NMR (Nuclear Magnetic Resonance) Spectroscopy User Handbook

Note: *Please read through the document and follow the rules and directions. The following rules MUST be followed, breaking them may lead to suspension of your account. Breaking the rules several times may lead to indefinite suspension of perpetrators from the NMR room, and NMR samples of that student will be handled by the NMR GSA.*

Rules and Regulations

1. Sign in into the NMR sign in book.
2. When taking ANY sample out of the holder, label them with the date, holder number, and username of the student, if you can't provide this information, DO NOT TOUCH ANY of the NMR tubes that do not belong to you. Failure to label the NMR tubes and/or taking out NMR tubes which are queued or running will result in immediate account suspension.
3. ONLY TAKE YOUR NMR TUBES, we have cameras, we know who has and does this.
4. Pick up your samples, you are given 2 weeks to collect the samples, after that they will be discarded.
5. Wipe the NMR tubes and spinner with kimwipes, keep the case with the spinners always closed if it's not in use. Dust settles, leading to cleaning of probe which will take between 2-4 days.
6. Do not write on NMR tubes or caps, if you need to label samples then use paper, cut a small hole and place NMR tube through it.
7. If you break someone else's NMR tube, let them know, clean up the mess properly, and make sure all of the glass is cleaned up.
8. If you want to take your sample out, do so properly! Otherwise, you do disrupt the current runs, and this will lead to malfunctions of the autosampler. Failure to take your samples out properly will result in temporary suspension of account. (**See part II**)
9. Use the correct amount of solvent and center the NMR tube based on the amount of solvent you added.
10. Clean up after yourself.
11. Do not use the NMR Thursday mornings from 8-10 am, this is due to the liquid nitrogen refill.

12. Always check the computer screen and notebook to see if anyone took your samples out before going to pick anything up from the sample holder.
13. Be careful when placing and picking up samples, do not bump into other samples because you risk accidentally pushing the NMR tube down further which will result in it getting stuck in the NMR.
14. Delete your experiments once you are finished, come check the NMR to make sure all of your experiments run, if some fail, you need to rerun them. If an experiment failed, you must delete it yourself, otherwise no one can use that holder.
15. Keep in mind that each NMR tube contains products, some need to recover this product so it is essential for you to label any samples you take out, and only take the NMR tubes you bring down.

Using proper amount of solution

Note: The easiest way to use and remember how much solution you should use is remembering the two finger rule. Hold the bottom of the NMR tube with two fingers, making sure the edge of your bottom finger is at the bottom of the NMR tube. You should have more solvent than one finger width, less than two (see figure below).

- In order to have an NMR spectrum, the sample should be sufficiently soluble. The greater the solubility of the sample in the deuterated solvent the better the spectrum is. **Do not** leave any suspended particles in the sample. If your solution is turbid, first filter it and then introduce in the NMR instrument.
- Introducing the correct amount of solvent is also an important step in sample preparation. The amount of solvent in your NMR tube should be sufficient to ensure that the meniscus level is located at a certain position above the shimming detection region of the sample. It is recommended to dissolve 2 to 10 mg of your compound in between 0.6 to 1 mL (based

on the size of your NMR tube) of solvent so that the sample depth is about 4 to 5 cm in the tube. Thus, a better signal-to-noise ratio with a normal amount of shimming is obtained. (A small amount of solvent will give a bad spectrum, and large amount of solvent a good spectrum, but it's a waste of expensive deuterated solvent).

