NMR Reservation Policy (https://nmr.unix.fas.harvard.edu/login.php)

(Effective September 6, 2015 till further notice)

Time	DD2-600	I500B, I500C	M400, M400B	I500	EPR500/MPMS	J400
11 PM 9 AM	B	B	B	B	E	B
3 PM	C	C	C	A	E	F
7 PM	C'	C'	C'	A'	E	F
11 PM	D	D	D	D	E	D

A. 10 minutes maximum time per block, reservations can be made after **12 AM** of the same day.
A' 10 minutes maximum time per block, reservations can be made after **11 AM** of the same day.
B: no limit in time per block, reservations can be made after **12 PM** of the same day.
C: **30 minutes** maximum time per block, reservations can be made after **12 AM** of the same day.
C':**30 minutes** maximum time per block, reservations can be made after **11 AM** of the same day.
D: **2 hours** maximum time per block, reservations can be made after **12 PM** of the same day.
E: ***No limit.***
F: Walk-on (30 minutes maximum per user)

GENERAL RULES

1. Every research group is entitled to have a block of time in any category.
2. No two consecutive blocks that exceed the time limit when combined may be reserved or used by the same user.
3. No one is allowed to reserve the same time on more than one instrument.
4. Maintenance (NMR) has the highest priority in all instrument times (i.e., No one should cancel reservations made by Operator).
5. Reserve only the time you are sure you need. Cancellations should be made as early as possible. Any rule violation, excessive reservation, and no-show will be noted and used as a basis for applying restrictions on the user's right of using the instruments.

Safety Measures for Working in the CCB Magnetic Resonance Laboratory (CCB MRL)

1. **The CCB MRL is restricted to only trained users and those with permission.**
2. **No one with pace maker is allowed to enter the lab.**
3. **Those people with stents, metal clips, or metal nails installed in the body should consult with the lab staff before entering the lab. (In general, it is safe if the stents have been installed for more than a year, and if any other metal devices are made of stainless steel.)**
4. **No food or drink is allowed in the lab.**
5. **Lab coat and gloves should be taken off before entering the lab.**
6. **Credit cards, cell phones, mechanical watches, ID cards should be removed from your pocket and placed on the work bench before approaching the magnet for sample exchange.**
7. **Do not carry any loose magnetic metal objects with you when approaching magnets.**
8. **When the fire alarm or the oxygen level alarm goes off, exit from the lab immediately.**
9. **Wear safety glasses when performing cryogen filling to magnets (for lab staff only).**

Sample Handling

- **Use the highest quality NMR tubes available from the stockroom.**

- **Do not dry tubes in oven. Use compressed air or nitrogen to blow-dry instead. If you really need to use oven, set the temperature to below 100 degree C and place the tubes upright in a flask. Do not lay tubes flat on the metal rack.**

- **Clean the outside wall of your sample tube with Kimwipes and alcohol before inserting it into the spinner turbine. Grab the top part of the tube so the grease and dirt on your fingers will not get onto the tube wall.**

- **Hold the spinner by grabbing its upper part. Avoid touching the lower part of the spinner and the two white reflectors on the upper part. Clean the lower part of the spinner with Kimwipes if needed.**

Instructions for Operating Varian Mercury, Unity/Inova, and Agilent DD2 NMR Spectrometers
(Draft #1)

I. Preparatory Steps

1. Log in at the RESLOG terminal by entering your code and select the instrument you want to use.

2. **Clean your fingers and the outside wall of your NMR sample tubes with Kimwipes**.

3. Move the mouse to turn on the screen, which should display the VNMRJ program.

4. Insert your own sample tube into the spinner by pushing it straight down through the o-ring without twisting or rotating the tube and adjust the tube depth with the sample depth gauge. On the Command Line, type **e** [Enter] to eject the reference sample from inside the magnet and remove it. Put the spinner with your sample in the magnet bore on top of the magnet, and type **i** [Enter] to lower the sample into the probe. (**Touch the magnet with your free hand before you take/put the sample from/onto the top of the magnet. This will discharge any static electricity you might pick up from rubbing against the chair, especially when the room air is dry**.)

