100 MHz & 60 MHz

User Manual

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nanalysis

This book was carefully produced to describe the setup and operation of the Nanalysis benchtop NMR spectrometers. However, the authors do not claim that the information herein is completely free of errors. Readers are advised to keep in mind that all text and figures, including instructions, explanations, data, illustrations, or procedural details may contain inaccuracies, and Nanalysis will not be held liable for any damages to persons, property, or data that result from use of the information contained herein.

Table of Contents

1.0 Introduction

Thank you for purchasing your Nanalysis benchtop NMR spectrometer! This permanent magnet based Nuclear Magnetic Resonance (NMR) spectrometer is a pulsed Fourier Transform (FT) NMR spectrometer containing:

(1) a thermally regulated permanent magnet (1.4T/60 MHz or 2.35T/100 MHz);
(2) an electronic shimming system;
(3) a digital radio frequency (RF) transmitter/receiver subsystem;
(4) a suite of pre-programmed pulse sequences whose acquisition parameters are easily edited by the user;
(5) a touchscreen computer for digital data acquisition and signal processing with optional versatile pulse-programming language interface;
(6) a progress indicator to easily assess the state of data acquisition; and
(7) a connectivity panel.

In order to minimize the spectrometer footprint and maximize its portability, the Nanalysis benchtop NMR spectrometers are completely contained in a compact, all-in-one enclosure.

1.1 Benchtop NMR Spectrometers

NMR is a spectroscopic characterization technique in which a sample of interest is placed into an external, static magnetic field (B_0) and pulsed with an oscillating magnetic field (B_1) at a specified angular frequency (ω) that is dependent on an isotope's gyromagnetic ratio (γ). This is known as the Larmor Frequency.

$$\omega = \gamma B_0$$

The principal difference between the Nanalysis low-field benchtop NMR spectrometers and the higher field superconducting magnets is the method used to generate the static magnetic field. Instead of generating a magnetic field using a cryogenically cooled superconducting solenoid, the 60 and 100 MHz benchtop NMR uses carefully engineered permanent rare-earth magnets.

1.2 Operation

In some respects, operation of Nanalysis' benchtop NMR is similar to cryomagnet-based systems. For example, samples are typically prepared in standard 5 mm NMR tubes to a total sample volume of 0.5 to 0.7 mL in a deuterated solvent (section 3.1). Acquisition of NMR is performed with onboard software, with a highly automated, simplified NMR acquisition interface designed to reduce the complexity of system operation. This interface provides the option for manipulation of basic acquisition parameters prior to data collection and spectral processing during the spectral work-up (section 4). These simple manipulations will be easily recognized by experienced users and easy for new users to learn and adopt.

1.3 Using this Manual

This document is a resource to facilitate the incorporation of your benchtop NMR into routine laboratory use. This manual contains:

(1) instructions for installing the spectrometer (section 2)
(2) specific details of the operational features of the NMR GUI interface (section 3 – 8)
(3) guidance for easy data acquisition (section 3 and 4)
(4) information on optional software packages (section 7)
(5) troubleshooting tips (section 9)
(6) answers to some frequently asked questions (section 11)

Within the discussion of operational features, this manual will frequently refer to the use of buttons from the user interface. In an attempt to simplify this discussion, buttons will be referred to by name and bolded (*e.g.*, **Go**, **Status**) and tabs by name and italicized (*e.g.*, *1D*, *Shim History*, *Customer Service Agent*).

2.0 Getting Started

2.1 What's in the Box?

Each Nanalysis benchtop NMR shipment includes:

- ☑ 1 spectrometer
- ☑ 1 converter + 1 power cable
- ☑ Solvent reference kit
- ☑ 1 sample warmer + power cord
- ☑ Tube holder/guide and depth gauge *(100 MHz only)*
- ☑ 2 broken sample removal tools (*60 MHz only*)
- ☑ 1 quick-start guide

In addition, each spectrometer includes a one-year, single-site spectral processing license. The user can choose between a license for Mestrelab's MNova or ACD/Labs' Spectrus. These third-party processing software packages are powerful tools for advanced processing and spectral manipulation.

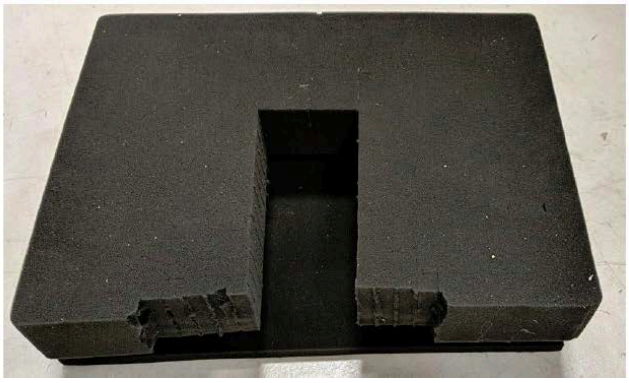
2.2 Unpacking and Moving your Benchtop NMR Spectrometer

While the benchtop NMR is mechanically robust, it is a precision instrument and must be handled accordingly. Care should also be taken to ensure that the spectrometer is not inverted for lengthy periods of time, and it is treated gently. In order to generate a strong magnetic field homogenous and stable enough for NMR applications, it requires the use of very precise mechanical and electrical means. To ensure that these systems are not damaged upon shipment. The spectrometer will be transported in a wooden crate and labeled as fragile. This helps to ensure that the unit is handled with care and that the instrument remains at the correct orientation.

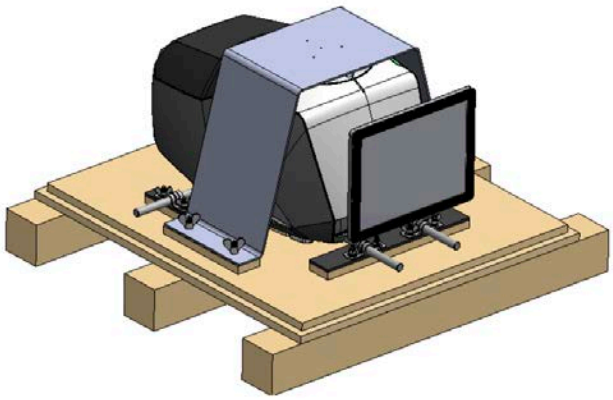
For 60MHz: Inside the wooden crate is a black, hard plastic case that will be useful for storing or relocating the instrument. It is recommended that two strong individuals lift this black case out of the wooden crate. This black case may have wheels for your convenience for storing. Regardless, it should *NOT* be rolled when the instrument is inside. Instead, it should be carried to the desired location before the case is opened and the NMReady removed. When moving the NMReady between locations, it does not need to be put back into the wooden crate. However, it should not be wheeled around in the black case. We recommend keeping this packing material to simplify any moves in the future.

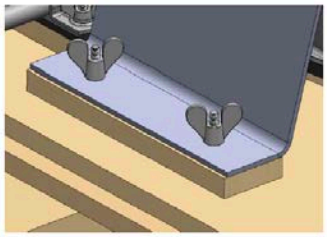
For 100MHz: The instrument is palletized and bolted to a wood crate. Upon receiving your instrument, inspect the crate for damage inspecting all shock and tip sensors.

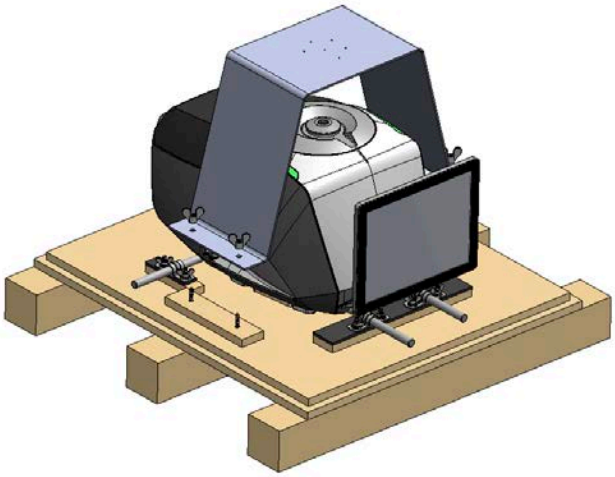
To remove the instrument, release the 8 latches and remove the crate lid by lifting it straight up, being cautious to not drag it along the instrument. Once the lid is off, remove all packed supplies (*i.e.*, power bricks, cables, accessories, etc.) and remove the protective foam from around the external monitor. It is recommended that this foam be kept along with the wooden packing material if a warranty service is required.



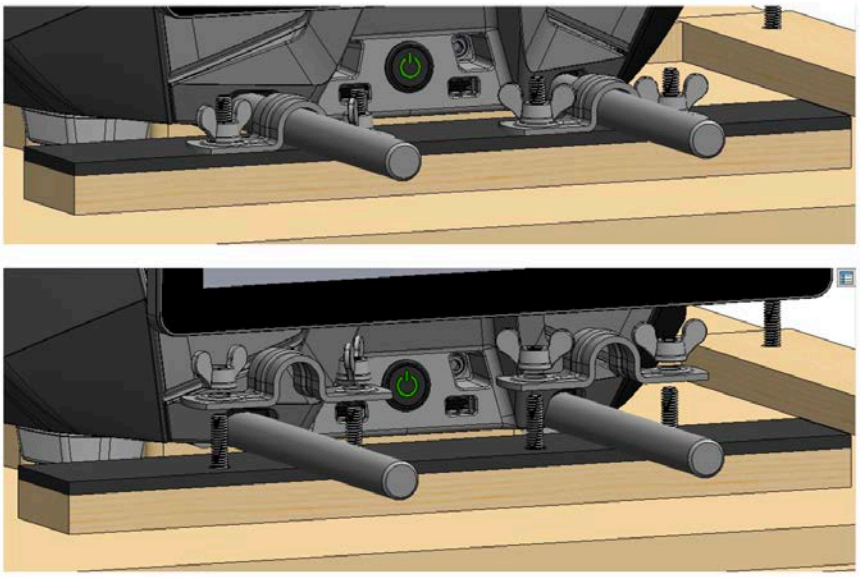
The instrument will be secured with a metal cover. Remove the large wing nuts and lift the metal support up and over the instrument, being cautious not to scrape it.







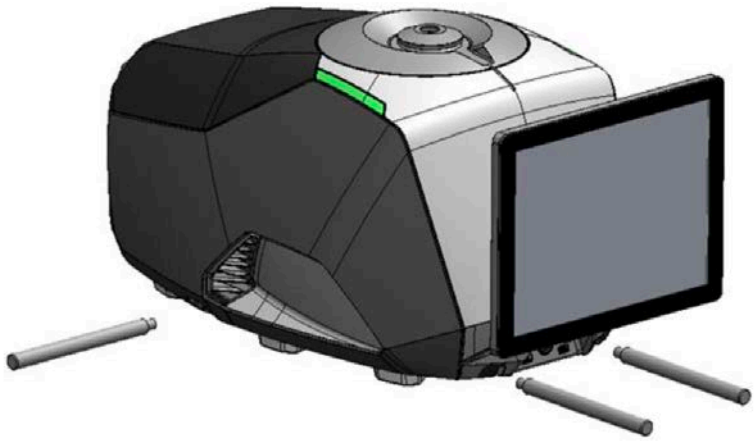
The instrument is secured to the crate through metal rods on the front and side. These can be loosened by removing the small wing nuts and lifting the clamps off the handles. Please retain this hardware for future packing usage. Prepare to lift the instrument from the crate with 4 strong people.



Please note that the instrument weighs 240lbs. Please ensure that all 4 lifting participants are capable of lifting heavy loads and can keep the instrument level. Tipping of the instrument during lifting may cause participants to experience heavy loads, resulting in injury and/or dropping of the instrument.

The instrument should ONLY be lifted by the carrying handles. Do not lift the instrument directly by its panels as they are not intended to be load baring and using them as such may result in damage to the instrument.

One the instrument is placed in the desired location, remove the carrying handles by unthreading them from the enclosure frame. Please store the handles for future use to relocate or pack the instrument.



2.3 Siting the Benchtop NMR

To facilitate optimum performance of your new benchtop spectrometer, please follow the guidelines detailed below:

2.3.1 Choosing a location in your lab

There are three environmental factors that can affect the performance of the spectrometer:
(1) ambient temperature gradients and fluctuations;
(2) magnetic field interference; and
(3) excessive vibrations.

To minimize these effects, the spectrometer should be located away from drafty areas (*e.g.,* directly under air conditioning vents or near open doors), moving metal (*e.g.,* elevators, machine rooms), or stray static or time-varying magnetic fields (*e.g.,* poorly shielded NMR spectrometers, stir plates, vacuum pumps, rotary evaporators). While it can be okay to have the spectrometer located nearby other laboratory equipment (*e.g.,* liquid chromatography or infrared spectrometers), one should be conscientious of the type and/or nature of this instrument. Stir plates should be situated a suitable distance from the spectrometer.

The spectrometer has vibration-absorbing feet that reduce most vibrational interference. However, in areas where vibration is particularly problematic, it may be beneficial to rest the spectrometer on a heavy wooden or cement benchtop.

2.3.2 Laboratory temperature specification

It is recommended that the instrument be located in a controlled laboratory environment. More specifically, we suggest a temperature range of 18-26 °C (64-78 °F), with a maximum hourly temperature variation of +/- 1.5 °C (+/- 2 °F). The relative humidity should be within 15-80 %.

2.3.3 Supplying power to the Spectrometer

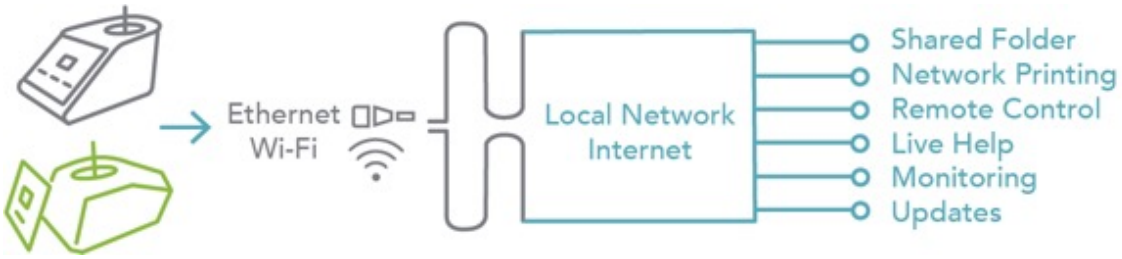
Nanalysis NMR Spectrometers can be plugged directly into a standard (120/240 VAC, 50/60 Hz) power outlet. For the 60MHz the connectivity panel is located on the side of the instrument, for the 100, the power cord can be secured in the back. However, for locations with frequent power fluctuations, it is strongly recommended that an uninterruptable power supply (UPS) be used to help maintain the longevity of the instrument. To prevent damage to the instrument, or injury to the user, it is strongly recommended that only the power cords provided with the instrument are used to power the spectrometer, autosampler and sample warmer. If there is consistently interference observed in acquired NMR spectra at a specific location, plugging the spectrometer into a dedicated circuit (often identified by an orange-coloured power receptacle) may help.

2.3.4 Networking the Spectrometer

The instrument is equipped with both a Wi-Fi and an Ethernet port (on the bottom right-hand side of the spectrometer) and it is highly recommended that the instrument be located near an Ethernet wall port.

Although the spectrometer can be operated as a stand-alone instrument, there are numerous features that utilize a network connection. This connection enables users to:

1) print directly to a network printer (section 6.4.2.2)
2) export data to a shared network folder (section 6.4.2.2)
3) remotely access the instrument (*e.g.*, from a laptop) (section 6.4.2.2)
4) access advanced customer service over the Internet (section 6.5.1)



Advanced customer service via the Internet can be used as the first line of defense for troubleshooting and correcting any problems that may arise with your instrument.
Features of advanced customer service include:

1) the ability to have customer service agents remotely see your spectrometer's screen and control its operation for troubleshooting or training purposes. This feature is initiated by selecting **Share your screen** on the *Customer Service Agent* tab after pressing the **Help** button. Please see section 6.5.1 for a more detailed discussion.
2) the ability to update the software on your spectrometer.
3) the ability to request that a Nanalysis customer service agent logs and analyzes operational details from your spectrometer.

It should be noted that the connectivity features described above might require some setup from your IT department. The following details should be shared with IT staff to ensure they understand how the benchtop NMR spectrometer behaves as a networked device.

1) Screen sharing uses the ConnectWise protocol (formely known as ScreenConnect) to remotely share the screen. The 60 MHz instruments use port 8041 by default and the 100 MHz instruments. Use port 443 by default. The Nanalysis customer service team cannot open a connection to the instrument from outside the firewall.
2) The instrument is setup to obtain its IP address dynamically from the network using DHCP. If a static IP address is required, please contact your account representative for instructions.
3) The instrument runs Linux (Ubuntu) as its operating system.
4) For security reasons, the MAC ID of the instrument may be required. If so, the MAC ID of the instrument can be provided by your sales representative.
5) New printer drivers can be installed on the instrument by the end user. They are downloaded using HTTP over port 80.

Nanalysis' customer service is always happy to communicate with your IT department regarding networking the instrument. More information can be found regarding networking in section 6.4.2.2.

Please note that Nanalysis Corp. will not be able to provide remote updates/customer support if you are unable to connect to the network. However, we can still provide assistance. Please submit offline inquires for customer support via email at *service@nanaylsis.com*.

2.4 Powering up for the first time

Once the location of your instrument has been designated, the first step is to plug the system either directly into the main power or into an uninterruptable power supply (UPS) that is plugged into the main power line. To prevent damage to the instrument, or injury to the user, it is strongly recommended that only the power cords provided with the instrument are used to power the spectrometer, autosampler and sample warmer. Once the machine is plugged in, the sound of the fan will be immediately evident. To initiate the power-up sequence of the CPU, press the power button on the right-hand side of the instrument and wait for the software to load. When powering down the instrument, it should be allowed to fully complete the shutdown process before the instrument is unplugged.

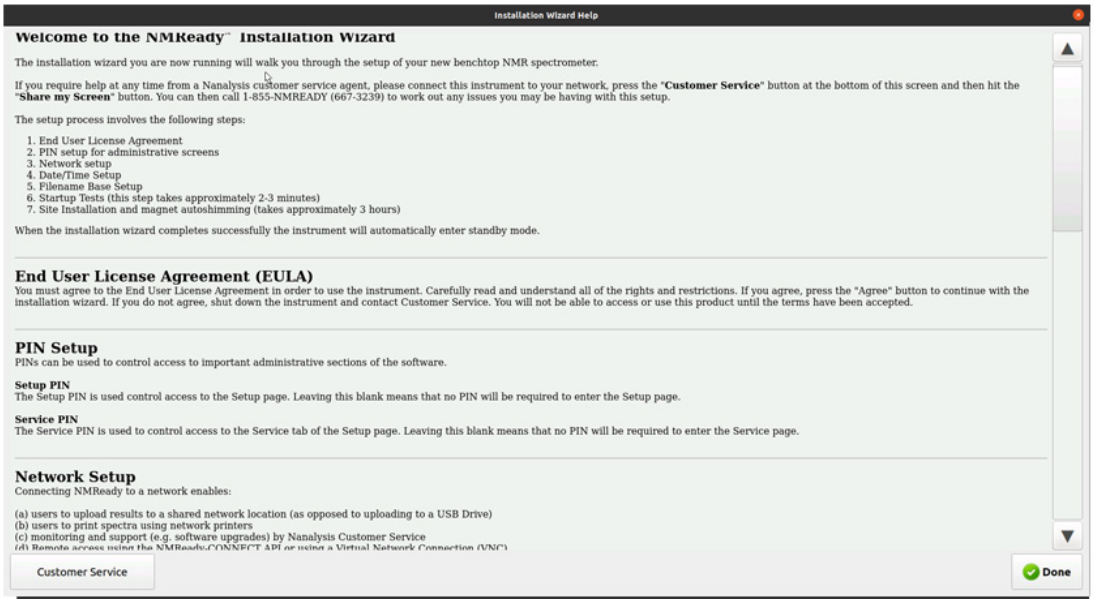
2.5 Temperature Stabilization

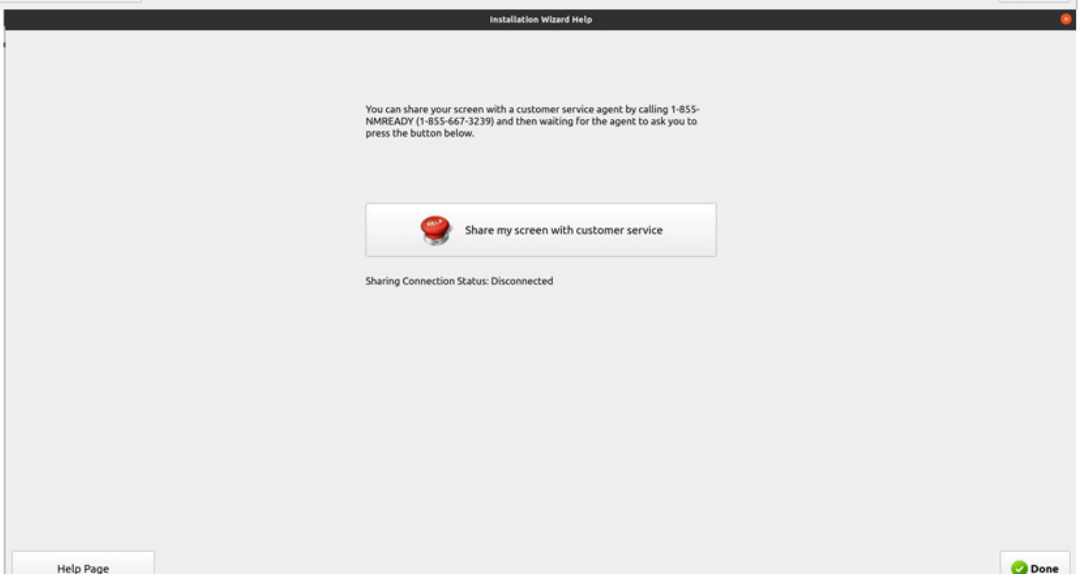
The magnet operates at a set-point temperature (typically 32.00 ± 3 °C). Depending on the initial temperature of the system, when it is powered up it may take a few hours for the system to reach temperature and stabilize internally. The average time required for a 60 MHz spectrometer is 4.5 hours, whereas 8 hours are required for the 100 MHz. The magnet temperature is monitored internally throughout this procedure and is displayed on the screen so its progress can be tracked.

In an effort to ensure that the internal magnet temperature is not suddenly perturbed once it has stabilized, we have also included a sample warmer. Optimal performance is achieved when the samples are warmed for approximately 1 minute prior to introduction into the magnet. This will help prevent temperature gradients and drift effects that can temporarily degrade magnet performance. However, use of this accessory is not absolutely necessary (section 3.1). Well-resolved spectra can be acquired without using it.

2.6 Initializing the system

All newly shipped instruments are configured to automatically run a setup procedure (the NMR Installation Wizard) that will guide you through the system initialization. This initialization takes about five to seven hours for the 60MHz, and ten to twelve hours for the 100MHz and consists of heating the magnet, signal optimization, solvent configuration and extensive shimming procedures. Once this script has reached completion, the spectrometer is ready for use. Screenshots from the wizard are shown below.





There is no need to remain in front of the spectrometer for the full duration of the installation process. Intermittent supervision, however, is recommended. While there is no need to contact Nanalysis' customer service about this process, it is strongly recommended that we are contacted to help ensure your instrument is performing to specification.

This can be done by selecting the **Help** button in the bottom left-hand corner of the installation wizard, then the **Customer Service** button in the bottom left-hand corner of the Welcome page, and then finally selecting **Share my screen with customer service**. This does require a network setup. Please see section 2.3.4 to learn more.

If it is required to run this procedure again, the installation process can be accessed from **Setup** ➔ *Service* ➔ *Maintenance* ➔ **Site Installation** (see section 6.4.6.1).

2.7 Regulatory Specifications

60 MHz

Product deployment environment: Laboratory
Pollution degree: 2
Protection class: Power Supply Class 1
Environmental rating: Standard
Installation category: II
Altitude: 2000 m
Humidity: 0-90% (non-condensing)
Electrical supply: 13.5 VDC
Indoor use only
Operating Temperature range: 18-26 °C
Follows the: CE mark, the CSA mark and FCC Declaration of Conformity

100 MHz

Product deployment environment: Laboratory
Pollution degree: 2
Protection class: Power Supply Class 1
Environmental rating: Standard
Installation category: II
Altitude: 2000 m
Humidity: 0-90% (non-condensing)
Electrical supply: 19 VDC
Indoor use only
Operating Temperature range: 18-26 °C
Follows the: CE mark, the CSA mark and FCC Declaration of Conformity

3.0 Acquiring NMR Data on Nanalysis' Benchtop NMRs

3.1 Sample Preparation

Given the inherently lower sensitivity of a 60 MHz spectrometer versus a higher field instrument, it is recommended that samples be prepared at higher concentrations than NMR users may be accustomed to. It has been found that ^{1}H samples prepared at concentrations of ≥0.1 M have similar acquisition times to experiments typically run on high-field instruments. We recommend typical sample volumes of 0.5–0.7 mL. For getting high quality spectra on heteronuclides, such as ^{13}C{^{1}H} data, we recommend more concentrated samples (*e.g.*, 0.5 M). More dilute samples, of course, are suitable but more signal averages and larger run times are required.

A sample warmer is included with the spectrometer. Spectra can be run on samples at room temperature, but for optimal results, it is recommended that the user allow the sample to warm up to the spectrometer's internal temperature in the sample warmer for 1–2 minutes prior to acquiring NMR data. This ensures that temperature drifts are not introduced to the magnet due to sample temperature gradients. It is not absolutely necessary that the sample warmer be used, but its use can insure optimal performance in a short time period. This can save time if many samples are to be run one after the other.

3.2 Benchtop NMR Operation

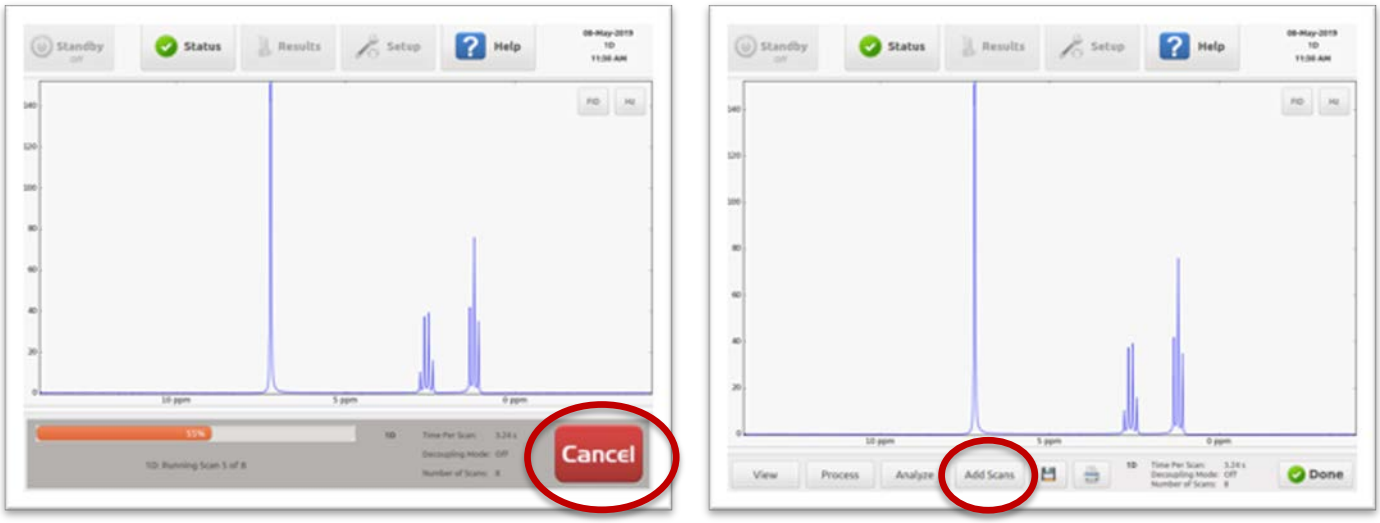
The basic parameters for an experiment can be defined directly from the quick select buttons on the bottom of the main screen, including nuclei, experiment, solvent, and number of scans. At the top of this screen, and most other screens, the Task Bar appears. Functionality available from the Task Bar is described in detail in section 6. The present chapter describes basic operation of your instrument, including selection of acquisition parameters and initiation of an acquisition.

Depending on the NMR model purchased, the nuclei available under the **Observe Nucleus** button will differ. The 60e/100e will provide ^{1}H, while the 60PRO/100PRO usually includes ^{1}H and either ^{7}Li, ^{11}B, ^{13}C,

^{19}F or ^{31}P as was specified by the user at the time of purchase. Unless requested, there wilk be the standard deuterium (^{2}H) **Lock Nucleus**, but there is also a proton lock (^{1}H) feature if desired.

By accessing the orange **Experiment Settings** button, other experiment parameters can also be easily manipulated, as is discussed in section 3.2.2.

