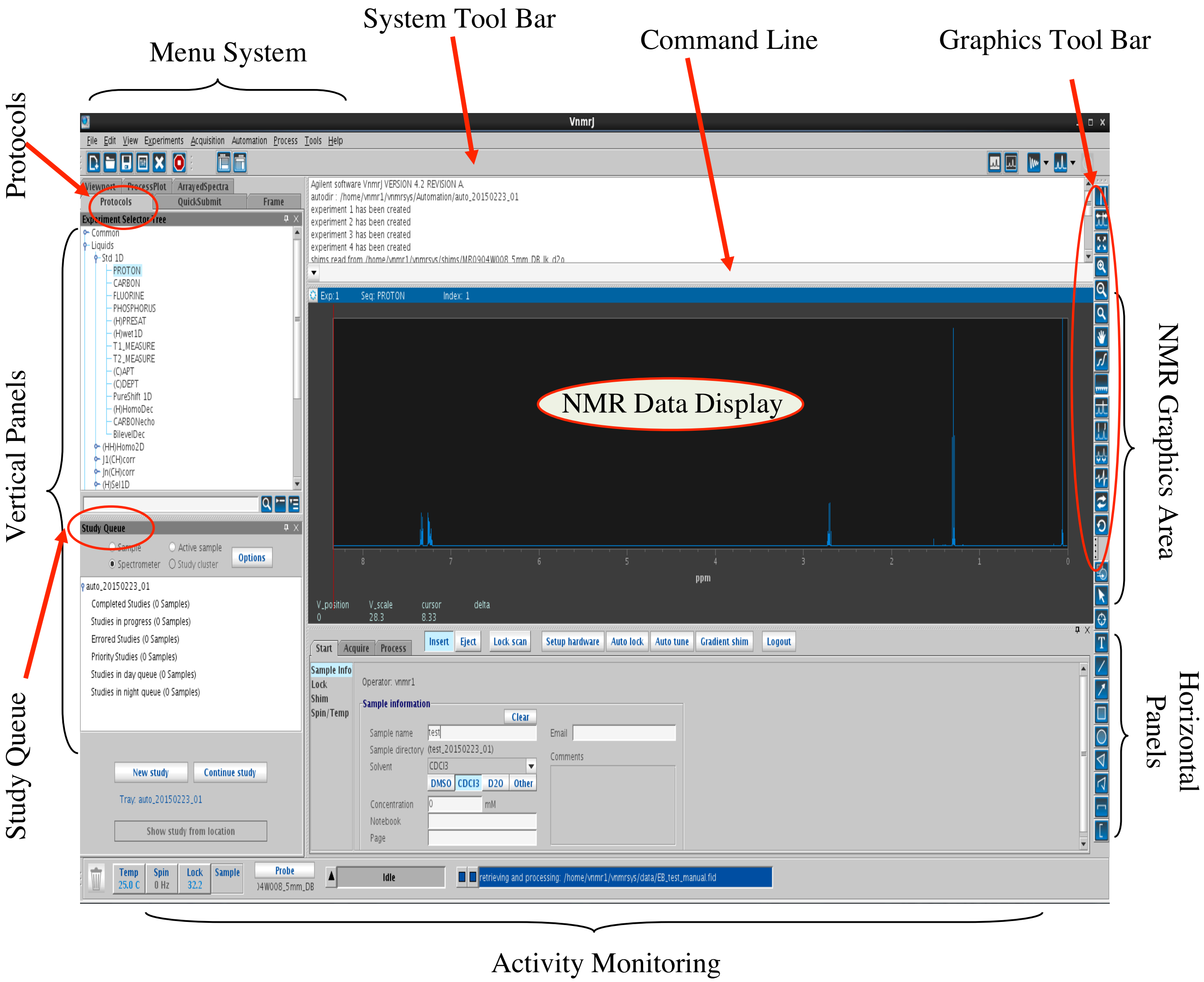
MRL Spectroscopy Facility



```
Agilent software VnmrJ VERSION 4.2 REVISION A.
autodir : /home/vnmr1/vnmrsys/Automation/auto_20150223_01
experiment 1 has been created
experiment 2 has been created
experiment 3 has been created
experiment 4 has been created
shims read from /home/vnmr1/vnmrsys/shims/MR0904W008_5mm_DB_lk_d2o
```

V_position 0 V_scale 28.3 cursor 8.33 delta

Operator: vnmr1

Sample name: test
Sample directory: (test_20150223_01)
Solvent: CDCl3
Concentration: 0 mM
Notebook:
Page:
Email:
Comments:

Temp 25.0 C Spin 0 Hz Lock 32.2 Probe)4W008_5mm_DB

retrieving and processing: /home/vnmr1/vnmrsys/data/EB_test_manual.fid

Varian Solution NMR Automation Procedure

(VNMRS Machines running VNMRJ 4.2 under Red Hat Enterprise 5.1)

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Materials Research Laboratory, UCSB

Version 1.1, Last Modified Wednesday, Oct. 10th, 2016

Safety Issues

- ⚠ If you, or people working with you, have magnetic metal implants, please consult your doctor for possible effect of magnetic field;
- ⚠ For those who have pacemakers, please stay away from NMR magnets;
- ⚠ Remove from your pocket anything ferromagnetic or vulnerable to magnetic field:
    - ➢ Your wallet, bank cards, credit cards, and any cards with magnetic stripes;
    - ➢ Electronics: cell phone, mp3, ipod, etc.;
    - ➢ Mechanic watches;
    - ➢ Keys and other magnetic items.

Table of Contents

I. Sample preparation

1. NMR tubes of **5mm** in OD and **7”** long are used and available from:
   i. Chemistry Department Stockroom, UCSB (Phone: x2107)
   ii. Aldrich
   iii. Wilmad
   iv. Newera

   Please get tubes for 600MHz or higher (500MHz grade tubes will work).

2. Samples are dissolved in **deuterated** solvents (~0.1 mM and 50 mM in concentration for ^{1}H and ^{13}C, respectively, and at least 0.6 mL in volume or 56 mm in height) for three purposes:
   i. Deuteration removes solvent ^{1}H signals which would otherwise dominate the ^{1}H spectrum.
   ii. Deuterons provides a lock signal.

   Lock is a deuterium NMR process that the spectrometer uses to prevent the magnetic field from changing during the course of NMR experiments, thus locking the spectrometer.

   iii. Deuterons provide chemical shift reference for NMR spectra, rendering addition of reference standards such as TMS unnecessary.

Label your samples with your name and your advisor’s name. This helps us take care of unknown samples.