Cleaning and positioning the NMR tube in the spinner

- The NMR tube must be placed properly in the spinner, before introducing it in the NMR machine (Figure 2). The spinner is a cylindrical tube, made out of Kel-F or other polymer with a rubber band or rubber O-ring at one end, which holds the tube well in place. Before introducing the tube in the spinner, it should be cleaned with Kimwipe or equivalent wipes. If not some grease and other chemicals on the outside of the tube may contaminate the spinner and even cause not to keep it properly in place. **Therefore, wiping off the tube is very important.**
- It is important to introduce the tube into the spinner carefully, by gently gliding it inside, since otherwise it might break. On the other hand, if the tube slides too loosely inside the spinner, it might be that the latter is contaminated or too old. In this case clean the spinner, use another one or ask for help to clean or replace it.
- The NMR tube must be positioned in the spinner so that the sample depth does not exceed the maximum allowable depth inside the NMR probe (Figure 2). If the maximum allowable depth is exceeded, the tube or probe or both might break (the tube should not stick out of the spinner too much or otherwise hit part of the probe). In order to avoid this mistake, always use a sample depth gauge that indicates the proper sample depth for a specific NMR probe (the maximum sample depth is usually only a function of the diameter of the NMR tube that the probe is designed to accommodate).

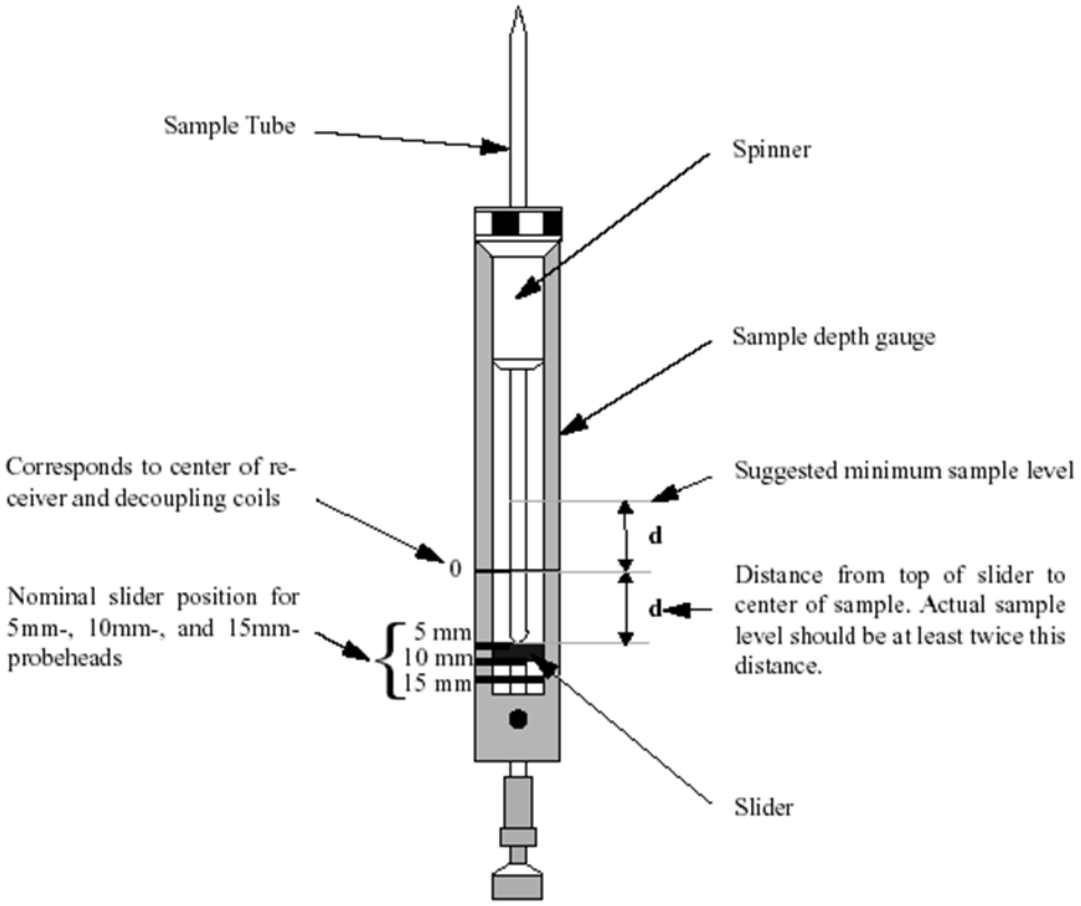


Figure 2: Sample positioning using sample depth gauge.