5. Recall the default shim set by typing **rts** [Enter], and enter the shim filename (usually **cdcl3**), then type **load='y' su** [Enter] to load the new shim values to the shim coils. *This can also be done by clicking the following tabs:* ***Start***, ***Shims***, ***Load Default Shims*** *in the Parameter Panel.*

6. Click on the **Start, Lock,** and **Lock Scan** tabs to display the lock signal. Turn the lock off by clicking on the **Lock Off** tab. Adjust **Z0** to set the field on resonance (indicated by a cosine wave with 0 frequency, i.e. a straight step-up line). Turn the lock on by clicking on the **Lock On** tab (refer to the table in the red binder to make sure the lock power is not too high to saturate the lock signal).

The step size of all adjustment tabs can be changed by pressing the middle mouse button.

7. Shim the field by clicking on the **Shim** tab, and use the left and right mouse buttons to adjust Z1, Z2 to maximize the lock level (no need to adjust other shims like z3, z4, x, y, xz, yz, xy, etc.).

Autolocking can be done by clicking on the **Start**, **Standard**, **Find Z0** buttons. Deuterium gradient shimming can also be used to shim the field. To do this, just click on the **Start**, **Standard**, **Gradient Autoshim** tabs. It should take less than 2 minutes to finish the task. If it takes longer, you can abort it by selecting on the pull-down menu **Acquisition**, **Abort Acquisition**, and adjust the shims manually.

II. Simple 1D 1H NMR experiment

<u>Setting up Acquisition</u>

1. On the Command Line, select the experiment number by **jexp*n***, where *n* is the experiment number. (Note that the data currently in this experiment number will be overwritten by the new experiment.) Click on the **Start** tab then the **Standard** tab to select the solvent and enter sample information if needed (the data will be saved in the DATA directory using the filename entered at the end of the experiment if you use StudyQ).

2. Click on the experiment that you wish to run in the **Experiment Selector** window. Click on the **Acquire** tab then either the **Default H1** or the **Acquisition** tab to adjust the spectral width and the number of scans. You can also use the command **H1** to load default parameters for H1 NMR (command **C13** is used to load default parameters for C13 NMR).

3. Type **ga** [ret] to start the acquisition (or **go** [Enter], which will not do wft after the data acquisition is done.).

4. When the message **BS 1 Completed** is displayed, type **wft** to see the spectrum after **bs** scans of the signal are collected. Repeat this every time the message **BS n Completed** is shown to check the progress of the signal accumulation. When you see the spectrum is good enough, type **sa** or **aa** to stop the data acquisition. (Command **df** displays the accumulated FID. Command **ds** displays the processed spectrum.)

Processing Data

5. Click the **Process** tab and select any tab to display the processing parameters and make necessary changes.

6. Type **wft** [Enter] to weight the FID and transform it into spectrum.

7. . Type **aph** (or **aphx**) [Enter] to phase the spectrum. If the phase is still not right, use manual phasing: click on the **Phase Mode** icon; left click on the upfield side of the spectrum; hold the left or the right mouse button and move the mouse up and down to phase the selected peaks (left for coarse and right for fine adjustment); release the mouse button; left click on the downfield side of the spectrum; hold the left or the right mouse button and move the mouse up and down to phase these peaks. After finishing these manual phase adjustments, type **ds** [Enter].

8. Spectral Referencing: You do not need to do spectral referencing if you have selected the correct solvent file. However, if you still need to do it, click the left mouse button when the cursor is moved to near the peak to be assigned, type **nl** [Enter] to set the red vertical cursor line right on top the peak, type **rl(#p)** to assign the chemical shift for this peak, where **#** is the chemical shift in ppm. Command **dscale** or clicking the **Show/Hide Axis** icon displays the chemical shift scale for the spectrum.