II. Sign onto Logsheet

Enter

1. your name
2. your advisor’s name and department
3. your recharge account number (in the format: 8-4xxxxx-xxxxx-3)
4. your start time
5. (**Do this at the end of experiment:** your stop time and duration of experiment)
6. (**Do this at the end of experiment:** Status of instrument and report problems if any)

III. Start VNMRJ Software

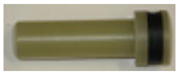
1. Make sure that the spectrometer is idle by looking at the computer. If yes, proceed to Step 2 below (if no, either wait, talk to the user, or do something else)
2. Login into your NMR account:

   Type your username, hit the Enter key

Type your password, hit the Enter key again

3. Double click on the VNMRJ icon on the desktop, the last VNMRJ layout from your previous login session will appear.

IV. Preparation: Load Samples into Auto-Sampler

1. Put your sample in a green spinner , measure depth with the golden depth gauge  or the rack for spinners, and clean the bottom half of the sample tube with a napkin while holding the top half of the tube;

Spinner

Sample should cover the opening, min. 0.7ml

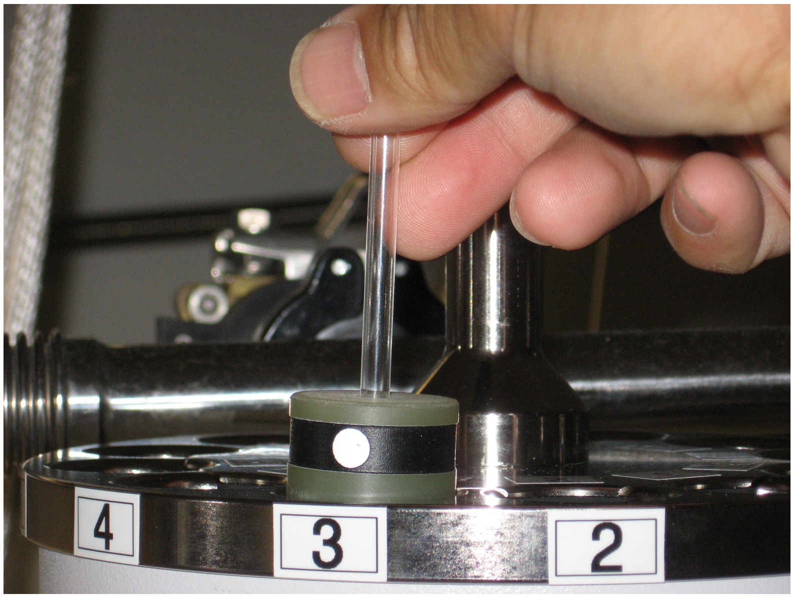
2. (Important information) Slot 12, aligned with the yellow triangle label at the bottom front of the sampler, is reserved for the standard idle sample (pure $CDCl_3$). Don't load any sample into this slot.

   The standard idle sample will be loaded into the magnet **automatically** at the end of an automation run.

Front View

Top View

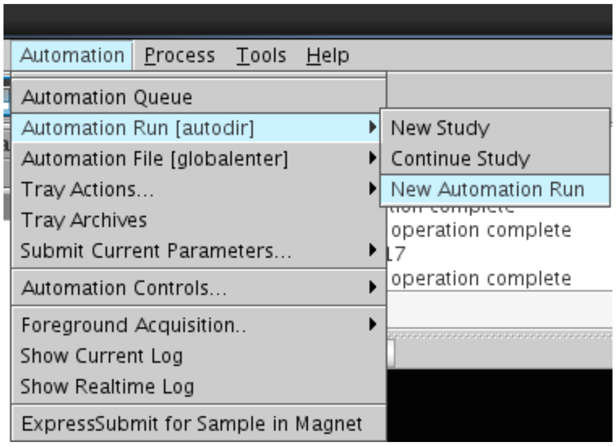
3. Go to the magnet and load your samples onto the sampler in the clockwise order: the 1st sample goes to slot 1, the 2nd to 2, the 3rd to 3, and so on.



⚠ Be very careful to align the sample tube with the slot holes when removing or loading samples.

V. 1D ^{1}H and ^{13}C NMR Setup and Data Acquisition

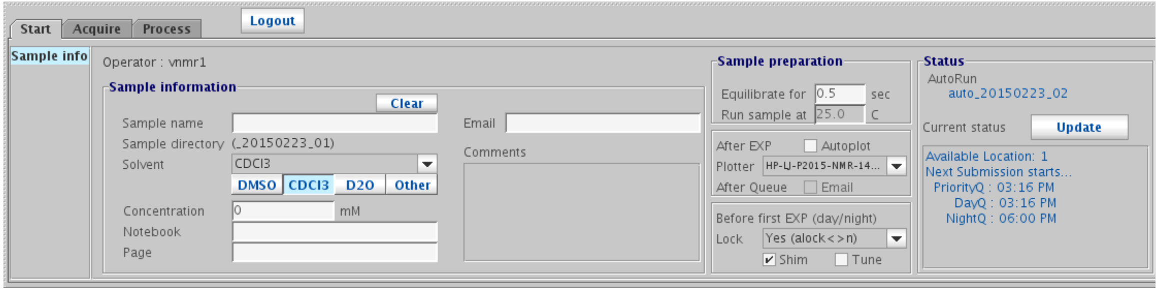
1. Choose New automation run from the Automation menu to create a new automation session.



2. Click on New study in the Study Queue panel to go to the "Submit mode". You will create ONE "New Study" for every sample you have.

3. In the Horizontal Panel, click Start tab to show the "Sample Info" page below.

Start → Sample Info page



Operator : vnmr1

Sample directory (_20150223_01)

Solvent CDCl3

Concentration 0 mM

Equilibrate for 0.5 sec

Run sample at 25.0 C

Plotter HP-LJ-P2015-NMR-14...

Before first EXP (day/night)

Lock Yes (alock<>n)

☑ Shim ☐ Tune

AutoRun auto_20150223_02

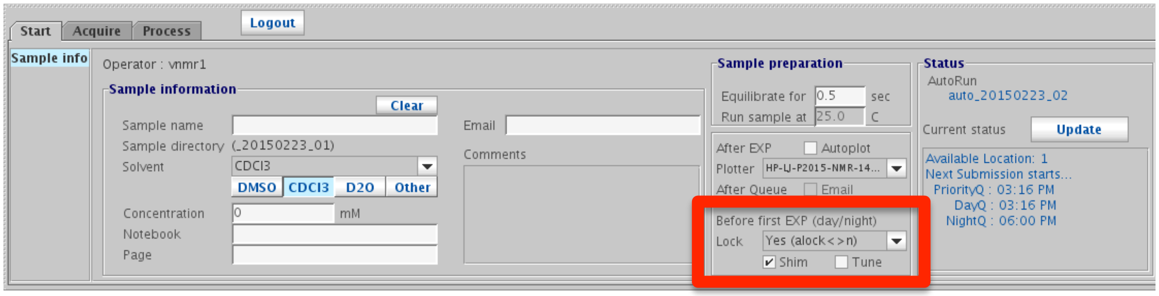
Current status

Available Location: 1
Next Submission starts...
PriorityQ : 03:16 PM
DayQ : 03:16 PM
NightQ : 06:00 PM

☐

- Fill out the Sample name box with a meaningful name for the sample (Letters and Numbers ONLY plus underscore _. No spaces and special characters).