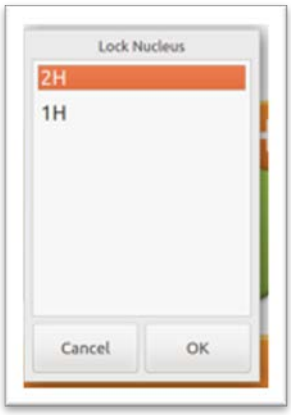
Once the parameters have been set, the **Go** button on the main screen will initiate the experiment. It is important to note that it is also possible to set up multiple experiments by using the **+** button located at the top right corner of the **Go** button (only visible for instruments with queuing enabled). This is discussed more extensively in section 7.1. For a standard experiment acquire, once **Go** has been selected, the instrument performs a quick lock and automatic receiver gain adjustment before the pulse protocol begins. The first scan will be presented (post Fourier transform) on the screen, and the spectrum will be updated with S/N improvements every additional four scans. If more scans were selected than are required, the experiment can be halted via the **Cancel** button in the bottom right-hand corner. Conversely, more scans can be added once acquisition is complete by selecting **Add Scans**.

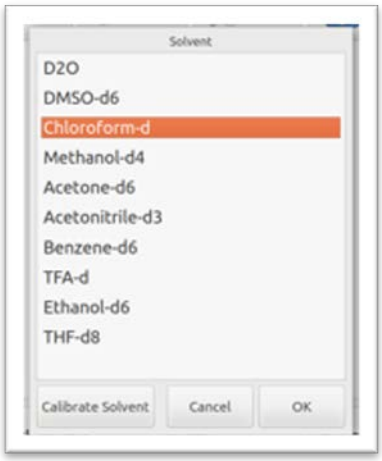


The final data will appear in the main panel of the screen ready for analysis, acquiring more scans, saving or exporting.

3.2.1 Quick Select Buttons

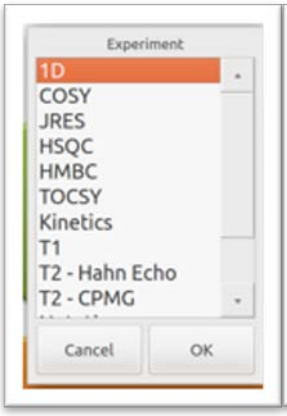
The quick select buttons across the bottom are: (1) **Observe Nucleus**; (2) **Lock Nucleus**; (3) **Solvent**, (4) **Experiment**; and (5) **Number of Scans**. The **Observe Nucleus** button allows you to select the nucleus you wish to observe. In the picture below, ^{1}H and ^{13}C are shown as options. The user can change the selection by simply selecting the desired observe nuclei. If non-*deutero* locking options are enabled, they can be selected here by choosing ^{2}H (standard deuterium lock) or ^{1}H (*proteo* lock) in **Lock Nucleus**. As is typical with NMR Spectroscopy, deuterated solvents are recommended. If enabled by your Nanalysis customer service representative, proteo-lock is used in the same manner as deuterium lock by selecting ^{1}H in **Lock Nucleus**.

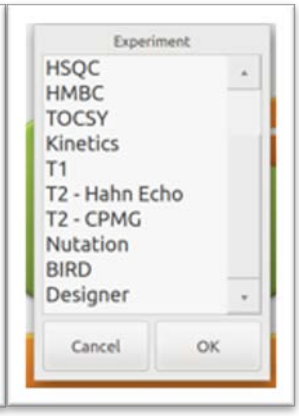




The solvent the NMR sample was prepared in should be specified on the **Solvent** button. The instrument is configured with a common solvent list upon shipping, however, this can be modified and customized to the users' preference as is discussed in section 6.4.2.5. Once this button has been selected, a pop-up menu will appear, and the appropriate solvent can be selected directly from this list, followed by **OK** to confirm selection. This allows the benchtop NMR to perform an internal calibration and set the lock signal at the correct frequency (*e.g.*, Chloroform-d δ = 7.24ppm). The calibrate solvent button is not commonly used, but it can be useful if the event that the user receives a 'lock lost' warning in the Status window (see section 11.1.9).

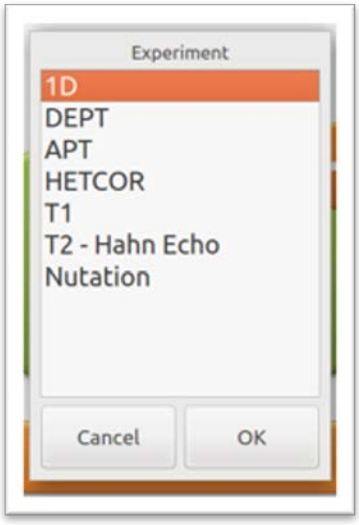
The experiment you wish to run can also be defined in the main screen by selecting an experiment from the bottom right-hand side of the touch screen **Experiment** selector. When you select this button, a pop-up window will appear in the center of the screen with the options available to you. Nanalysis' benchtop NMR spectrometers are compatible with a variety of experiments. Depending on the experimental package that came with your instrument and the nucleus you are observing. For proton detected experiments, for example basic experiment lists could include basic 1D, spin-lattice relaxation (T_1), a variety of spin-spin relaxation (T_2- Hahn Echo, T_2-CPMG), Nutation (used to calculate the precise 90° pulse angle), COrrelation SpectroscopY (COSY), J-REsolved Spectroscopy (JRES), TOtal Correlated SpectroscopY (TOCSY), Heteronuclear Single-Quantum Coherence (HSQC), Heteronuclear Multiple Bond Correlation (HMBC), solvent suppression, designer (for advanced pulse programming) and kinetics.



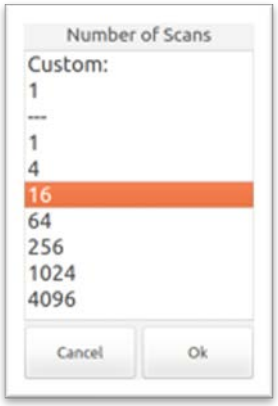


For carbon detected experiments, for example basic experiment lists could include basic 1D, spin-lattice relaxation (T_1), spin-spin relaxation (T_2), Nutation (used to calculate the precise 90° pulse angle), Distortionless Enhancement by Polarization Transfer (DEPT), Attached Proton Test (APT),

HETeronuclear CORrelation spectroscopy (HETCOR), nutation, and designer. The individual experiments will be described in more detail in section 3.2.2.

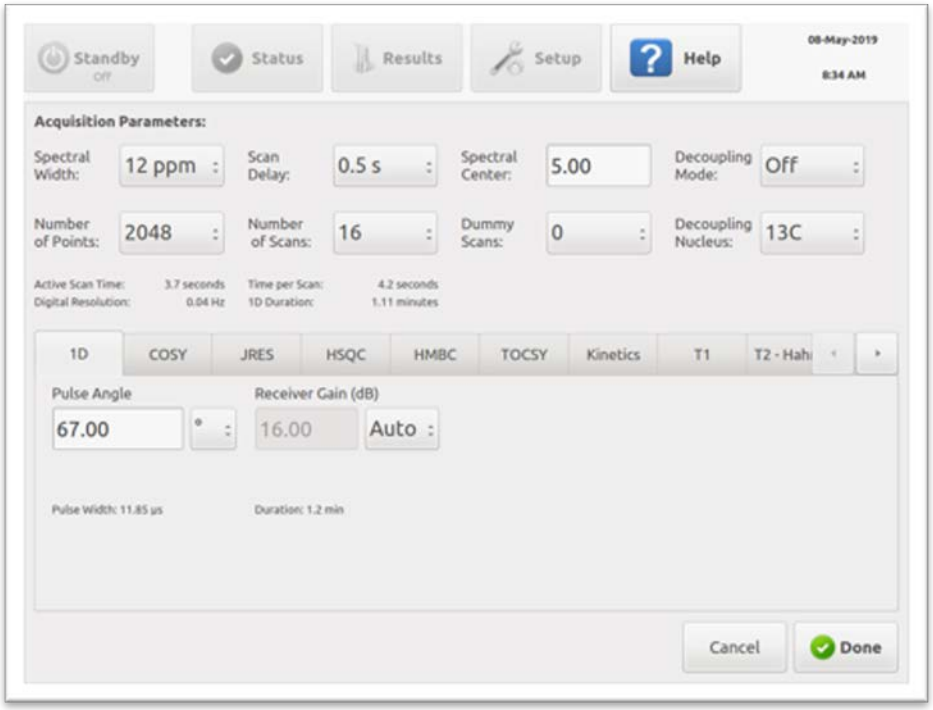


Finally, the number of scans that will be performed in an experiment can be selected from the **Scans** box from a preset list or by selecting the word **Custom** and using the pop-up keyboard to input the exact number of scans you desire. The scans in the list are multiples of four as the instrument uses a phase alternating pulse sequence (PAPS), however it is not necessary to customize to a multiple of 4.



3.2.2 Experiment Settings

The **Experiment Settings** menu, located immediately below the **Go** button, will open a new window with a more detailed selection of acquisition parameters. The top half of this screen has basic acquisition parameters: (1) spectral width; (2) number of points; (3) scan delay; (4) number of scans; (5) spectral center, (6) number of dummy scans, (7) decoupling mode and (8) decoupling nucleus. The last two parameters are only available in certain configurations (*e.g.*, $^{1}H/^{13}C$).



3.2.2.1 Spectral Width

The spectral width (SW in ppm) is the range of frequencies over which NMR signals will be observed. Depending on what nucleus is being observed, the spectral width will change considerably (*e.g.*, ^{1}H: 0 to 12 ppm; ^{13}C: 0 to 220 ppm). Please note, that resonances outside the selected spectral width may still be observed in the spectrum as 'fold-back' peaks. If these are observed, reacquire the spectrum with a wider spectral window.

3.2.2.2 Number of Points

The number-of-points parameter controls the number of data points that are acquired per free-induction decay (FID) by the spectrometer. This number is generally an integer power of 2, *e.g.*, 512, 1024, 2048, etc. Together with the spectral width (SW), this parameter affects the active scan time and the digital resolution. These are reported on the right-hand side of the screen. The active scan time is the time during which the spectrometer is busy acquiring and processing data while the digital resolution is the number of Hz per digital point.

A smaller digital resolution can enable greater definition of finer features; however, it also takes longer to collect scans. A balance should be struck between time per scan and the number of points required. For complex valued points,

$$\text{Acquisition time (AT)} = (\#\text{ of points}) / (\text{SW in Hz}).$$

(Note that some authors discussing this relation define "number of points" to be the number of real values obtained, which is twice the number of complex valued data points). For most applications, 0.05–0.10 Hz resolution is more than sufficient unless you require very well-defined peak shapes, such as in a

quantitative application. It is also possible to improve the effective resolution of displayed data by using zero-filling during post processing of the data (see section 4.1.3.4) without increasing the acquisition time.

3.2.2.3 Scan Delay

The scan delay is the interscan delay time in seconds. That is, it is the time the NMR sample is given to relax to its equilibrium magnetization before the sample is pulsed again. Longer scan delays can afford more accurate integrations and are necessary for quantitative NMR (qNMR) applications. However, depending on your application, for basic structural elucidations, integration values of peaks can be approximate, and shorter scan delays will suffice. For example, a 1 second scan delay is often sufficient for basic structural elucidation and will provide a good estimate of the relative integrals of each signal.

For qNMR, a useful rule of thumb is that the scan delay should be five times greater than the longest T_1 for the signals of interest. Typically, 10–20 seconds are sufficient for proton NMR. Aromatic groups can be slow to relax, so if comparing alkyl to aryl composition typically longer scan delays give more accurate relative integrals. ^{13}C NMR signals typically have long T_1 and need longer scan delays than ^{1}H NMR signals, especially when ^{1}H decoupling is not used.

3.2.2.4 Number of Scans

The number of scans can also be changed from Experiments Settings window. Signal averaging improves the signal-to-noise ratio (SNR). Typically, this improvement is proportional to the square root of the number of scans used.

SNR can be estimated in a spectrum by comparing the amplitude of a peak (A) and the root-mean-square (rms) noise within a relatively flat region of the spectrum. A crude estimate of the rms noise is about (1/2.5) times the peak-to-peak noise, and so the formula

$$\text{SNR} = (2.5A) / (\text{peak-to-peak noise})$$

is helpful as a crude estimate of the SNR.

3.2.2.5 Spectral Center

The default setting for spectral center is half of the spectral width minus 1 (*e.g.*, if the spectral window is 12 ppm, the center will be 5 ppm). This is pretty typical for ^{1}H and ^{13}C where typically one would scan from approximately 0 ppm to 12 or 220 ppm, respectively. However, for nuclei like ^{19}F or ^{31}P, this can be easily manipulated depending on the type of sample being observed.

3.2.2.6 Dummy scans

In some cases, it is helpful to pulse on the sample a few times before data is acquired, so that incomplete relaxation of the sample's spin system in later scans does not render signal strength appreciably different from that of the initial scans. Dummy scans permit the spin system to reach a steady state prior to acquisition of data. This parameter can be set to 0, 1, or 2 to allow for attainment of steady state, and this may be useful, particularly in 2D experiments.

3.2.2.7 Decoupling Mode

Using the drop-down menu for heteronuclides, like ^{13}C and ^{31}P, the decoupling can be set to '**On**', '**Inverse Gated**', '**Gated**', or '**Off**' (see figure 1) '**On**' is the most basic setting when the decoupler is on for both pulsing and decoupling and is the default setting for a 1D $^{13}C\{^{1}H\}$ NMR experiment. This results in a spectrum that is proton decoupled and has Nuclear Overhauser Effect (NOE) enhancements and is therefore not quantitative. An 'inverse gated' spectrum is both decoupled and quantitative, as the decoupler is only applied during acquisition. A 'gated' spectrum shows carbon-proton multiplicity and therefore can be used to aid with structural elucidation, while having the benefit of NOE sensitivity enhancement. 'Off' acquires a 1D heteronuclear spectrum without any involvement of the proton channel.

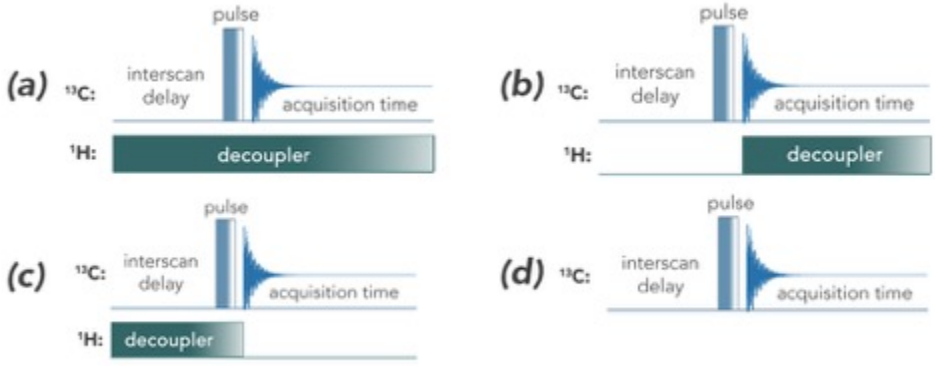


Figure 1: Pictorial depiction of decoupling modes *(a) on; (b)* inverse gated; *(c)* gated; and *(d)* off.

The **Pulse Width** is displayed in either degrees (°) or microseconds (µsec) to represent the angle the bulk magnetization is tipped to or length of time the RF pulse must be applied to reach this tip angle, respectively. The **Receiver Gain** can be left in auto or a fixed value can be selected. For both fields, the user can use the dropdown menu located on the right to change between the units.

On the bottom half of this screen, one can access additional experiment specific parameters as will be discussed below. As aforementioned, depending on the instrument purchased these tabs may differ between instruments.

3.2.2.8 COrrelation SpectroscopY (COSY)

The COSY experiment is a homonuclear 2D experiment meant to help a user determine which spins in a molecule are coupled together. The experiment is very simple. It consists of a single 90° RF pulse, followed by a specified time interval (t_1), followed by a second 90° pulse and detecting the FID (figure 2). The time interval between the pulses is incremented on successive iterations of the pulse sequence, and the data are collected into a matrix.

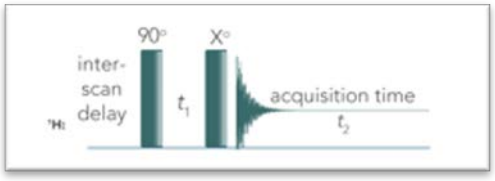


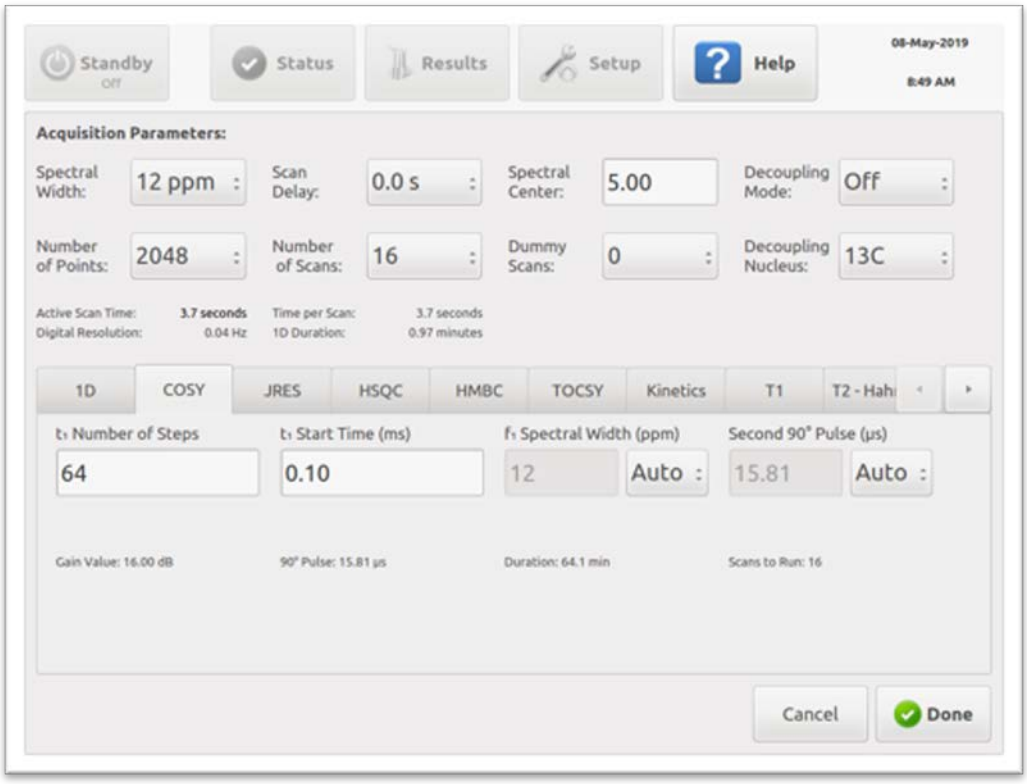
Figure 2: Pictorial depiction of COSY pulse sequence.

A given nuclear spin in a sample molecule evolves during the interpulse delay, and this evolution includes a contribution from its coupling to other, nearby spins. Thus, the resonance due to the given spin will be cyclically modulated during τ by the couplings. A Fourier transform with respect to the incremented time period encodes these couplings in cross peaks.

When the information is Fourier Transformed, the normal coherence of each type of nucleus is detected on the diagonal, and the magnetization transfer from spin-spin coupling partners is detected as these off-axis cross peaks. The appearance of cross peaks is evidence of proximity of the spins within the molecule.

There are many COSY variants described in the NMR literature. The instrument runs a standard COSY-90 (i.e., X=90° in figure 2) and displays and stores its data in magnitude mode.

To acquire COSY data select COSY from the **Experiment** quick select button on the main page. In **Experiment Settings** you can set the appropriate parameters. First, select a **Number of Points**, the more points (*e.g.*, 256 or 512) offer better resolution in the f_1 dimension and therefore better structure resolution, whereas fewer points offer magnitude COSY's where correlations are noted but fine structure is not resolved. We would recommend that you use 256 as the **t_1 Number of Steps**. If the resolution is not sufficient you can increase it in a subsequent experiment. If desired, modify the **Tau Start** time, but the default of 0.1 ms is suitable for the majority of samples. This defines the time (t_1) between the first and second pulses. The **f_1 Spectral Window** will automatically default to that selected in **Spectral Width** to ensure, as the resultant spectrum is symmetric. The **Second 90° Pulse** is calculated by the instrument in a nutation experiment. If the user does not run a nutation experiment on their sample prior the instrument automatically uses the last 90° pulse, which is suitable for most samples.



Once these parameters have been added, select **Done** to exit Experiment Settings and return to the main page, followed by **Go** to initiate the experiment.

3.2.2.9 JREsolved Spectroscopy (JRES)

The JRES experiment is meant to decouple chemical shift from coupling information. This can be a very useful experiment, particularly at low-field. The projection of chemical shift is shown in the f_2 dimension and the coupling information is displayed in the f_1 contour map. The pulse program itself is also quite simple, it consists of 90° pulse followed by a mixing interval delay, t_1 and a 180° pulse, t_1, and then data acquisition (figure 3). The time interval between the pulses is incremented on successive iterations of the pulse sequence, and the data are collected into a matrix.

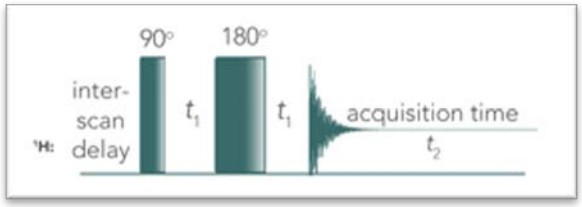
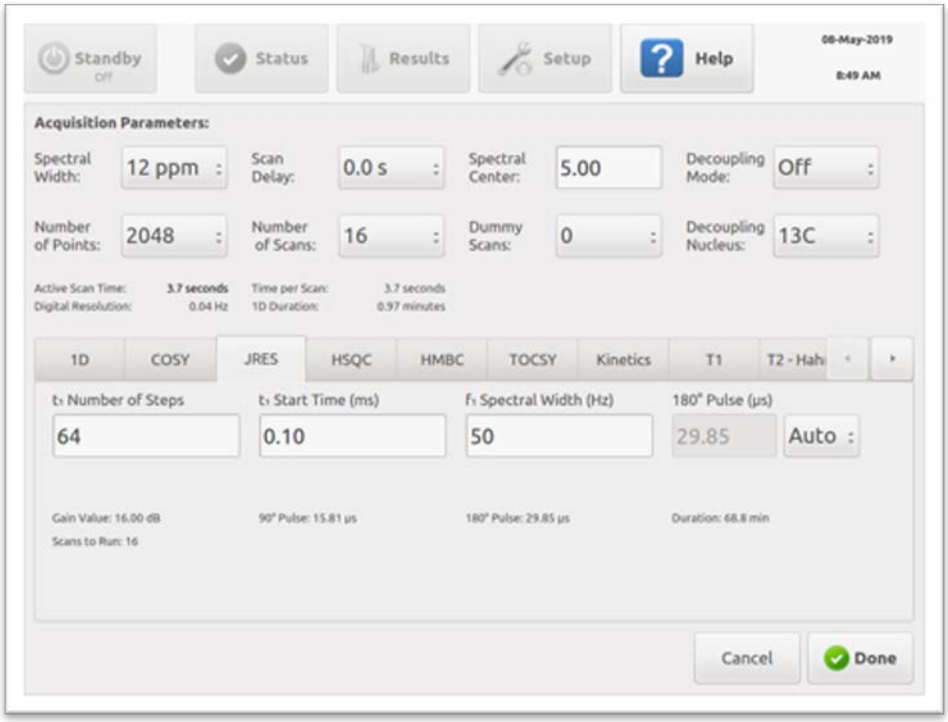


Figure 3: Pictorial depiction of JRES pulse sequence.

The data can be collected with fewer rows than a COSY without affecting the resolution of the cross peaks. We typically recommend **t_1 Number of Steps** of 32 or 64. If desired, modify the **Tau Start** time, but the default of 0.1 ms is suitable for the majority of situations. The **f_1 Spectral Width** is the width of *J*-couplings that will be displayed and, for most proton spectrum this should be between 20 and 60 Hz. Septets may require larger windows.

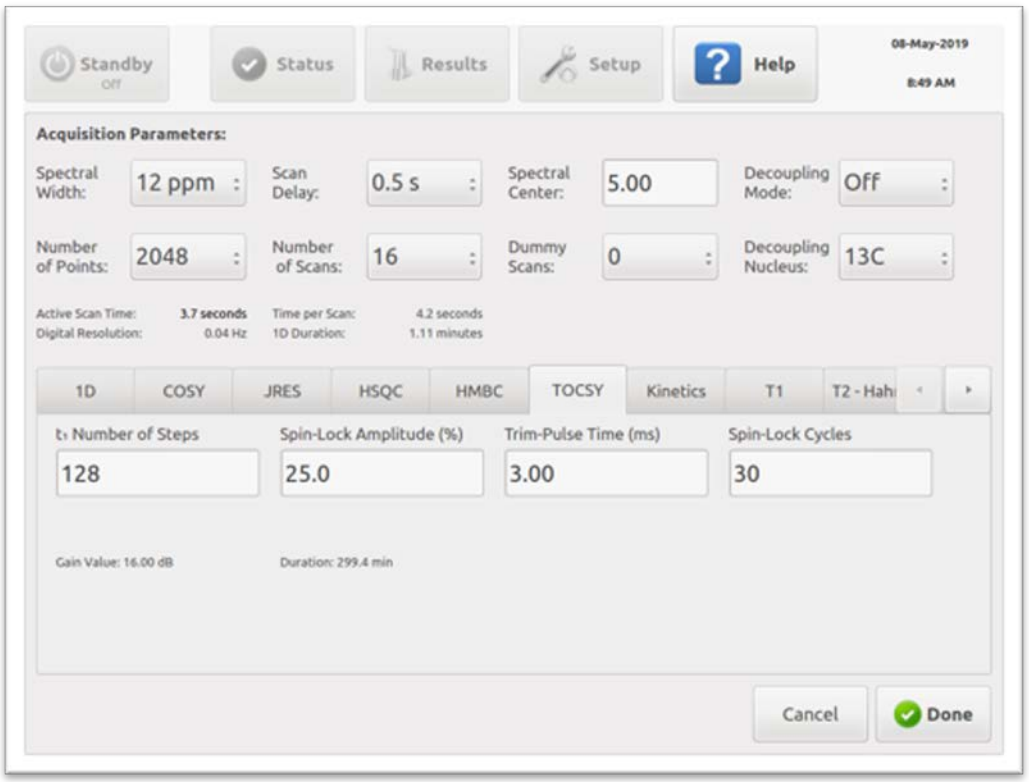


3.2.2.10 TOtal Correlation Spectroscopy (TOCSY)

The TOCSY experiment is a homonuclear 2D experiment similar to the COSY, however instead of only showing molecular fragments that are directly bonded, this correlation experiment shows all relationships in a spin system whether or not they are directly coupled. It consists of a single 90° RF pulse, followed by a specified time interval (t_1), followed by spin lock series framed by 'trim pulses' applied for time τ_m before detecting the FID (figure 4). This procedure is run for a specified number of steps where t_1 is run from a short to a long period of time.

Figure 4: Pictorial depiction of TOCSY pulse sequence.

To set up the experiment, alter the 1D acquisition parameters as is desired for each step, the number of which is set in **t_1 Number of Steps**. The TOCSY specific parameters that can be modified primarily address the nature of the spin-lock pulse. The **Spin-Lock Amplitude (%)** is a measurement of the relative amplitude of the spin-lock pulse relative to the amplitude of the initial 90° pulse. This is run at lower amplitude to reduce the amount of power being applied during the pulse sequence. The **Spin-Lock Cycles**, represent the number of composite rapid pulses applied during the spin-lock τ_m time. The **Trim-Pulse Time** is the duration of the pulse applied at the beginning and end of the spin lock pulsing sequence. It is included in the τ_m time. This pulse is used to remove magnetization that is transverse to the spin lock axis prior to the spin lock sequence.



3.2.2.11 Heteronuclear Single Quantum Coherence (HSQC)

The HSQC (heteronuclear single quantum coherence) experiment is a 2D experiment that can be used to correlate resonances in a ^{1}H spectrum with those that is directly bonded to in the spectrum of a heteronuclide, such as ^{13}C. This can give key structural clues, since cross peaks in the 2D data appear at positions (f_1, f_2) that correspond to directly bonded ^{1}H-^{13}C pairs. In the HSQC, the free-induction decays (FIDs) are recorded on the ^{1}H channel, and so the "direct" dimension f_2 frequencies, horizontal in the spectral display, are proton chemical shifts. The vertical f_1 dimension is the result of a Fourier transform with respect to time values, t_1, that are incremented one FID to the next in the manner common to 2D spectroscopy.

Full analysis of the pulse sequence, show below in figure 5, is best considered from the perspective of product operators and is beyond the scope of this discussion, but a few words here are useful to help in selecting parameters for acquisition. The key evolution that takes place during t_1, and gives rise to peak position along f_1, is due to ^{13}C chemical shift. Therefore, select an f_1 spectral width and an f_1 spectral center that encompass the full range of anticipated ^{13}C chemical shifts. This can vary from sample to sample but may typically be about 200 ppm, centered at about 100 ppm, unless it is expected from chemical considerations to be narrower in the ^{13}C domain.