Integration

9. Type **cz** [Enter] first to clear previously defined integral regions. Click the **Show Integral Regions** icon. Two new icons will appear to allow you select regions and adjust Lvl/Tlt of the integrals. To define new regions, click the **Define Integral Region** icon, then use the left mouse button to click on any place on the integral line to cut the line (the right mouse button can be used to undo the previous cut).

10. To adjust the baselines of the integrals, click the Integral **Lvl/Tlt** icon, move the cursor to the up-field region of the spectrum and click the left mouse button, press and hold the left or right mouse button and move the mouse up and down to adjust the level (**Lvl**); then move the cursor to the down-field region, click and hold the left mouse button and move the mouse up and down to adjust the tilt (**Tlt**). Type **isadj** to set the largest integral to full scale. Place the left cursor line onto the integral that you want to define its value, and click **Process, Integration** tabs**,** select **Single Peak** and change the **Integral Area** to a value representing the number of protons included in this integral region, then click the **Set Integral Value** tab.

11. Command **dpir** (or **dpirn**) displays the integral values on the screen.

Baseline Correction

12. If the spectral baseline shows linear drift, use command **dc** to correct it. To correct more irregularly uneven spectral baseline, command **bc** can be used after the spectral integration reset points are selected (as performed in the following step). You can also try the command string: **region bc cz**. (These can be done by clicking **Process**, **Default**, and any of the three tabs in the **Baseline Correct** category.

Plotting Spectrum

13. Use the two red cursor lines (controlled by the left and the right mouse buttons, or the parameters **cr**, for the left cursor, and **delta**, for the distance between the two cursors) to define the plot limits, and click on the **Expand** button. The middle mouse button is used to adjust the amplitude of the spectrum. You can also use parameters **sp** and **wp** to define the plot limits (**sp** is the high-field end, and **wp** is the width of plot, e.g., **sp=-0.5p wp=10p** covers the region from –0.5 ppm to 9.5 ppm).

14. Type **plot** to plot the spectrum. This is similar to typing a string of commands **pl, pscale, ppa** (or **pap**)**, pir, page**). You can also click on the **Process**, **Plot**, **Plot Spectrum, Plot Spectrum Scale, Plot Parameters, , Plot Page buttons.** (By clicking on the **Plot Preview** button in this sub-window, a pdf page with all the signals selected to plot will be displayed, and can be saved for publication purpose.)

Printing Peaks

15. Click on the **Show/Hide Threshold** icon to display a yellow threshold line, use the left mouse button to adjust its position so all the peaks to be printed are above this line.

16. Type **pll page** to print the peaks that are higher than the threshold line. You can also plot the chemical shifts of the peaks on the plotted spectrum by including the command **ppf** in the string of plotting commands (Command **dpf** displays these chemical shifts with the spectrum on the screen.)

(These can also be done by clicking the appropriate tabs by selecting **Process**, **Plot** tabs.)

Saving Data

17. Use the pull-down menu **File**, **Save As…** or the **Save As…** icon to save your data in your own folder.

You can create your own directory within your group directory by clicking on the File **Open …** icon, selecting your group folder and clicking the **Create New Folder** icon.

*You can also run experiments by using the **Study Queue** feather of the VNMJ.*

1. *Click on the **New Study** tab, then click on all experiments you wish to run on the sample in the magnet from the **Experiment Selector** window.*
2. *For each experiment, move the mouse cursor onto the experiment name in the **Study Queue** window, click the right mouse button and select **Open Experiment**. You can then adjust the parameters you wish to set up by clicking on appropriate tabs.*
3. *When all parameters are set correctly, click on **Submit** button. All the experiments will be carried out in the order entered.*

III. Advanced 1D NMR Experiments

A. Experiments with arrayed parameters

1. Enter more than two values, separated by a comma, for the parameter to be arrayed. E.g. typing pad=0,600,600,600,600 will set up five experiments using 0 sec, 600 sec, 600 sec, 600sec, 600sec for pad, respectively. This will run five spectra with 10 minutes delay between each spectrum - a convenient way to monitor the chemical reactions in the solution.