- Choose the Solvent Solvent CDCl3 ▾ DMSO CDCl3 D2O Other your sample is using.
- The rest of the text boxes are optional.
- **Tune the probe**:
    - **When to check the ☐ Tune box:**
        - When aqueous solvents are used, or
        - When running any other nuclei than ^{1}H, or
        - When salt concentration is high, or
        - When running variable temperature exps



Operator : vnmr1

Sample information

Sample name

Sample directory (_20150223_01)

Solvent CDCl3 ▾ DMSO CDCl3 D2O Other

Concentration 0 mM

Notebook

Page

Email

Comments

Sample preparation

Equilibrate for 0.5 sec

Run sample at 25.0 C

After EXP ☐ Autoplot

Plotter HP-LJ-P2015-NMR-14... ▾

After Queue ☐ Email

Before first EXP (day/night)

Lock Yes (alock<>n) ▾

☑ Shim ☐ Tune

Status

AutoRun

auto_20150223_02

Current status

Available Location: 1

Next Submission starts...

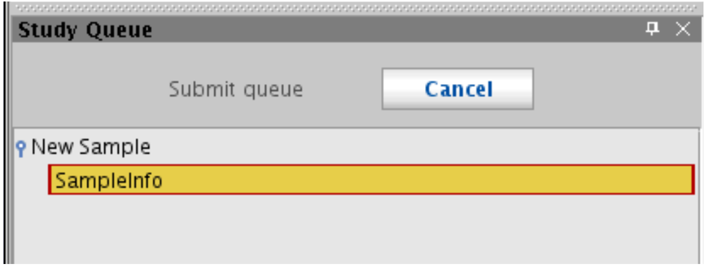
PriorityQ : 03:16 PM

DayQ : 03:16 PM

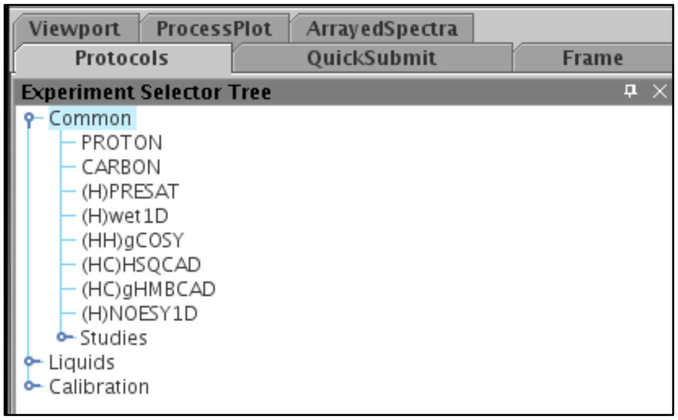
NightQ : 06:00 PM

If you would like to do NMR on ^{13}C, ^{11}B, ^{19}F, ^{29}Si, ^{31}P, etc. please come talk to Shamon (CNSI Room 1528) for complete instruction.

If you want to change the information on the **Start → Sample Info** page later on, click on SampleInfo in the Study Queue panel:

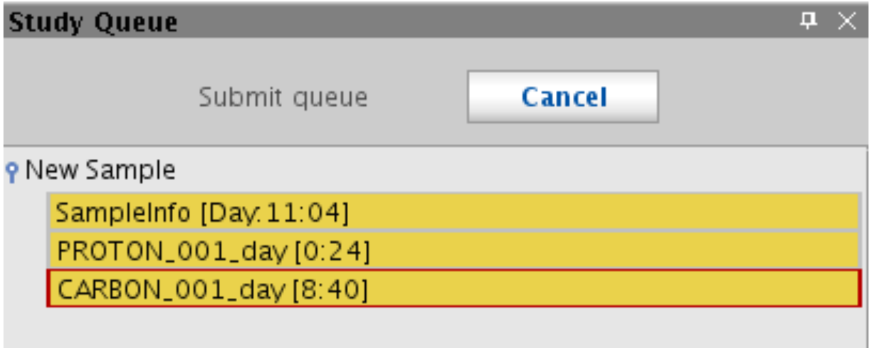


4. Select experiments with double-clicks from the lists under **Experiment Selector** in the vertical panel of VNMRJ window. To add NMR experiments to the **Study Queue**, double-click the desired experiments.



To delete an experiment from the Study Queue, right-click and select DELETE.

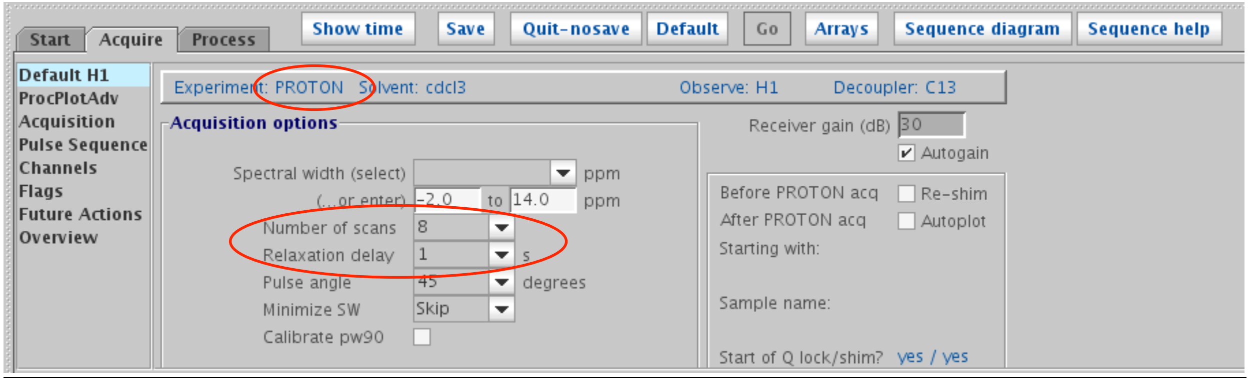
If other 1D NMR's and 2D NMR's are to be run, do basically the same thing.



