NUCLEAR MAGNETIC RESONANCE RESEARCH RESOURCE

NMR-3 User Guide

Department of Chemistry
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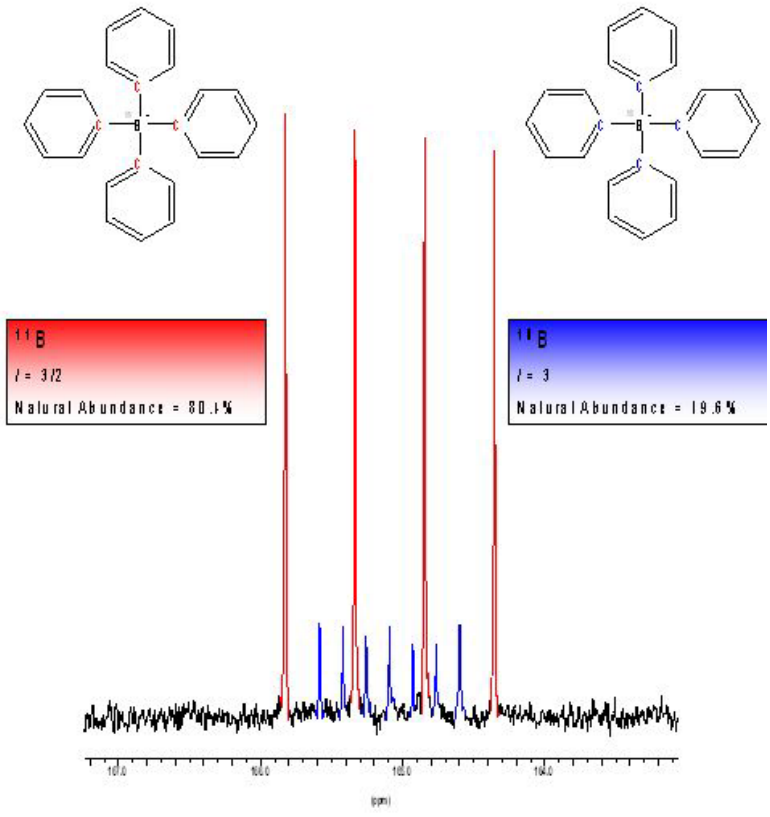

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1. Introduction

The Nuclear Magnetic Resonance Research Resource (NMR-3) is located on the ground (Room 127) and fourth floors (Rooms 426-431) of the Department of Chemistry, Dalhousie University. The facility is currently home to five Bruker NMR spectrometers. Our flagship instrument is an AV-III 700 MHz system (installed '09, jointly owned and operated with the NRC Institute for Marine Biosciences) equipped with 5 mm and 1.7 mm TCI cryoprobes. This state-of-the-art instrument provides researchers with access to 700 MHz NMR data at the current maximum limits in both mass and bulk sensitivity. The facility also operates an AVANCE 700 MHz spectrometer dedicated to the study of solids (installed '04, Room 127). This spectrometer represents, after the 900 MHz system at the NRC-Ottawa, the highest field NMR spectrometer dedicated to solid-state NMR applications in Canada. The facility is also home to an AVANCE-500 (installed '03, Room 431, liquids only), a DSX-400 (installed '04, Room 127, solids only), and an AVANCE-300 (purchased used, installed '10, Room 431, liquids only). Further details regarding this equipment will be provided below. The facility operates with 2 Ph.D. level staff members. Dr. Mike Lumsden (100% full-time, Room 428, (902) 494-1635) is the Facility Coordinator and provides high-resolution NMR services and support. Dr. Ulrike Werner-Zwanziger (50% part-time, Room 127, (902) 494-8085) is the solid-state NMR Coordinator and provides solid-state NMR services and support. The Facility Director currently is Professor Jan Rainey. NMR-3 not only supports the NMR needs of scientists at Dalhousie University but also at numerous other universities and companies within the Atlantic Region of Canada.

In any given year, the NMR-3 facility provides NMR access for up to 100 undergraduate students, graduate students, post-docs, and faculty. With such a large and diverse user base, a set of policies and procedures has been adopted to ensure everyone has equal and fair access to the resources of the facility as well as to ensure the safety of its users. *In order to become a trained hands-on user of the NMR-3 instrumentation, you will have to read and understand the contents of this document in its entirety.* Please be aware that one component of your NMR training will require you to write a short quiz on the contents of this manual. You are also encouraged to provide feedback regarding any of the policies and procedures.