2. Alternatively, type **array('parameter',#steps,starting,increment)** or just type **array**, and you will be prompted for the four arguments.
3. All FIDs can be transformed into spectra together by **wft**.
4. Command **dssa** displays all spectra in stacked form with offsets determined by **ho** (horizontal offset) and **vo** (vertical offset).
5. Command **dssh** displays all spectra horizontally.
6. Use **pl(start,finish,step)** or **plarray** to plot the spectra.

B. APT (Attached Proton Test) Experiment

1. Run a regular C13 spectrum, if time permits.
2. In the Experiment Selector, select Std 1D, then (C)APT to load parameters.
3. Adjust **One Bond XH Coupling** in Acquire, Pulse Sequence group, and **nt** (multiple of 4) if needed.
4. Type **ga** or click on the light-green **Acquire** button to start. Phase the spectrum so that CHn peaks are up and CHn+1 peaks are down or vice versa.

C. DEPT (Distortionless Enhancement by Polarization Transfer) Experiments

1. Run a regular 1D C13 spectrum if time permits.
2. Type **DEPT** or select **(C)DEPT** button in the Experiment Selector group to load proper parameters.
3. Change **One Bond Coupling**, **nt** (multiple of 16), and **d1** (relaxation delay for protons) if needed.
4. Choose appropriate experiment in the **Multiplicity Selection** group
5. Type **ga** or click on the light-green **Acquire** button to start the experiment.
6. Type **adept dssa** to analyze the spectra, and **pldept** to plot them.
   The full analysis of the DEPT result can also be done by clicking on the **Process** button in the Process, Basic group.
   (If the DEPT135 is run, only one spectrum is obtained and no need to do step 6.)

D. NOESY1D (Gradient-enhanced 1D NOE Experiment) (can only be done on the 500s and the 600)

1. Take a normal 1D 1H spectrum. Save it in the other experiment number by **mf(n,m).**
2. Type **NOESY1D** [Enter] or select **(H)NOESY1D** in **Common** group or **NOESY1D** in **Liquids**, **(H)Sel1D** group to load relevant parameters.
3. Use the two cursors to enclose the peak region to be irradiated (inverted), and click on the **Select** button, which is in the **Acquire**, **Defaults** window.
4. Adjust **nt** and **mix** (NOE Mixing Time, typically 0.5 sec) if needed, and click the **Acquire** button to start the experiment.

E. TOCSY1D (can only be done on the 500s and the 600)

1. Take a normal 1D 1H spectrum.
2. Type **TOCSY1D** [Enter] or select **(H)TOCSY1D** in **Common** group or **TOCSY1D** in **Liquids**, **(H)Sel1D** group to load relevant parameters.
3. Use the two cursors to enclose the peak region to be irradiated (inverted), and click on the **Select** button, which is in the **Acquire**, **Defaults** window.

4. Adjust **nt** and **mix** (Spinlock Time, typically 0.5 sec) if needed, and click the **Acquire** button to start the experiment.

VI. Proton 2D gCOSY experiment

1. In exp*n* (*n* =1-9), take a simple 1D H1 spectrum. Use the two cursor lines to select a narrower spectral region with all the peaks included; then, type the command **movesw** to fix the new spectral window. Recollect a 1D H1 spectrum to make sure the new region is properly selected.

2. Move to the next experiment number by **jexp*m*** (*m*=*n*+1). If exp*m* is not existent, type **cexp(*m*)** to create it.

3. Type **mp(*n,m*)** [Enter] to copy the parameters in exp*n* to exp*m*. Go to Experiment Selector table and click the **(HH)gCOS**Y tab to load all parameters used in the gCOSY experiment. Type **su** [Enter].

4. Check the total time needed for the experiment by typing **time** [Enter]. Adjust **nt**, if needed, then start the experiment by typing **go** [Enter].