5. Double click on node PROTON_001_day [0:24] in the **Study Queue** to load parameters for ^{1}H. If successful, you will see a new graph in the NMR Data Display area and the pages associated with the Acquire tab in the Horizontal Panel, as shown below.

   Change Number of scans and Relaxation delay as necessary by clicking on the Acquire tab and then on Default H1.

Acquire tab → Default H1 section



Start | Acquire | Process

Show time | Save | Quit-nosave | Default | Go | Arrays | Sequence diagram | Sequence help

Default H1
ProcPlotAdv
Acquisition
Pulse Sequence
Channels
Flags
Future Actions
Overview

Experiment: PROTON Solvent: cdcl3 Observe: H1 Decoupler: C13

Acquisition options

Spectral width (select) ppm
(...or enter) -2.0 to 14.0 ppm
Number of scans 8
Relaxation delay 1 s
Pulse angle 45 degrees
Minimize SW Skip
Calibrate pw90 ☐

Receiver gain (dB) 30
☑ Autogain

Before PROTON acq ☐ Re-shim
After PROTON acq ☐ Autoplot
Starting with:

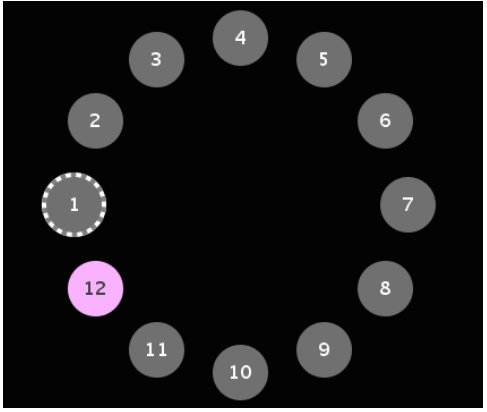
Sample name:

Start of Q lock/shim? yes / yes

6. Do the same for ^{13}C: double click on node CARBON_001_day [8:40] in the Study Queue to load parameters for ^{13}C.

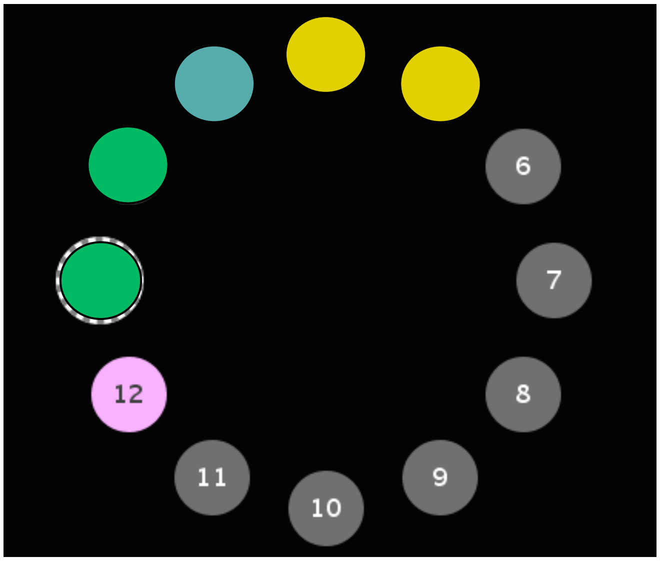
7. In the Horizontal Panel, click on the Acquire tab and then on Default C13 to show the default ^{13}C parameters. Change Number of scans and Relaxation delay as necessary.

8. Click on ⁙ at the top left corner of the Graphics Display area and then on the numbered circle where the 1st sample is located (slot 1) in the tray display to have it selected: (ℹ: ⁙ and X toggles the tray display and NMR data display)



9. Click on Submit to start data acquisition. The auto-sampler will start changing samples by lifting up the CDCl3 standard from the magnet, rotating counter-clockwise, and loading the sample in the 1st slot to the magnet. Followed by auto-tune, auto-lock, gradient-shim, and data acquisition.

ℹ Color codes in the tray panel:



🔵 Sample Active

🟡 Sample in Queue

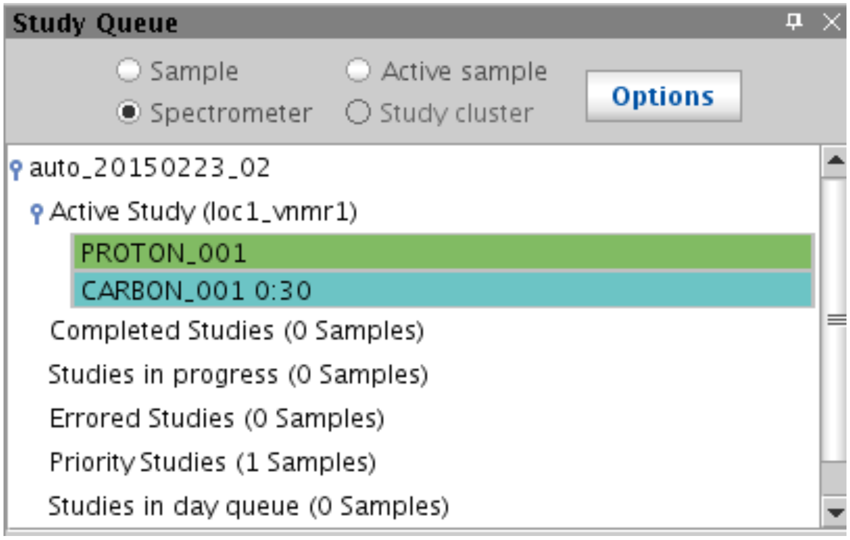
🟢 Sample Finished

🔴 Sample with Error

- ℹ To stop a running experiment and move on to the next one in the queue, use Stop-Save-Resume or Stop-Discard-Resume under the Automation → Automation Controls menu.
- ℹ To abort a whole automation run, use Abort Automation and then Resume Automation (or the machine will hang in the automation and nobody can use it) under the Automation → Automation Controls menu.

10. While waiting for all experiments for Sample 1 to finish, you may setup the experiments for Samples 2, 3, etc. by clicking on [New study] as necessary and then repeating steps 3 to 11 above to create a new Study Queue for a new sample.

11. To view progress of experiments or process the finished experiments, look in the Study Queue window and make sure "Spectrometer" is checked:



auto_20150223_02
Active Study (loc1_vnmr1)
PROTON_001
CARBON_001 0:30
Completed Studies (0 Samples)
Studies in progress (0 Samples)
Errored Studies (0 Samples)
Priority Studies (1 Samples)
Studies in day queue (0 Samples)

ℹ Same color codes as in the tray display: 🟩 finished, 🟦 running, and 🟨 waiting.