2. The Spectrometers

2.1 Liquid-State NMR Spectrometers

2.1.1 AV-III 700: The Bruker AV-III 700 MHz spectrometer is a state-of-the-art high-resolution spectrometer. It is a three-channel instrument with single-axis Z-gradients and a standard-bore UltraShield Plus 16.44 Tesla magnet. The spectrometer is controlled with a Linux workstation running TopSpin 2.1. For high-throughput studies, the system has a SampleJet autosampler, which in turn is equipped with a sample cooling feature for the temperature control of biological samples waiting for analysis. The SampleJet has 47 positions for single tubes as well as 5 positions for 96 well plate format tube racks, allowing for the handling of batches of up to 480 sample tubes! NMR studies involving ultra-stable temperature control is provided with a BCU-X refrigeration unit and a BVT-3000 temperature controller. The spectrometer is equipped with 2 cryo-probeheads, both with the autotune & match accessory (ATMA). A 5 mm version provides ultra-high bulk sensitivity for proton observation but also extremely high sensitivity for ^{13}C studies. The 1.7 mm probehead provides state-of-the-art sensitivity for mass limited samples.

2.1.2 AVANCE 500: The Bruker AVANCE 500 spectrometer is a three-channel instrument with single-axis gradients and a narrow bore Bruker Ultrashield 11.75 Tesla magnet. To minimize room and building vibrations, the magnet is supported by integrated anti-vibration rubber pads. The spectrometer is controlled from a PC workstation running TopSpin v. 2.1. The system is protected with a 11-kVA uninterruptible power supply (UPS) and is fed –70 °C dewpoint compressed air from a desiccant air dryer. This ultra-dry air is routed through a Bruker BCU05 air-chiller to produce -5 ºC air which is then re-warmed by the probe's heater before reaching the sample, greatly improving temperature stability during NMR experiments. The "500" has three 5 mm probes, all with a single axis Z-gradient coil. The TXI probehead is an inverse probe with superior ^{1}H sensitivity, lineshape, and water

suppression capabilities. It is capable of decoupling both ^{13}C and ^{15}N, making it a powerful tool for structural elucidation of biological systems. The workhorse probe, used for all experiments performed in automation, is a 5 mm BBFO SmartProbe, installed in 2012. This is a double resonance probe with the inner coil tunable from ^{15}N up to ^{19}F and a dedicated outer ^{1}H decoupler coil. The unique engineering of this probe, along with a corresponding upgrade to the spectrometer shim hardware, results in spectacular ^{1}H sensitivity and lineshape for a 5mm X-detected RT probe. This probe permits routine acquisition of ^{19}F experiments in automation (without any re-cabling), either with or without ^{1}H decoupling. Also, this probe is equipped with Bruker's ATMA feature (*a*uto-*t*une and *m*atch *a*ccessory). Finally, the instrument is equipped with a 5 mm BBO probehead with ATMA. This probe has an inner coil tunable from ^{109}Ag up to ^{31}P and a dedicated outer ^{1}H decoupler coil. Currently, this probe is used infrequently; its capabilities and performance having been replaced by the superior SmartProbe late in 2012. The specs for these three probes are summarized in Table I. For efficient sample throughput, the spectrometer is equipped with a sample changer from Bruker called "B-ACS" (*B*ruker *A*utomation *C*ontrol *S*ystem) which can accommodate a queue of 60 samples. In order to perform variable temperature NMR experiments, the spectrometer has a Bruker BVT-3200 temperature controller (essential for temperatures above and below ambient) along with a 25 L liquid nitrogen dewar and a nitrogen exchange coil which are used to generate cold nitrogen gas for NMR experiments below ambient temperature. Training courses are available for hands-on access to the AVANCE 500.

2.1.3 AVANCE 300: The Bruker AVANCE 300 spectrometer is a two-channel instrument with single-axis gradients and a narrow bore Bruker 7 Tesla magnet. To minimize room and building vibrations, the magnet is supported by a TMC vibration dampening platform. Like the AVANCE 500, the spectrometer is software controlled by Bruker's TopSpin 2.1. The spectrometer has a 5mm BBFO probe equipped with the ATMA accessory and a Z-gradient coil. This probe is used to observe nuclei ranging in frequency from ^{15}N up to and including ^{19}F. The outer decoupler coil is dedicated to ^{1}H. The specs for the 5mm BBFO probe are summarized in Table I. In 2012, a Bruker SampleXPress Lite autosampler was installed. This autosampler can accommodate a queue of 16 samples. At the time of this writing, the SampleXpress Lite is in use every week day beginning at 4 pm and ending 10 am the next morning as well as on week-ends. Also of note is the inclusion of a Bruker BVT-3200 temperature controller which enables variable temperature NMR experiments. Low temperature experiments also require a source of cold nitrogen gas, which can be generated on the 300 using an evaporator and 25 L liquid nitrogen dewar. A training course is available for hands-on access to the AVANCE 300.