Processing Data

5. The spectrum can be examined before the data acquisition is actually finished if **proc1** is set to **ft** (if **proc1** is set to **lp**, you can temporarily change it to **ft**, and change it back to **lp** after the data acquisition is finished). To generate the spectrum, type **wft2d** [Enter], or click on the **Autoprocess** button. The resultant 2D spectrum will be displayed. (Command **dconi** will re-display the 2D spectrum.)

6. The amplitude of the spectrum can be adjusted with the middle mouse button in two ways: (1) by moving the mouse cursor to the spectral region and click the middle mouse button, (2) by clicking on the blue (increasing 20%) or red (decreasing 20%) arrow icon on the right side of the spectral region.

7. The expansion of the spectrum can be done by defining the lower left and the upper right corners of the selected region with the left and the right mouse buttons followed by clicking the **Zoom In** icon.

Plotting the Spectrum

8. To plot the 2D spectrum displayed, type **plcosy('pos',*i,j,k*)** [Enter], where *i* is the number of contour levels to be plotted (usually >10), *j* is the intensity ratio of every adjacent levels (usually between 1.2 and 1.5), and *k* is the experiment number where the 1D proton NMR spectrum is stored and is to be used as the projection in both F1 and F2 axes.

VII. Other 2D NMR Experiments

In general, if there is a macro file for the experiment to be performed, you can simply start by clicking on the icon corresponding to the experiment (e.g. gHSQC, gHMQC, gHMBC, NOESY, TOCSY, ROESY, etc.) after running a regular 1D spectrum with desired parameters. To display the pulse sequence of this experiment, type **dps** [Enter].

By default, **gHMQC** and **gHSQC** will set up H/C hmqc and hsqc experiments with **sw1** set to cover about 0-160 ppm; and **gHMBC** will set sw1 to 0-220 ppm.

By default, **NOESY** will set up the experiment with mixing time **mix**=0.5, which can be changed according to the molecular size to optimize the NOE cross peak intensity.

Some 2D experiments (e.g. gHSQC, gHMQC, NOESY, TOCSY, ROESY) generate phase-sensitive spectra (you can tell from the parameter **phase**: if **phase** is **arrayed**, it is phase sensitive, if **phase** is **1**, it is magnitude mode), which need **wft2da** (instead of **wft2d**) command to transform into spectra, and these spectra often require phase corrections. The following is one convenient way to do it:

Phase Correction of Phase-sensitive 2D Spectra (e.g. NOESY, gHSQC, gHMQC, TOCSY, ROESY)

1. Display the 2D spectrum with command **dconi**.
2. Use the left mouse button to select a row across a large peak on the upper right corner.
3. Type **ds** to display this selected row and phase the peak with only the 0^{th} order phasing parameter.
4. Type **dconi**, and select another trace across a peak on the lower left corner of the 2D spectrum.
5. Type **ds** to display the second row selected. Phase this new peak with only the 1^{st} order phase correction (do not change the 0^{th} order phase). – This is done by clicking on the **Phase** button, left-clicking on the peak region selected in step 3 above immediately followed by left-clicking on the region containing the new peak and phase this new peak.)
6. Type **dconi** and see if all the rows are phased.
7. To phase columns, rotate the 2D matrix by clicking on the **Rotate** icon; then follow the above steps to phase the new rows.

- If you cannot perform phase correction, check the parameter **pmode**, it should be set to **full**.
- For NOESY spectrum of small molecules, always phase the diagonal peaks to negative so that the NOE cross peaks are positive.

Reference Assignment of Heteronuclear 2D spectra (e.g. gHSQC, gHMQC, gHMBC,)

1. Select a peak whose chemical shifts on both axes are known by using the left mouse cursor.
2. Type **rl(*m*p) rl*1*(*n*d)**, where ***m*** is the chemical shift of the peak on the F2 axis and ***n*** is that on the F1 axis. Note that **d** has to be typed after ***n***. You can also go to **Process, Display** group and enter appropriate chemical shift values in **Referencing F1** and **F2** blanks for the cursor position.