- The NMR tube must be placed inside the spinner so that the detection region is in the middle of the distance determined by the meniscus at the top and the bottom of the tube (Figure 3). Once again use the sample detection gauge provided to see where the detection region is. The above directions for placing the tube can be disregarded only when the tube cannot go any lower, because it is down to the maximum allowable sample depth. If the sample is not positioned properly (particularly in the case of insufficient solution volume), shimming will be very difficult.

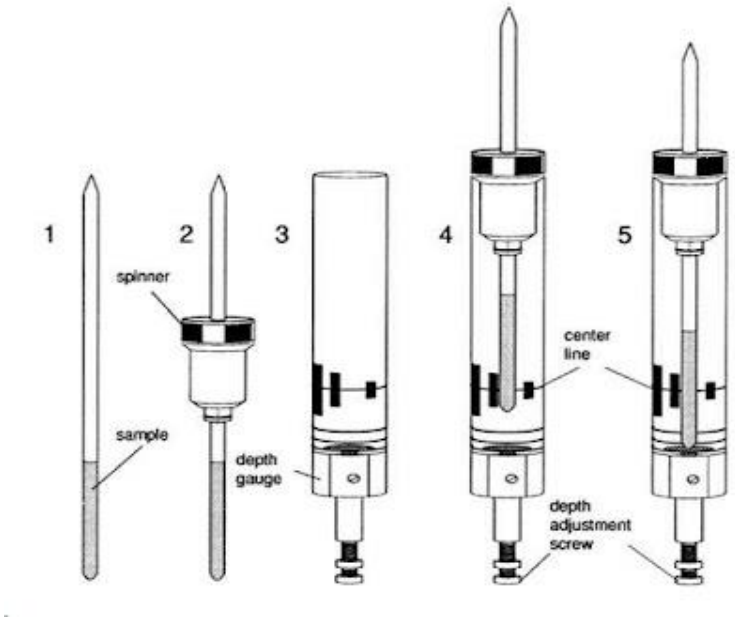


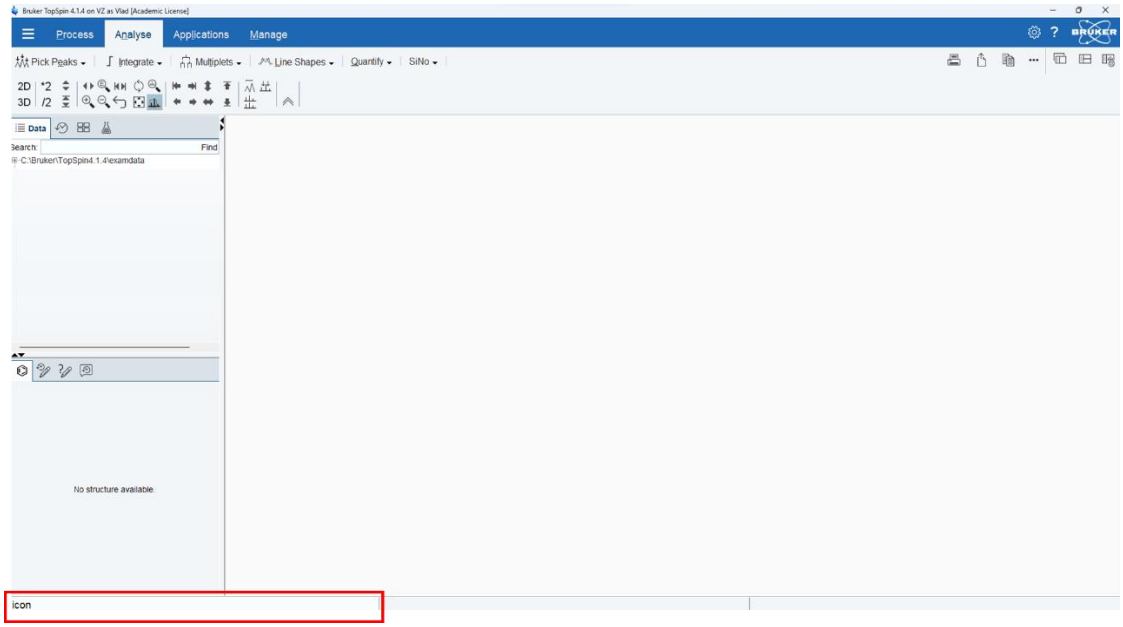
Figure 3: Illustration of properly gauged NMR sample.