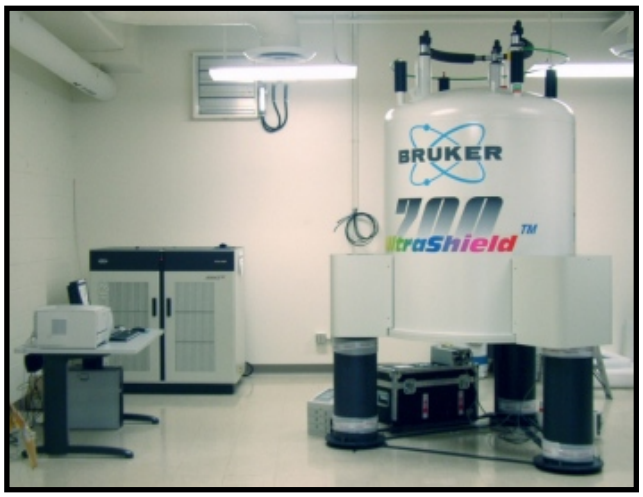
Table I: NMR-3 High Resolution Probe Specifications

Specification	300 BBFO (5 mm)	500 TXI (5 mm)	500 BBFO (5 mm)	500 BBO (5 mm)
^{1}H S/N (2 ppm)	135 (2 ppm)	900 (200 Hz)	730 (200 Hz)	330 (2 ppm)
^{13}C S/N (ASTM)	100	N/A	250	220
^{31}P S/N (TPP)	100	N/A	180	120
^{15}N S/N (Formamide)	15	N/A	40	32
^{19}F S/N (TFT)	180	N/A	550	N/A
^{1}H Lineshape (spin)	6 / 12	5 / 10	N/A	N/A

^{1}H Lineshape (static)	N/A	7 / 14	7 / 14	N/A
^{1}H Resolution (static)	0.6 Hz	0.45	0.8	N/A
^{1}H π/2 pulse (μs)	<15	<8	<12	<15
^{13}C π/2 pulse (μs)	<10	<12	<10	<10
^{31}P π/2 pulse (μs)	<12	N/A	<14	<15
^{15}N π/2 pulse (μs)	<15	<33	<15	<15
^{19}F π/2 pulse (μs)	<15	N/A	<15	N/A

2.2 Solid-State NMR Spectrometers

2.2.1 AVANCE 700: The Bruker AVANCE 700 MHz NMR Spectrometer is a modern instrument dedicated to the study of solid materials. It operates under XWINNMR from a Linux workstation. Together with the 16.4 T magnet, the instrument includes an H-F/X CP/MAS probe, suitable for the study of ^{1}H, ^{19}F, and other nuclei (X) with Larmor frequencies between ^{13}C and ^{31}P (176-283 MHz). The probe permits spinning speeds up to 35 kHz in 2.5 mm rotors. Also available is a VTN CP/MAS H/X probe for X nuclei frequencies between ^{15}N and ^{13}C (71-176 MHz) with spinning speeds up to 15 kHz in 4 mm rotors. In addition, we have two triple-resonance probes; specifically a TriGamma™ double-broadband H/X/Y MAS probe employing a 3.2 mm MAS system. These probes offer the capability for heteronuclear X/Y correlation techniques. The second triple resonance probe is a 4 mm design specifically for ^{11}B/^{29}Si correlations but also allows correlations between other nuclei close to ^{11}B and ^{29}Si. Our most recent probe addition is a 5 mm probe head dedicated to the observation of nuclei that resonate at low Larmor frequencies ("low gamma nuclei" such as ^{39}K, ^{43}Ca, ^{67}Zn, ^{91}Zr, ^{107}Ag, ^{25}Mg, and $^{47/49}$Ti). To acquire data on this spectrometer, please contact Dr. Ulrike Werner-Zwanziger.