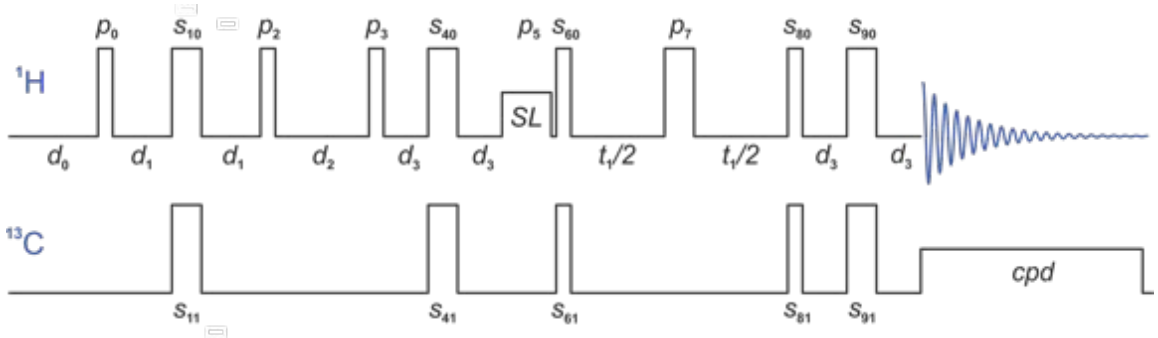
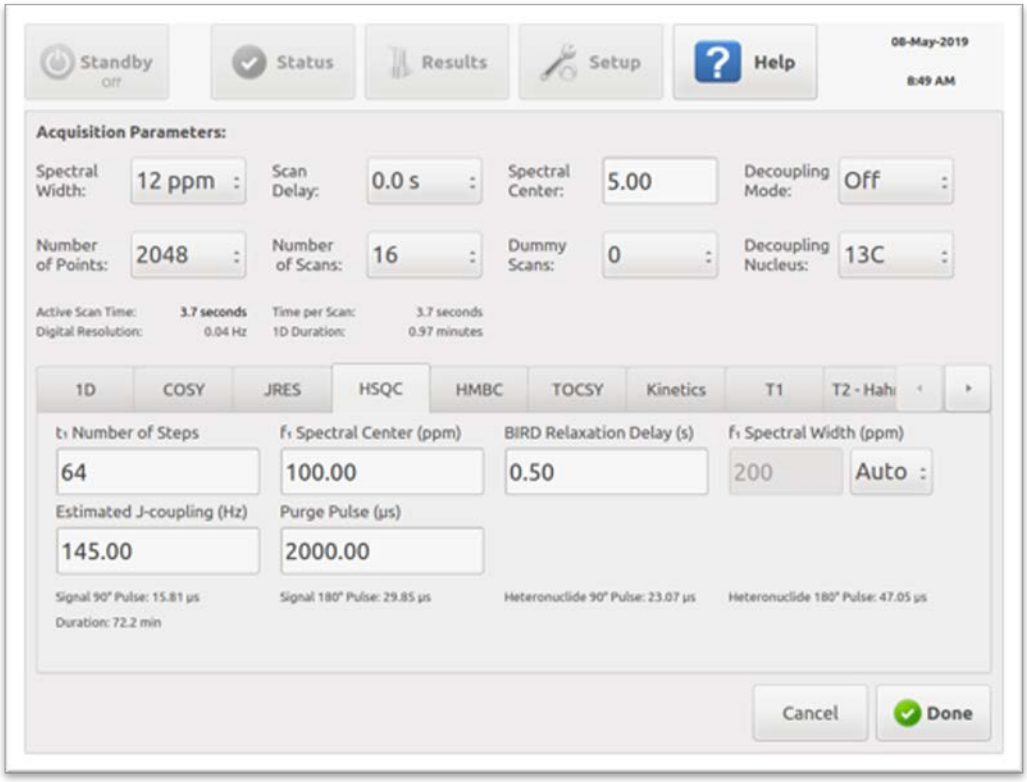


Figure 5: Pictorial depiction of HSQC pulse sequence.

The parameters shown in the top menu (**Spectral Width**, **Number of Points**, **Scan Delay**, **Number of Scans**, **Spectral Center** and **Dummy Scans**) refer to directly detected acquisition of the ^{1}H FIDs and should be selected to encompass the full range of anticipated ^{1}H chemical shift. A relatively low # of points (512) can be used, as full resolution of the homonuclear *J*-couplings in the proton spectrum is not as important as the location of the correlation peaks. The ^{13}C spectral width and center is addressed by using the automatic 200 ppm specified in **f_1 Spectral Width** and 100 ppm in **f_1 Spectral Center** or setting your desired value.



Select the **t_1 Number of Steps** based upon the desired resolution over this range – 64 or 128 is common. This period of evolution is preceded and followed by short pulse sequences that transfer spin coherence between ^{1}H and ^{13}C, the so-called 'INEPT' sequence (Insensitive Nuclei Enhanced by Polarization Transfer). The **Estimated J-coupling** is set at 145 Hz as a compromise between sp^3, sp^2, and *sp* hybridized carbon centers.

An important consideration in acquiring HSQC spectroscopy is suppression of artifacts having to do with very large but unwanted ^{1}H resonances from protons bonded to ^{12}C, which are excited by the pulse sequence but are not subject to the coherence transfers that precede and follow the t_1 evolution period in the pulse sequence. This suppression is achieved primarily with an 8-phase cycle, and so the number of scans selected will be a multiple of 8. It can also be helpful to select 6 or 8 dummy scans to help reach a steady state magnetization for the sample. The NMReady-60 HSQC program includes an optional "BIRD" (BIlinear Rotation Decoupling) filter as part of the further suppression of the spurious non-^{13}C-bonded ^{1}H magnetization as well as a purge pulse. If desired, the **BIRD Relaxation Delay** can be optimized for the sample by running a BIRD experiment from the **Experiments** menu. In particular,

small molecules with long ^{1}H relaxation times can benefit from somewhat longer BIRD relaxation delays – 0.5 s is used as a default. The **Purge Pulse** is similar to the spin lock series applied in TOCSY (section 3.2.2.10), and the user can modify the length that this is applied (in μsec).

Finally, the cross peaks can be displayed as doublets with **Decoupling Mode** turned '**Off**', or, for improved effective SNR, decoupling on the heteronuclide channel can be applied by selecting '**Inverse Gated**' from the decoupling mode menu.

3.2.2.12 Heteronuclear Multiple Bond Correlation (HMBC)

The HMBC experiment is a 2D experiment that can be used to correlate resonances in a ^{1}H spectrum with those that are coupled to, but not necessarily directly bonded to a heteronuclide, such as ^{13}C. This can give key structural clues, since cross peaks in the 2D data appear at positions (f_1, f_2) that correspond to ^{1}H-^{13}C pairs connected by up to four bonds. In HMBC, the free-induction decays (FIDs) are recorded on the ^{1}H channel, and so the "direct" dimension f_2 frequencies, horizontal in the spectral display, are proton chemical shifts. The vertical f_1 dimension is the result of a Fourier transform with respect to time values, t_1, that are incremented one FID to the next in the manner common to 2D spectroscopy.

Full analysis of the pulse sequence, show below in figure 6, is best considered from the perspective of product operators and is beyond the scope of this discussion, but a few words here are useful to help in selecting parameters for acquisition. The key evolution that takes place during t_1, and gives rise to peak position along f_1, is due to ^{13}C chemical shift. Therefore, select an f_1 spectral width and an f_1 spectral center that encompass the full range of anticipated ^{13}C chemical shifts. This can vary from sample to sample but may typically be about 200 ppm, centered at about 100 ppm, unless it is expected from chemical considerations to be narrower in the ^{13}C domain.

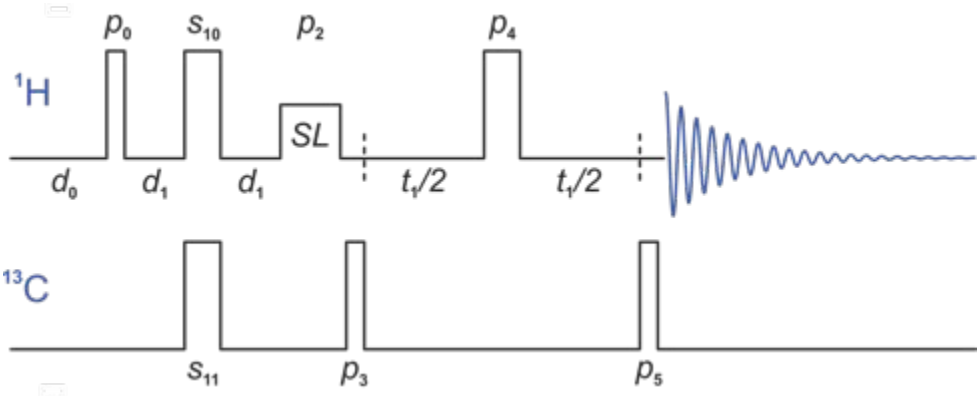
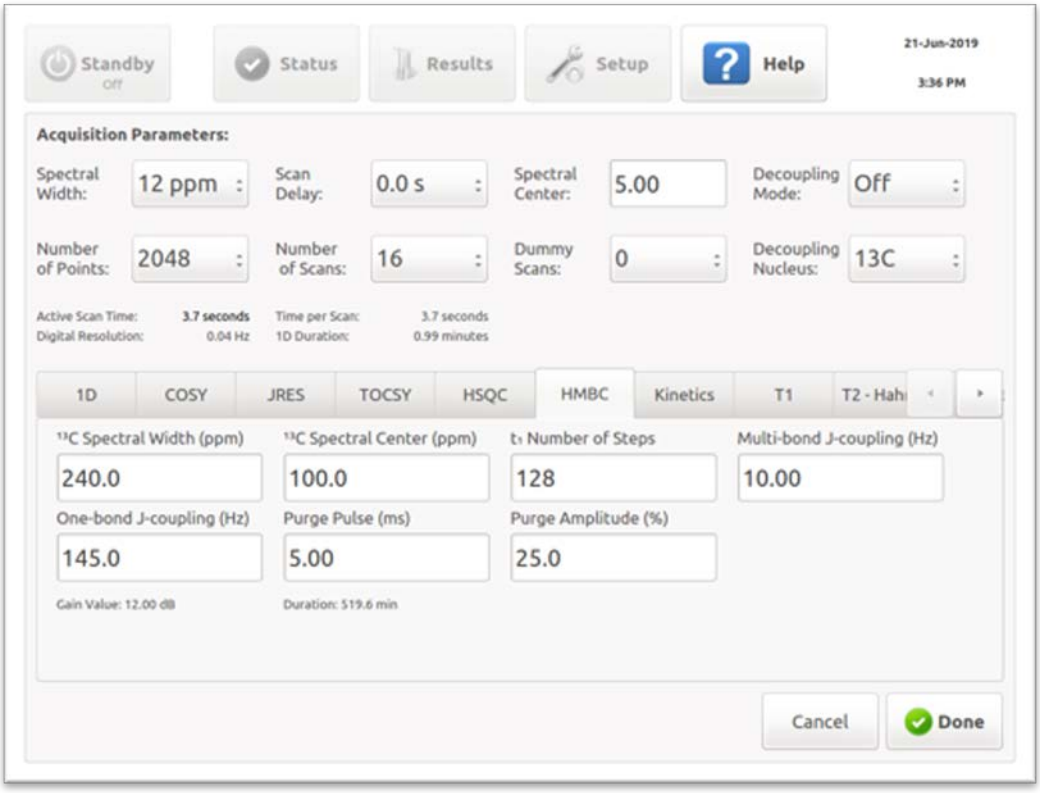


Figure 6: Pictorial depiction of HMBC Pulse Sequence.

Similarly, to HSQC (section 3.2.2.11), the top half of the experiment tab allows the user to modify the spectral parameters of the ^{1}H NMR experiment (**Spectral Width**, **Number of Points**, **Scan Delay**, **Number of Scans**, **Spectral Center** and **Dummy Scans**). A relatively low # of points (512) can be used to reduce overall data size and processing time, as full resolution of the homonuclear *J*-couplings in the proton spectrum is not as important as the location of the correlation peaks. The ^{13}C spectral width and center is addressed by using the automatic 240 ppm specified in **^{13}C Spectral Width** and 100 ppm in **^{13}C**

Spectral Center or setting your desired value. Unlike the HSQC, it is customary to run the experiment with **Decoupling Mode 'Off'**. This helps to distinguish between $^{2}J_{CH}$ and $^{3}J_{CH}$ correlation peaks, which appear very close to the central ^{1}H chemical shift position, and $^{1}J_{CH}$ correlation peaks, which remain split in the f_2 domain by the ~ 150 Hz coupling.



For the NMReady-60 HMBC pulse program, the purge pulse is used to suppress the resonances from protons bonded to ^{12}C. The length of that **Purge Pulse**, as well as the **Purge Amplitude** can be optimized as desired.

3.2.2.13 Spin-Lattice Relaxation, T_1

The spin-lattice, or longitudinal, relaxation time (T_1) relates to the time required for a sample to return to thermal equilibrium, with its bulk magnetization vector (*i.e.*, *z*-magnetization) along the static field direction after it has been perturbed with an RF pulse. In other words, it quantifies the time required to reestablish full magnetization of the sample. Often this relaxation exhibits an exponential decay. In that case, T_1 is the time constant for that decay.

The experiment is performed through an inversion-recovery pulse sequence (figure 7), whereby the sample is given a long relaxation delay before it is pulsed by 180°. This 180° pulse inverts the *z*-magnetization, and this is followed by a tau time (τ) where the bulk magnetization begins partially to relax back toward equilibrium. It is then pulsed by 90°. Any *z*-magnetization that remains will be detected during the acquisition period. For short τ times, we see a full intensity spectrum, but it is in the negative direction (as the bulk magnetization is still largely inverted). As the τ time is increased on successive iterations of the pulse sequence, the intensity of the spectrum decreases until a point where

$\tau = T_1 \ln 2$, and we do not observe the resonance of interest at all. As this time is passed and longer τ times are used, the bulk magnetization moves into the positive *z*-direction, and we observe a normal spectrum that increases in intensity as the τ time lengthens. Accurate knowledge of the T_1 time can assist the experimenter in setting the optimal pulse angle for rapid recycling and/or the recycle delay between pulses especially in cases where accurate integrations are necessary. It can also be helpful in studies of larger molecules, such as polymers, where it can correlate with timescales of molecular motion.

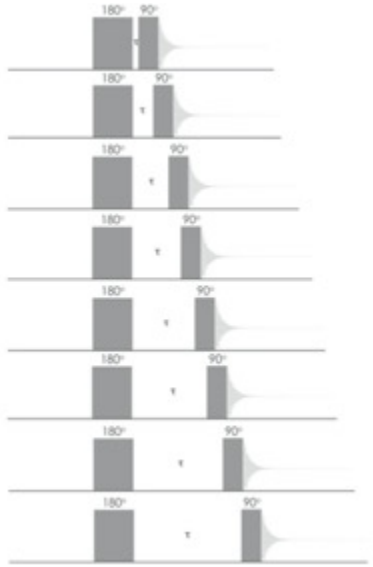
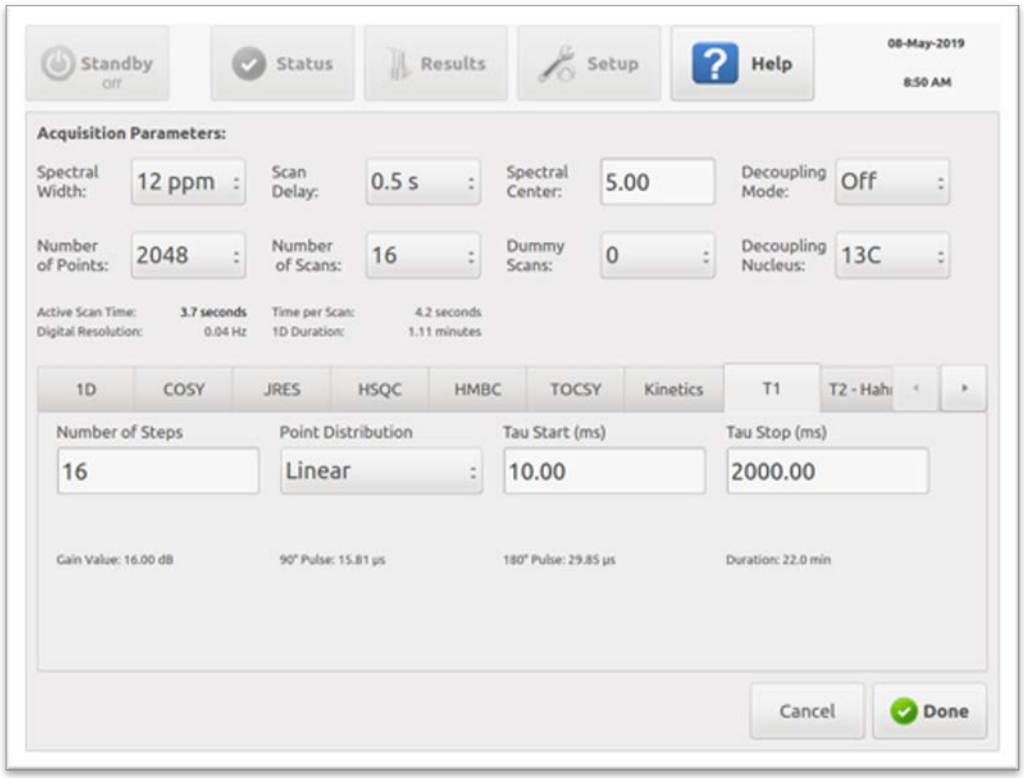


Figure 7: Pictorial depiction of inversion recovery pulse.



Before running a T_1 experiment it is necessary to run a 1D spectrum, followed by phase and baseline correction. Then you should manually integrate the protons for which you want to determine the T_1 value. You don't have to save this spectrum, the integral areas as stored in the memory of the instrument. If the relaxation times of the protons of interest are very different, we would recommend running separate T_1 experiment for each signal.

To set up the experiment, select *T_1* from the **Experiment** button on the bottom right-hand side of the main page. In **Experiment Settings** you can set the appropriate parameters. First, select a **Tau Stop** time that is longer than the expected T_1 for the material of study. Then select a short **Tau Start** time to establish an accurate base for the analysis. These numbers represent the length of time between the 180°and 90° pulses in inversion recovery sequence depicted in the first and last acquires depicted in figure 7. The **Number of Steps** is the number of acquires that occur between the first and the last acquire. For example, figure 7 shows 8 steps. The distribution of these points can be **Linear** or **Logarithmic** over the chosen time interval. To change between these sampling options, just press the button and selected the desired sampling method from the dropdown menu. Once these parameters have been added, select **Done** to exit Experiment Settings and return to the main screen. From the main screen, select **Go** to initiate the experiment.

As the experiment progresses, a graph will be displayed showing signal intensity of the user integrals *vs.* τ. This graph will automatically scale itself as the data is collected. If integrals were not selected prior to the T_1 experiment, the spectra will still be processable in a third-party software.

Common mistakes made while acquiring T_1 data: (1) not setting a long enough scan delay in acquisition parameters. It should be at least 5 times greater than the longest T_1; (2) not centering the window properly. Sufficient data points should be collected on both sides of the anticipated T_1 value; and (3) **Tau Stop** is not appropriate for the experiment (ideally you have to use a **Tau Stop** that is roughly four times longer than the T_1).

3.2.2.14 Spin-Spin Relaxation, T_2

The spin-spin, or transverse, relaxation time, (T_2) quantifies the time required for the bulk magnetization to dephase after an RF pulse due to interactions with other spins. Nanalysis benchtop NMR spectrometers provide three types of experiments to measure T_2 relaxation in a sample. The T_2 data may be of interest as is, or it may assist the experimenter in acquisition parameter selection in subsequent experiments, particularly parameters related to line width and number of points.

The user can run a Hahn Echo T_2 experiment, a standard CPMG (Carr-Purcell-Meiboom-Gill), or a CPMG-FSE (CPMG fast spin echo). The standard CPMG is useful to determine the T_2 separately for each resonance of an organic molecule dissolved in solution. The Hahn Echo experiment is useful for solids and/or semisolids with short T_2 times. The CPMG-FSE is useful for samples that feature a single resonance, such as aqueous solutions containing paramagnetic relaxation agents, which can change the transverse relaxation rate $1/T_2$ of the water resonance in proportion to dissolved particle concentration.

For the Hahn Echo, the experiment is performed through a spin-echo sequence, where the sample, at equilibrium, is pulsed with a 90° pulse to create transverse magnetization. The spectrometer waits a specified time (τ), before performing a 180° pulse and waiting the same time (τ) before collecting an FID (figure 8a). The amplitude of the resulting echo is recorded as a function of 2τ, which is incremented on successive iterations of the experiment. The CPMG is similar, but it uses a string of refocusing pulses (figure 8b), whose number is incremented, and a fixed interpulse delay. In CPMG-FSE, there is no incrementation shot-to-shot of either the number or length of the echo intervals. Rather, a series of echoes Is recorded in one shot of the pulse sequence, with the echo acquisitions interleaved between the applied echo pulses. This allows the experiment to take much less time, but the resulting brevity of each interleaved echo places severe restrictions on the effective resolution of the data one can acquire with CPMG-FSE.

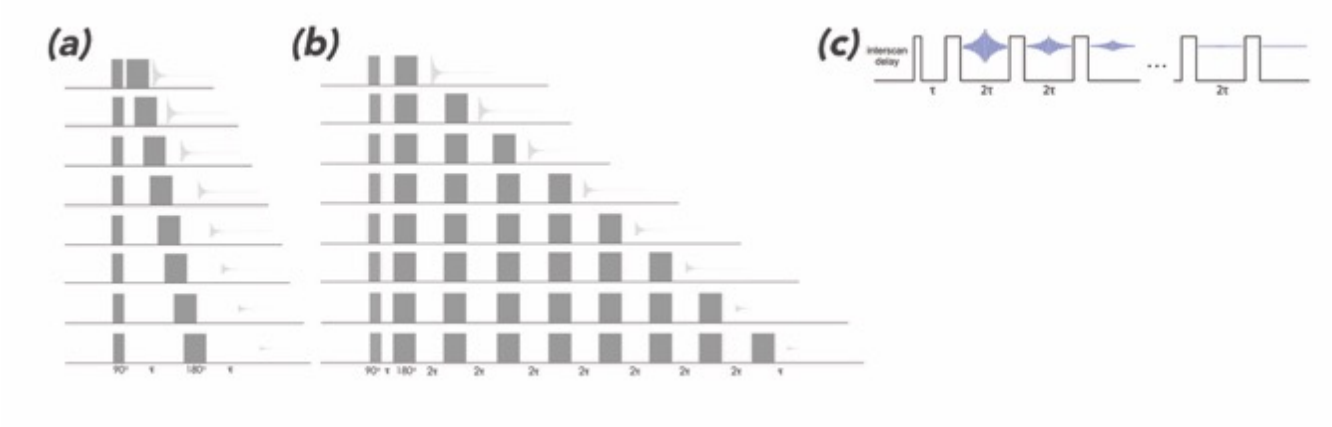
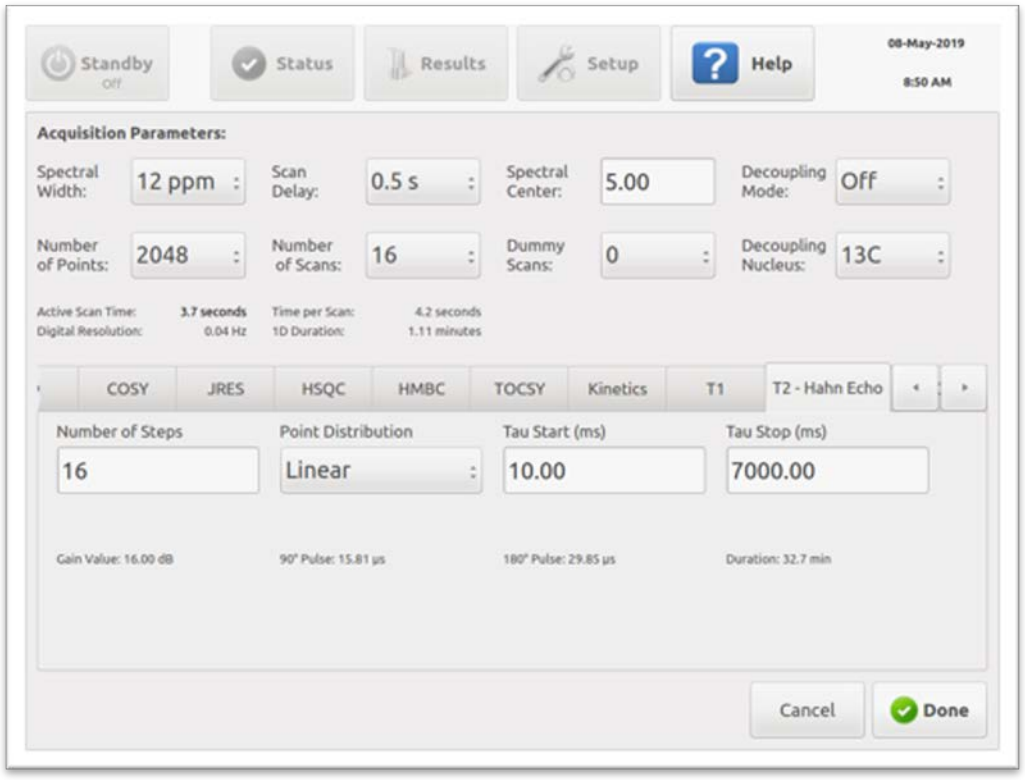


Figure 8: Pictorial depiction of T2 pulse sequences.

Much like a T_1 experiment, to measure the T_2, first you have to run a 1D spectrum and select the peaks for which you want to determine the T_2 values. These peak regions can be selected using the manual integration feature (see section 4.1.4.1). Then, select one of the T_2 experiments from the **Experiment** button on the bottom right-hand side of the main page.

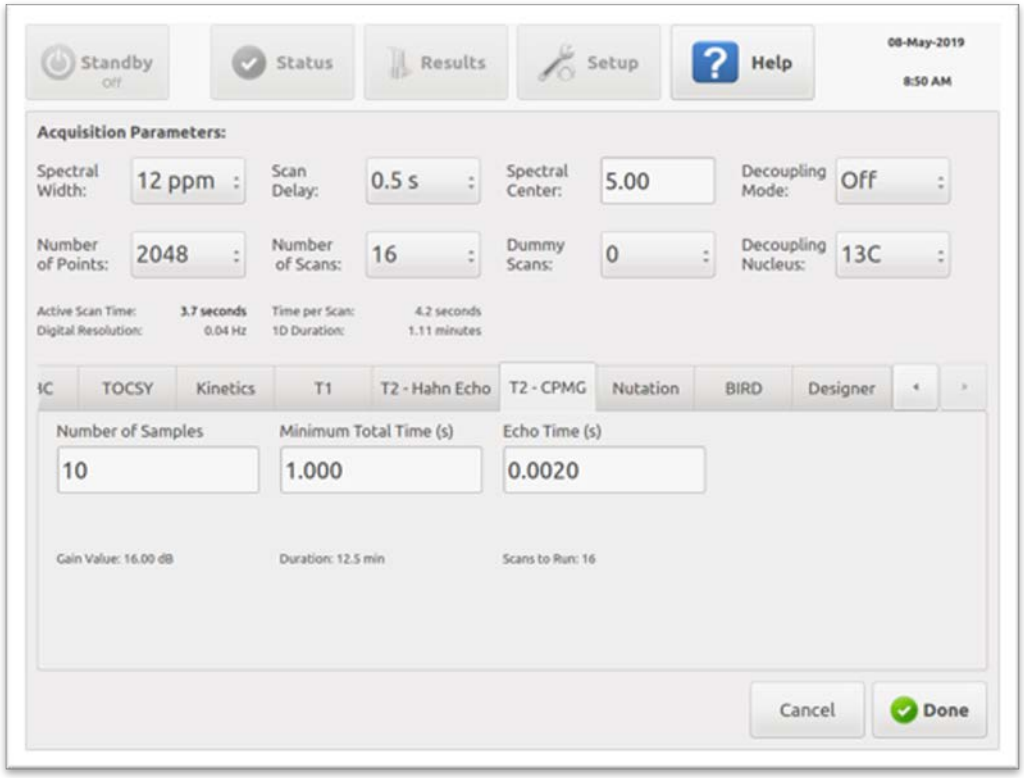
In **Experiment Settings** you can set the appropriate parameters for the experiment. The top half of the window shows standard 1D parameters, and it should be noted that to run a successful T_2, the Scan Delay should be set to at least 5 times the T_1 value of the sample. For CPMG, make sure that the full spectral range of the sample will be visible and that the number of points is compatible with the desired resolution of the full spectrum.