- Wiping off the tube one more time, after inserting inside the spinner is also an important step (do this carefully so that the adjustment with respect to the spinner is not disturbed). This is necessary, since others might have skipped wiping off the tube and thus introducing unwanted grease and chemicals on the inside of the spinner wall. As sliding the tube inside the spinner, it might collect some of them and contaminate the probe, thus making sample spinning difficult.

Student Training: Acquiring and Processing NMR Spectra

Part I – Running a sample (^{1}H NMR)

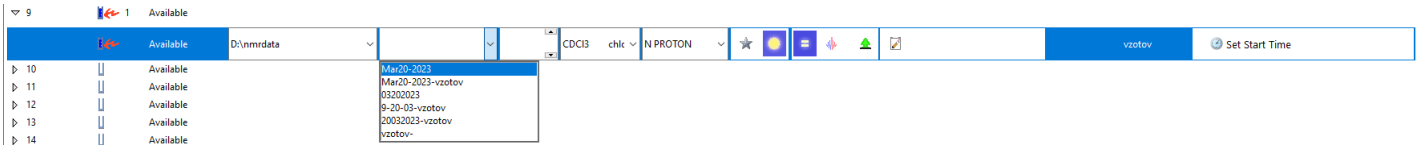
1) If you are starting up the computer or the program, log into the UND Chemistry account on windows, topspin will open automatically, or you can do this manually.
2) Once topspin is open the following screen is opened, in the command line type in icon, example shown below:



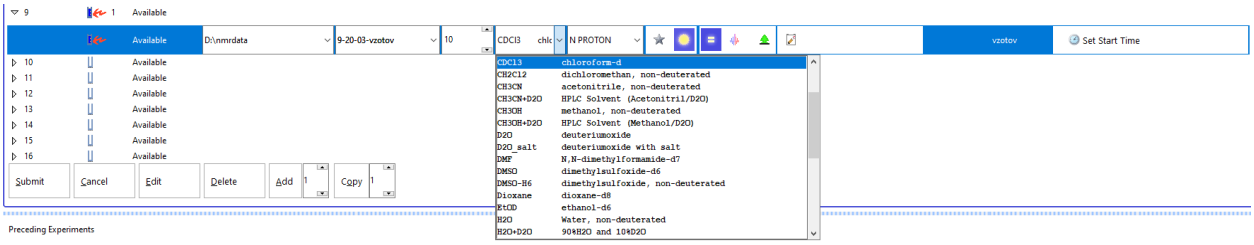
3) The Icon-NMR page will open with the following choices: Routine, Automation, Configuration. Click on automation and a new page will open.

4) Go to the sample holder entry corresponding to your sample and type in the needed information, click submit.

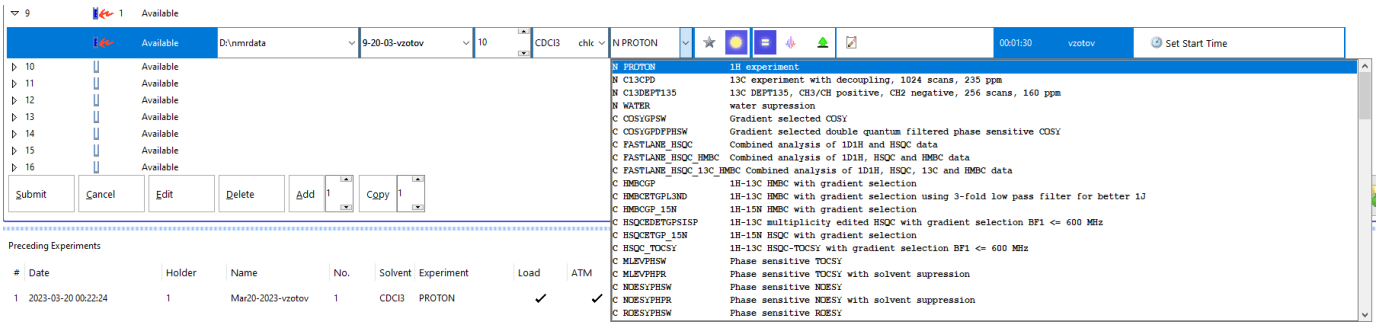
   a) First choose the file name, or write your own, this is the folder the currently taken NMRs will be in.