2.2.2 AVANCE DSX-400: The Bruker AVANCE DSX-400 MHz spectrometer was originally installed in 1997 at Indiana University. Professor Josef Zwanziger transferred the spectrometer with him from Indiana and made it available for NMR-3 users. Reinstalled in 2004 in room 127, this spectrometer is also exclusively dedicated to the NMR of solid samples. This spectrometer is equipped with 5 probeheads:

- A triple resonance multinuclear VT CP/MAS probe with exchangeable inserts. Using rotors with 4mm diameter, this probe can spin up to 15 kHz.
- A multinuclear VT MQ/MAS probe tuning from ^{69}Ga to ^{31}P, capable of proton and fluorine NMR and optimized for minimum fluorine background. With 2.5 mm rotor diameters, the samples can spin up to 35 kHz.
- A multinuclear VT CP/MAS probe tuning the X nuclei frequency between those from ^{13}C to ^{31}P. This double resonance probe features 4mm rotor sizes spinning up to 15 kHz. This is a real workhorse for standard applications on this spectrometer. In addition to the usual MAS housing, we can insert a boron depleted housing.
- A VT CP/MAS probe tuning from ^{15}N to ^{31}P for the X-nuclei in conjunction with ^{1}H/^{19}F cross-polarization. Its 7mm rotor design gives more sample space for materials with less sensitivity on the cost of spinning only up to 8 kHz.
- A high power probe for wide line NMR applications including exchangeable rf coils and matching inserts to allow the probe to tune from ^{109}Ag to ^{31}P. This probe is capable of extended variable temperature applications up to 300 ^{o}C.

This spectrometer operates under XWINNMR from a SGI workstation. Low and high temperature operations are available. Access to this spectrometer again is granted through Dr. Ulrike Werner-Zwanziger.

3. Facility Access

3.1 Liquid-State NMR Access: Hands-on access to liquid-state NMR studies is currently available on the AVANCE 500 and 300 MHz spectrometers. Training and scheduling is coordinated by Dr. Lumsden. The list of courses, at the time of this writing, includes courses for using the AV-300 and for automation on the AV-500. In addition, more specialized training is available that includes using reactive sample slots on the AV-500 as well as variable temperature NMR experiments on both of these spectrometers. Note that in addition, a classroom tutorial on processing NMR data using Bruker's TopSpin software is offered (when there is sufficient demand). Spectrometer access can only be obtained after you've registered for and successfully completed the appropriate training course. By default, training is provided each January, May, and September, with the arrival of large numbers of new researchers, as well as at other times throughout the year when demand warrants. The training forms are available via our website at http://www.dal.ca/diff/nmr3/training.html. Please note that the forms must be completed and submitted online. You are asked to register for training only when you actually have a need for NMR results in your research program. All too often in the past, people have completed the training but not needed NMR spectra for months down the road. By that time, you will have forgotten most things you learned and will require re-training. Such a scenario is an inefficient use of your time as well as the staff of NMR-3. Please note that spectrometer access privileges are automatically removed when a researcher has not used a given spectrometer for a period of 6 months or longer. Access can only be reinstated once the researcher attends a "refresher" training session.

Both the AV-500 and 300 MHz spectrometers are equipped with automation hardware and software. When this equipment is in use, no spectrometer reservation is required for access. Researchers simply submit their samples and experiments to the automation queue and wait for their data. Wait times are highly variable and are obviously a function of the demand on the spectrometer at the time of submission. **All** non-autosampler usage must be reserved in advance. Reservations are made using the web-based booking software called FACES. Once trained on a spectrometer, Dr. Lumsden will create a FACES account in your name allowing you to reserve time on that spectrometer.

Access to the AV-III 700 MHz instrument is currently available but specialized and must be coordinated through Dr. Lumsden. This instrument is located at the Institute for Marine Biosciences (IMB) and consequently additional access security measures are in place. Experiments performed on this instrument tend to be more complex and are typically performed on very dilute samples or macromolecular systems not easily studied at 500 MHz.

3.2 Solid-State NMR Access: Due to the complexity of solid-state NMR experiments, solid state NMR data are typically obtained as service or collaborative measurements. Hands-on access is only granted to experienced users after substantial training one-on-one. If you need solid-state NMR data for your research, please contact Dr. Ulrike Werner-Zwanziger (Ulli.Zwanziger@dal.ca) to discuss further details.

3.3 Dal Chemistry Access: Upon successful completion of your training course(s), researchers are permitted access to the facility resources 24 hours a day, 7 days a week. The room housing the liquid-state NMR spectrometers is protected with a numeric keypad lock (access code is provided during training). Researchers with lab/office space within Dal Chemistry will already have the necessary building access and so no further steps will be required for around-the-clock access. For those researchers not working within Dal Chemistry, after-hours building access can be coordinated through the Chemistry administration office. An online form will first need to be submitted by your research Supervisor, specifying why this access is needed and for how long. Once this form is received, you will be contacted to setup a building access code.