For the Hahn Echo, first, select a Tau Stop time that is longer than about 5 times the expected T_2 for the material under study. Then select a short Tau Start time to establish an accurate base for the analysis and a sufficient Number of Steps to span the time space with acceptable granularity. The distribution of these points can be Linear or Logarithmic over the chosen time interval. To change between these sampling options, just press the button and select the desired option from the dropdown menu. Once these parameters have been added, select **Done** to exit Experiment Settings and return to the main screen. From the main screen, select **Go** to initiate the experiment.

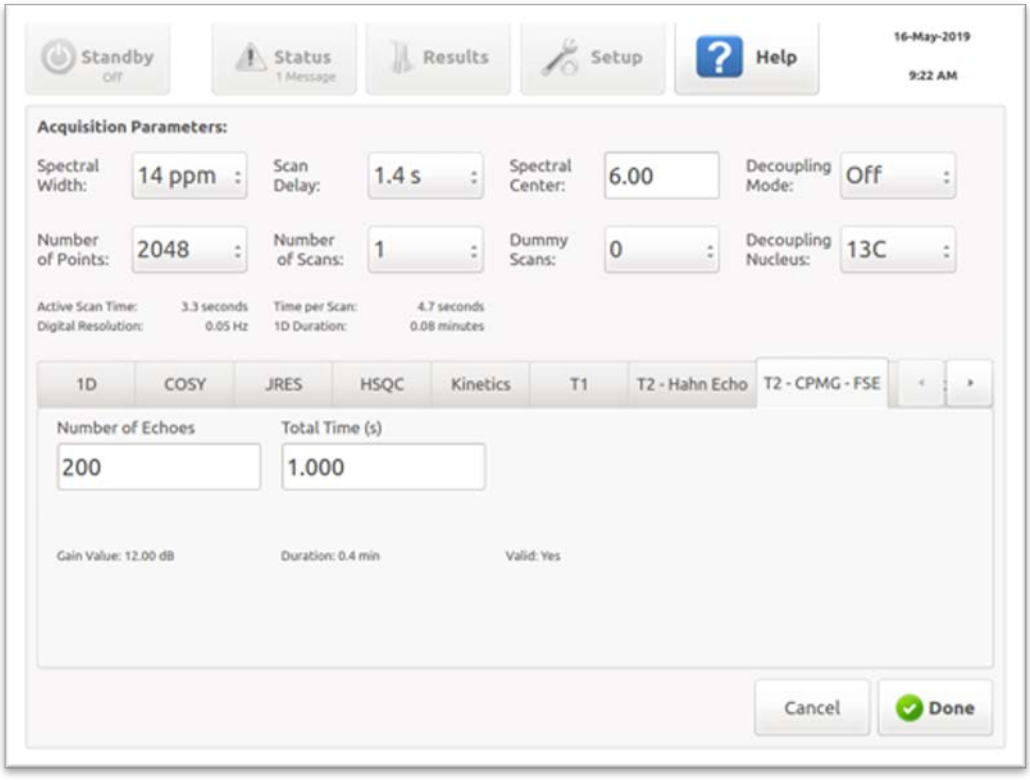


For the CPMG, first set the **Minimum Total Time** to a value that is roughly 5 times the expected T_2 of the longest relaxing resonance in the sample. Then, set the **Echo Time**. A good default Echo Time is about 2 ms (0.002 s) for typical liquids containing ^{1}H resonances, including resonances that might be subject to *J*-modulation. Since the software limits the number of echoes to at most 3000, use a longer Echo Time with samples with very long T_2s. Use a shorter Echo Time if needed for quickly relaxing samples. Finally, select the **Number of Samples**. This determines the number of times an FID will be acquired during the iteration sequence.

For example, if Minimum Total Time = 1.000 s, Echo Time = 0.001 s, and Number of Samples = 10 are specified, *eleven* spectra will be recorded (a "zeroth" spectrum is recorded in addition to the specified ten), the first after 100 echoes, the second after 200 echoes, … and the tenth after 1.000/0.001 = 1000 echoes. (But note that each spectrum is an average of scans, the number of which is determined by the **Number of Scans** parameter specified in the top menu.)



For CPMG-FSE, after setting the **Scan Delay**, **Spectral Center**, **Number of Scans**, and **Dummy Scans**, one need only set the **Total Time** and **Number of Echoes** in the *T2 - CPMG - FSE tab*.



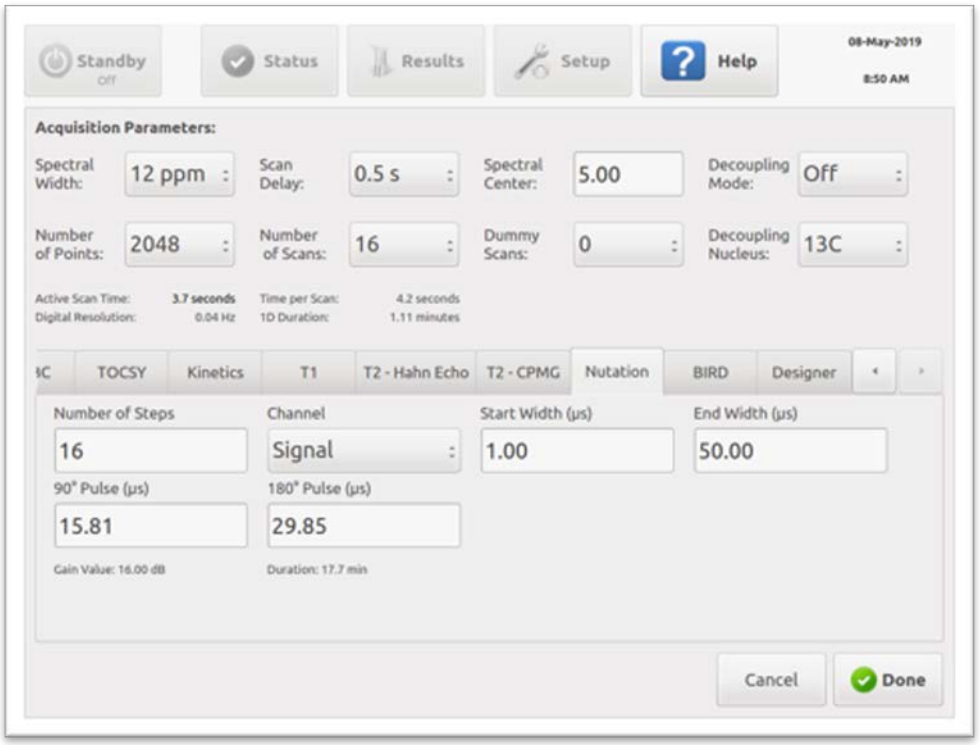
As each of the T_2 experiments progress, a graph will be displayed showing signal intensity *vs.* τ. This graph will automatically scale itself as the data is collected. If integrals were not selected prior to the T_2 experiment, the spectra will still be processable in a third-party software. In CPMG-FSE, the maxima in the echo trains are displayed as soon as the first scan is completed. The data are adjusted as the phase cycle proceeds.

Common mistakes made while acquiring T_2 data: (1) not setting a long enough scan delay in acquisition parameters. It should be at least $5T_1$; (2) not centering the window properly; and (3) **Tau Stop** is not appropriate for the experiment (ideally you have to use a **Tau Stop** that is roughly four times longer than the T_2). Sufficient data points should be collected on both sides of the anticipated T_2 value.

3.2.2.15 Nutation

A nutation experiment can be used to determine the optimal 90° pulse angle for each spectrometer. To run a nutation experiment, select **Nutation** in the experiment quick select button on the main page. Then select the appropriate parameters in the **Experiment Settings** window. This feature takes a series of spectra by varying the time of the pulse applied from the **Start Width** to the **End Width** in the defined by the **Number of Steps**. The NMReady-60 uses the 180° null as the point of reference so **End Width** should be greater than twice the expected 90° pulse width. The **Start Width** should be short enough to provide an accurate base for the analysis and the number of points should span the time between the start and end width such that the results aren't too granular.

The average 90° pulse on the NMReady-60 varies, due to a variety of conditions (*e.g.*, the hardware, quality of the shims, nuclei observed) but for proton is typically is between 12 – 30 µs. The **90° Pulse** and the **180° Pulse** will auto populate after the experiment is completed.



The display shows the amplitude of the largest peak in the spectrum as a function of applied RF pulse time. The pulse-time value of the highest point is the '90-time', the time it takes for the RF pulse of the applied power to nutate spin magnetization by 90°. This pulse time is used in T_1, T_2, and COSY experiments and should be entered into the '90° pulse' box by selecting the number pad adjacent to the box after the experiment concludes. Sometimes it is more convenient to measure the '180-time', the time it takes for the RF pulse to produce a null in the response, and to use half this value as a best estimate of the 90-time. If the nutation parameters have been selected to include this null point, the 180-time value will be indicated by a cursor when the experiment ends, and the numerical value of the 180-time will be displayed in a box near the upper right-hand side of the display.

Please note that the 90° pulse angle will not be the same for every nucleus that can be observed. In the above screenshots, the ^{13}C 90° pulse angle is 20 µsec while the ^{1}H 90° pulse angle is 15 µsec. One can also determine the nutation of the lock channel by selecting **Channel** and selecting '**Lock**'.

3.2.2.16 Distortionless Enhanced Polarization Transfer (DEPT)-trio

The DEPT experiment involves a more complex, two-channel pulse program. It is useful to quickly collect and assign peaks in ^{13}C data based on their phase, which is determined by the final ^{1}H NMR pulse (X°) (figure 9).

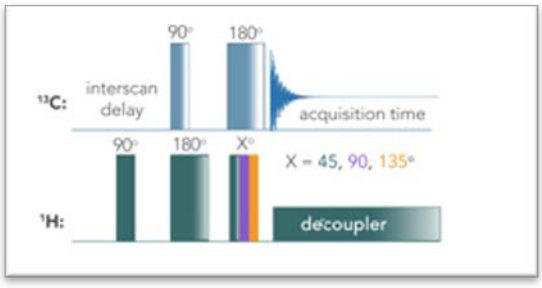
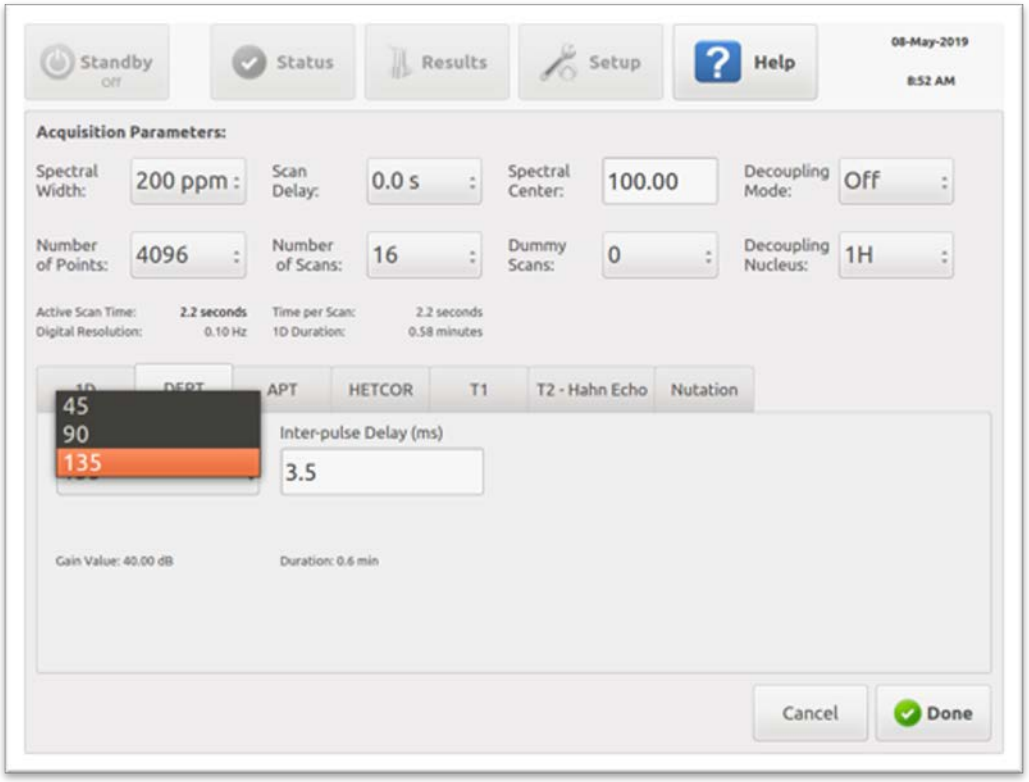


Figure 9: Pictorial depiction of DEPT pulse program.

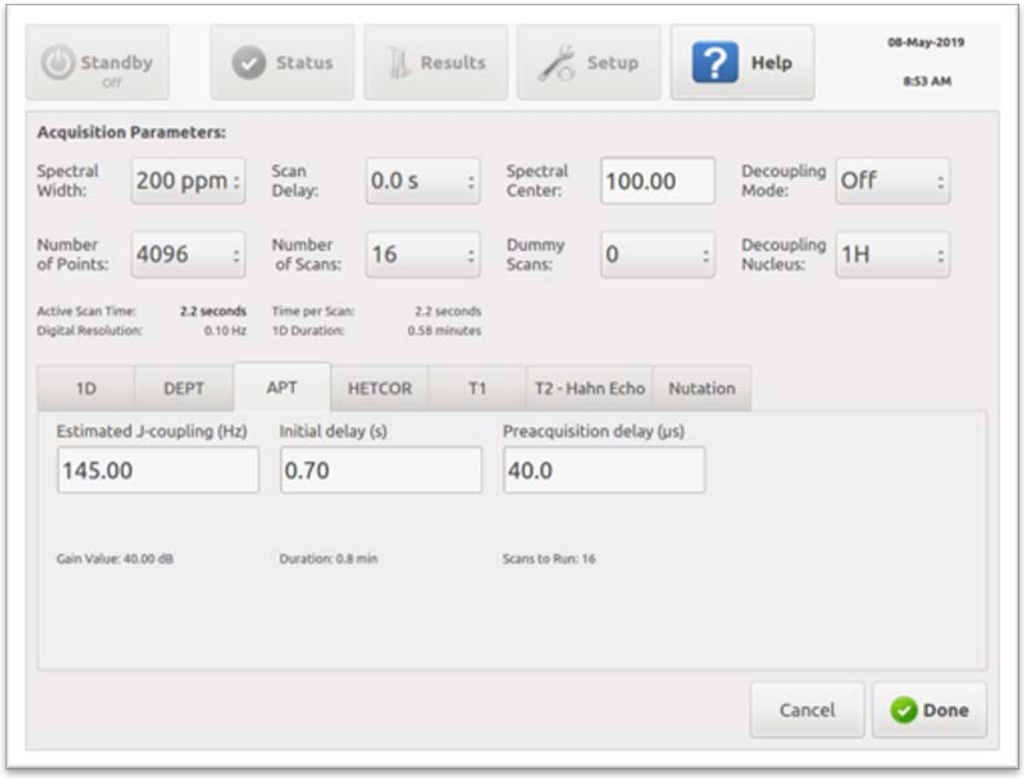
DEPT-45 gives positive phases to CH, CH_2 and CH_3 carbon resonances. DEPT-90 only show CH resonances, DEPT-135 inverts CH_3 and CH resonances while leaving CH_2 and any remaining quaternary resonances that may be seen with a positive phase. One of the problems with DEPT, although it generates a good SNR $^{13}C\{^{1}H\}$ NMR spectra quickly, it is that it masks quaternary carbons so cannot be used as a stand-alone spectrum for structural elucidation. The **decoupling mode** for a DEPT experiment should be **Inverse Gated** and the type of DEPT experiment can be setup by selecting the desired θ Pulse Angle (45, 90 or 135).



3.2.2.17 Attached Proton Test (APT)

The Attached Proton Test (APT), like DEPT, is useful for distinguishing between C, CH, CH_2, and CH_3 groups. The APT only benefits from NOE-type signal enhancements, so it is typically a longer experiment than the DEPT-trio. However, it does give information about the quaternary carbons, which the DEPT-trio does not.

To set up the experiment, select T_1 from the **Experiment** button on the bottom right-hand side of the main page. In **Experiment Settings** you can set the appropriate parameters. The standard 1D ^{13}C parameters can be chosen here if the user wishes to modify the default parameters (**Spectral Width**, **Number of Points**, **Scan Delay**, **Number of Scans**, **Spectral Center**, **Dummy Scans**). The APT specific parameters are located on the bottom half of the page, where the **Estimated J-coupling** can be optimized for the molecule, depending on the nature of the structure.



3.2.2.18 HETeronuclear CORrelation (HETCOR)

The HETCOR (heteronuclear correlation) experiment is useful for identifying C,H-correlations of directly bonded atoms. While this experiment has been largely superseded by "inverse detection" experiments like HSQC (section 3.2.2.11) and HMBC (section 3.2.2.12), it can be useful in a teaching context or in cases where t_1 noise artifacts render inverse detection experiments nonoptimal.

In HETCOR, the free-induction decays (FIDs) are recorded on the ^{13}C channel, and so the 'direct' dimension f_2 frequencies, horizontal in the spectral display, are carbon chemical shifts. The predominant evolution during the period t_1 is due to ^{1}H chemical shift, and this determines the position in the vertical f_1 dimension of the correlation peaks (see figure 10).

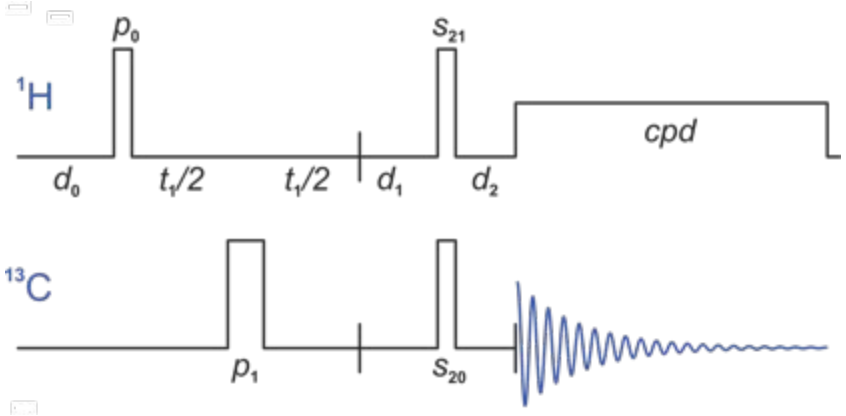
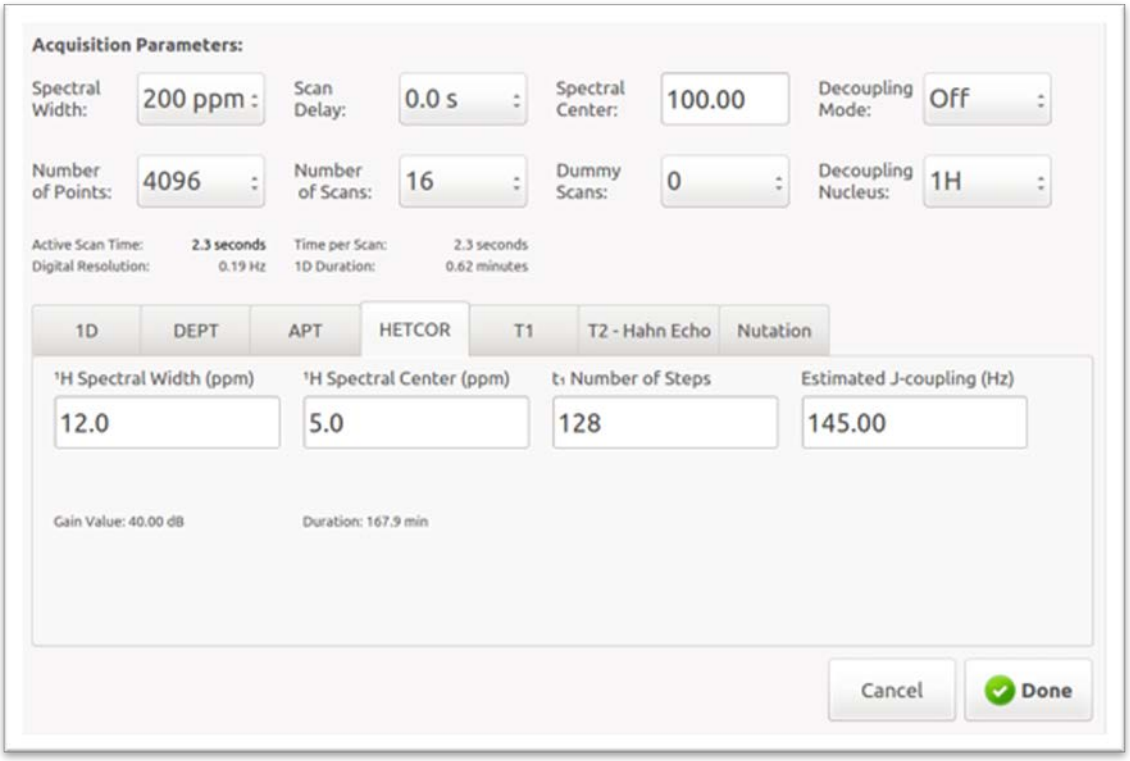


Figure 10: Pictorial depiction of HETCOR pulse program.

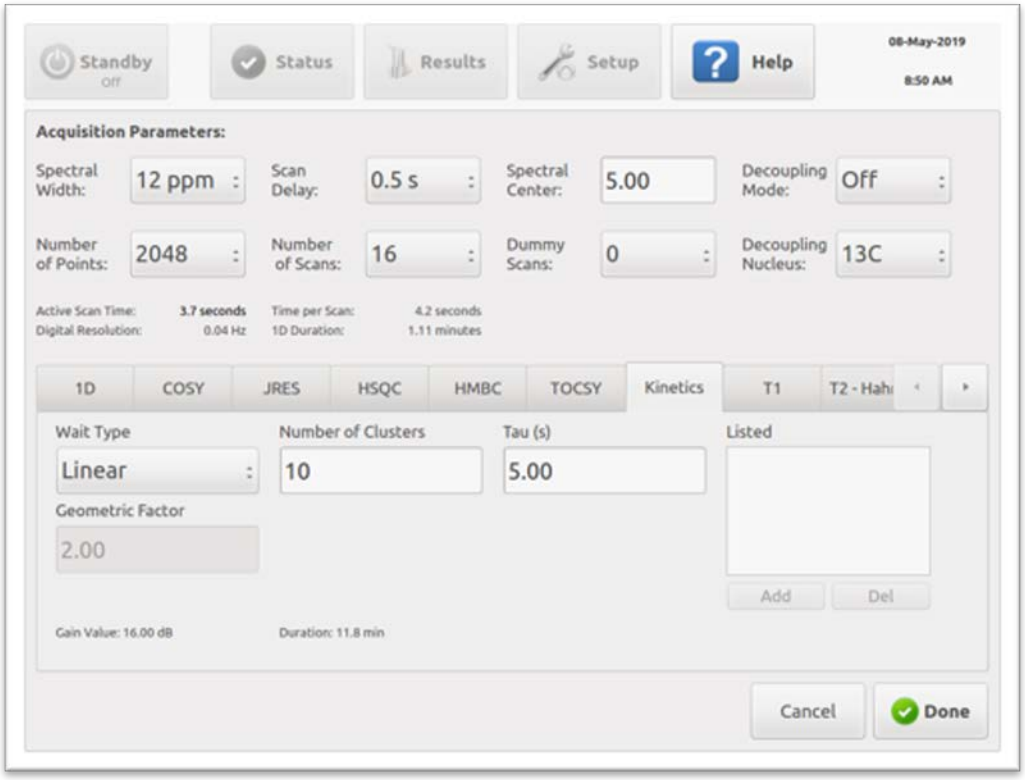
As the HETCOR is carbon-detected, the top half of the experiment tab allows the user to modify the spectral parameters of the ^{13}C NMR experiment (**Spectral Width**, **Number of Points**, **Scan Delay**,

Number of Scans, **Spectral Center**, **Dummy Scans** and **Decoupling Mode**). A relatively small number of points (512) can be used to reduce demands on data storage and per-scan acquisition time. The **^{1}H Spectral Width** and **^{1}H Spectral Center** are entered into their respective inputs in the HETCOR settings tab. **t_1 Number of Steps** can be modified to improve resolution in the f_1 domain or to shorten total experiment time as desired. The default value for **Estimated J-coupling**, 145 Hz, is near the average of one-bond J-couplings for sp^3 and sp^2 carbons and can be adjusted as desired



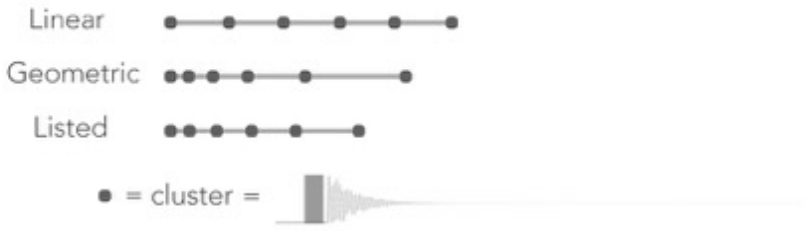
3.2.2.19 Kinetics

The optional kinetics module allows the user to acquire a series of 1D experiments over a specified time interval. As per the 2D experiments, the top of the screen allows the user to configure the appropriate settings for a 1D experiment, whereas the bottom half of the screen allows the user to set up the number of 1D experiments that will be run and the frequency with which the will be acquired. The user can select: (1) **Wait Type**; (2) **Tau**; (3) **Number of Clusters**; and (4) **Geometric Factor**.



The **Wait Type** options are **Linear**, **Geometric** and **Listed** and they can be selected from the dropdown menu. The wait is governed by the time **Tau** in seconds. The **Tau** can be changed by clicking in the white box below, and a keyboard will pop-up. **Linear** provides access to equally spaced intervals (*e.g.*, it waits 5 minutes between all 1D experiments). **Geometric** increases the time over the duration of the experiment (*e.g.*, when **Tau** is set to 5 minutes and the geometric factor is 2, the first wait time will be 5 minutes, the second wait time will be 10 minutes, the third 20 minutes, the fourth 40, etc.). Finally **Listed** allows you to set the frequency with which the experiments are taken (*e.g.*, run first experiment, wait 1 minute, run second, wait 2 minutes, run third, wait 3 minutes, etc.).

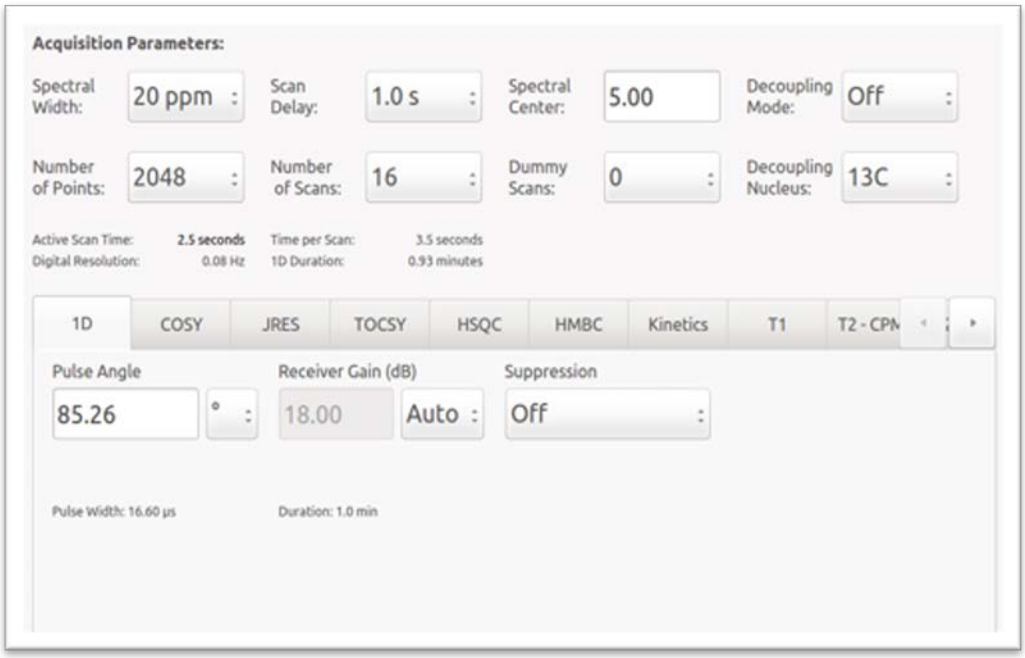
The **Number of Clusters** refers to how many times the specified 1D experiment is run; by selecting '6' there will be 6 1D experiments are run in succession with the defined time interval.