ℹ You don't have to wait until the whole Study Queue (sample) finishes in order to process data. For example, you can process the Proton data while waiting for Carbon to end by double clicking the Proton node PROTON_001.

ℹ To toggle between the normal graphics display and the tray display, click on ◌ or X near the top-left corner of the graphics display area.

14. At the end of the Automation Run, the sample tray will rotate multiple times to Sample Position #12. It will load the deuterated solvent sample into the magnet **automatically**.

VI. Finishing up

YOU ARE NOT FINISHED WITH THE SPECTROMETER UNTIL YOU DO THE FOLLOWING.

a. Set solvent to $CDCl_3$ on the **Start → Standard** page as shown:

b. Click on Find z0 on the **Start → Lock** page and wait for finish:

Start → Lock

c. Exit VNMRJ by clicking the X in the upper right of the screen.

d. Log off your account: in the bottom left corner of screen click on the logout button .

and hit Log Out in the popped up window.

e. Important: On the logsheet, record your stop and duration times, and the spectrometer status. Report problems if any.

f. Remove your samples from the lab and clean the space you have used.

VII. Data Processing Workstation

- Please go to NMR Processing Room (CNSI Room 1522) and use the computer titled “Varian NMR Data Processing”

1. Login
    a. Type “vnmrp” as a username and input password “4epp967”

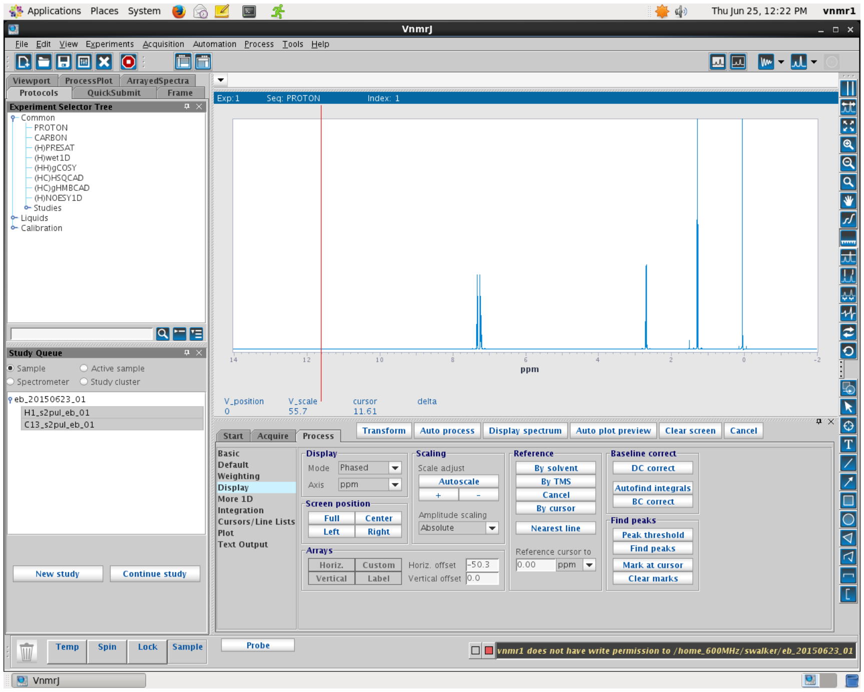
2. Click “New Home_600MHz” icon on the desktop to access files

3. Open “VNMRJ” software on desktop

VIII. NMR Data Processing

1) Load your NMR file:

Choose “Open” → Your username → VNMRsys → Data → Your experiment → Open the .FID file (Not the folder!)



V_position 0
V_scale 55.7
cursor 11.61
delta

Mode: Phased
Axis: ppm
Amplitude scaling: Absolute
Horiz. offset: -50.3
Vertical offset: 0.0
Reference cursor to: 0.00 ppm

vnmr1 does not have write permission to /home_600MHz/swalker/eb_20150623_01

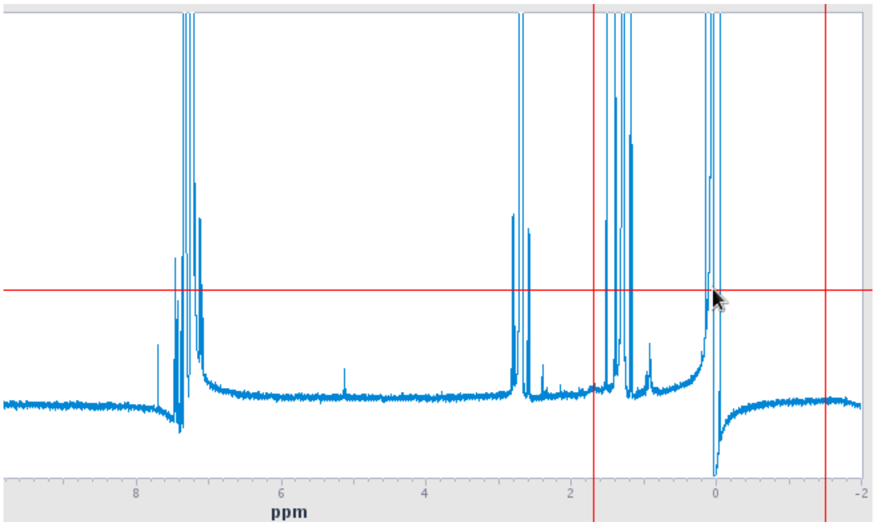
2) Click on near the upper right corner of VNMRJ 4.2 to display the Spectrum and Spectrum Tool Bar. (Optional: you can click on near the upper right corner to view the FID and the FID Tool Bar).