4. NMR Data Processing & Analysis

4.1 Processing Stations and Software: For those who wish to do their data analysis at NMR-3, the facility has two Windows desktops available in Room 430. On these systems, users can access both TopSpin 1.3 as well as 2.1. Other software packages available include 1D/2D processing along with powerful $^{1}H/^{13}C$ prediction software from ACD/Labs (Advanced Chemistry Development Inc) and gNMR for the simulation of complex second order spectra. On the newer Windows 7 desktop, researchers have access to a node-locked processing license for TopSpin 3.2 as well as Dynamics Center 2.2, a new package for the analysis of experiments such as relaxation, diffusion, kinetics, etc. Together, these software products provide the facility's

users with powerful tools for analyzing and understanding their NMR research data. A networked HP LaserJet P1606dn is also available in Room 430 for printing and plotting. Within the solid-state NMR facility, simulations are possible using several open source programs such as dmfit, Simpson, Quest, etc. These programs allow the fitting of spectra to determine isotropic and anisotropic chemical shift values as well as quadrupole parameters for magic-angle-spinning and static applications.

4.2 Remote Processing Options: Researchers wishing to work up their NMR data at home or in their offices can take advantage of floating licenses available for both TopSpin 1.3 (6 seats) and 2.1 (3 seats) for Windows XP or Vista. Installation instructions can be found on the facility website here. For TopSpin users running Windows 7 or 8 on their home or office computers, these floating licenses will not be of any use. However, a fairly inexpensive ($99 USD) 3-year student version of TopSpin3 is available for these systems via the Bruker Online Store. In terms of third party software products, note that ACD/Labs offers a free academic/non-commercial version of their ACD/NMR Processor available for download here. Kirk Marat's popular SpinWorks program is also freely available for download. Commercial software products include Mestrelab Research's Mnova NMR (free trial available) as well as Acorn NMR's NUTS package.

4.3 Remote NMR Data Retrieval: NMR experiments obtained on the AVANCE 300 and 500 spectrometers are automatically archived to one of the facility's data stations. This system also is an ftp server, allowing users "around-the-clock" remote ftp access to their NMR data. Ftp login details are provided during spectrometer training sessions and instructions are available on our website for the use of the built-in ftp client within Windows. Researchers may of course also retrieve their data electronically by bringing a thumb drive to the facility and copying the data directly from the server. Shortcuts to the data folders are available directly on the desktop for this purpose.

5. Facility Rules & Guidelines

5.1 Spectrometer Usage Rules: The NMR-3 spectrometers have individual guidelines governing access and usage. These guidelines change from time-to-time depending upon the time of year and the needs of the individual research groups. Email notification is provided when the signup rules are modified. The most up-to-date rules can always be found on our website at http://www.dal.ca/diff/nmr3/scheduling.html. In addition to the posted access rules, spectrometer usage is governed by the following general rules of conduct:

- Only authorized (trained) researchers are permitted to use an NMR-3 NMR spectrometer independently.
- Researchers are not permitted to use the NMR spectrometers without first signing up for the time in FACES (the only exception is when samples are submitted to an autosampler). Using a spectrometer without a reservation is a serious problem as it interferes with the planning of other potential users. Furthermore, it reduces "usage statistics", which are an important component of funding applications for NMR equipment and infrastructure.
- Continuing to use a spectrometer beyond your reserved time because there is no booking following yours is also strictly forbidden. All usage must have a corresponding FACES reservation, no exceptions.
- Extending your available time beyond the allowed allotment by consecutively signing up in FACES in your name and then somebody else's name, such as a lab colleague, is strictly forbidden.
- Users are not permitted to use the spectrometers during liquid nitrogen or helium fills.
- Users must never use or alter another user's NMR data.
- Users of the AV-500 and AV-300 spectrometers are permitted to use only the parameter sets starting with 1d_ or 2d_. No other parameter sets are available for routine users.
- Ensure that the door to the spectrometer room closes behind you when you leave the NMR facility.
- Please report unusual spectrometer behavior to the facility coordinator as soon as you notice it.

5.2 Computer Usage Rules: The NMR-3 spectrometer workstations as well as the processing stations are governed by the following usage rules:

- The computers in NMR-3 are available for NMR acquisition, processing and data analysis only.
- Users are not permitted to browse the web or check email on any of the NMR-3 computers. The only exception to this rule is users can check the reservation schedule on the FACES website.
- Get in the habit of backing up your NMR data frequently. The computers at NMR-3 are all purged of user data 2-3 times per year.

- Usage of the data stations cannot be reserved in advance; they are available on a first come – first served basis only.
- Plugging usb flash/thumb drives into the spectrometer workstations is strictly forbidden – these are permitted on the processing stations only.