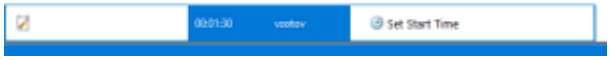
   b) Choose the sample number (automatic goes by 10s, manually you can type in 1, 2, 3… n), and choose your solvent.



   c) Then choose your experiment, most common being the top 3 experiments: proton, carbon, and DEPT135.

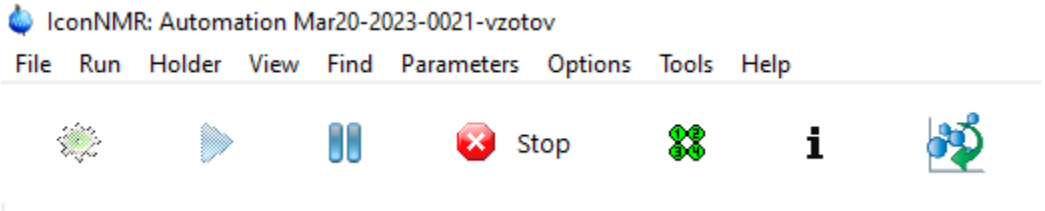


d) Each experiment has a preset number of scans such as PROTON being set to 16, C13CPD at 1024, and C13DEPT135 at 256. This gives the resolution of your spectra, the difference between your signal and baseline. This can be changed by clicking on the = sign and a drop bar opens, shown below, there you have several options including NS (number of scans) along others which are described later in the manual. The run time of the experiment is shown below. *Any experiments which take longer than 30 minutes MUST run overnight*.



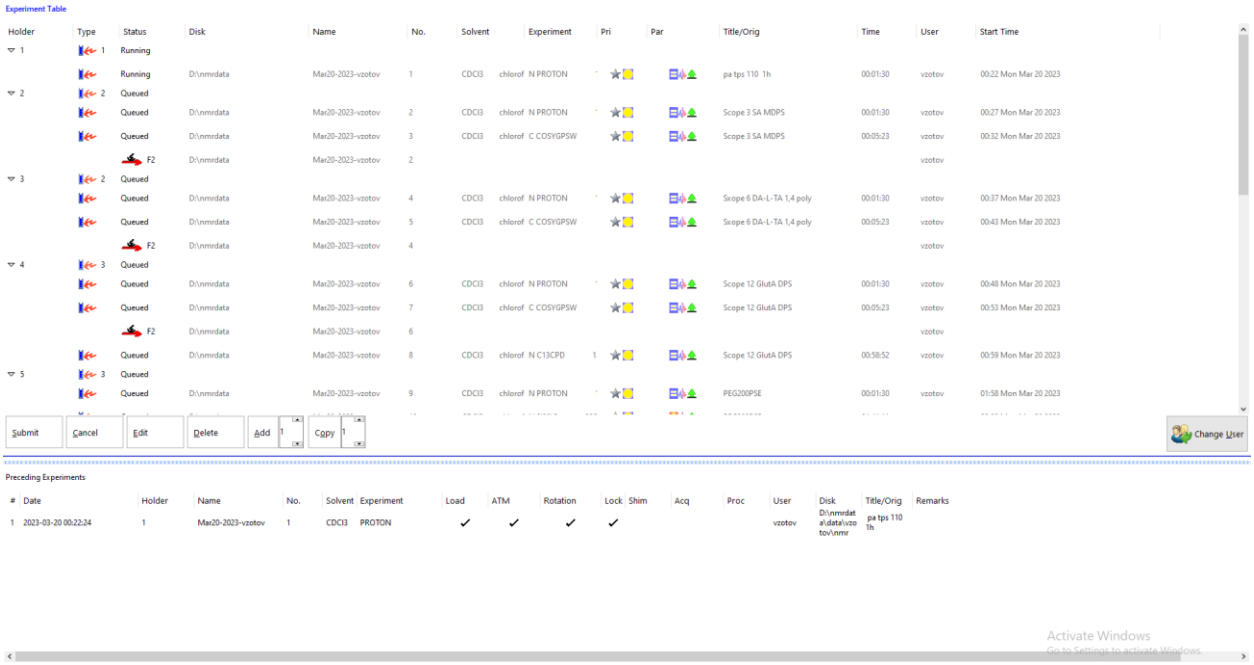
e) Once everything is filled out click submit (shown below). To add more samples simply click add, the number to the right of add gives an option of how many experiments to add. Copy will copy and add THE WHOLE set of experiments for one sample holder, and copy it to the sample holder below. If you need to fix any mistakes on a submitted experiment, simply click on the experiment, click cancel (cancels the experiments) then edit (allows you to edit the parameters filled out).

