

AN400 Operating Procedures

1. Log in to IconNMR by double-clicking on your group's name on the user list, entering its password and pressing Enter. Do not click on the **[Lock IconNMR]** button at the bottom. If you click on **[Lock IconNMR]** by mistake, click on **[Change User]** on the opposite side of **[Submit]** to get back. If you don't see the user list when you start, click on **[Change User]** to bring it back.
2. Go to IconNMR's top menu, click on **{Holder}** -> **{Delete Completed}** to clear the completed samples. Samples in the queue and the currently running sample will remain.
3. Insert your sample in a Bruker spinner and adjust its position in the spinner with the Bruker sample depth gauge. **Never use Bruker spinners or depth gauge on the Agilent MR400 or use Agilent/Varian spinners or depth gauge on the Bruker AN400.**
4. Put your sample in an available (green, gray, or red) and optimal position:
 - a. If the instrument is idle, put your sample 2 positions up from the center, *e.g.*, if the center position is 5, put your sample in 7. **Do not put your sample in the center position or the one before it.**
 - b. If there are samples running and waiting, put yours after the last one in the queue. Note that samples should be arranged **counter-clockwise** in the tray and one after another, *i.e.*, **do not skip positions**. The tray rotates clockwise.
5. Double-click on your sample's position, then
 - a. If there are samples already in the queue, check and make sure there is enough time left for your sample (see Additional Information <10.h> and <10.t> below)
 - b. Under **Disk**, select your data storage location from the drop-down list or append your subdirectory name to the data path: /opt/nmrdata/<your group>/<your sub dir>
 - c. Under **Name**, enter your sample name as you would on the other instruments
 - d. **No.** is the experiment number. It will start at 1 and be incremented when you add more experiments for the same sample. If the sample name already exists, it will increment the experiment number based on that sample instead of starting from 1.
 - e. Under **Solvent**, select your sample's solvent from the drop-down list, default is CDCl₃.
 - f. Under **Experiment**, select one experiment from the drop-down list, default is PROTON.
 - g. Under **Pri**, the Sun/Moon icon stands for DayQ/NightQ. Default is DayQ. The star icon stands for priority and is disabled for everyone.
 - h. Under **Par**, click on the [=] sign and change the parameters based on your sample and experiment. See Additional Information <10.l> for parameter setting recommendations. Note that you must change at least one parameter (even with the same value) and press Enter or OK in order to show the experiment time under **Time**.
 - i. Under **Title**, you can enter something more descriptive for the sample and click on **[Set Title]**
 - j. **Start Time** shows the experiment's start time which is not user changeable
 - k. To add more experiments for the same sample, click on **[Add]** and fill in the required information as above.

6. **Make sure the sample position is selected**, not the individual experiment, and click on **[Submit]**. If an experiment is selected when you click on submit for a multi experiments sample, only the selected experiment is submitted, and you will have to submit the remaining experiments for that sample.
7. If you have another sample to run, repeat step 3 - 6. Otherwise, you are done.
8. When done, click on **[Change User]** which is located on the opposite side of the **[Submit]** button. **Do not close IconNMR or TopSpin, and do not log out of the iconsu Linux user account!!!**
9. Sign the logbook – PRINT your name (not just your initials) and fill in the rest of the required information. You are required to sign the logbook every time you use the instrument.
10. **Additional Information:**
 - a. Both 7" and 8" NMR tubes can be used on this instrument, but **tubes that are longer than 8" cannot be used**. Please be reminded that **7" tubes cannot be used on the MR400**. So to keep things simple, I recommend phasing out 7" tubes and only order 8" tubes in the future.
 - b. Shigemi tubes for Agilent/Varian instruments cannot be used on Bruker instruments due to the differences in the lengths of the bottom filling, which is 15mm for Agilent/Varian and 8mm for Bruker. However, Shigemi tubes for Bruker should be usable on Agilent/Varian instruments.
 - c. **Bruker and Agilent/Varian spinners are not interchangeable** – never use a Bruker spinner on an Agilent/Varian instrument or vice versa.
 - d. Samples running on the Bruker AN400 must be positioned using the Bruker sample depth gauge, and samples running on the Agilent MR400 must be positioned using the gold Agilent/Varian sample depth gauge. **Damage to the probe will occur if you put a sample positioned with the Agilent depth gauge inside the Bruker AN400's magnet.**
 - e. Sample position color coding: When IconNMR starts, all positions are green and available, grey is deleted finished samples and available, blue is currently running, and pink is waiting in queue for both DayQ and NightQ samples.
 - f. **Do not put samples in or remove samples from the center position or the position before it.**
 - g. To remove the sample in the center position, you must either wait for another sample to finish and the tray to advance, or if yours is the last sample in the queue and the manual tray advance button lights up in blue, push that blue button once to advance the tray by one position. **Do not touch any other buttons!**
 - h. **Do not put samples in or remove samples from the tray while the instrument is changing samples.**
 - i. The following information is shown at the bottom of the screen from left to right:
 - *Sample: 15* – Current center sample position
 - *300.2K* – Sample temperature, normally set at 27C
 - *Busy until: Sun 14:59* – This would be the start time for the next sample submitted and allow you to figure out if there is enough time left for your sample. It would show "No Jobs!" if the instrument is idle.
 - *Day: ##:##* – This is the total time for all the samples in DayQ including the currently running sample