5.3 Access Issues: Given the tremendous diversity in the access requirements of its large user base, it is impossible that a single set of signup rules will be ideal for each user. Compromises are inevitable and access to any given spectrometer may not be perfect for your research needs. If you need clarification on any of our signup policies and the background rationale, feel free to speak to a staff member. However, if access problems under the current guidelines are demonstrably hindering your research progress, it is important that the coordinator of NMR-3 be made aware of this. Analogously, if you notice an infraction of the signup guidelines, it is important that you inform the coordinator. The coordinator is solely responsible for the day-to-day operations of the facility, including scheduling of time on the spectrometers, and it is his/her responsibility to address these concerns in a timely fashion. It is important to be aware that the director is not involved with these everyday issues. If **and only if**, you are unable to resolve your problem/concern after discussions with the coordinator are you invited to contact the NMR-3 Users' Committee in writing, outlining in detail the nature of your concern and your attempt(s) to resolve it. At the time of this writing, Professor Mark Stradiotto at Dalhousie University is the chair of the NMR-3 Users' Committee (Mark.Stradiotto@dal.ca).

6. Safety in the NMR Facility

In a modern NMR facility containing superconducting magnet(s), some serious safety hazards exist. In general, these risks can be divided into 3 separate categories: (a) hazards due to magnetic fields (b) hazards due to the presence of cryogens (c) NMR sample related hazards. Each of these will be discussed further below. In light of these hazards, access to the hands-on liquid-state spectrometers of NMR-3 is exclusively through the main door (marked Rooms 426-431) off the 4th floor hallway, no exceptions. Appropriate warning signs about these perils are placed at this main entrance as important reminders.

6.1 Stray Magnetic Field Hazards: Superconducting magnets possess extremely strong magnetic fields inside the canister, necessary for NMR spectroscopy. Unfortunately, they can also possess sizeable magnetic fields outside the canister. These external fields, known as "stray" magnetic fields, emanate in all directions from the magnet canister and are particularly strong vertically (above and below the magnet). Consequently, dangerously strong attractive forces are present in the vicinity of the magnet towards any ferromagnetic materials/equipment present. These forces increase in strength as the mass of the equipment increases and as you move closer to the magnet with the equipment. Due to stray fields, a number of hazards exist:

- Serious injury or even death can occur to people with certain implanted medical devices or hardware such as cardiac pacemakers, surgical clips, or prostheses. These individuals must stay away from the magnet at all times! A colleague will have to physically insert/remove your NMR samples for you.
- If brought too close to the magnet, ferromagnetic tools and equipment could be pulled out of your hands and become attached to the side of the magnet. Such an event obviously presents a serious danger to all personnel within NMR-3, as well as to the magnet itself. Items such as screwdrivers, keys, wrenches, gas cylinders, metal chairs, fire extinguishers, metal rulers, etc. must be kept away from the magnet at all times. This list is by no means meant to be exhaustive! The picture to the right shows the aftermath of a 2015 accident at the University of New Mexico where a construction worker got too close to a 500 MHz magnet with a welding pack. The accident caused the magnet to quench.

- Although not a threat to human life or health, please note that magnetic media such as ATM/credit cards and some computer storage disks may be damaged if brought too close to the magnet. Also in this category are analogue watches (digital watches are fine).

Stray magnetic field strengths are quantified in units of Gauss (G). To put this unit in perspective, the strength of the Earth's magnetic field is, on average, 0.5 G (it varies over the surface of the earth) whereas the strength of a small fridge magnet is on the order of 50 G. To provide a visible reminder of this invisible hazard near the NMR-3 superconducting magnets, the location of the radial (horizontal) 5- and 10-G lines are marked on the floor with yellow (5 G) and red (10 G) tape. In addition, yellow plastic chain-link fencing is located at the position of the 5 G line as another reminder. In general, a safe zone exists outside the 5 G line; all items identified above in the description of hazards must remain outside the 5 G line and thus within this safe zone. Please note that even when positioned within the safe zone, **moving** ferromagnetic equipment/materials (filing cabinet drawers, trolleys, etc) in the vicinity of the magnet adversely affects field homogeneity and thus the quality of the data from any NMR experiment in progress. Such movement must be well outside the 5 G line, typically at a distance from the magnet of 3 to 9 m.

The magnet manufacturers provide detailed specifications as to the location of the stray field lines around their magnets. Generally, the stronger the magnet, the further a given line extends from the magnet center. However, much variation exists depending upon the vintage of the magnet, its level of "shielding" as well as whether the magnet has a standard or wide bore. The data in Table II provide the position of the radial stray fields associated with the existing 5 superconducting magnets at NMR-3.