5) Then go to the top left corner and click on the “Start Run” button, and make sure only “start with sample 1” is checked off.



6) The experiment will begin, and you will get an email of the spectra once it is finished.

Before you leave make sure the page looks similar to the image below and click on change user.



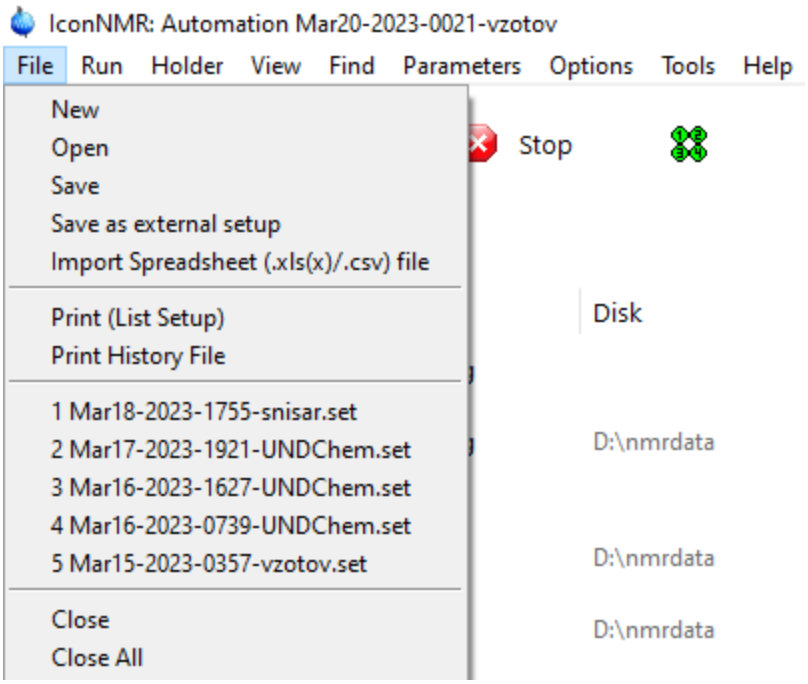
7) Once you are finished, make sure to sign into the logbook on the computer, and report what sample holder you are using to make the recovery of the NMR tubes easier.
8) If you take a sample out make sure to label it with date, holder number and username MINIMUM, the more information you provide the easier it will be. If you can't provide this information, please do not take the samples out!

   You can find the information by looking at the "pending experiments under the "[submit] [cancel] [edit] [delete] [add] [copy] buttons, see figure above. You will see "date, holder, name, No. solvent, etc., you can scroll down until you find the holder out of which you are taking the sample out.