- *Night: ##:##* – This is the total time for all the samples in NightQ including the currently running sample
 - *User: iconsu* – Currently logged in user
- j. On Bruker instruments, 1D spectral window is defined by SW (spectral width in ppm) and O1P (center of spectral window in ppm). For example, to have a -2ppm to 14ppm spectral window, you would set SW=16 and O1P=6. For 2D HSQC and HMBC, the C13 spectral window is defined by 1SW and 1O1P.
- k. **Acquiring 1D data in blocks and checking its progress REMOTELY (not locally):** set NS to be the block size and TD0 the number of blocks. Total number of scans = NS x TD0. For example, to run a 13C with 1600 scans in blocks of 16, set NS=16 and TD0=100. This is the only way to check your long 13C experiment before it finishes.
- l. List of experiments and recommended setting for the most relevant parameters. Note that all 2D experiments are set up to optimize the 1H spectral window automatically and acquire with nonuniform sampling which will cut the experiment time by half.
- **PROTON** – SW (default 16ppm; if changed, change AQ back to its default value), O1P (default 6), NS (default 16, 1 minute)
 - **C13CPD45** (normal 13C) – SW (default 240ppm; if changed, change AQ back to its default value), O1P (default 110ppm), NS (default 128, 5minutes; a 10mg sample with MW < 500 will need about 300 scans, 12 minutes)
 - **C13DEPTQ-135** – SW (default 240ppm; if changed, change AQ back to default value), O1P (default 110ppm), NS (same as a normal 13C)
 - **F19CPD45** – SW (default 240ppm; if changed, change AQ back to its default value), O1P (default -85ppm), NS (default 32, 1 minute; needs about 25% more scans than 1H)
 - **P31CPD45** – SW (default 300ppm; if changed, change AQ back to its default value), O1P (default 50ppm), NS (default 64, 2 minutes; it's about 380 times more sensitive than 13C)
 - **B11CPD45** – SW (default 410ppm; if changed, change AQ back to its default value), O1P (default 0ppm), NS (default 128, 4 minutes; it's about 150 times more sensitive than 13C, but with much broader linewidths)
 - **gCOSY60-NUS** – NS (≥ 2 , depends on concentration and size of coupling constants), 1TD (200 – 400, depends on degree of 1H signal overlapping and/or spectral width). Default NS=2, 1TD=200, 6 minutes.
 - **gHSQC-PS-NUS** – NS (≥ 2 , depends on concentration), 1TD (200 – 400, depends on degree of 13C signal overlapping and/or spectral width), 1SW, 1O1P (1SW and 1O1P define the 13C spectral window and should be optimized to include only 13C's with directly attached 1H). Default NS=2, 1TD=256, 1SW=140, 1O1P=75 (spectral window 5 to 145ppm), 12 minutes.
 - **gHMBC-NUS** – NS (4 to 6 times the NS for HSQC), 1TD (400 – 800, depends on degree of 13C signal overlapping and/or spectral width), 1SW, 1O1P (1SW and 1O1P define the 13C spectral window and should include ALL 13C's). Default NS=16, 1TD=512, 1SW=240, 1O1P=110 (spectral window -10 to 230ppm), 1 hour 15 minutes.
 - **NOESY-NUS** – NS (10 to 50 times the NS for a normal 1H, depends on size of NOE), 1TD (200 – 500, depends on the degree of 1H signal overlapping, spectral width, and 1H