- Toggle 1 or 2 cursors
- Show Full Spectrum
- Reset to Full Display
- ZOOM IN
- ZOOM OUT
- ZOOM Mode
- Move Spectrum
- Integrate
- Show/Hid Axis
- Peal Threshold
- Find Peaks
- Display Integrals
- Phase Mode
- Redraw
- Return

3) Phase the spectrum:

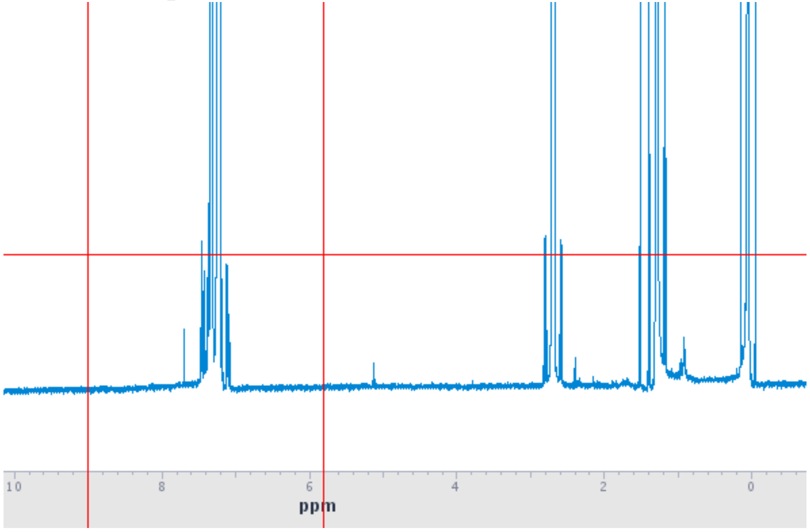
   a) To better see the phase, scale up the spectrum vertically using Autoscale + − on the **Process/Display** page. + and − are scaling up and down vertically.

   b) Click on in Graphics Tool Bar and then on the rightmost region of peaks on the spectrum. The region (~2 ppm wide) will be marked with two vertical red lines and one horizontal red line as below:



c) Place the mouse cursor on the horizontal red line between the two vertical lines, click-and-hold the right or left mouse button and move the mouse back and forth for fine or coarse phase adjustment, respectively, of the phase for the rightmost peak(s).

d) Once the peak(s) are phased, click on the leftmost region of peaks (~2 ppm wide) and use the right or the left mouse button for phase adjustment of the leftmost peak(s).



e) Repeat steps b) to d) if necessary.

f) After phasing, click on 🔄 in the Graphics Tool Bar to refresh spectrum display and on Autoscale on the **Process/Display** page to normalize the spectrum if necessary.

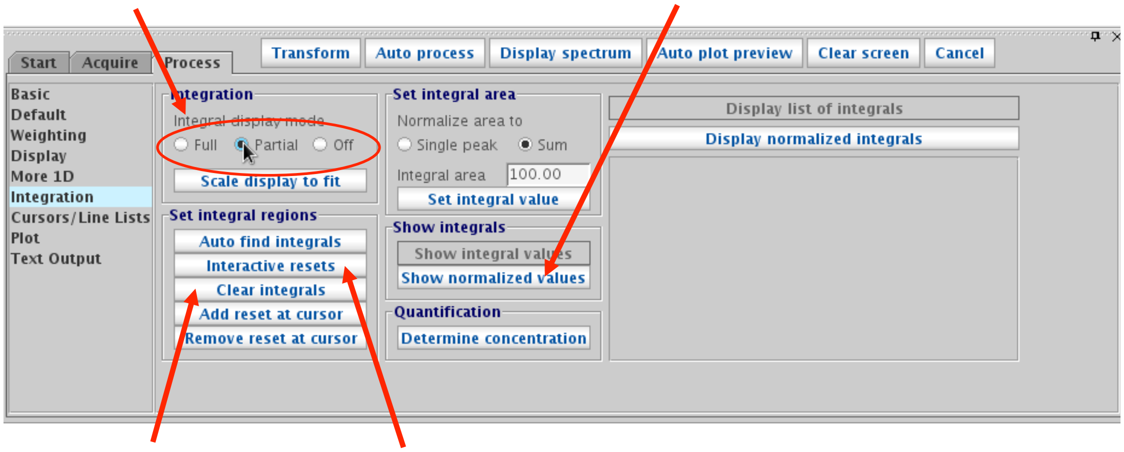
4) Baseline correction for accurate integration: on the **Process/Display** section, click on **DC correct** to correct the baseline DC offset and then on **BC correct** to get rid of the curvature or wobbling of the baseline.

5) Integration: click on Integration to go to the **Process/Integration** page:

<u>Process/Integration page</u>

Step a

Step d

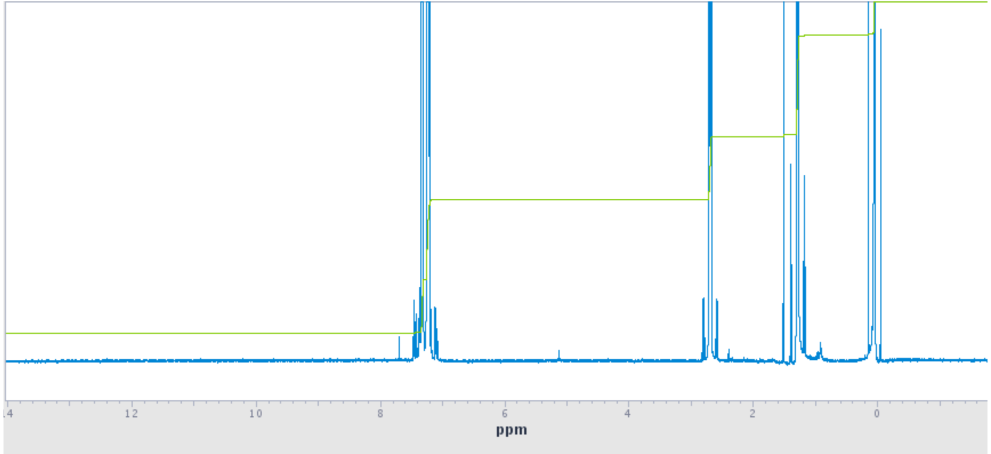


Step b

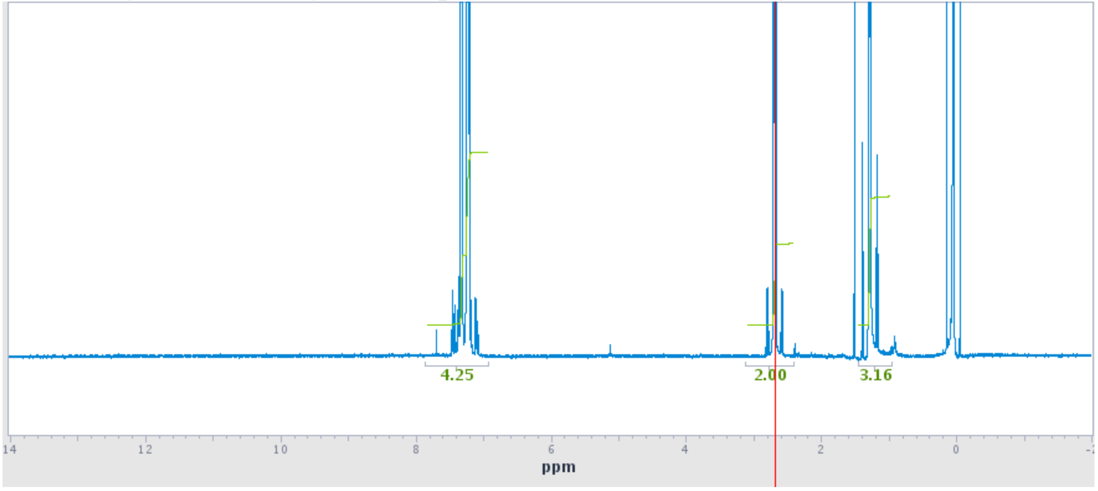
Step c

a. Make sure Integral display mode ○ Full ● Partial ○ Off is properly checked.