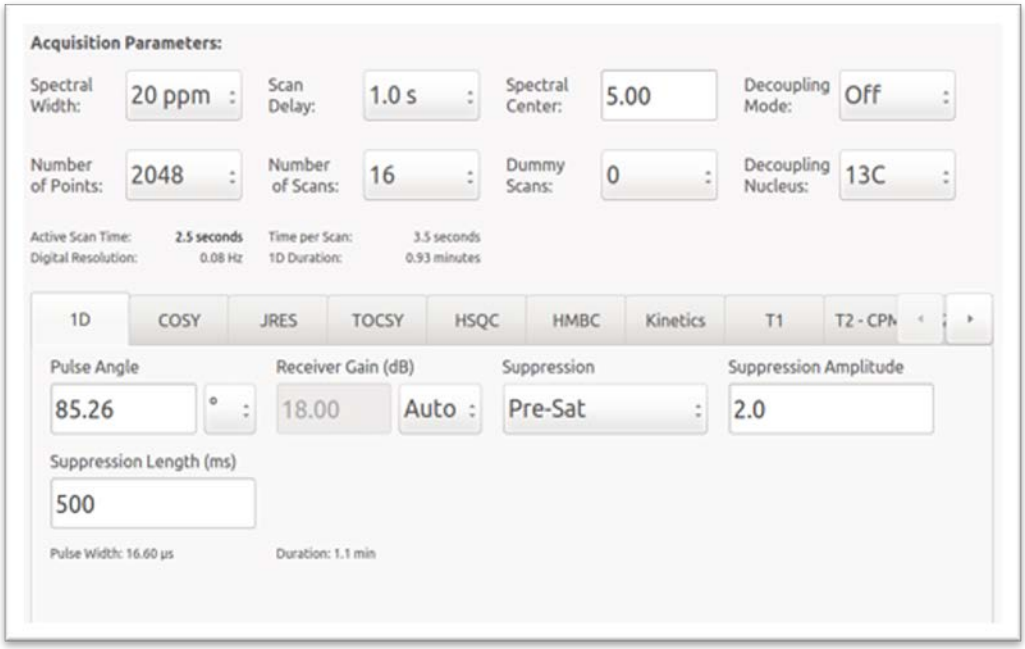
3.2.2.20 Solvent Suppression

Solvent suppression can be useful to decrease the intensity of a large signal and help lower the dynamic range of the spectrum. Our instrument comes with two solvent suppression options, pre-saturation (Pre-Sat) and Delay Alternating with Nutation for Tailored Excitation (DANTE).

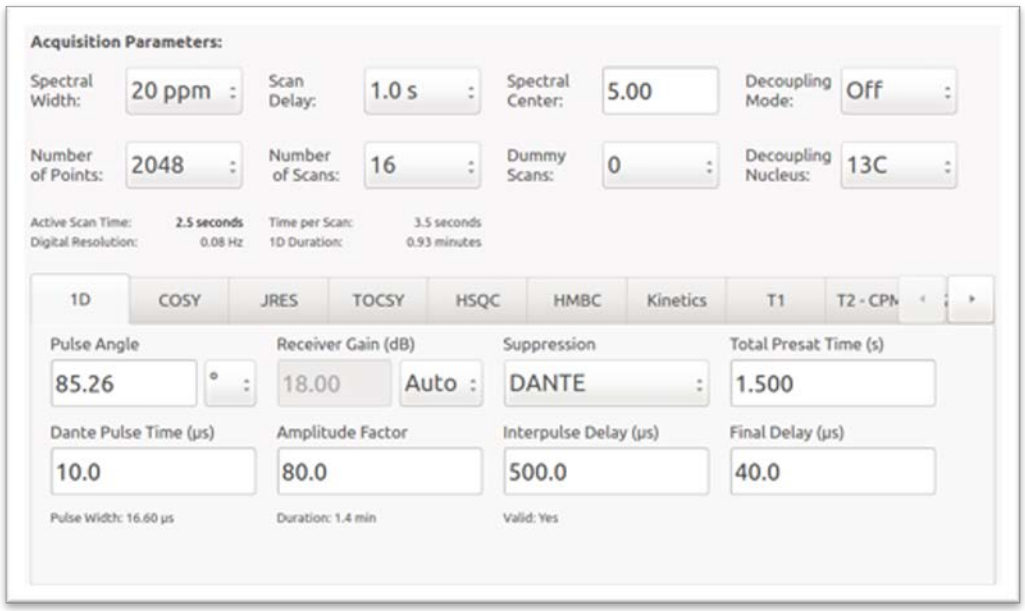
If solvent suppression is enabled in your instrument you can choose the suppression method with the **Suppression** dropdown menu.



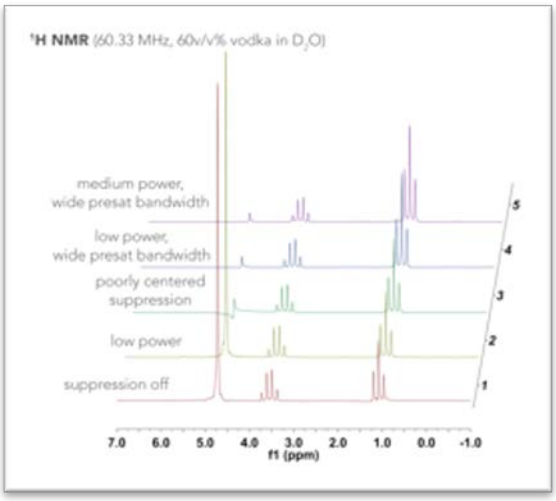
To get solvent suppression one must first acquire a 1D spectrum and correct the phase. Subsequently you should set the **spectral center** such that it is centered on the peak you wish to suppress. Selecting the Pre-Sat suppression method will bring up two parameters that can be adjusted (**Suppression Amplitude** and **Suppression Length**). The suppression length is roughly inversely proportional the width of the peak being suppressed, and the amplitude controls the power of the pre-saturation pulse.



Selecting the DANTE suppression method will bring up five parameters that can be adjusted (**Total Presat Time**, **Dante Pulse Time**, **Amplitude Factor**, **Interpulse Delay** and **Final Delay**). In DANTE, a train of very short, weak pulses is applied to saturate and suppress the solvent resonance before a final pulse excites the remaining nuclear spins in the sample. The **Total Pre-sat Time** parameter controls the total time over which the DANTE train is applied. The **DANTE Pulse Time** is the time for each pulse, and the **Amplitude Factor** controls the amplitude of the pulses (as a percentage of the full-power 90 pulse). The **Interpulse Delay** is the time between pulses in the DANTE train, and the **Final Delay** is the length of the last delay that precedes the regular 90 pulse.



For an example of suppression please see the illustrative stacked plot below for a 60% vodka sample in 40% D_2O.



3.2.2.21 Experiment Designer

This allows users to design their own pulse programs, customizing the number of pulses, timing, phases etc. For more information please contact your Nanalysis customer service representative.

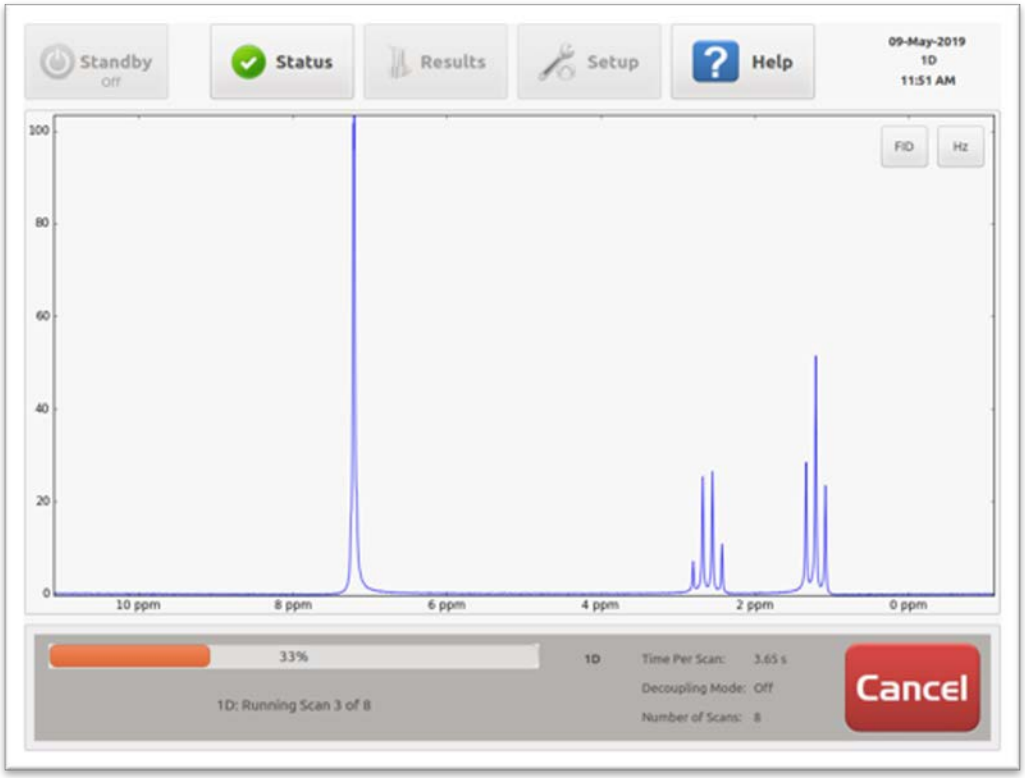
4.0 Data Processing

During a 1D acquire, the first Fourier Transformed spectrum of one transient will be displayed on the screen and the display will update every additional four scans so one can visually watch the signal-to-noise ratio improve. For 2D data, there is no change to the display, with the exception of the progress bar, until the data collection is complete. For both 1D and 2D experiments, once the experiment is finished, the data is displayed on the screen, and processing and analysis buttons become active.

4.1 1D Data Processing

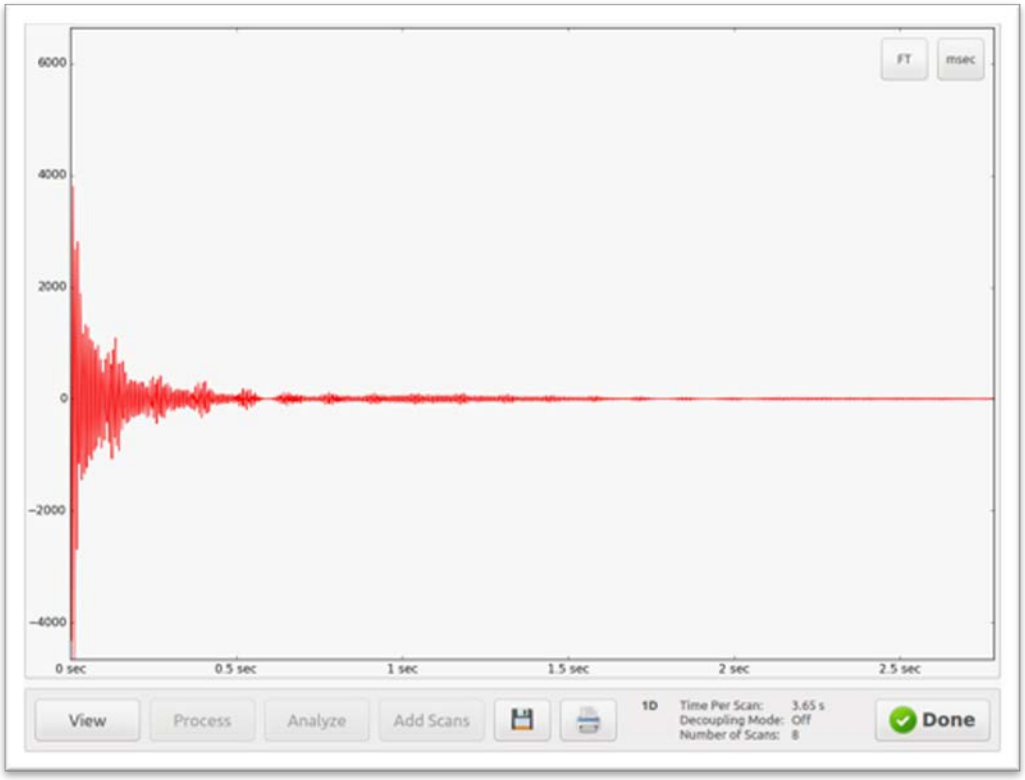
4.1.1 Visual Appearance

After **Go** has been selected in the main window, the user sees the acquisition and processing window. The spectrum appears in the main portion of the screen and a bar appears across the bottom of the screen with a progress bar and text that tells the user what the instrument is performing (*e.g.*, 1D: Auto Gain, 1D: Running scan 4 of 8). There is some information about experiment configuration and a **Cancel** button. If pressed the experiment will stop adding FID and show a spectrum displayed with the number of scans it had run prior to being canceled. Once the experiment has completed, the spectrum will auto-scale itself, and the bottom title bar will disappear.



4.1.1.1 FID/FT Toggle 

On the top right-hand side of the spectrum will be an FID button. If pressed, instead of viewing the Fourier Transformed spectrum, the user can see the time domain data.



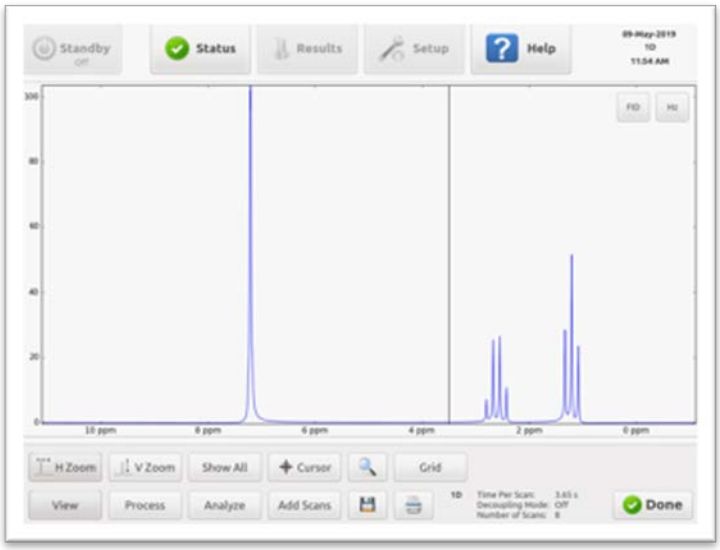
4.1.1.2 PPM/Hz Toggle PPM / Hz

A spectrum can be viewed with the chemical shift displayed in ppm (parts per million) or in Hz. This toggle button is located the upper right-hand corner of the screen. The spectrum defaults to ppm because these chemical shift values are independent of the field strength at which the spectrum was acquired. However, coupling constant determination is simplified when viewed in Hz.

4.1.2 View

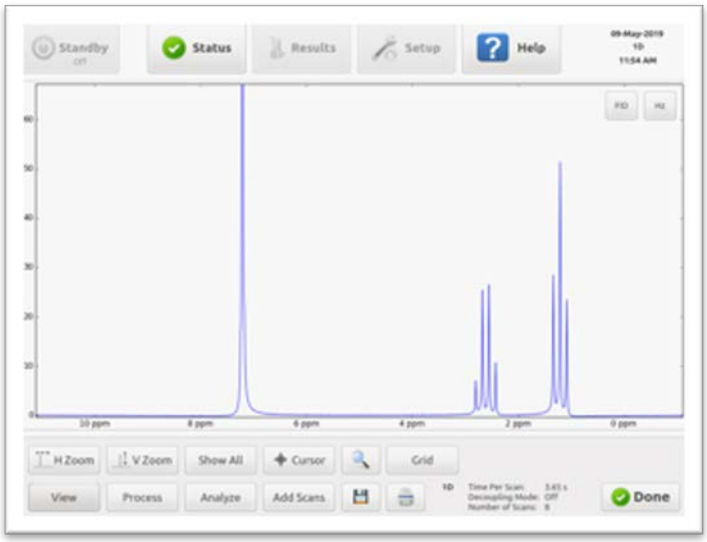
4.1.2.1 Horizontal Zoom (H Zoom) H Zoom

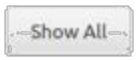
To zoom into a specific chemical shift range horizontally along the *x*-axis of a spectrum, **H Zoom** and touch the screen on either side of the area in which you want to zoom in on. Remember, this touchscreen does not respond to gestures like a smart phone (*e.g.*, there is no two-finger "spread" feature). After selecting **H Zoom**, you need to touch the screen twice to define the left and right boundaries.



4.1.2.2 *Vertical Zoom (V Zoom)* 

To adjust the height of the displayed peak(s), first press the **V Zoom** button (vertical zoom). This is using a two-touch zoom. To use, touch the part of the screen that you want to be the top of the screen. A line will appear horizontally across the spectrum. Then touch the bottom of the screen to highlight what you wish to include within the window. The spectrum will be then scaled accordingly.

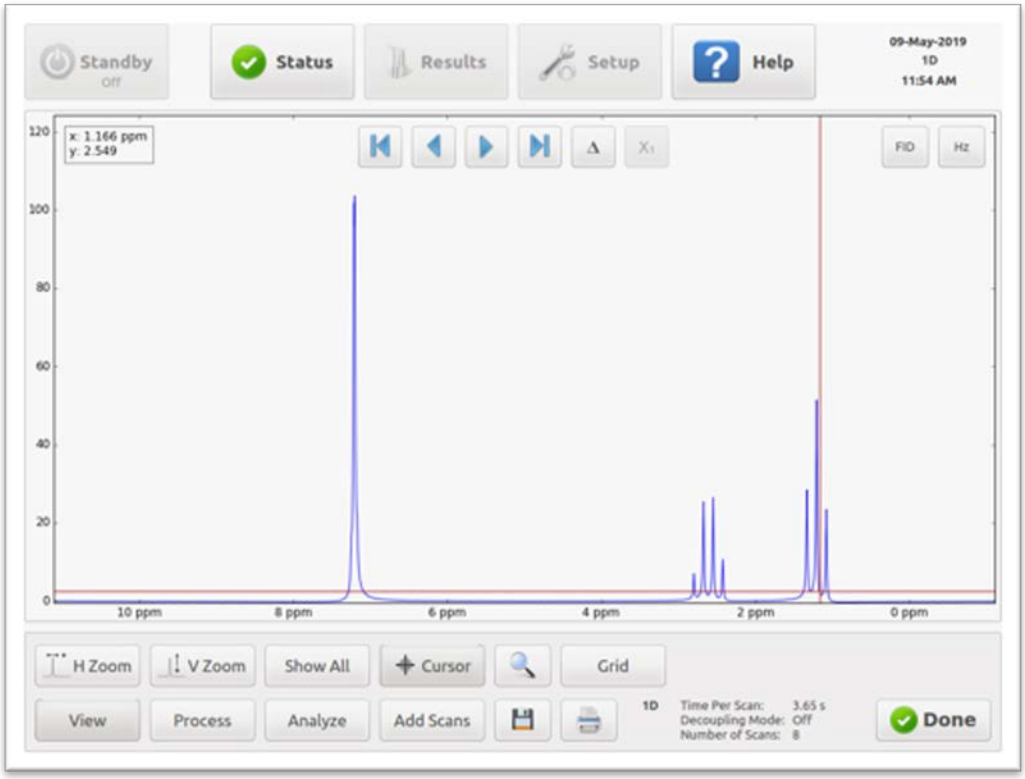


4.1.2.3 *Show All* 

The **Show All** button restores the original size of the spectrum.

4.1.2.4 Cursor 

Touching the **Cursor** places a cursor on the screen, the center point of which is the intersection of the horizontal and vertical lines. This can be adjustable by touch. The cursor can be used to quickly ascertain the position of a resonance or, by switching to the Hz mode, used to quickly determine coupling constants. The x coordinates (in ppm or Hz) and the y coordinates (arbitrary intensity units) is visible in a box in the top left-hand corner of the spectrum.

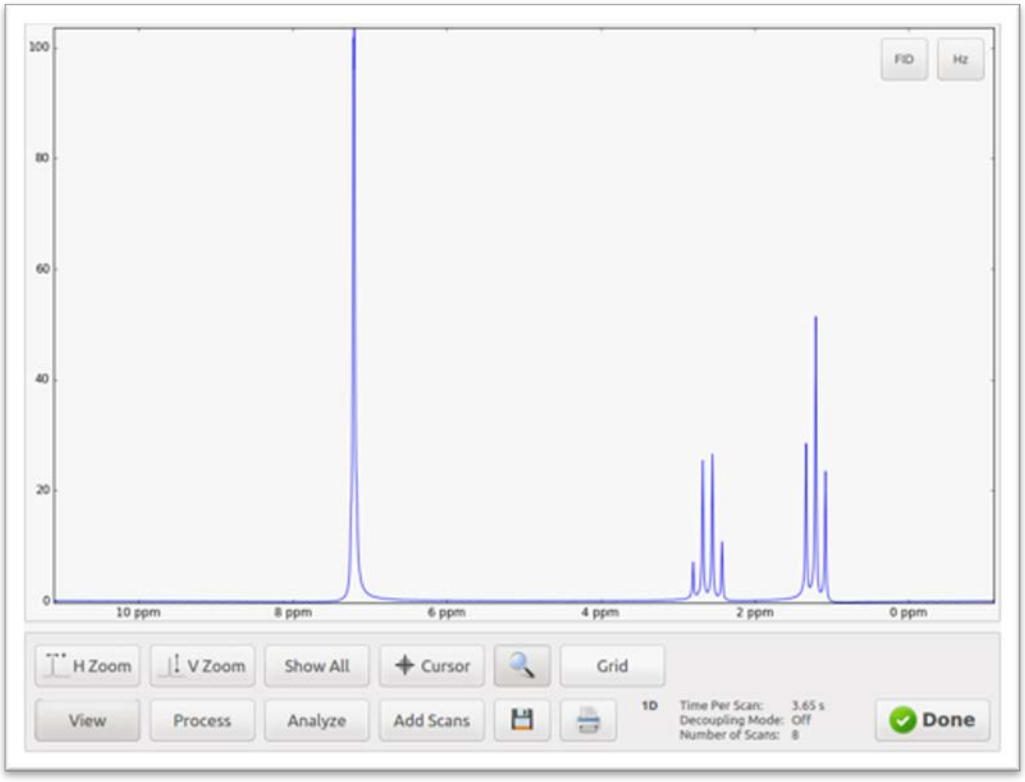


The **Cursor** function also has a Snap to Peak and Nudge functions that are presented as CD player style controls at the top of the spectrum display. The Nudge function shifts the cursor position left and right by small amounts using the **Forward** () and **Reverse** () buttons. The **Snap to Peak** (left or right) function is enabled by using the **previous track** arrow () and **next track** arrow () on the control display.

To assist in determining coupling constants, the **Cursor** function has a dual cursor display. The dual cursor display is enabled with a delta button, and the **X1** and **X2** toggle buttons alternatively lock the position of one or the other of the dual cursors for fine adjustment.

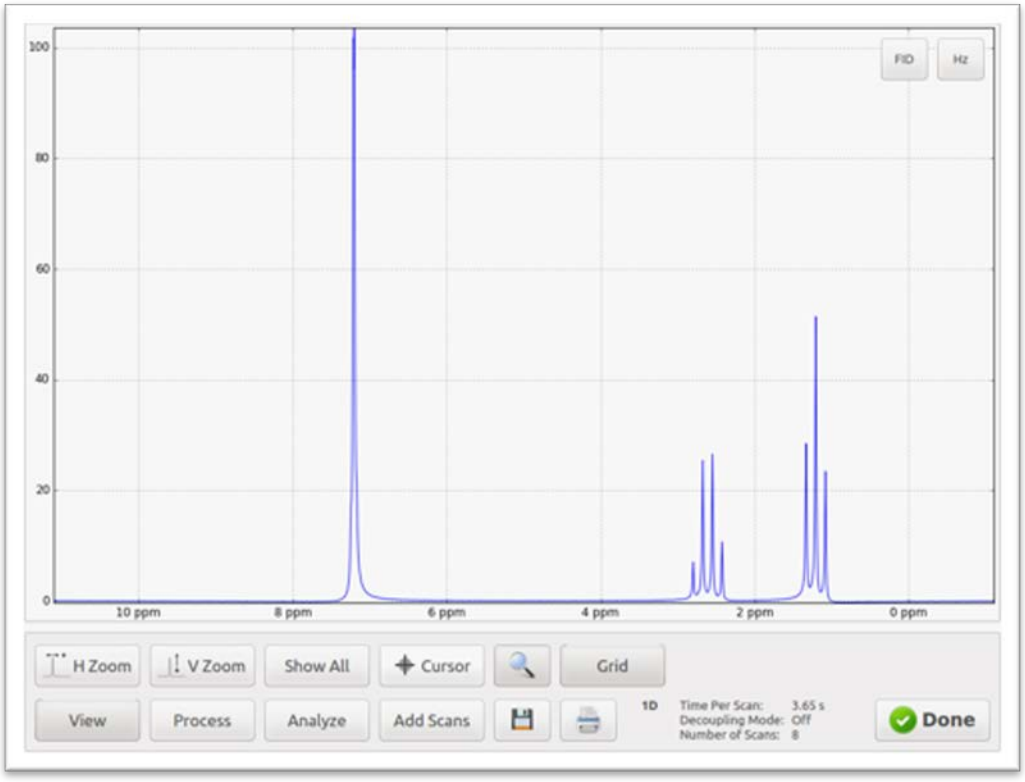
4.1.2.5 Full Screen

By selecting the  magnifying glass icon, one can expand the spectrum on the screen by removing the top task bar.



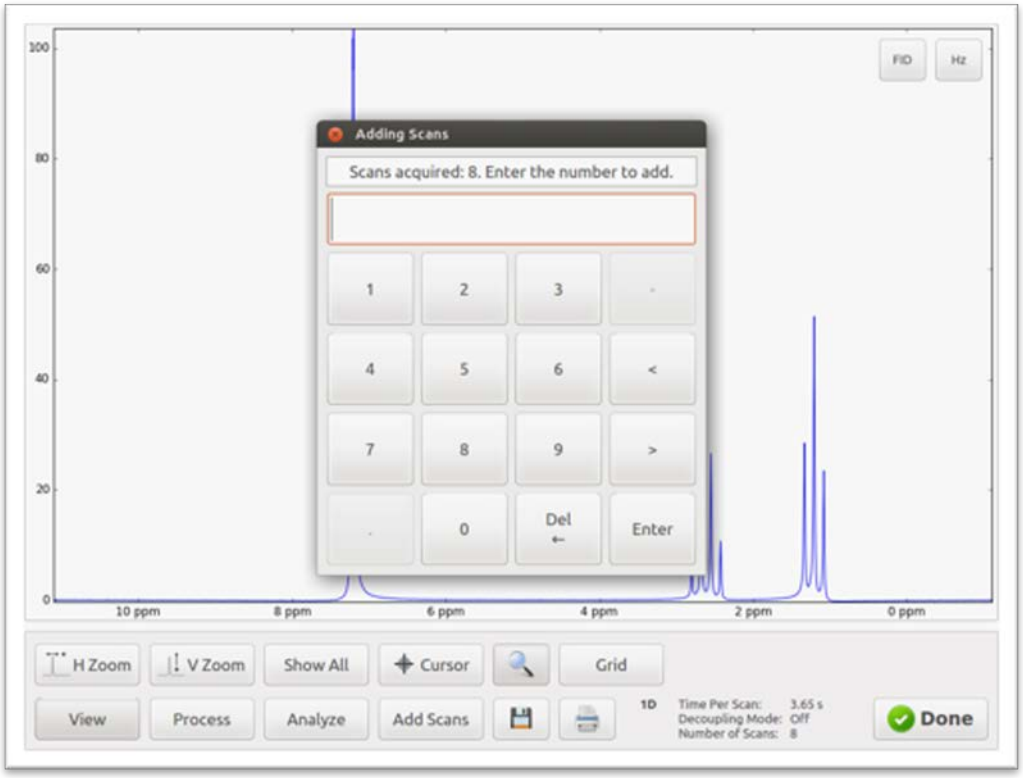
4.1.2.6 Grid

If desired, the user can put on a grid to help visualize the spectrum by selecting **Grid**. The spacing of the grid lines will depend upon the zoom of the spectrum, the more zoomed in the spectrum is the closer the lines will appear.



4.1.2.7 Add Scans

This feature allows you to continue the data collection and signal averaging, if, for example, you do not feel there is a sufficient signal to noise ratio. Simply select the **Add Scans**, input the number of scans you wish to add and hit **Enter**. This will continue the data acquisition. This button can be used after phasing, etc., although it is not required that the data be analyzed prior to continuing.