To plot the heteronuclear 2D spectrum, use the command **plhxcor** instead of **plcosy**:

plhxcor('pos|neg',*i,j,k,l*)

where the first argument is used to select either positive or negative peaks (or both if ignored), ***i*** is the number of contour levels (e.g. 20), ***j*** is the intensity ratio of adjacent levels (e.g. 1.3), ***k*** is the exp # with the H1 spectrum for plotting the F2 projection, and ***l*** is the exp # with the X spectrum for plotting the F1 projection (setting ***k*** or ***l*** to -1 suppresses the projection).

To plot the actual projections of the 2D spectrum, go to **Process**, **Plot** tabs, click on **Projection Horiz Plot** and/or **Vert Plot** tabs, then use the command **pcon('pos|neg',*i,j*)** and **page** to plot the 2D spectrum. Or, you can click on any icon for plotting that particular signal in the **Process**, **Plot** group, ended with **Plot Page** (to plot the spectrum on the local printer) or **Plot Preview** (to be the spectrum in pdf format, which can be saved and used for publication purpose).

Connecting to NMR Data Storage- Windows:

Note: If you are using a wireless network connection, it will be necessary to download, install and run VPN before connecting to the server. It is available for download from the HUIT download web site. VPN is not needed for a wired connection.

1. Request a Harvard RC computing account from: https://account.rc.fas.harvard.edu/request
2. Click on the Windows start logo on lower left hand corner of screen.
3. Click on “Computer" or "My Computer”.
4. a. Windows 7: Select “map network drive” on the top of the screen.
   b. Windows XP: Click on the “tools” menu and select “map network drive”.
5. In the “drive” list select a drive letter (you can choose any available letter).
6. In the "Folder” box type: \\nmrfs.rc.fas.harvard.edu\nmr_small\nmrdata
(To connect every time you log on to your computer, check the “Reconnect at logon” box).
7. Click “Finish”.
8. When prompted, please type in your “rc” username and password. Your username should be entered as: rc\(your username)
(NOTE: click on “Remember my Credentials” if you want your username and password to automatically be entered every time you log on).
9. NMR data should appear on the "drive" that you selected in step #5.

Connecting to NMR Data Storage- MAC OS X

Note: If you are using a wireless network connection, it will be necessary to download, install and run VPN before connecting to the server. It is available for download from the HUIT download web site. VPN is not needed for a wired connection.

1. Request a Harvard RC computing account from: https://account.rc.fas.harvard.edu/request
2. Minimize all open windows and single-click anywhere on the background to make sure ‘Finder’ is the active application on the top left of the screen.
3. Once you see ‘Finder’ in the top right corner, click the ‘Go’ button. Scroll down and click ‘Connect to Server’.
4. In the "Server Address" box type in:
smb://nmrfs.rc.fas.harvard.edu/nmr_small/nmrdata and hit the "Connect" button".
5. Enter the username and password in the next box that you received from RC computing, and hit the "Connect" button (be sure to use the exact username given to you by Harvard RC computing. If a different name appears, delete it and use the RC username). Your username should be entered as: rc\(your username)
6. NMR data should now appear on your desktop.
7. Now to allow your computer to automatically connect to the NMR data every time you log in to the computer, click the system preferences icon on the dock at the bottom of your screen.
8. In the system preferences menu click on “**Users and Groups**” or "Accounts"
9. In the “**Users and Groups**” window click “**Login Items**”. (Make sure that the lock on the bottom left hand corner is unlocked. If it is locked, click it, and input your credentials).
10. Locate the drive you want to connect at login on your desktop, left click and drag into the window that says “**These items will open automatically when you log in**”.
(Step 7- 10 may vary slightly by OS version).

(~/nmr_large/eprdata for EPR data; ~/nmr_large/MPMS for MPMS data.)