b. Click on **Clear integrals** to clear automatic integrals and all peaks are integrated as a whole:



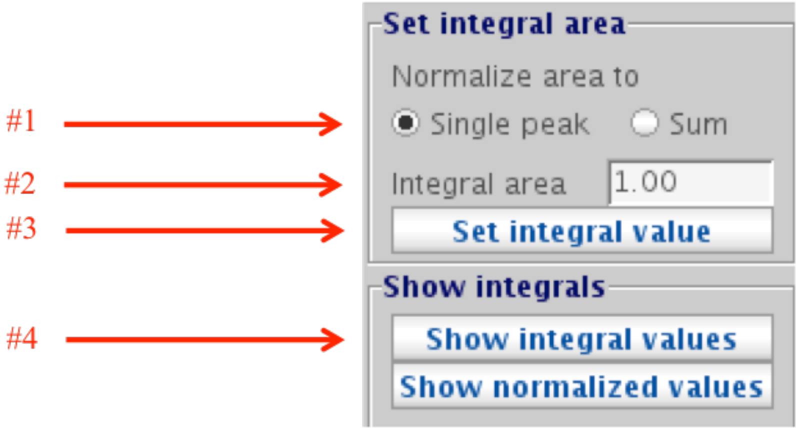
c. Click on Interactive resets and left-click on both sides of a region to be integrated. Repeat left-clicks on other regions of interest.



d. To show integrals on spectrum, click on Show normalized values.

To calibrate the integrals to a single peak:

- Go to activate the single-cursor mode by clicking on in the Graphics Tool Bar so that you see a vertical red line on the spectrum. Place the cursor over the peak you want to calibrate.



Set integral area

Normalize area to

● Single peak ○ Sum

Integral area 1.00

Set integral value

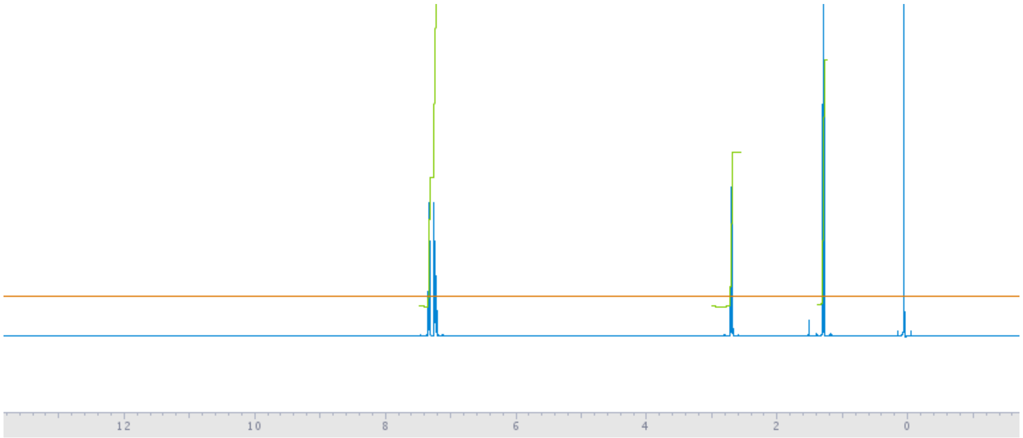
Show integrals

Show integral values

Show normalized values

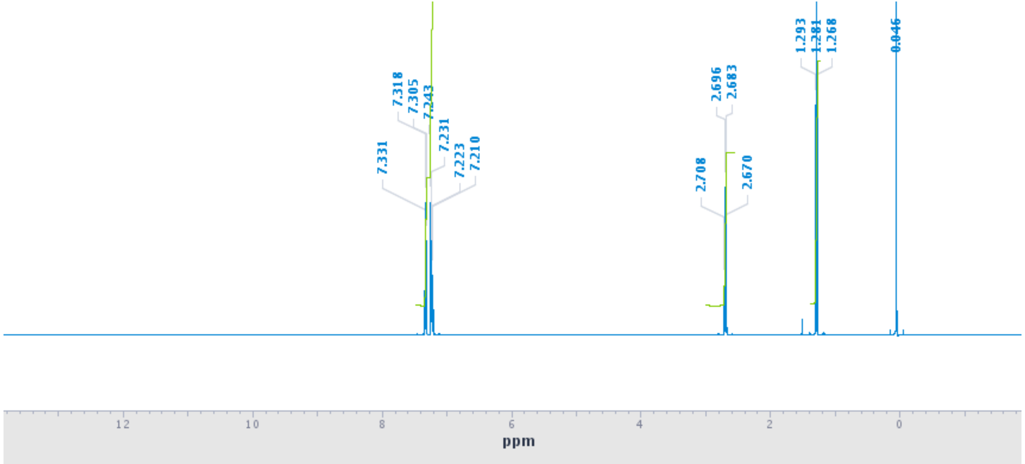
6) Peak Picking:

i. Click on Peak threshold on the **Process/Display** page to show the threshold (a horizontal yellow line) on the spectrum. Only those peaks taller than the threshold will be picked up.



ii. Place the mouse cursor on the yellow line and use the left-mouse-button to drag the line up or down.

iii. Click on Find peaks on the **Process/Display** page to label peaks with their chemical shifts on the spectrum.