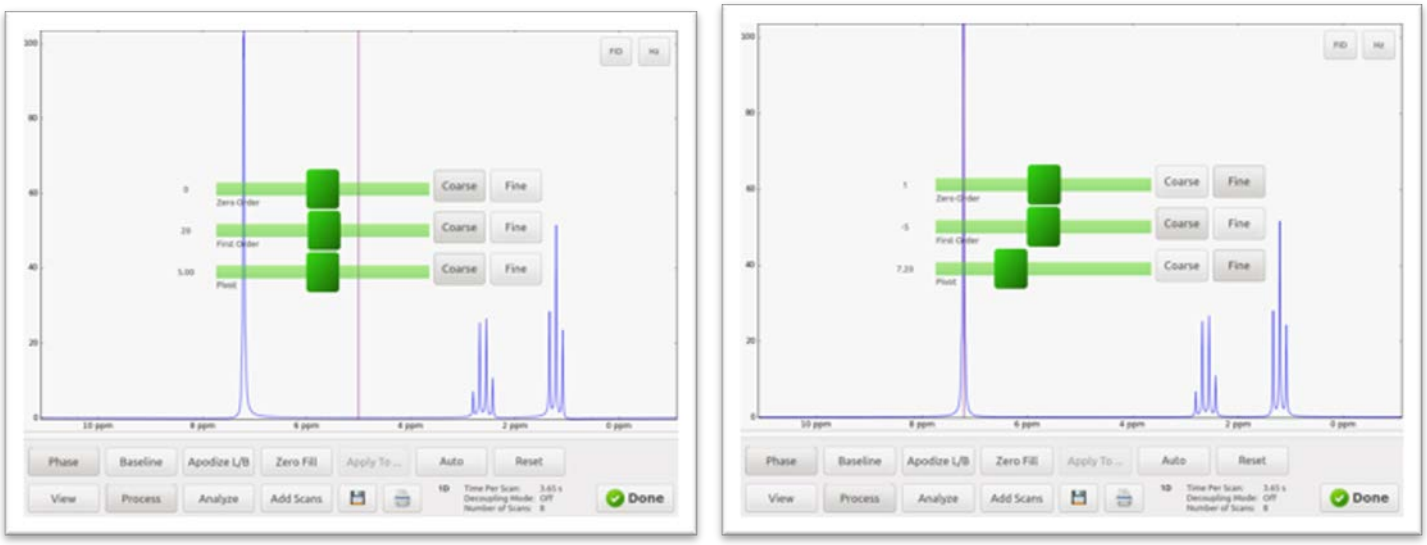
4.1.3 Process

4.1.3.1 Phase

Nanalysis benchtop NMR spectrometers have onboard tools to adjust the spectrum. The **Phase** can let you modify the spectrum either automatically or manually using the onscreen controls. To phase automatically, simply press **Auto**. The manual phase adjustment is performed using on-screen sliders by either dragging the cursor or tapping on the lighter green side rail. Tapping on the side rail is recommended because it offers slightly better control over the adjustment. These adjustments can be made in **Coarse** and **Fine** to regulate the amount of correction made for each tap or slide.

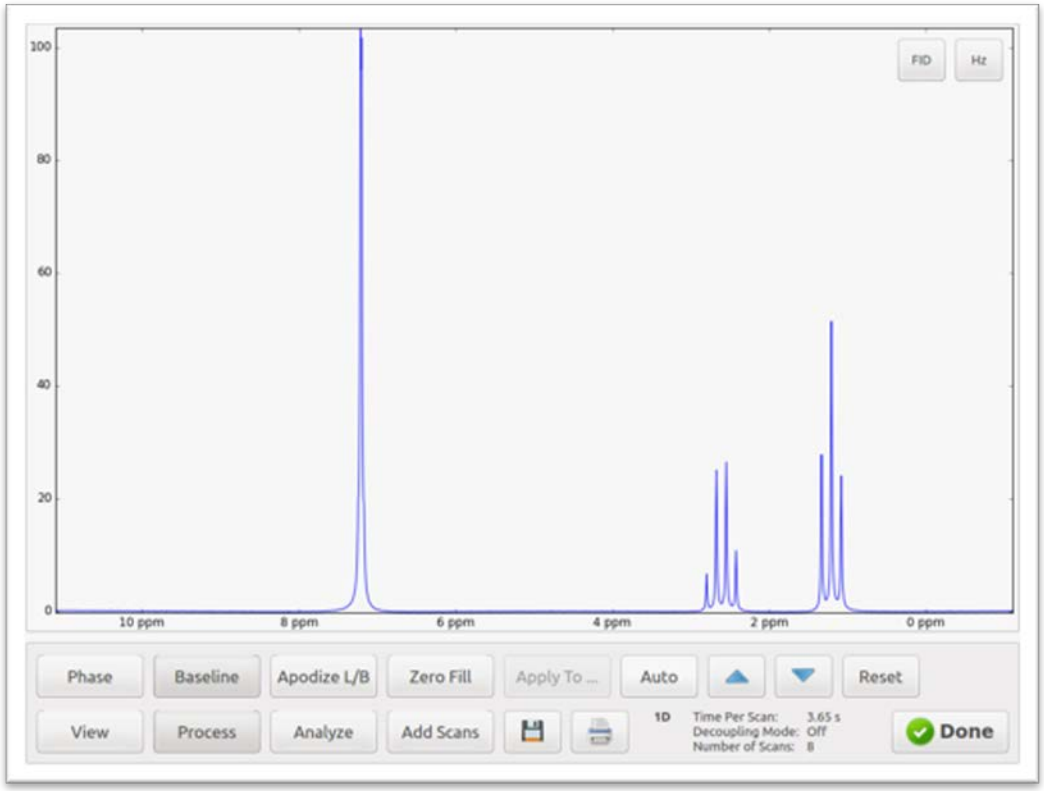
If the user does not change the **Pivot** point, the center point is automatically used. It is usually easier to phase, particularly with a wider spectral window if the Pivot is on the largest peak. To move, drag the slider or tap on the light green side rail to make **Coarse** and **Fine** adjustments.

A spectrum is well phased when the base of the peak is symmetric, and the peak intensity is located entirely above the baseline.



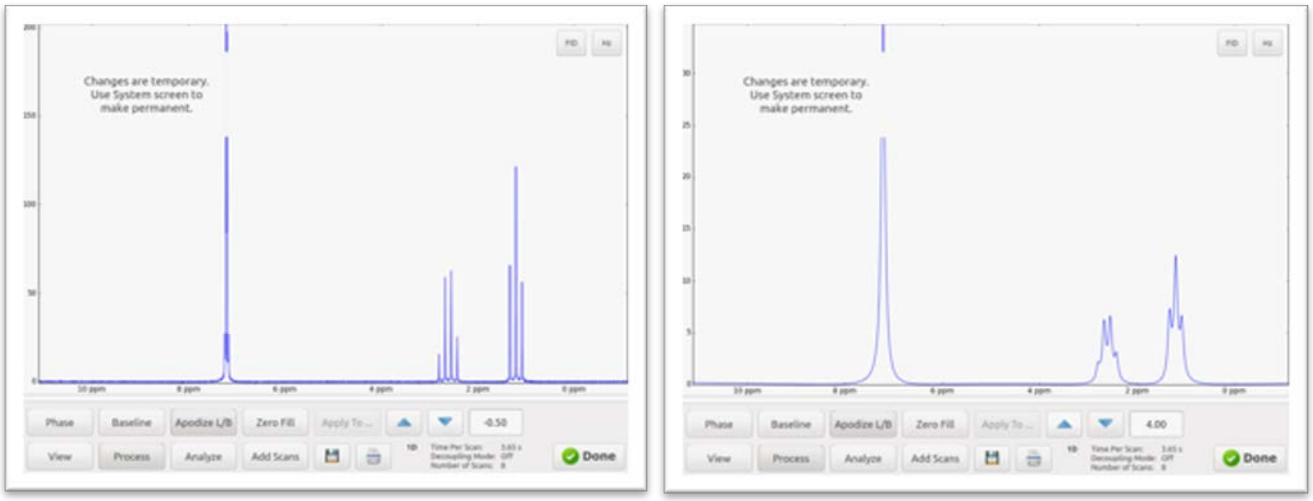
4.1.3.2 Baseline

OneTouch NMR software employs a robust auto-baseline correction that can be done automatically depressing the **Baseline** button and then by selecting **Auto** or manually by using the **up** and **down** arrows. If the autocorrect or manual correction does not improve the baseline, the **Reset** function restores the original baseline settings.



4.1.3.3 Apodize L/B

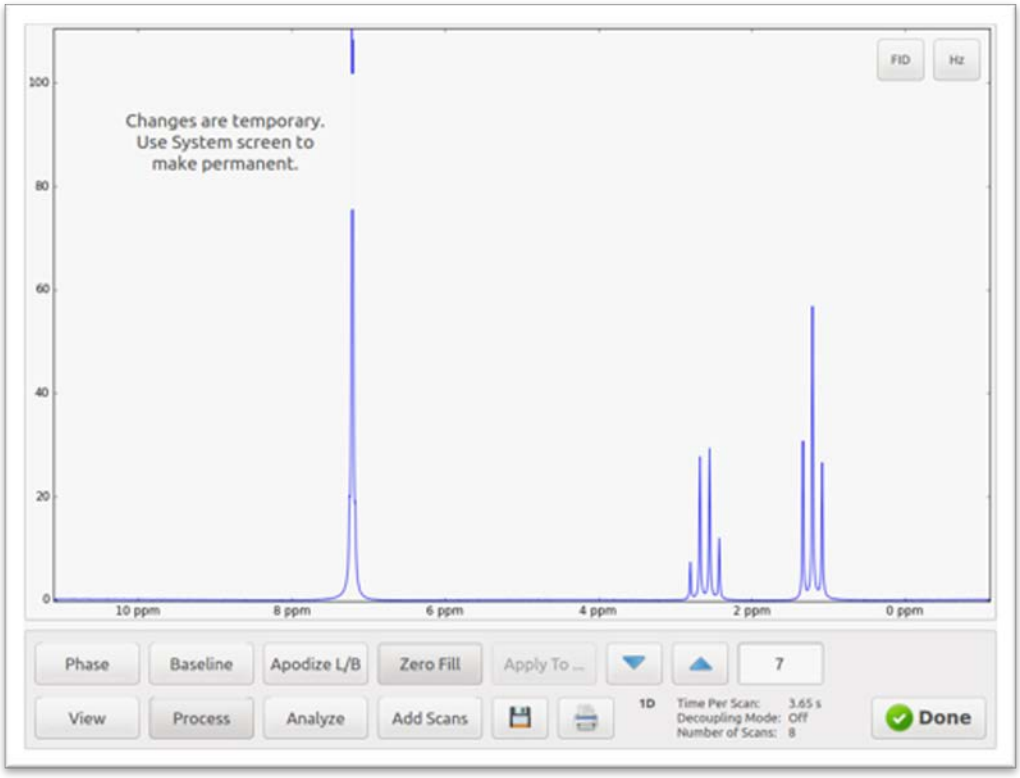
Line broadening increases or decreases the smoothness of the spectral lines and the baseline, so for a well-resolved spectrum, optimize the apodization to can improve the look of the spectrum. By selecting the **Apodize L/B**, arrows will appear to the right-hand side of the bottom bar. Please note that depending what nuclei you're observing the instrument will have a different default apodization that can be optimized after the spectrum is done acquiring (*e.g.*, 0.1 Hz for ^{1}H, 3.0 Hz for ^{13}C). When optimizing, a negative apodization can be applied, which allows better resolution in the peaks but at the expense of the baseline, this will look nosier. However, if a positive apodization is applied, the baseline looks smoother, but the peaks broaden out, and multiplicity could be lost. This is illustrated in the two figures below where the spectra shown here are shown at -0.3 Hz and 4.0 Hz to see the effect on the spectra.



4.1.3.4 Zero Fill

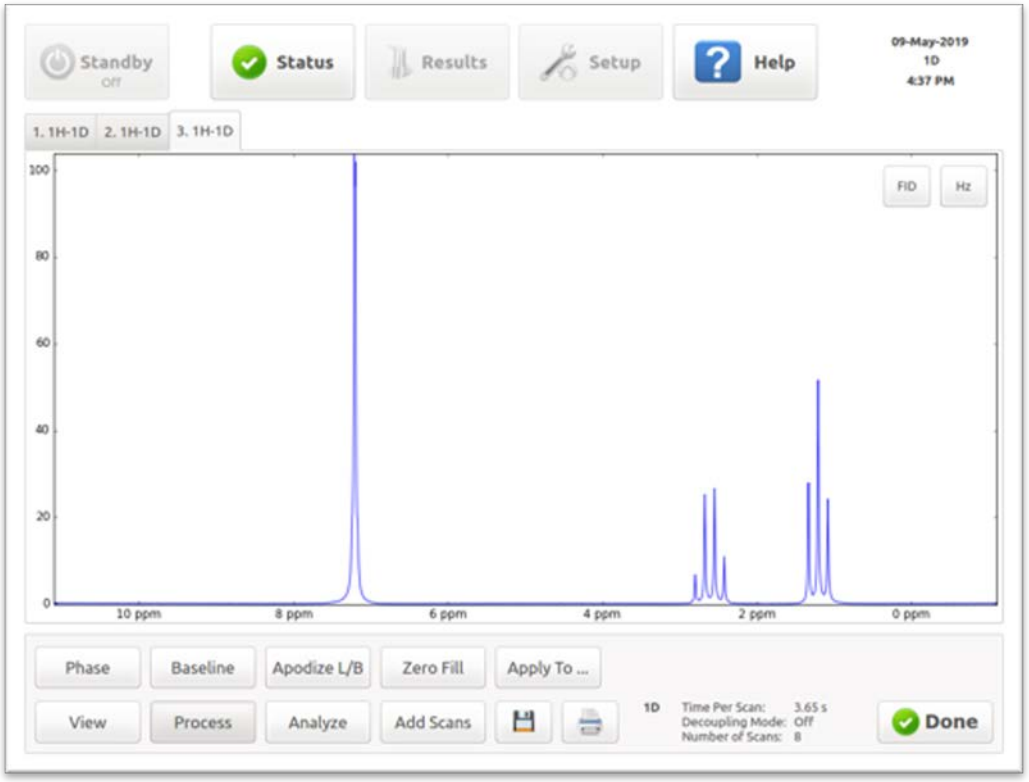
Increasing the zero filling enhances the digital resolution of the spectrum. Enhanced digital resolution will make feature-to-feature measurements more accurate. Increased zero filling can aid in accurate coupling-constant determination.

The integer value of the zero-filling constant represents the number of multiples of the number of points (e.g., if number of points is 1024 then setting zero filing to 1 will process 2048 points, setting it to 3 will process 4096 points. The instrument defaults to 7x zero fill, but by using the arrows, this can be changed to 0, 1, 3, 7 and 15, as is desired.



4.1.3.5 Apply To...

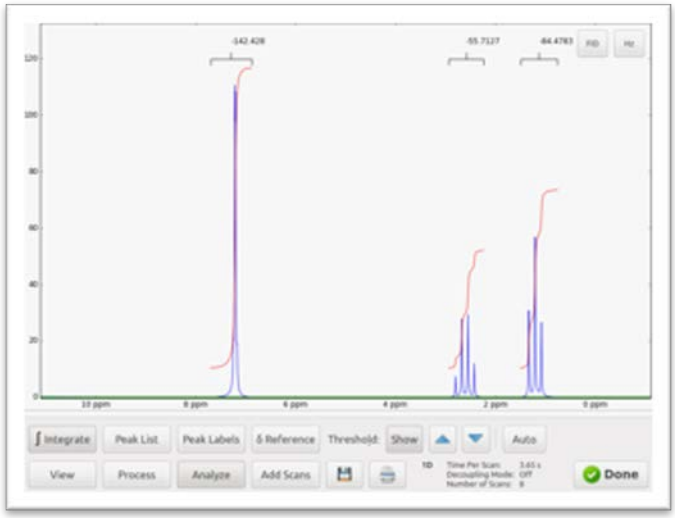
The **Apply To...** button becomes active when using the AUTOsample-60 (section 10.0) or queueing (section 7.1). This allows the user to apply the same **Phase**, **Baseline**, **Apodize L/B** and **Zero Fill** parameters to a series of spectra.

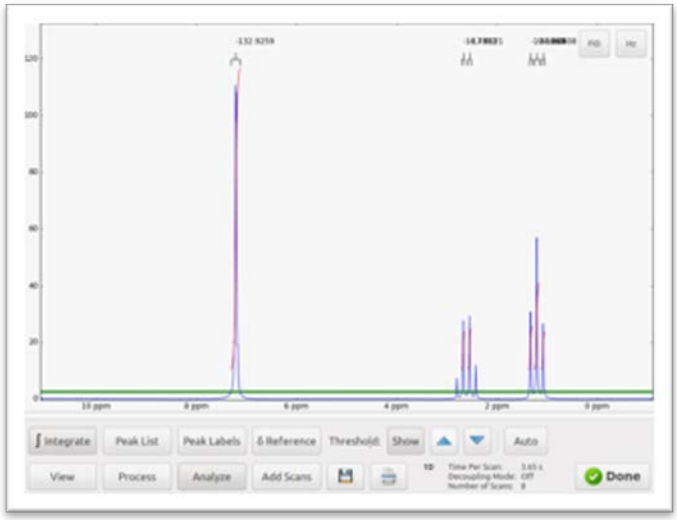


4.1.4 Analyze

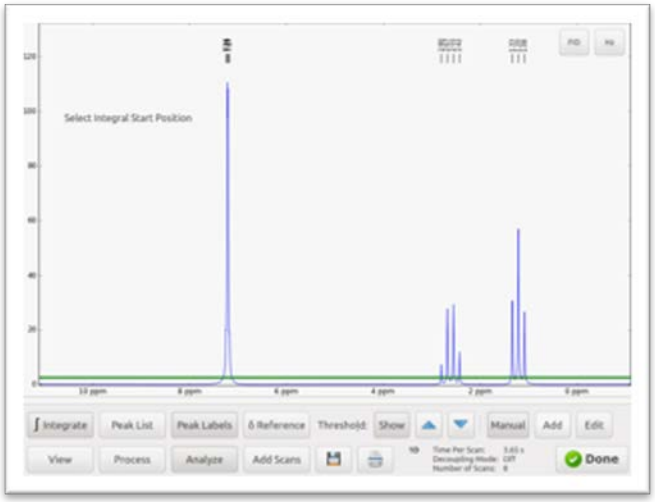
4.1.4.1 Integrate

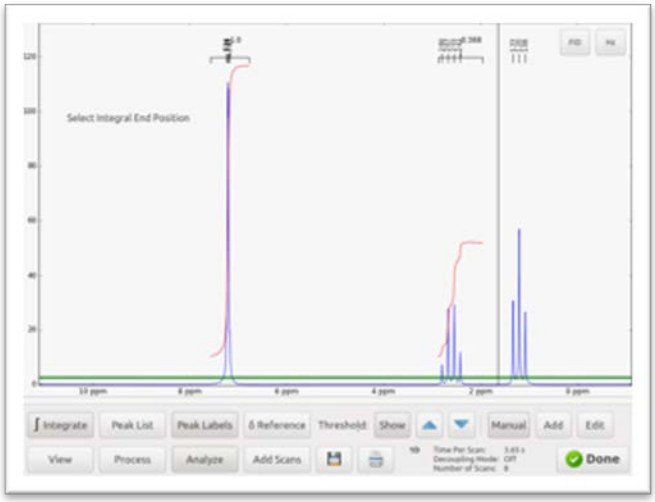
Touching the integrate button brings more options to the right hand-side of bottom bar. One can choose to **Show** the threshold. When selected a green line will appear on the screen showing the lower threshold of the integration. Using the **Up** and **Down** arrows, you can include more or less of the peak in an integration region. This is the threshold **Auto** integration.





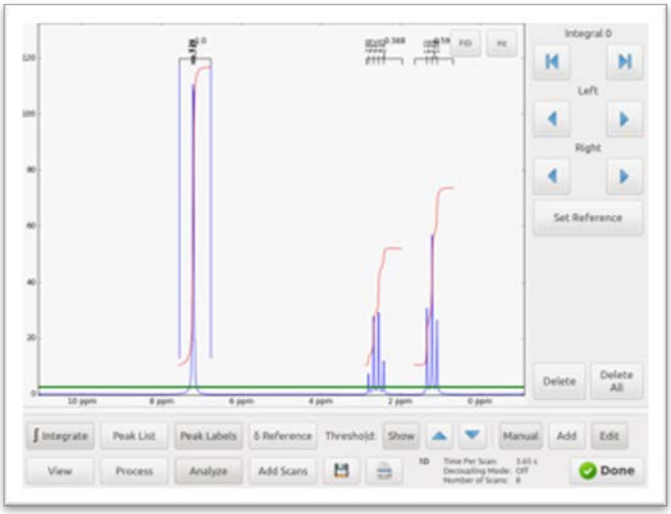
To perform a manual integration, select the **Auto** button, and it will toggle to **Manual**. Two additional buttons will then appear, by selecting **Add** you can select the bounds of each desired integral. One can click on the left-handed boundary of the area you wish to integrate and then the right. A red integral and numerical value will appear on the screen. Repeat this procedure for each desired integral.





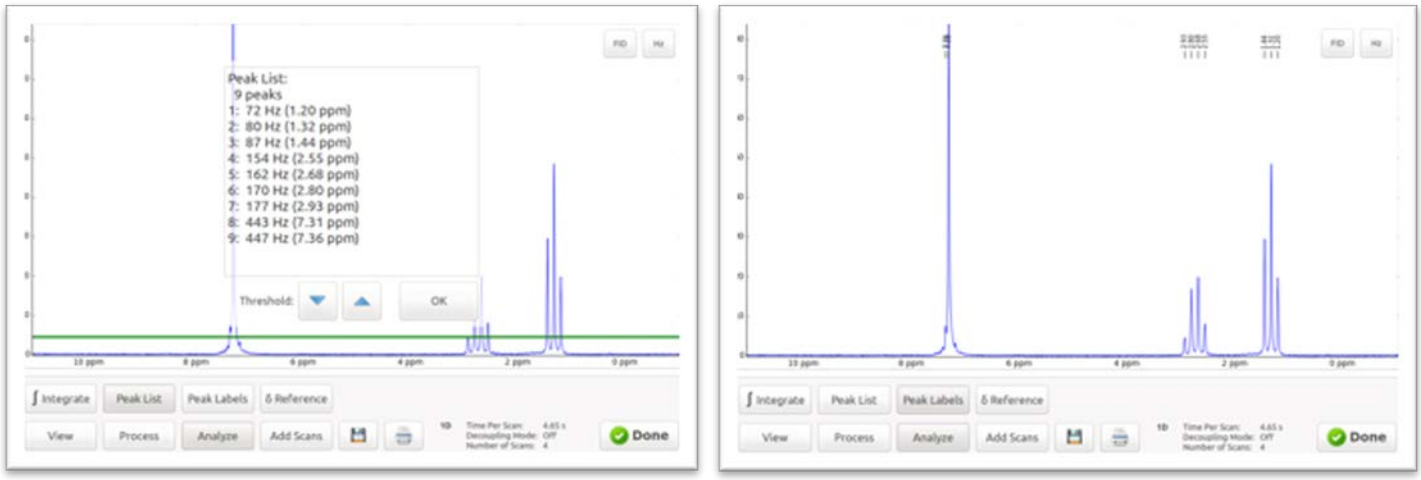
Selecting **Edit** allows the user to: (1) make modifications to the integral region; and (2) set the normalized value for an integral. To jump back and forth between the integrals, use the skip keys under the word **Integral**. The first integral you selected will be referred to as 'Integral 0', the second 'Integral 1' and so on. The active integral will be highlighted with blue bars on either side.

The nudge arrows under the words **Left** and **Right** allow the integral boundaries to be moved in small increments to make the integral region larger or smaller as is required. The arrows under Left will move the left boundary, while the arrow under Right can be used to adjust the right boundaries. Once the integral has been adjusted to size, selecting **Set Reference** will pop-up a keyboard. This will allow you to input the relative integral of that region. For example, the integral of a $–CH_2–$ resonance can be set to 2, and the other integrals will adjust relatively.



4.1.4.2 Peak List / Peak Labels

Touching the **Peak List** button places a list of all detected peaks on the screen in both Hz and ppm. This is governed using the threshold and can be adjusted as is desired. The peak list can be used for comparative purposes or to measure coupling constants. By pressing the **Peak Labels** button, labels with chemical shifts will be placed on top of each signal.



4.1.4.3 Chemical Shift Reference

The instrument monitors the deuterium channel to lock to ensure that there is a minimal drift. This process affords reasonably accurate chemical shifts. However, to ensure that the observed chemical shifts are precise, they can be further referenced upon workup if desired.

Touching the δ **Reference** button brings up two options to set the chemical shift.

4.1.4.3.1 Clear Ref

This function automatically corrects the observed chemical shifts to the pre-determined position as described in the configuration file for a loaded solvent.

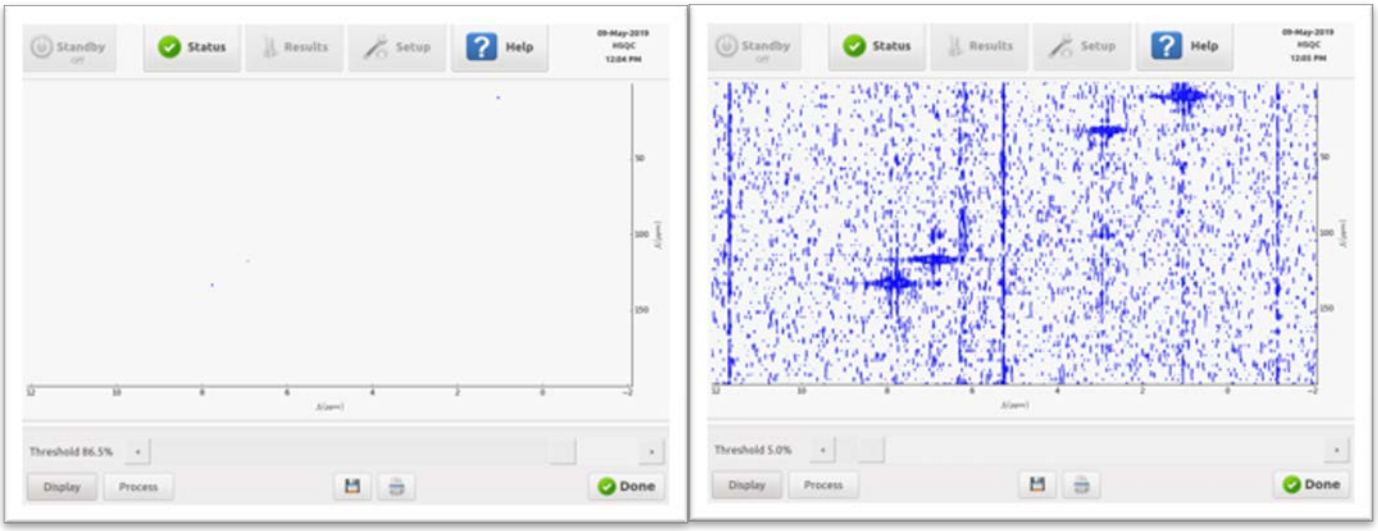
4.1.4.3.2 Keypad

To use **Keypad** to set a reference chemical shift, simply touch the peak you wish to use as a reference peak (*e.g.*, tetramethylsilane, Me_4Si or TMS, $\delta = 0.00$ ppm) and press **Keypad**. A keypad will pop-up on the screen, and the appropriate chemical shift can be entered manually (*e.g.*, TMS $\delta = 0.00$ ppm, DMSO $\delta = 2.50$ ppm).

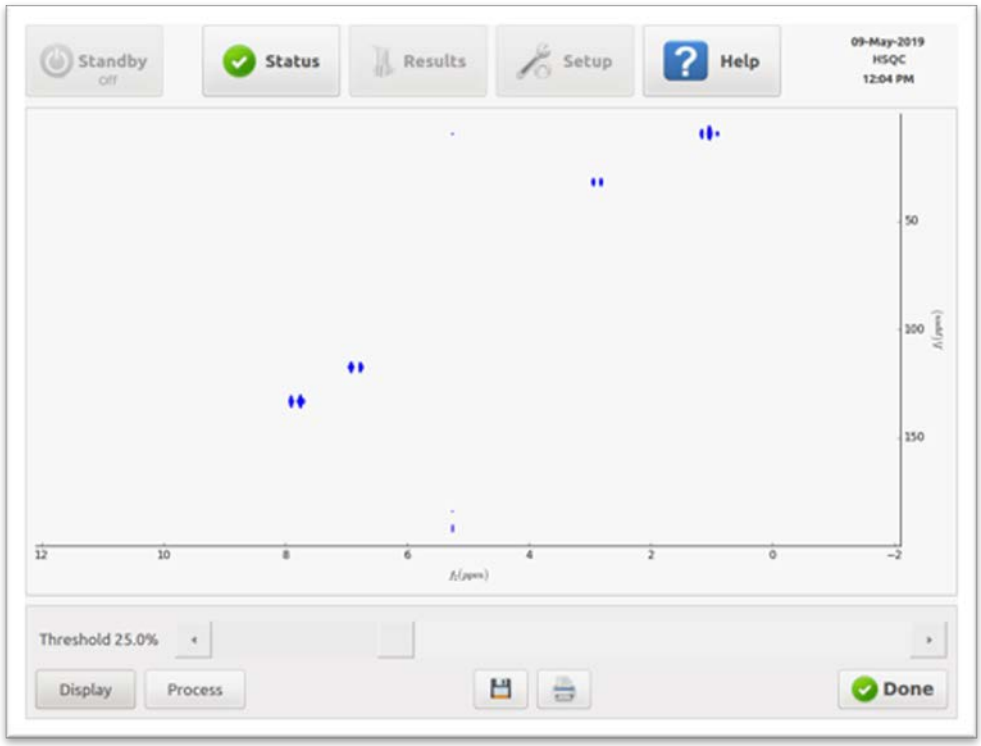
4.2 2D Data Processing

4.2.1 Display

The **Display** window allows the user to change the relative threshold of the displayed data, by decreasing the threshold more correlations will be observed (picture on the right below), by increasing the threshold only strong correlations will be seen and weaker ones won't be observed. If the threshold is too high, correlations will be masked by spectral noise. This is shown for an HSQC, but the principle holds for all 2D data processing.

