder you created "ddmmyyX" and open "HP Data" folder, replace the data files with the new HP data files you want to analyze. Open "Reference Data" folder and replace the data files with the new thermal data files you want to analyze. (see below)

Usually, a data set acquired using Pable sequence contains 64 .dx files of the HP data (depending on the number of scans you set) and 64 .dx files of the reference data. You want to copy all the 64 files of the HP data into the HP folder and only the last accumulated .dx file of the thermal data into the Reference data folder.

4. Then open MatLab and select the path of the folder "ddmmyyX" and open "test_jcampread.m" file, it will look like below. Once, you open the .m file hit "Run".

"Run"   "test_jcampread.m"

5. MatLab function will start running and process all the data. First thermal spectrum is analyzed and plotted, when completed you will see a pdf created with the thermal spectrum. *eg.*
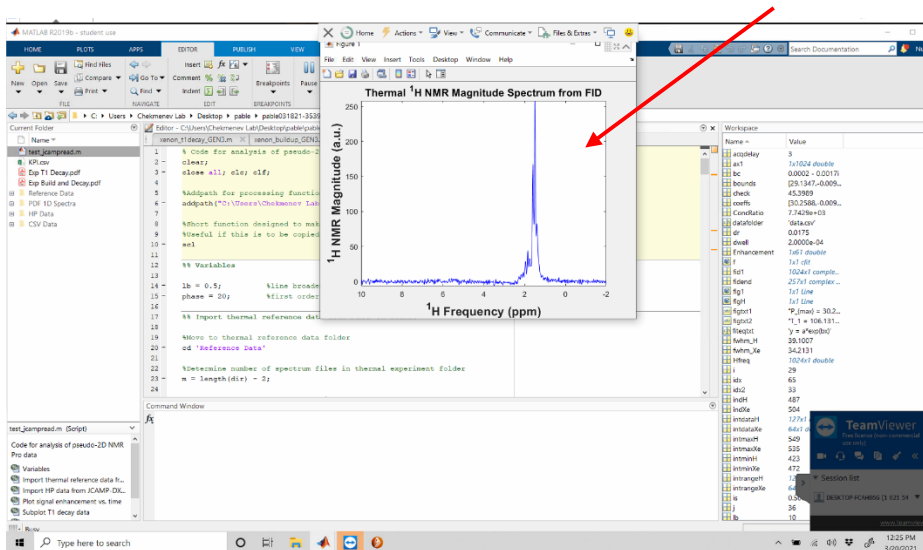