- 1H spacial distance), **D8** (0.5 to 1 sec for small molecules). Default **NS**=16, **1TD**=256, **D8**=0.6, 1 hour 40 minutes.
 - **ROESY-NUS** – **NS** (10 to 50 times the NS for a normal 1H, depends on size of NOE), **1TD** (200 – 500, depends on degree of 1H signal overlapping, spectral width, and 1H – 1H spacial distance). Default **NS**=16, **1TD**=256, 1 hour 30 minutes.
 - **TOCSY-NUS** – **NS** (similar to COSY), **1TD** (200 – 400, depends on degree of 1H signal overlapping and/or spectral width), **D9** (With the default of 80ms, you may see cross peaks from 1H up to 5 or 6 bonds away in the same spin system. A setting of 20 - 30ms will limit the cross peaks to mainly from geminal and vicinal 1H's). Default **NS**=2, **1TD**=256, 9 minutes.
 - **C13IG45** (for 13C integration) – **SW** (default 240ppm; if changed, change **AQ** back to default value), **O1P** (default 110ppm), **NS** (at least 2 -3 times the NS for a normal 13C). Default **NS**=256, 10 minutes.
 - **N15IG** – **SW** (default 500ppm; if changed, change **AQ** back to default value), **O1P** (default 200ppm), **NS** (45 times less sensitive than 13C, so needs lots of scans!)
 - **PROB11DEC** (1H with B11 broadband decoupling) – similar to a normal 1H
 - **PROF19DEC** (1H with F19 broadband decoupling) – similar to a normal 1H
 - **PROP31DEC** (1H with P31 broadband decoupling) – similar to a normal 1H
 - **WATER** – Not tested yet
- m. Double-clicking on a finished experiment inside the “Preceding Experiments” window will display the spectrum in a pop-up TopSpin window where you will only be able to zoom in and out and change vertical scale and nothing else. Don't attempt to do anything else in this restricted TopSpin window. Click anywhere inside IconNMR to return to IconNMR.
- n. Partially completed 1D (set up with TD0) and 2D data can not be displayed in TopSpin during their acquisition, but can be viewed remotely in Mnova. Remote viewing is the only way to check your data before the acquisition completes.
- o. Submitted samples can be deleted before they start. Just select the sample position and click on **[Delete]**.
- p. Experiments within a submitted sample can be deleted individually before they start. Just select the experiments, instead of the sample, and click on **[Delete]**.
- q. Submitted experiments in the queue cannot be edited/changed. The only way to change a submitted experiment is to delete that experiment (not the sample), add it back and make the needed changes, and resubmit that experiment (not the sample).
- r. Experiments can be added to a submitted sample as long as it has not finished. Just select the sample and click on **[Add]**, then submit the added experiment (not the sample).
- s. Stopping an experiment: Due to potential data loss and concern with automation queue not being restarted properly, users will **not be allowed to stop an experiment**.
- t. Schedule and Rules:
- Weekdays:**
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| 8:00AM – 6:00PM | DayQ, 30 minutes per user (see the one-hour rule below) |
| 6:00PM – 8:00AM | NightQ, unlimited. Submission starts at 4:00PM |

Weekends:

8:00AM – 6:00PM(Sat.)

DayQ, 60 minutes per user (same one-hour rule applies)

6:00PM(Sat.) – 8:00AM(Mon.)

NightQ, unlimited. **Submission starts at 1:00PM on Saturday**

- The one-hour rule: One hour *after the finish* of your previous sample(s), you may submit another 30 minutes of samples. There is no limit on how many 30 minutes blocks one can have in a day as long as the one hour rule is followed.
- You must check and make sure there is enough time left in DayQ or NightQ for your experiments before submitting your samples (see <10.i> on how).
- The total number of NightQ samples submitted by all users before 6pm on weekdays and Saturdays should be no more than 12, so that DayQ users will have at least 12 sample positions available.
- Please observe the NightQ submission start time. I intend to check, and early submissions will be removed without notice.
- Use of this instrument is on a first-come, first-served basis. No reservation is required or allowed. If you have a need that cannot be met because of the above policy, please let me know so that alternative arrangement or a one-time exception to this policy can be made.