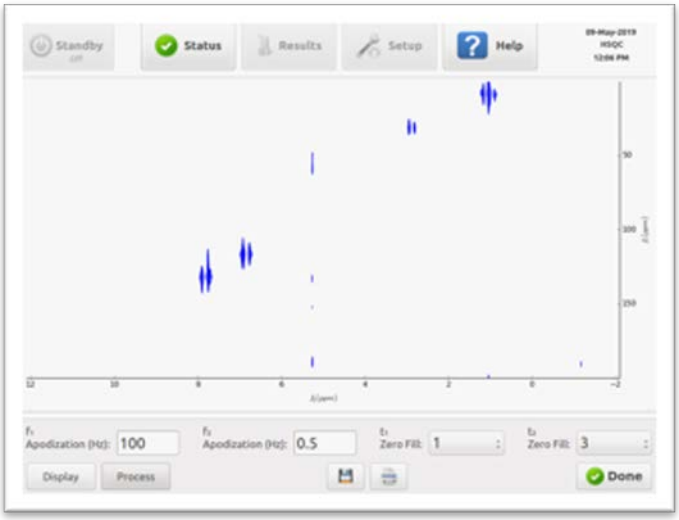


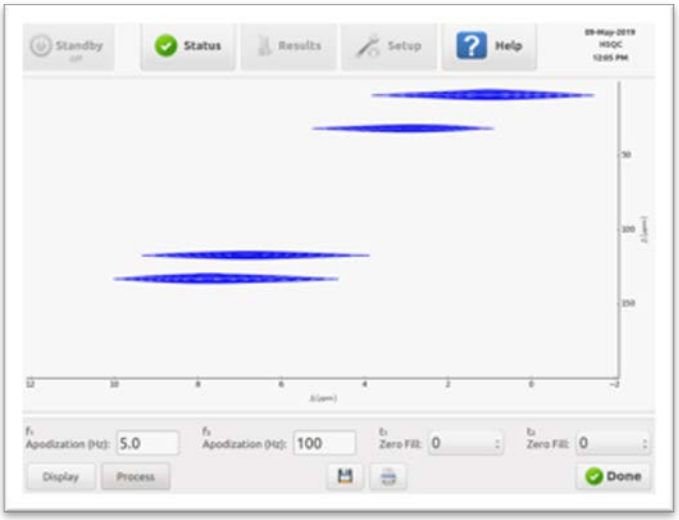
The user should optimize the threshold to see the maximum number of correlations without making either the cross peaks or noise artifacts (*e.g.*, T_1 noise) too dominant.



4.2.2 Process

The **Process** window allows the user to change either **f_1 Apodization** and **f_2 Apodization** and **t_1 Zero Fill** and **t_2 Zero Fill**. Increasing the **f_1 Apodization** will increase the effective 'height' of the peaks, while increasing the f_2 Apodization will change the width of the peaks, this is illustrated below by showing apodization to 100 Hz in both dimensions. Like in the 1D spectra, increasing the apodization reduces noise artifacts, but it does so at the expense of fine resolution of points. Applying a negative apodization or increasing the zero fill will help the user see observe better resolution in both dimensions.



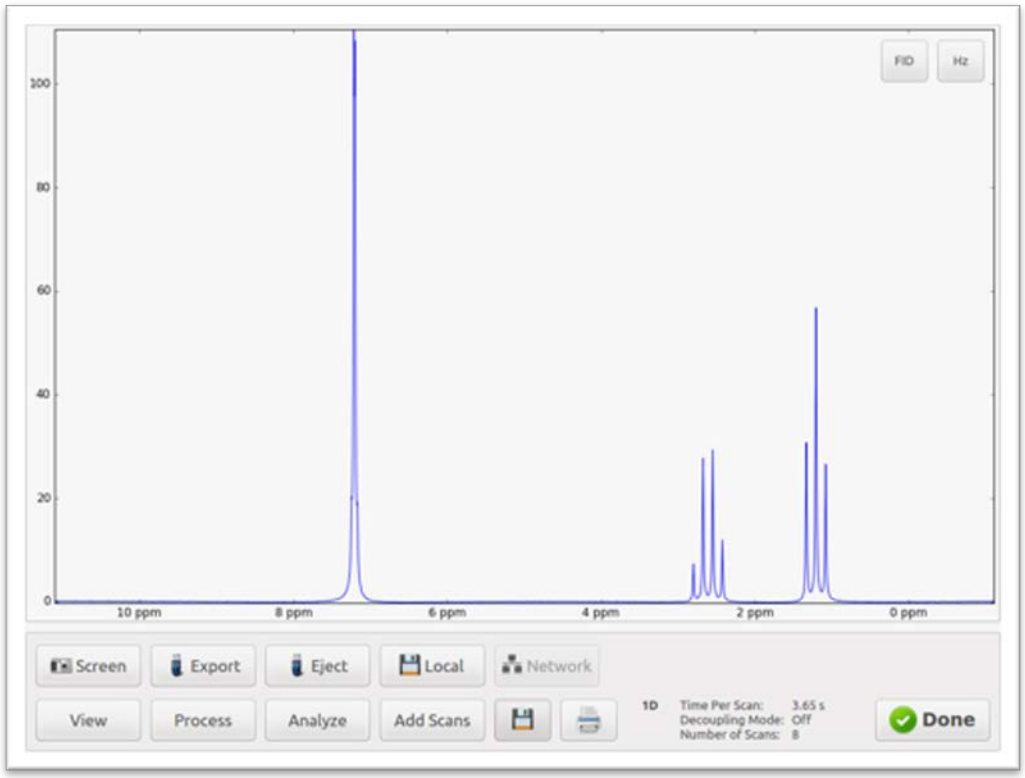


5.0 Saving and Exporting Data

After data processing is complete, the data can be saved directly to your benchtop NMR (as either a dx, pdf or a png file, see section 6.3.2 and 5.1), exported to a network folder or sent to a network printer once those features have been enabled. If buttons are greyed out, it means that the connectivity is not currently active.

5.1 Save

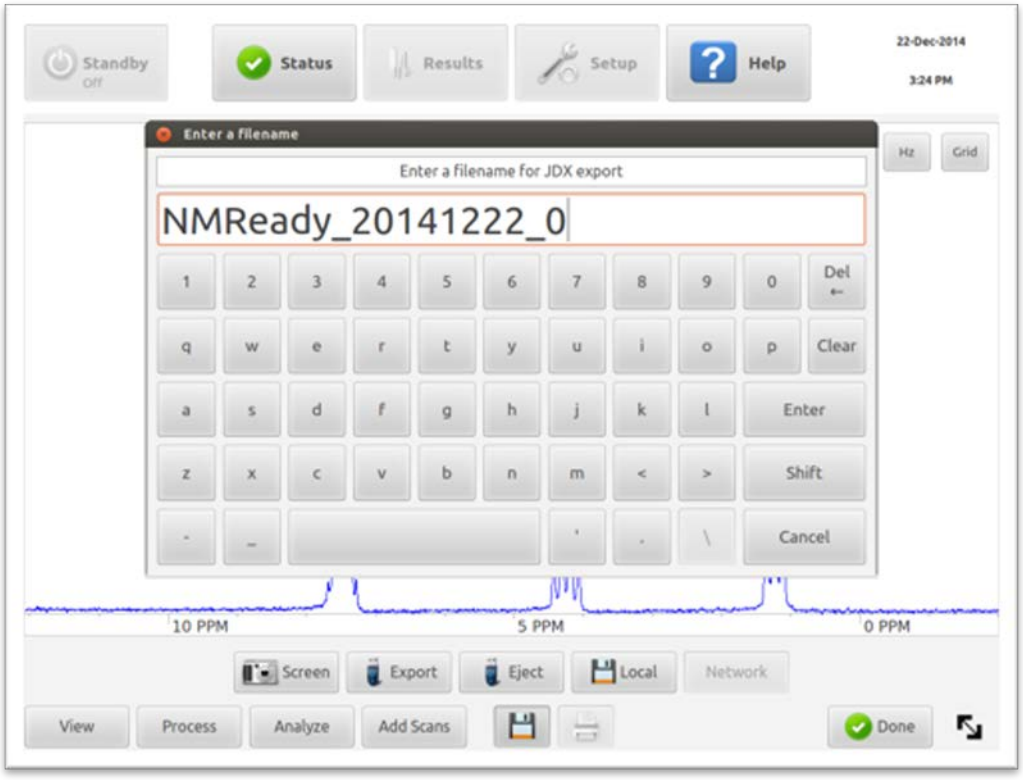
Pushing the disk icon will offer five options: (1) to save a .dx file locally to the spectrometer; (2) to save a dx file directly to a USB key, and then export; (3) to save a dx file to an existing network folder; (4) to save a png screen capture locally; and (5) print to pdf (discussed in section 5.2).



Local dx files are accessible from the **Results** button (section 6.3) on the task bar and can be reloaded for further processing into the OneTouch NMR software or exported to third-party software NMR processing tools.

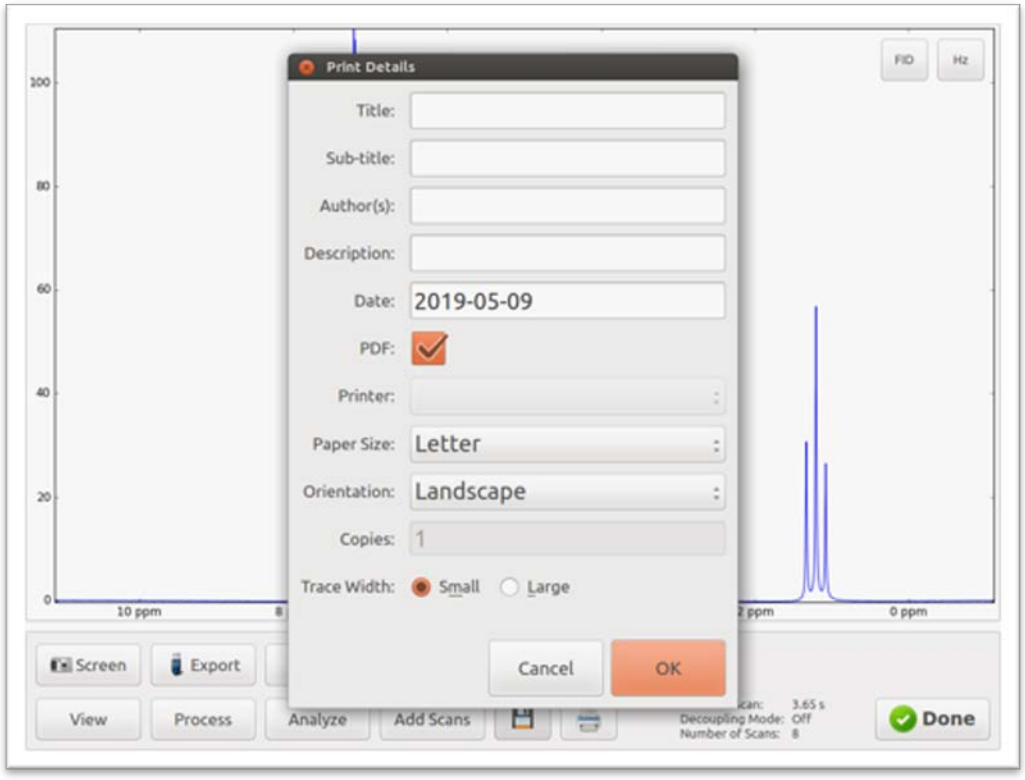
Files saved as **Screen** shots can be accessed from the **Results** screen as well, but they cannot be reloaded on the NMReady-60. They can be exported and included in a report, for example, but as an image file they can no longer be processed.

Once the user has selected the saving and/or exporting option of choice a keyboard will pop-up with the file route name (see section 6.4.2.6) and the date.



5.2 Print

The printer icon sends the current spectral image (including integrations and peak picks, if they were accessed during the spectral workup) to a suitable network printer. When the printer icon is selected, a Print Details window will appear allowing the user to customize fields including **Title**, **Sub-title**, **Author(s)** and **Description** and selected the desire **Printer**, **Paper Size** and **Orientation**.



The user can check the **PDF** box if they'd like to save as a pdf. The image will print in a frame and will be accompanied by experimental parameters. In order to ensure that your peak picks and integrations are accurate, ensure that the threshold is positioned correctly for each spectrum.

6.0 Nanalysis User Interface: The Task Bar

The main screen of the user interface is divided into two sections from which the spectrometer can be operated.

1) The task bar is located at the top of the main screen and can be accessed from most windows in the user interface. This bar allows easy navigation of instrument maintenance, access to saved results, and online help manuals and/or Nanalysis customer service through given options: **Standby**, **Status**, **Results**, **Setup** and **Help**.

2) Access to experimental configuration and control are only possible from the main screen. This includes access to experiment settings and parameters required to set and start an experiment. This was discussed in section 3.0.

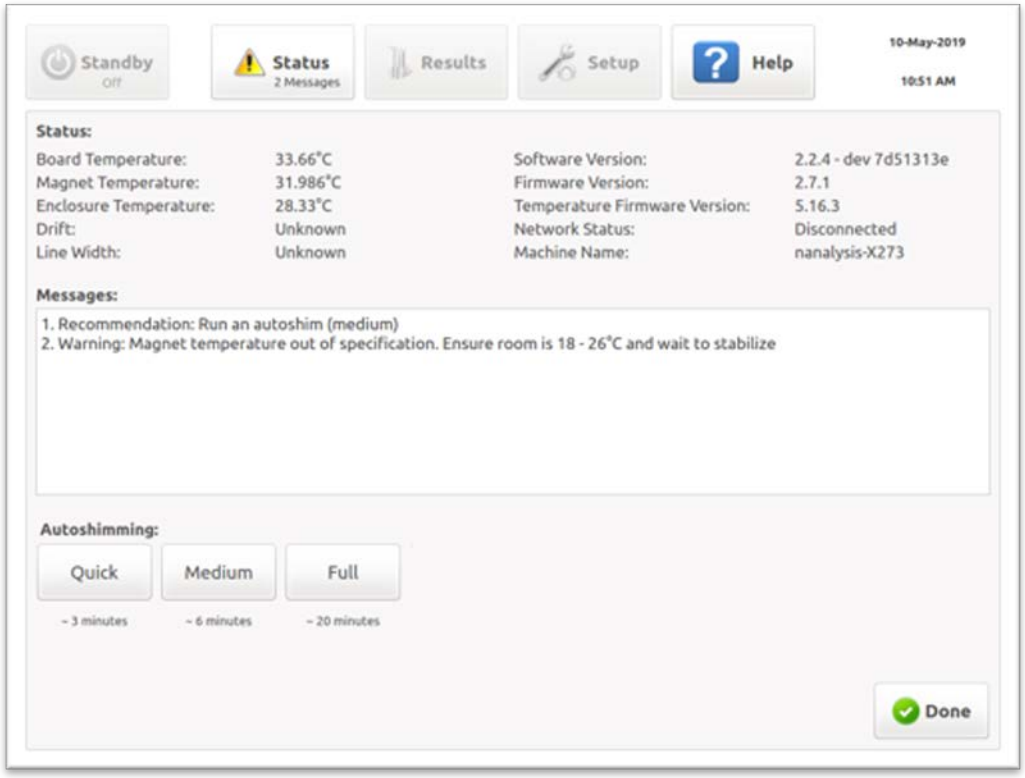
6.1 Standby

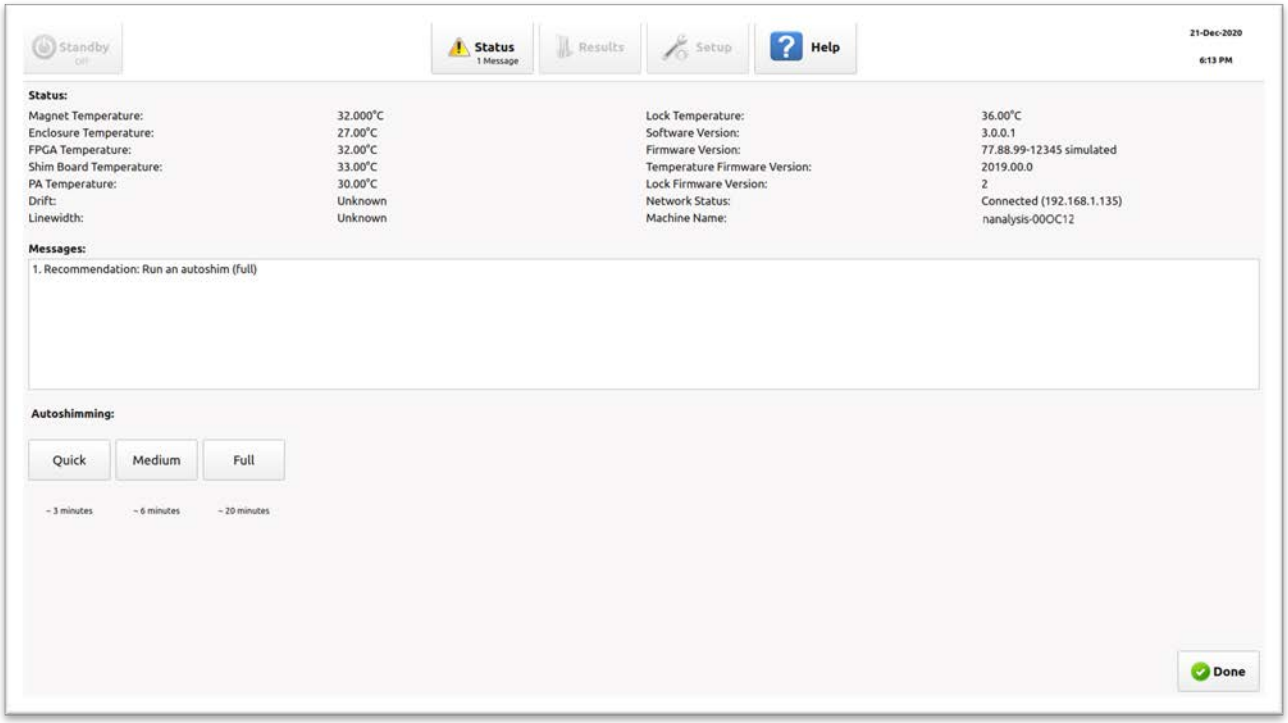
The standby button initiates a shimming routine that helps maintain line shape over extended periods during which the spectrometer is idle. This operation is addressed in detail in section 8.3.

6.2 Status

The **Status** button is located second from the left on the task bar and is visible from most operating screens. At a glance the system status provides information as to whether the spectrometer is operating normally () or requires adjustments ().

When selected, this button opens a window that displays information regarding the system's performance (*e.g.*, internal temperatures, frequency drift, line width at half height). It also provides easy access to three automated shimming procedures. In some cases, messages are meant to notify the user that a regularly scheduled tune or shimming procedure is required. Regular shimming is essential due to fluctuations in room temperature and the electromagnetic environment (*e.g.*, change in room temperature; a nearby device turns on/off). Depending on the instrument, this will change slightly between the 60 and the 100 MHz, shown respectively below.





This screen offers: (1) a means by which various performance metrics can be tracked; (2) advice on operations required to ensure that the instrument continues to run optimally; and (3) automated shimming routines to help the user maintain the instrument with ease.

6.2.1 Temperatures

The 60MHz tracks the temperature of the: (1) magnet; (2) electronics board; and (3) enclosure. Fluctuations are expected within the boards and enclosure, but the magnet temperature should remain constant. The 100 MHz tracks the temperature of the: (1) magnet, (2) enclosure; (3) FPGA; (4) Shim Board; (5) PA; and (6) Lock. Although the spectrometer, particularly the magnet, is well insulated, rapid or extreme temperature fluctuations will affect the resolution and overall performance of the instrument. Inconsistencies in performance can often be correlated to temperature, so these values are logged and monitored to provide the user with an accurate view of the situation.

6.2.2 Drift

Rapid temperature fluctuations can be an issue for consistent performance in spectrometers based on permanent magnet systems. This is evidenced in the drift, calculated in Hz/s. To counteract broadening effects that drift can induce in spectral lines, the NMReady is equipped with rigorous thermal control techniques. However, there is still some drift, and this parameter provides a measure of the performance of the thermal control mechanism under the ambient conditions.

6.2.3 Line Width

The line width (in Hz) displayed in the **Status** window represents the width at half-height of the NMR signal of the shim standard; it is not taken on the NMR line shape standard. If this value exceeds the recommended threshold that is required for suitable resolution, the value itself will turn orange, a warning will appear along with a recommendation for how to improve the performance.

6.2.4 Versions

In case of service, this information contains specific software and firmware version numbers that can be monitored and updated if necessary.

6.2.5 Network Status

The benchtop NMR is capable of being networked via its Ethernet connection. As discussed in section 2.3.4, we highly recommend that the user take advantage of this feature. The communications section reports the protocols that are used by the benchtop NMR spectrometer.

6.2.6 Machine Name

A short-hand identifier of the machines serial number is displayed here for the 60 MHz, X273 in the above screenshot or Nanalysis-OC012 for the 100MHz, and this can be useful to identify your machine if talking to a customer service representative.

6.2.7 Messages

When a warning appears on the **Status** button, the instrument will provide a recommendation to improve the overall performance of the instrument. These are based on numerous measurements of system parameters and performance characteristics. These messages can include recommendations (*e.g.*, 'Run an autoshim (full)', 'Ambient temperature instability since last shim. Run an autoshim'), a warning (*e.g.*, 'Magnet temperature out of specification. Ensure room is 18 - 26°C and wait to stabilize), or an error (*e.g.*, Magnet temperature out of tolerance).

The most common warning is the suggestion is for an updated autoshim. While adhering to these recommendations is advised, it is not an immediate requirement. If it is not feasible (*e.g.*, it is the end of an undergraduate laboratory experiment, it is the middle of a long reaction-monitoring run), and there is insufficient time to complete this task, it is not vital that it be addressed immediately. They are not indicative of a deterioration of the magnetic field but are scheduled in the software at timed interval. The real-time performance of the instrument can also be monitored in **Setup** ➔ *Manual Shim* (section 6.4.5) where the calculated 50% linewidth is a measure of the current, real time environment within the coil.

6.2.8 Auto-shimming

Shimming is an electronic means to modify the homogeneity of a magnetic field, using currents to correct for non-uniformity. To simplify the procedure, your benchtop NMR is equipped with three pre-set shimming procedures: quick, medium, and full. Each represents an increasingly comprehensive adjustment of the instrument's internal magnetic field. Depending on the solvent selected, most shimming will be done on the 'blue' reference standard. The blue tube contains H_2O/D_2O and a small quantity of paramagnetic relaxation agent to facilitate more rapid shimming.

6.2.8.1 Quick

It is recommended that a quick shim be completed every 4 hours. This makes very basic adjustments to the first order, linear gradients of the magnetic field (*i.e.*, x, y and z). This type of auto-shim typically takes about 3 minutes. The length of the autoshim will vary slightly between instruments and over an instrument's lifetime.

6.2.8.2 Medium

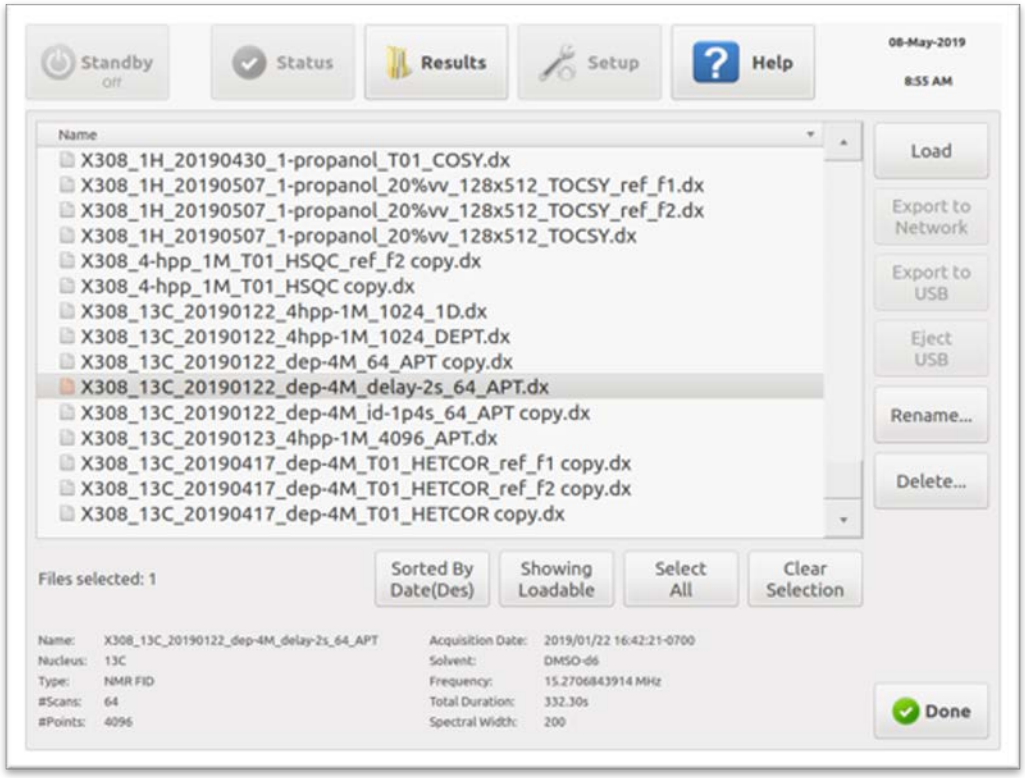
This is a pre-set shim profile meant to optimize the first and second order shim gradients. It is recommended that a medium auto-shim be performed every 10 hours and typically requires about 6 minutes to complete.

6.2.8.3 Full

Finally, a full shim performs a full electronic tune-up. This shims down to fourth order gradients. It takes approximately 20 minutes to complete and will be recommended by the system status function every 24 hours.

6.3 Results

The **Results** button is located in the center of the task bar. This provides access to the library of spectral data that was saved directly on the spectrometer. Files are automatically sorted by either alphabetical order or by date. To toggle between these options, select the **Sorted by Date(Des)** or **Sorted by Date(Asc)** to show the dx files descending or ascending order. Alternatively, you can sort alphabetically with **Sorted by Name(Asc)** or **Sorted by Name(Des)**.

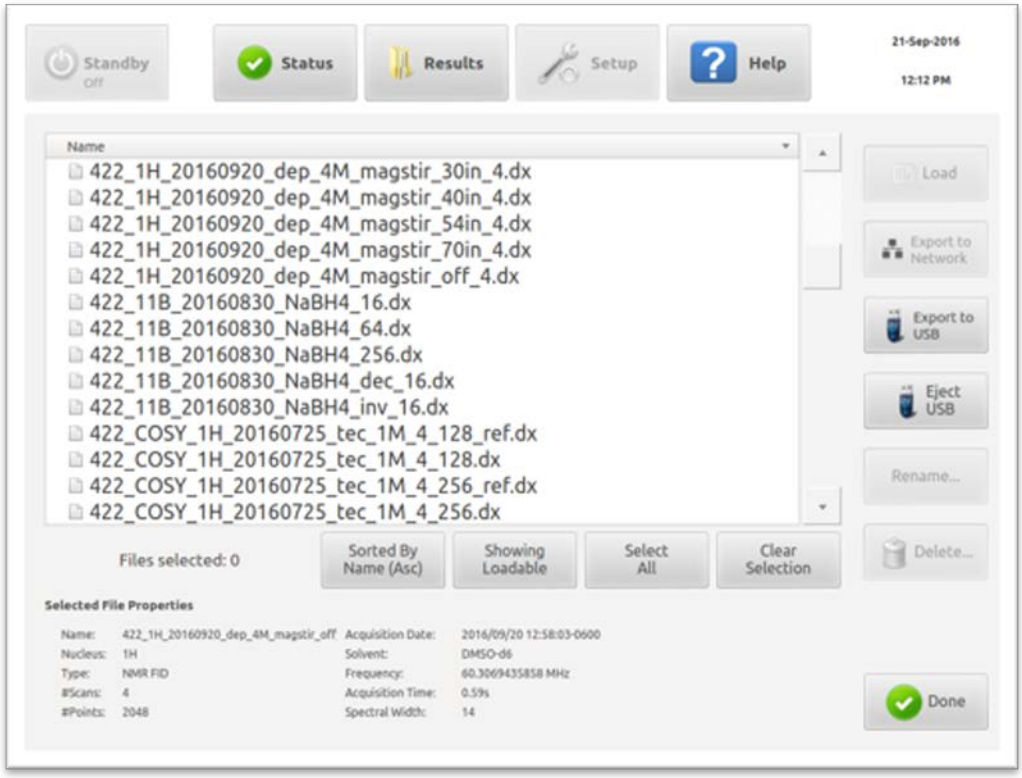


When the button **Showing Loadable** is visible, the data contained within this list is in the form of JCAMP-DX. This is the NMR spectral file standard and can be opened with the majority of NMR processing software tools (*e.g.*, Mestrelab MNova, Bruker Topspin, ACD/Labs Spectrus, JEOL Delta, Spinworks, etc.).