After processing of the thermal spectra is completed, it will start going through the HP files. Below is an example of one such spectrum during the analysis of the 64 spectral files.
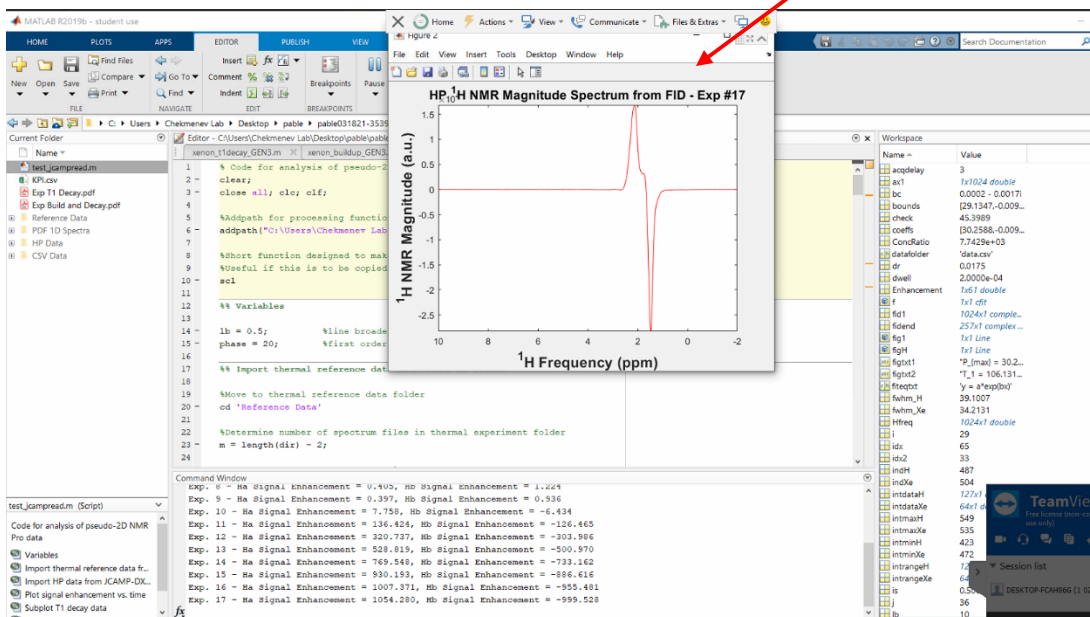
6. After completion, it will generate two other plots i) build up and decay curve of the data which plots the signal enhancement vs time and ii) a T1 decay plot. See below.
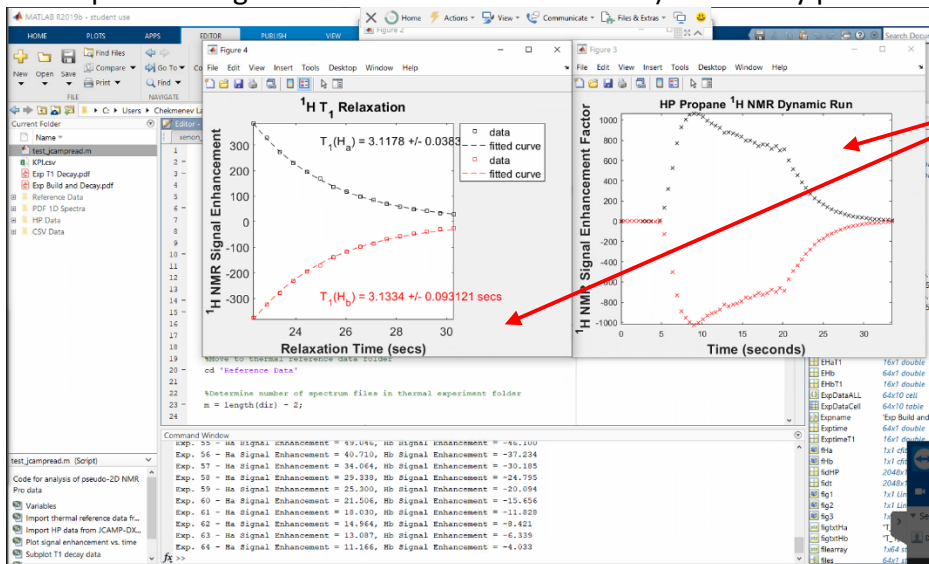
7. The folder now will have all the important data saved.
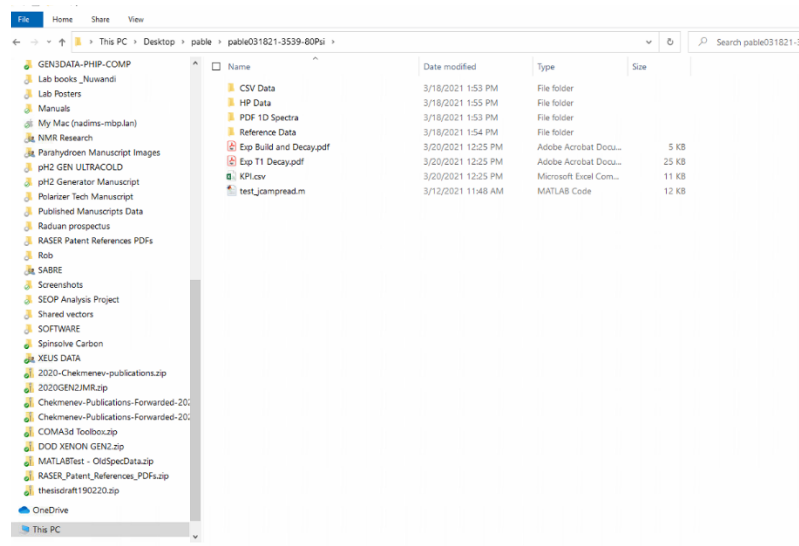
HP data folder will have all the HP files (signal.dx) that is copied from the spectrometer.