Part II- Removing samples manually

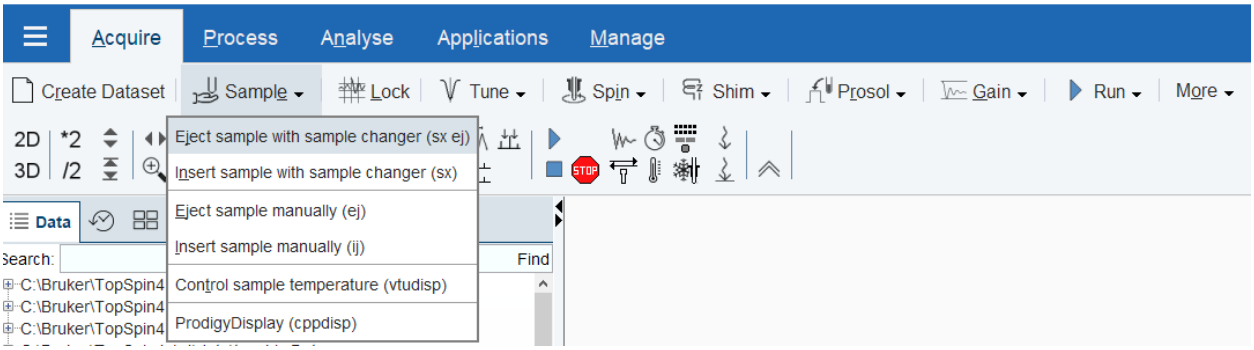
To remove a sample manually from the magnet, there are two ways of doing so.

1) First STOP any NMR experiment running, if you do not know the password of the person who started the run, click on file, and save, shown below. **Note the name of the automation you are saving and closing so you can reopen it!** This allows all of the experiments and runs saved on the current log to be pulled back up. Close the automation, then reopen it, click on the file, the example is shown in the picture below.

2) 

3) Make sure the experiments are stopped, then go to the NMR, click on the green button showing the ⬍ sign on the button, then click on the blue button with the ⊂ sign on it which will move the sample holder forward.

4) To do this automatically, go back to the main topspin page, and under acquire, click on the sample drop menu, choose the one with the sample changer, or type “sx ej” in the command line and click enter.



How to choose a suitable NMR tube and proper sample preparation for NMR analysis

NMR tube

- Standard NMR tubes range in price from a dollar to a two-digit number per tube. A better-quality tube usually will be more expensive, but you will avoid variations in wall thickness and/or outside diameter as well as some bends or bows that might result in poor spinning and shimming. A good quality tube can be seen as a predisposition to good NMR results. It

does not guarantee them, but using a cheap tube will often result in problems with the line shapes (due to poor spinning and shimming) and even breaking the tube (if it is significantly bent) and contaminating the NMR probe with your sample. **Do not** use chipped, cracked or deformed NMR tubes!

- Improper cleaning of the NMR tube can render a good quality tube useless. **Do not** leave tubes in a drying oven for a long time. This can distort the tube, due to the high temperature and flow under the force of gravity, and cause them to lose their cylindrical shape. It is best to clean the tubes with an appropriate solvent and finish with a few rinses, using HPLC-grade acetone. The acetone can be easily expelled from the tube with dry air, nitrogen or argon. The nearly-dried tube can also be placed flat in a glassware oven for approximately 10 minutes. For more information on proper cleaning procedures follow the link: http://www.wilmad-labglass.com/Support/NMR-and-EPR-Technical-Reports/Proper-Cleaning-Procedures-for-NMR-Sample-Tubes/

Using a deuterated NMR solvent

- Using a deuterated solvent has two advantages: deuterium is used for field frequency lock, thus making spectral resolution easier to acquire in less time; the proton signal from the solute is more pronounced compared to the solvent one (if a deuterated solvent is not used, the proton NMR signal from the solvent will overpower the signal from the solute). Therefore, it is highly recommended to use deuterated solvents as often as you can.

Departmental Rules Regarding NMR Access and Reservation

Access:

Each potential user needs to:

1. be recommended by his or her research advisor to the faculty member in charge of the equipment,
2. contact the faculty member in charge of the instrument and the GSA,
3. be trained by the faculty in charge of the instrument or by a person designated by that faculty such as the GSA (not by his or her research advisor), and
4. receive approval from the faculty responsible for the equipment.