Table II: Radial Stray Field Characteristics

	10 G Line (m)	5 G Line (m)
AVANCE 300	1.46	1.87
AVANCE 500 Ultrashielded	1.15	1.35
DSX 400	1.25	1.60
AVANCE 700	2.25	2.50
AV-III 700 MHz @ IMB	0.88	1.00

6.2 Hazards Related to Cryogens: The internal design of a modern superconducting magnet involves separate dewars containing liquid nitrogen (normal BP -196 ºC) and liquid helium (normal BP -269ºC). The presence of these cryogenic fluids in an NMR magnet room generates potential hazards. Under normal operating conditions, these two cryogenic liquids are safely contained inside the magnet and pose no risk despite the associated extremely low temperatures. NMR-3 staff must occasionally replenish these liquids since they slowly boil off – during these "fills" users are not permitted to use the NMR spectrometer.

Of broader concern is the risk of asphyxiation related to the boil-off of these fluids to produce gaseous nitrogen and helium. As mentioned, under normal operating conditions, there is a slow but continuous release of these gases from the magnet into the NMR room. Nitrogen boils off more rapidly than helium given the proximity to ambient temperatures (typical numbers are 250 and 15 mL/hour, respectively). Since the NMR rooms are large and air is constantly being exchanged, oxygen levels are easily maintained. During fills, somewhat larger but still safe volumes of gas are generated. Facility policy requires the doors to the room to remain open during these fills as an extra precautionary measure. The biggest risk of asphyxiation comes from an unlikely event associated with all superconducting magnets called a quench. When a quench occurs, the superconductivity of the magnet is lost for some reason, heat is generated, and the cryogenic fluids inside the magnet boil off rapidly. Helium will rise to the ceiling whereas nitrogen is denser than air and will fall. On the right is a picture posted on the web of a 700 magnet just after a quench occurred. Please watch this YouTube video from Bruker about quenches. Although quenches are rare, they can occur and so it is important for facility users to be aware of the threat and to know how to respond. **If you are in any of the magnet rooms and a quench were to occur (obvious from the noise of the expanding gas and the formation of a vapor cloud), your**

instructions are very simple - get out! Once you reach safety, please contact an NMR-3 staff member and let him/her know what has happened.

6.3 NMR Sample Related Hazards: Please take note of the follows rules which are designed to mitigate the risk associated with handling NMR samples:

- Many users will be dealing with toxic compounds or compounds of unknown toxicity. **You** are responsible for understanding all of the dangers associated with your compounds in case of a broken NMR tube and therefore a spill of the contents.
- Eye protection is required the entire time you are in the NMR facility.
- Nitrile/latex gloves as well as lab coats are not to be worn anywhere within the confines of the NMR facility. Your NMR tubes should be properly cleaned in your own lab before bringing it to NMR-3 for analysis. These items are available in the wet lab in the event of an sample-related accident requiring clean-up.
- Exercise caution when inserting your sample into a spinner. There have been cases over the years where students have used a tube which was a very tight fit in the spinner. When pushing down on such tubes, it is very easy to break it and seriously cut your hand. The proper approach is to grip the tube as near to the spinner as possible and use a twisting motion while inserting or removing the sample.
- No food or drink is permitted in the NMR facility, no exceptions!

7. Rule Violations & Disciplinary Action

NMR-3 facility rules and guidelines are put in place and enforced to give all users equal access to the NMR spectrometers and to help ensure the safety of the users and equipment within the facility. Therefore, it is extremely important that these rules are strictly adhered to. To help enforce this, penalties are in place for rule infractions. For a first-time offense, spectrometer access will be suspended for **1 week**. For each additional violation, you will be suspended for a total of **TWO MONTHS**. If it happens that the additional violation occurs within the initial 1-week suspension period, the two month suspension will be imposed consecutively, not concurrently.