Reference Data folder will have all the thermal spectral files (signal.dx)

CSV Data folder will have the processed FFT data files saved as .csv format.

PDF 1D Spectra folder will have all the spectral files generated after data processing, saved in the pdf format.

"Exp Build and Decay" shows the generated build up and decay curves (will be explained later)

"Exp T1 Decay" shows the generated T1 decay curve (will be explained later)

"test_jcampread.m" is the Matlab master file to run the code.

"KPI.csv", key performance indicator file shows all the result output values such as Ha, Hb integrals, enhancement values etc in an excel file. You can use this file to extract data you wish and plot them in different plotting software etc.

C) Instructions for Pseudo2D NMRPro JCAMP-DX Import and Analysis
   by Jonathan Birchall (Wayne State University, March 2021)

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====CONFIGURATION=====
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This folder should contain:

CSV Data - Empty folder for storing tabular processed spectrum data

HP Data - Place your JCAMP-DX hyperpolarized experiment files here. Include only signal files (ignore lock scans)

PDF 1D Spectra - Empty folder for storing graphical processed spectra

Reference Data - Place your JCAMP-DX thermal reference experiment file(s) here. Include only signal file(s) (ignore lock scans)

NMRPro_JCAMPread.m - MATLAB program for file importing and spectral analysis

README.txt - this file (:

Make sure to copy your HP and thermal experiment data in JCAMP-DX format to the appropriate folders before running the MATLAB script.

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===PROGRAM EXECUTION===
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Importing and processing should complete without user input.
Depending on result, user may opt to re-process under different conditions to achieve a more reliable result.

- Line broadening and phase correction angle can be configured from the %% Variables cell on lines 15 and 16 if necessary

- Occasionally, integration limits may need manually adjusting to account for peak broadening, phase change, etc. These should be done via inspection of a relevant spectrum with good SNR to find the desired x-coordinate(s), and then noting the row on which the relevant ppm value appears
    + Thermal reference spectrum on line 102 (bound1, bound2)
    + HP spectrum Ha peak on line 212 (bound3, bound4)
    + HP spectrum Hb peak on line 213 (bound5, bound6)
    + T1 calculation limits line 272 (bound3, bound4)

- Experiment total duration can be configured on line 247

- Plot co-ordinates for text data on the T1 decay curve can be adjusted by changing the xpos and ypos variables on lines 322 and 323.

Program can be run as many times as necessary with variable adjustments until desired reliability is achieved.

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=====COMMON ERRORS=====
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"Unable to open file" - Attempting to read a non JCAMP-DX file in Reference/HP Data. Make sure experiment data and nothing else
is contained within these folders.

"Index exceeds number of array elements" - An integration bound is set to be higher/lower than the number of data points present
(typically 1-2048). Change the bound variable to be within this range.

"Unable to write object to file" - Attempting to save data to figure/table that is currently open in e.g. Excel/PDF viewer. Close

file(s) and attempt again.

"Error using addpath (or cd) - no such filename or directory" - Check location of additional processing functions folder (gan_func)
and/or experiment folder names for spelling errors.

D) *Turning off the individual scan capability on 1.4T Nanalysis Spectrometer:*

11. Turn off individual-scan capability! (If left on, the individual scan capability will persist, and you may fill your disk with files.) by Hitting "Ctrl+P", " – this will now disable the Pable mode; one can Hit "Alt+Tab" to double check that the Pable mode is truly OFF.

12. Disable the debug key by doing the following:

(i)   press F12 on USB keyboard connected to the device,
(ii) then type "debug", an icon will appear that the debug has been toggled OFF, Hit "Ok"

13. toggle the observe nucleus to 129Xe and then back to 1H, toggle the Lock to 2H and then back to 1H, change solvent to "Generic 1-Peak", Change experiment to "1D"; the system is now back to the same operational mode.

14. Remove the files to a computer for processing (processing is shown under B) and C) above.

15. Disable debug keys or not as desired