If an uncertified student or other personnel is found operating the instrument without proper training and approval, he or she along with the person who provided the access will not be allowed to use the instrument. **This ban will be on 1st infraction for a week, on 2nd infraction for a month, on 3rd infraction the loss of access will be permanent.**

Reservation:

Weekday (Monday-Friday) Restrictions:

Day (8:00 AM - 6:00 PM):

Walk in times throughout the day, only run experiments under 30 minutes, if more time needed run the experiment and click on the sun to change it to the moon so it runs over night.

Evening (6:00 PM - 10:00 PM):

Can run longer experiments such as carbon and others, only experiments up to 1h.

Overnight (8:00 PM - 8:00 AM):

Can run longer experiments and large number of scans, be mindful of other users.

Weekend (Saturday-Sunday) Restrictions:

Day (9:00 AM - 5:00 PM): Can run longer experiments and larger amounts of samples back-to-back, up to 1h experiments.

Evening and Overnight: Can run any experiments up to 12h.

Note: Long-term and special experiments can be scheduled for an entire weekend.

If you need to use the NMR urgently contact the GSA or Dr. Du. Please contact Dr. Du to schedule days and times. Be mindful of the experiments running or set up overnight, do not mess with other peoples experiments without talking to the faculty in charge of the instrument, or the GSA.

A Few Final Notes:

- ✓ Take good care of the NMR machine, since NMR is one of the most versatile laboratory instruments, which plays an important role in our research/academic carrier.
- ✓ Follow the NMR reservation principles and be thoughtful when you reserve a time (https://sites.und.edu/chemistry/). If you need to perform a special analysis (such as high temperature, low temperature, and kinetic studies using NMR, other nuclei, etc.) please contact Dr. Guodong Du. (For more information on such experiments follow the link: http://www.columbia.edu/cu/chemistry/groups/nmr/index.html)
- ✓ When you reserve the time, be aware that people from the same groups are not supposed to have more than two (2) consecutive hours in the day time.
- ✓ When you run experiments overnight, make sure you come back in time (by 8:00am) to take care of your sample. It is also a good idea to always leave a note about what to do with your sample before you go home. At the same time, it is certainly a huge waste of NMR machine time if you terminate others' overnight runs prematurely. Be considerate.
- ✓ Make sure you cancel your reservation(s) ASAP, if you decided not to use them.
- ✓ Clean the NMR tube and the spinner with Kimwipes before inserting to the machine.
- ✓ Keep the place clean by removing used Kimwipes, unwanted printed spectra, etc., from the table. Recycle any unwanted paper in the box by the door.
- ✓ If you take someone's sample out, label it, and set it into the holders on the table. Please be courteous and take only your samples, and label others if you touch theirs
- ✓ Pick up your samples within a week of running the NMR or they will be thrown out.
- ✓ **If you feel someone is not following these guidelines, you can file a complaint to Dr. Guodong Du or Vladimir Zotov with a short description and/or screenshot of the incident. He will investigate and keep a record of the incidents. Repeated violations may lead to further actions, including suspension of access.**

WELCOME TO THE GROUP OF NMR USERS ☺

References:

1. Butler, E. Bruker: AVANCE Beginners: User guide version 004.
2. MIT, department of chemistry, Instrumentation facility – https://chemistry.mit.edu/facilities-and-centers/department-of-chemistry-instrumentation-facility-dcif/
3. Colombia University, Dept. of chemistry, NMR facility - http://www.columbia.edu/cu/chemistry/groups/nmr/index.html
4. Princeton University, Dept. of chemistry, NMR lab - http://chemists.princeton.edu/nmr/
5. Lake head University, Instrumentation laboratory - https://www.lakeheadu.ca/centre/lucas/laboratories/luil/facilities/nmr
6. The Hebrew University, Institute of Chemistry, NMR facility - http://chem.ch.huji.ac.il/nmr/techniques/expts.html
7. Wilmad-lab glasses, Proper Cleaning Procedures for NMR Sample Tubes: http://www.wilmad-labglass.com/Support/NMR-and-EPR-Technical-Reports/Proper-Cleaning-Procedures-for-NMR-Sample-Tubes/