8. Concerning High-Resolution NMR Samples

High resolution NMR spectroscopy of liquids demands the utmost attention to detail when it comes to preparing samples. This is particularly true when it comes to ^{1}H spectroscopy, where linewidths can routinely get down to a tenth of a Hertz in some solvents. To put this into perspective, 0.1 Hz for ^{1}H on the AVANCE 500 corresponds to a magnet field deviation of less than 0.2 parts per billion over the volume of the sample! In order to realize superior resolution such as this, you must observe the following:

- **Solution Homogeneity:** Do not allow particles of solids to remain in your NMR tube under any circumstances. If solubility proves to be a problem, filter your sample before putting it into the magnet. For the same reasons, gas bubbles in the NMR sample also severely degrade resolution and should be eliminated.
- **Sample Height:** It is critical that the height of the column of liquid in your NMR tube measure at least two times the height of the NMR receiver coil. If not, extensive shimming will be required. The precise height of the relevant receiver coil depends upon the combination of probe and nucleus you are observing. To keep this simple, a general rule of thumb for 5 mm NMR tubes is to use at least 700 μl of liquid which corresponds to a height of ~ 5 cm. Using more liquid than this is wasteful and will unnecessarily dilute your sample and hence your sensitivity. Keep the height of the liquid column consistent from sample to sample to minimize the time you spend shimming.
- **Tube Length:** The shortest commercially available NMR tube in a 5 mm design is 7” and that is the minimum acceptable height for use in NMR-3. If your tube breaks near the top, do not “cut it off” and continue to use it – this tube must be discarded. This rule is particularly important on the AV-500 in automation as tubes shorter than 7” may not be retrieved properly by the robotic sample changer, posing a serious risk for tube breakage.
- **Tube Quality:** There is evidence available to suggest that poor quality &/or mistreated NMR tubes can lead to damage of the delicate glassware inside the NMR probehead. Bruker has told me that one of our probeheads for the AVANCE 500 has a tolerance of only 190 μm between the NMR tube and the closest glass component inside the probehead! To help protect the probes, NMR-3 has a tube rule in place. Only Wilmad tubes are permitted with the minimum tube quality set at the Wilmad model 506-PP. Please be aware that this is not a 500 MHz quality NMR tube! 500 MHz quality NMR tubes from Wilmad cost on the

order of $30 Canadian per tube and, in our opinion, are not necessary for everyday research use. What is necessary is a tube of sufficient quality such that it poses no danger to the glassware inside the probehead and we believe the 506-PP serves this purpose. These tubes can be purchased through Dal Chemistry Stores.

- **Flame-Sealed Tubes:** Flame sealed NMR tubes are permitted in NMR-3 but these must not be spun. Of particular concern is when researchers wish to study flame-sealed samples at elevated temperatures. Please consult the NMR-3 Coordinator before performing any studies of this nature. In general, this is normally permitted but you will first be asked to "test" the NMR sample of interest by letting it sit in a water or oil bath at the highest temperature you wish to probe.
- **J-Young Tubes:** All sealed tubes containing valves (i.e. J-Young tubes) are permitted but must not be spun in any spectrometer. In addition, these tubes must be manually lowered and ejected from the magnets using a piece of string (there is no possibility to use these tubes with an autosampler). Please talk to the Facility Coordinator for further information and instruction.
- **Tube Labels:** NMR tubes are to be labeled either on the cap or along the side of the tube (just below the cap) using a marker. Paper or other "flag" labels are not permitted in NMR-3.
- **Concerning Spinners:** Always use the supplied depth gauge to position the sample in the spinner. Never touch any NMR spinner with your bare hands! Make sure the sample tube is held tightly in the spinner. If the tube is not held tight, it could move during insertion into the probe, causing problems with your NMR experiment and risking damage to the probe. Tiny pieces of kimwipe wedged between the spinner wall and the NMR tube wall for a tighter fit are not permitted under any circumstances!
- **Rinsing with Isopropanol:** Every NMR sample, as a final step before placing it in the magnet, must be rinsed with isopropanol and dried with a kimwipe.
- **Sample Spinning:** In order to help minimize the chance of damage to the probehead glassware, sample spinning is NOT permitted on the 300 and 500 MHz spectrometers.
- **Tube Care:** Clean your tubes immediately after you are done analyzing your sample. Always perform a final rinse with alcohol and allow to air dry. Using compressed air for quick drying and reuse of a tube is fine. Do not place high quality NMR tubes in a hot oven under any circumstances! Note that brand new NMR tubes straight out of the package should be cleaned before their first use.

9. Additional Remarks about Solid-State NMR

Solid-state NMR experiments are usually non-routine. If you need to acquire solid-state NMR data, please talk to the solid-state NMR coordinator, Dr. Ulrike Werner-Zwanziger (room 127). You will discuss with her what research questions you try to address and with which experiments this can be accomplished best, what methods will be used and which sample containers are best suited. Please bear in mind that Dr. Ulrike Werner-Zwanziger works only part time and is typically reached best in the mornings or using the contact information provided in Section 10.

10. Contact Information

NMR-3 Facility Contact Information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