IX. Appendices

1. Requirements for Access to the MRL NMR at CNSI

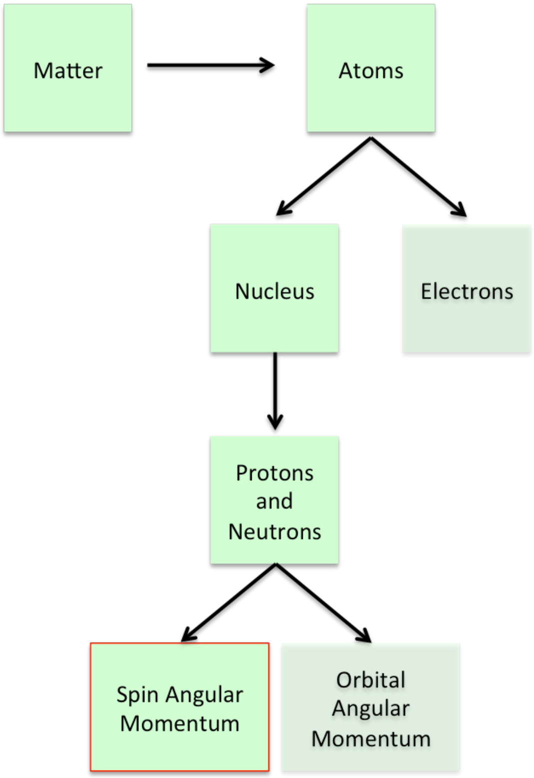
You have to pass the mini quiz within one month after training in order to be qualified for access to the NMR facility of MRL, which includes:

- Key Card for Lab & Building:
    1. Pass the MRL safety training;
    2. Fill out the CNSI access form: http://www.cnsi.ucsb.edu/facilities/building_services/access/access_application.pdf
    3. Take the form to Sylvia in 2066G, MRL
- Web Scheduling Account
- NMR Account

These requirements apply to both on- and off-campus users.

X. NMR Basic Principles

1. Spin



*Spin is a quantum mechanical phenomena that has no physical analog in classical physics. However, it will be helpful to visualize it as a small bar magnet that precesses about an axis.

*The existence of spin angular momentum is inferred by experiments, such as the Stern-Gerlach experiment, in which particles are observed to have angular momentum that cannot be solely accounted for by orbital angular momentum alone.

*Electrons, protons, and neutrons all have a value of spin +/- ½.

2. Common NMR Nuclei

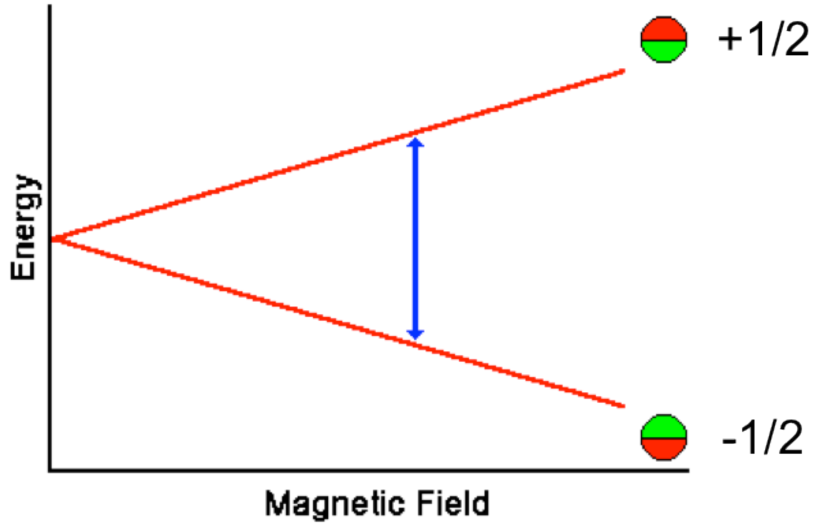
Nuclei	Unpaired Protons	Unpaired Neutrons	Net Spin	γ (MHz/T)
^{1}H	1	0	1/2	42.58
^{2}H	1	1	1	6.54
^{31}P	1	0	1/2	17.25
^{23}Na	1	2	3/2	11.27
^{14}N	1	1	1	3.08
^{13}C	0	1	1/2	10.71
^{19}F	1	0	1/2	40.08

Larmor Frequency Equation:

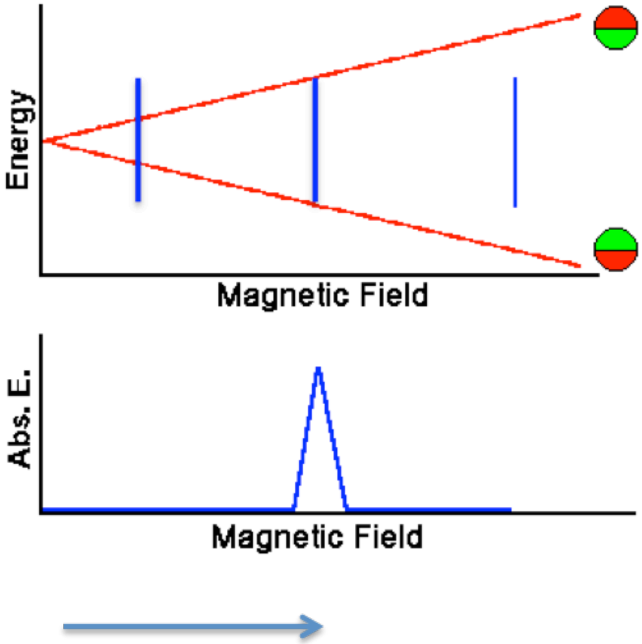
$$\nu = \gamma B_o$$

where γ is the gyromagnetic ratio (specific to each nuclei) and B_o is the magnetic field strength

3. Energy Level Diagram

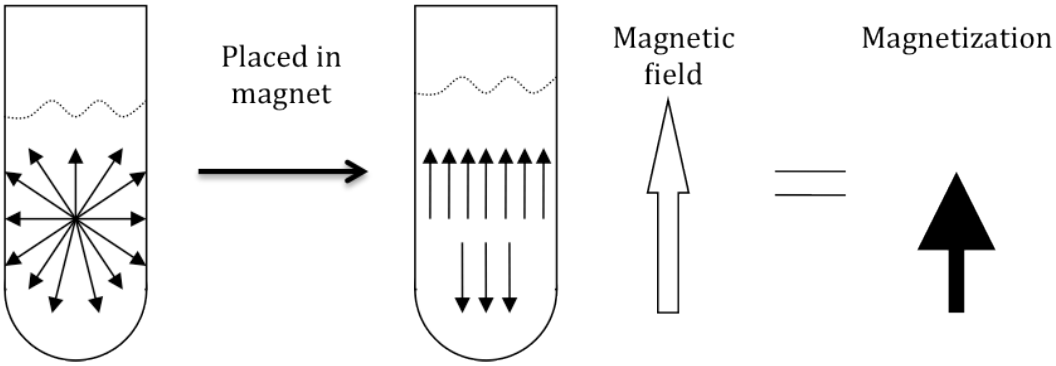


4. cw NMR



5. Magnetization

Alignment of nuclei in a magnetic field



6. Pulsed NMR, Relaxation, and Detection