To view pdf, png or csv files, one must press the **Showing Loadable** button to reveal **Showing All**. This allows these non-loadable files to be exported and managed from within the results window.

Please note that based on the file type, only the relevant buttons will be enabled (*e.g.*, .png files cannot be loaded back into the processing window for further processing and therefore **Load** will remain greyed out).

When a file is selected some parameters associated with the acquisition and data collection will appear at the bottom of the screen (*e.g.*, Name, nucleus (*e.g.*, ^{1}H, ^{7}Li, ^{11}B, ^{13}C, ^{19}F, ^{31}P), acquisition date, the solvent the spectrum was obtained in, # scans, time per scan and the Larmor frequency of the spectrometer. These can be seen in the below figure under the title 'Selected File Properties'.



This window also allows you to manage either one file, a series of files or all files by selecting the desired files in the list or hitting **Select All**. After the files are selected you can **Rename** or **Delete** saved files or export files. Please note that the relevant buttons will be greyed out until they are active (*e.g.*, **Export to USB** and **Eject USB** will remained greyed out until a USB key is connected to the system or **Export to Network** will remain inaccessible until a network is set up).

The instrument is powered by a Linux Ubuntu operating system. The spectrometer can store many spectral files before reaching its storage capacity. However, it is advisable to occasionally maintain this library, removing outdated files.

6.3.1 Load

Once files that are saved in JCAMP-DX (dx) format are highlighted, they can be re-loaded into the data processing screen by selecting **Load** from the top right-hand corner of the screen. Image files cannot be loaded back into the NMReady-60 data processing window for further manipulation.

6.3.2 Exporting Data

Data files of all file extensions can be exported. These files can then be accessed on an external computer, and the dx files can be further manipulated in any of the third-party software tools mentioned earlier.

Files can be exported one by one or in a batch. To select more than one file, select the top file of interest and drag your finger downwards towards the bottom file of interest. All successive files will be

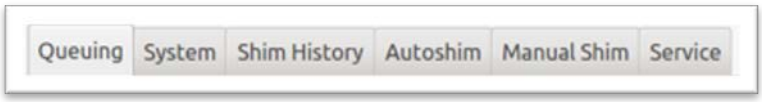
highlighted and can be exported at one time. A selected file can be deselected by touching its filename a second time.

To export to a network, the network folder must be previously set up via the **Setup** panel and *System* tab. The set-up of this folder will be further discussed in section 6.4.2.2.

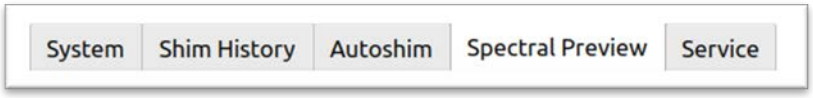
To export a file to a USB drive, the USB key must be inserted into the benchtop NMR. This will render the **Export to USB** and **Eject USB** keys active. Next, select the desired file and touch **Export to USB** button. This will export the files that were selected. **Eject USB** will properly disconnect the USB key and ensure that the files are not corrupted during the data transfer.

6.4 Setup

The **Setup** panel is the fourth feature on the top task bar. In 60 MHz benchtop NMR spectrometers, this panel contains access to six additional function tabs that enable the user to customize and use: (1) *Queuing*; (2) *System*; (3) *Shim History*; (4) *Autoshim*; (5) *Manual Shim*; and (6) *Service*.



In the 100 MHz, this tab contains five additional function tabs: (1) *System; (2) Shim History; (3) Autoshim; (4) Spectral Preview)*; and (5) Service. As this menu primarily offers access to parameters that can affect system performance, it is recommended that only selected site administrators operate this screen. If there is concern about certain users' access to this panel, it can be restricted with a PIN to allow only trained local administrators can access the features in this menu (for more information see section 6.4.6.4.



6.4.1 Queuing

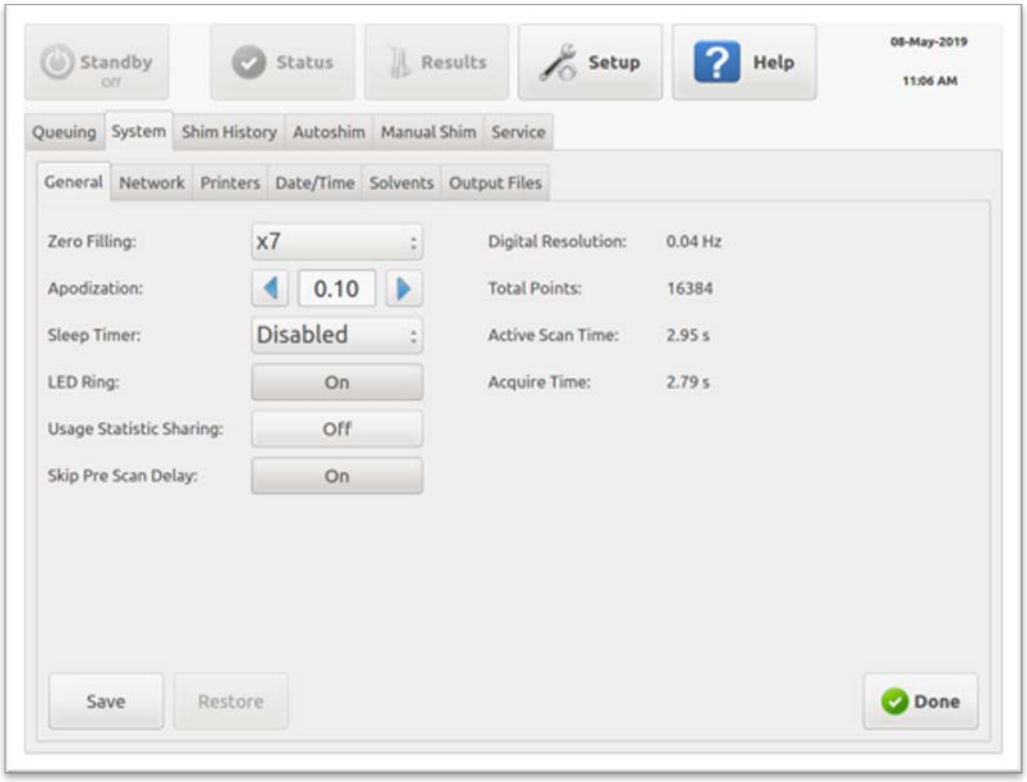
An optional software feature is queuing. This allows the user to run a series of experiments on the same tube automatically, or with the autosampler. This will be discussed more extensively in section 7.1 and 10.0, respectively.

6.4.2 System Tab

The systems tab contains a number of settings that control the operation of the instrument for all users, including: (1) *General*; (2) *Network*; (3) *Printers*; (4) *Date/Time*; (5) *Solvents*; and (6) *Output Files*. We recommend that only advanced users or local administrators make adjustments to the default parameters on this page.

6.4.2.1 General System Settings

This tab provides access to the default parameters that load upon experiment selection. By altering these parameters and hitting **Save** button the pre-loaded parameters are changed for each solvent.



These parameters include: zero-filling factor, apodization, sleep timer and the filename base. The LED ring can be turned **On** or **Off** by toggling the button, similarly one can choose to share generic usage statistics with Nanalysis with an **On** or **Off** toggle. There is also an option to skip the pre scan delay. It is recommended that the user does not use this.

Zero-filling the data prior to processing increases the digital resolution of the spectrum. The integer value of the zero-filling constant represents the number of multiples of the number of points. For example, if the number of points is 1024 then setting zero filling to 1 will process 2048 points, setting it to 3 will process 4096, and 7 will process 8192.

Apodization is a mathematical function (*i.e.*, exponential multiplication) that is applied to the raw FID data in order to change the NMR line shape (as discussed in section 4.1.3.3). The function can take many forms, however the most common is exponential. It can be positive, which gives a smoothing of the spectrum and broadening of the peaks, or negative that gives a narrowing of the peaks and an increase in the noise. Typical line broadening settings are 0–1 Hz for proton data with higher values for heteronuclide, but the user should be careful not to set this value so high that the lines are artificially broadened, and structural data lost. This parameter can be set both pre- and post-data acquisition.

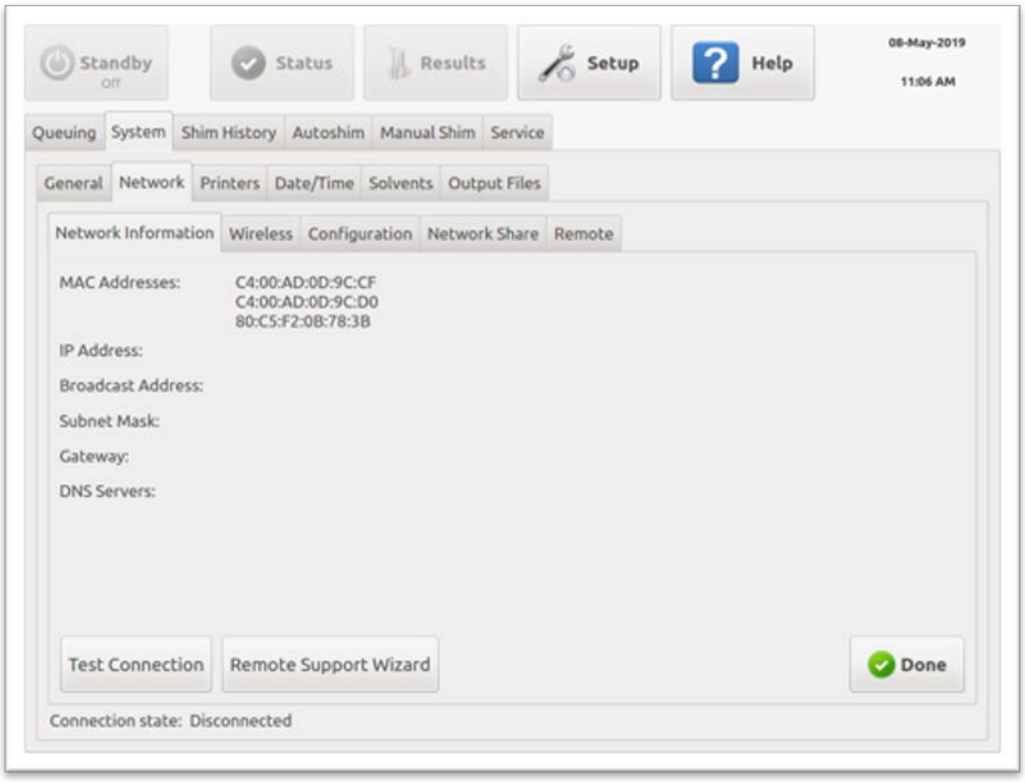
Sleep Timer sets the length of time the CPU will remain lit up and active after the touchscreen becomes idle. The system does not require that the sleep timer be used, but it is a power conservation method. Any time the screen has gone to sleep, touching it will turn it back on.

6.4.2.2 Network Settings

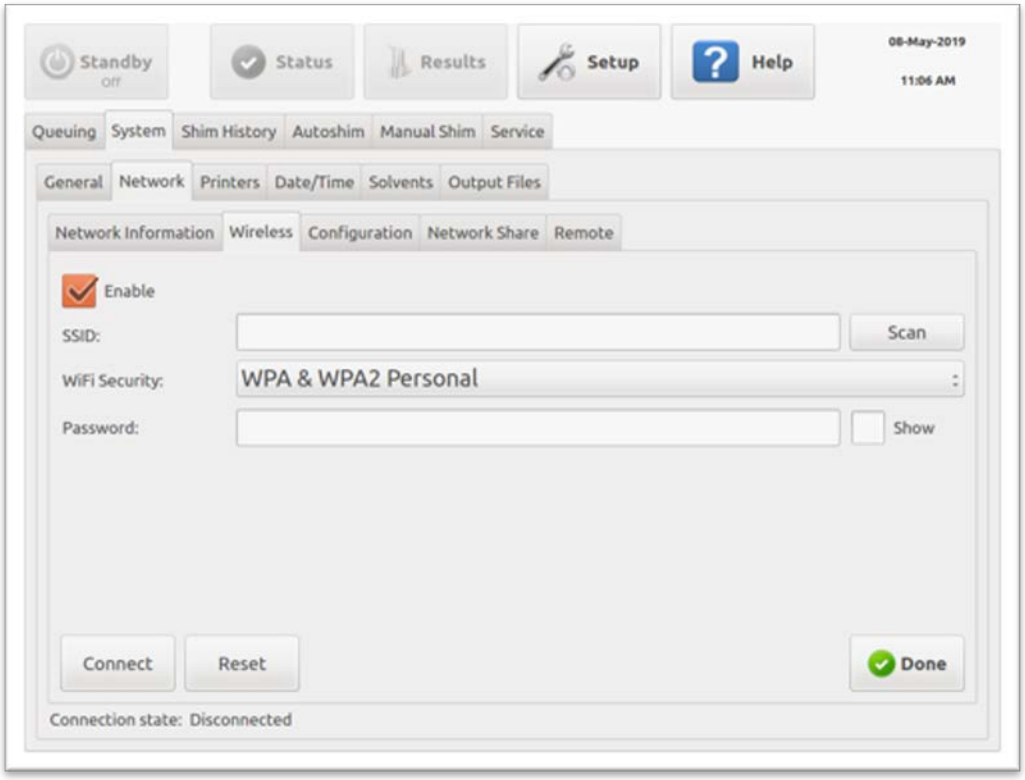
As discussed in section 2.3.4 connecting the benchtop NMR to a network enables: (1) access to a network folder for exporting data; (2) using a network printer; (3) software updates; and (4) advanced customer service.

The steps for connecting the instrument to a network are as follows:

1. Plug an Ethernet cable from an active wall port into the Ethernet port on the instrument.
2. Verify on the *Network Information* tab that an IP address has been obtained via DHCP.
3. Turn off DHCP by un-checking the checkbox
4. If there is no IP address, please contact your IT department. You may need to give the MAC id (**Hardware Address**) of the instrument to your IT department to get an IP address.
5. If you want to use the Internet-based network features (software updates and advanced customer service), press the **Test Connection** button to test the Internet connection from the instrument to Nanalysis customer service servers.

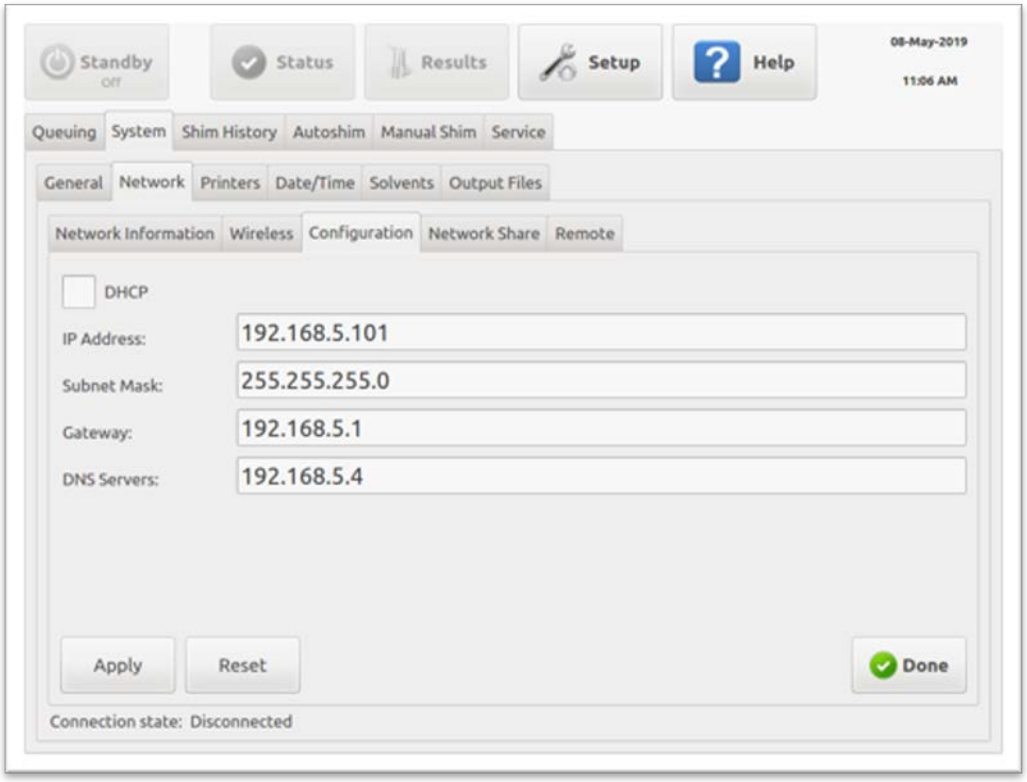


If Ethernet is not available, but Wi-Fi is available, a *Wireless* configuration tab is also available. To find a wireless network, select **Scan**, to select the Wi-Fi network that you would like to connect to. Then enter the password and select **Connect**.



If a static IP address is required (instead of DHCP) use the *Configuration* sub-tab and:

1. Obtain the IP address from the network administrator
2. Go to the screen **Setup** ➔ *System* ➔ *Network* ➔ *Configuration*
3. Enter in the IP address supplied by the network administrator along with the netmask, gateway and nameserver.
4. Double check that addresses have been entered correctly
5. Press the **Apply** button



Setting up a shared folder is very useful in that it provides a fast and simple way to export results from the spectrometer. There are two parts to this procedure.

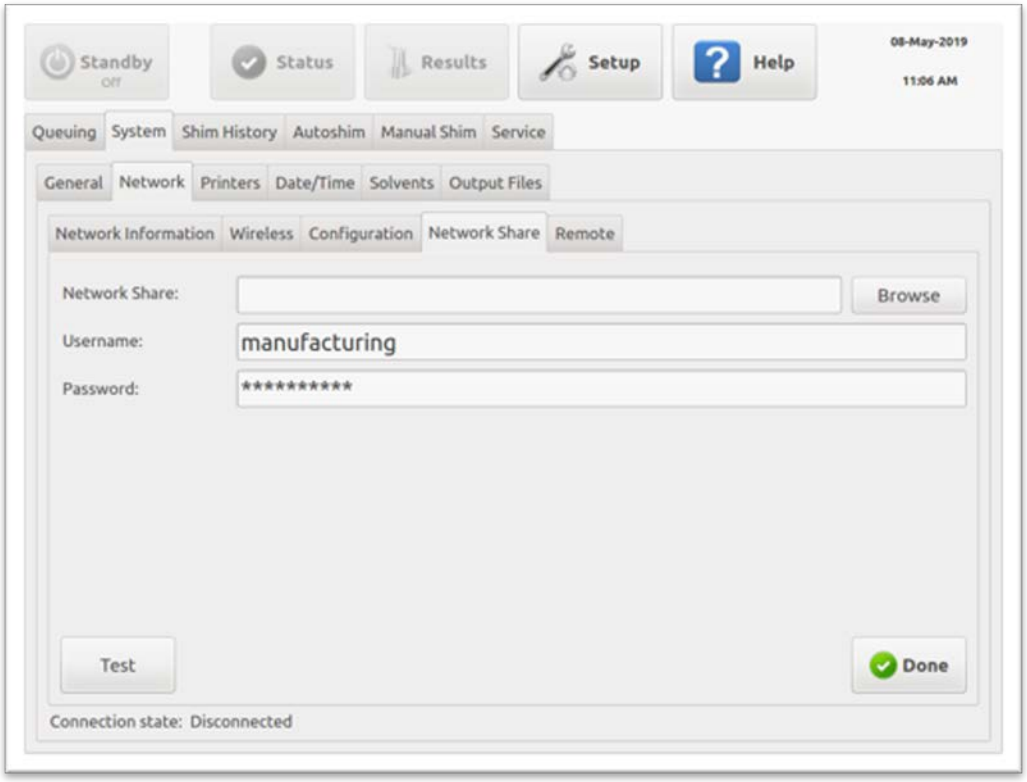
1. Request that your IT department:

- Create a 'share' on your local network for storing and sharing NMR spectra
- Create a specific account (*e.g.*, manufacturing) on your local network for use by the instrument
- Ensure the account has access to the shared spectra folder

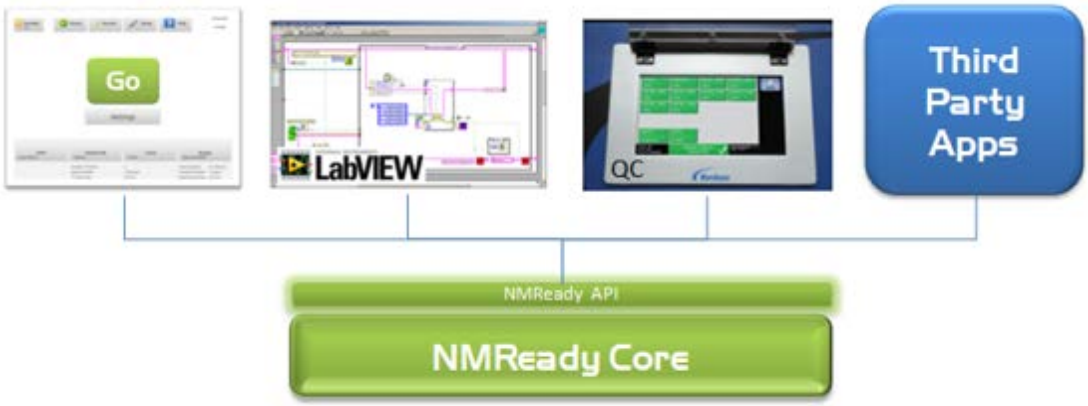
2. To setup file sharing on the instrument:

- Press the **Setup** button and navigate to the *System* ➔ *Network Share*
- Type in or **Browse** to the location of the shared folder
- Enter the username and password supplied by the IT department
- Test the path to the shared folder to make sure it is working (press **Test** button)

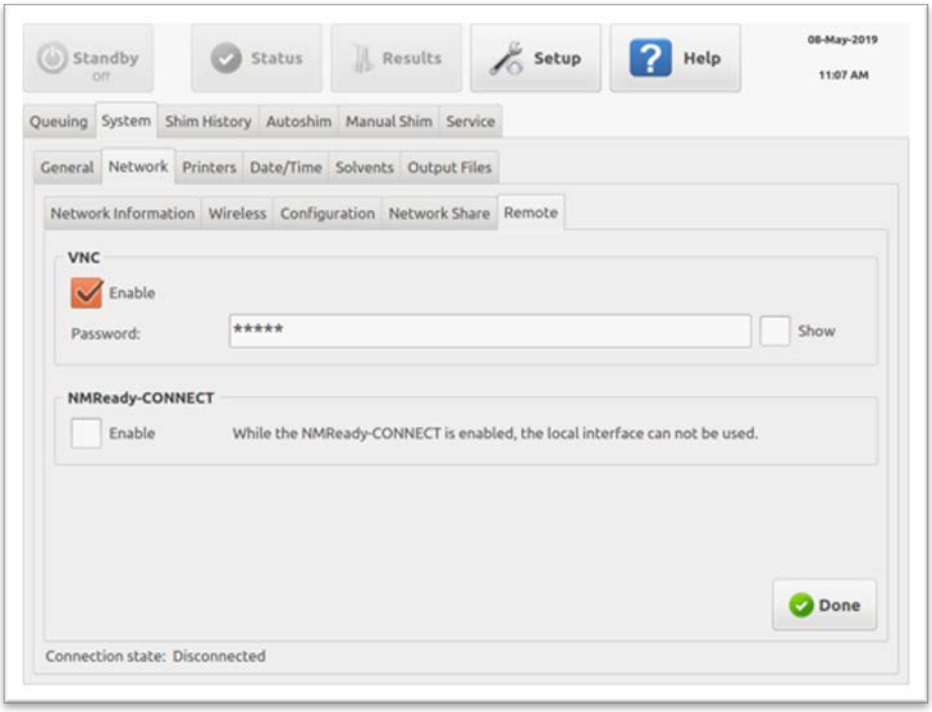
Once this is done, you can press the **Export to Network** button on the results screen and quickly upload files to the shared folder.



If enabled, the *Remote* sub-tab allows the user to use VNC to control the instrument remotely or provides easy access to the NMReady-CONNECT™. The latter provides access to the spectrometer's core functionality to provide an interface that a user can develop their own applications. Through this interface a user can connect the NMReady software architecture with third party applications/clients to create/perform custom monitoring and experiments. For example, one can control the spectrometer from instrumentation software such as LabVIEW to develop industrial green/red light (QA/QC) applications through Microsoft .NET or JSON protocols.

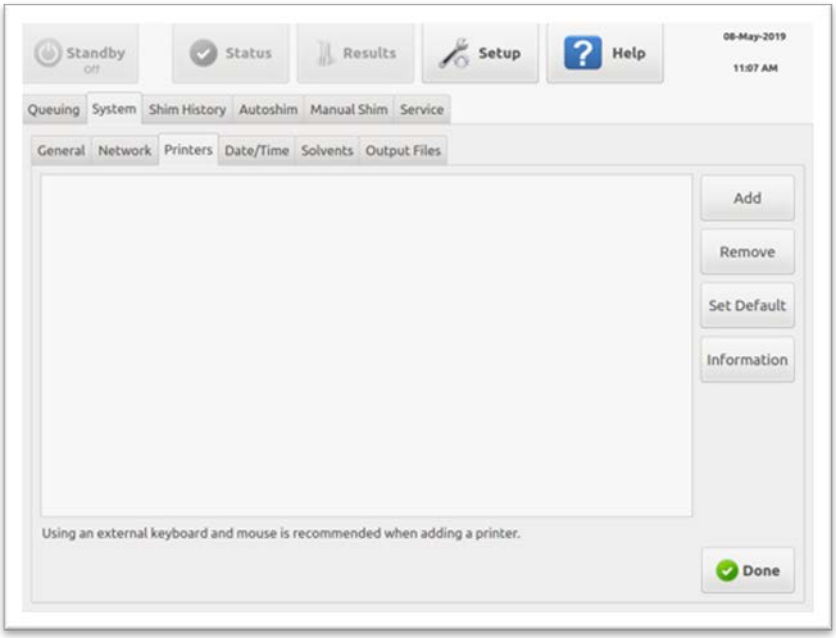


This functionality is not included in the basic instrument package, but if enabled can be easily accessed from **Setup** ➔ *System* ➔ *Network* ➔ *Remote*. If you are interested in more information about this functionality, please visit the product page (Nanalysis.com/nmready-connect), login to the developer website (Nanalysis.com/developer-zone) or notify your Nanalysis representative.



6.4.2.3 Printers

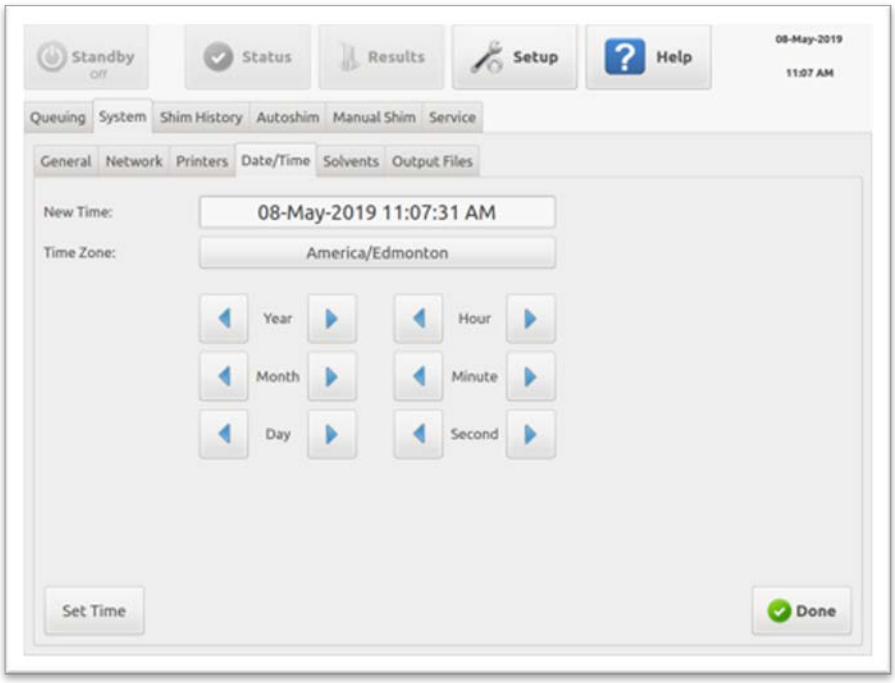
A network printer can be set up by pressing **Add**, after which a screen will pop up with available network printers. The desired printer can be selected by highlighting it and then hitting **Add**. Again, to ensure the printer is properly configured, the instrument must be restarted after the printer installation. To restart, click the power button on the right-hand side of the 60 or the front of the 100, chose **Restart** and wait for the instrument to reboot itself.



Please note that network printers are recommended above local printers (*i.e.*, those that are connected to the instrument's USB port) because local printers can introduce random loads on the system that can disturb NMR experiments. If you wish to install a local printer ***please contact your Nanalysis representative before proceeding***. The instrument most readily works with Lexmark, Canon or Samsung printers and any printer which does not come from the aforementioned list may not be supported by Nanalysis Corp.

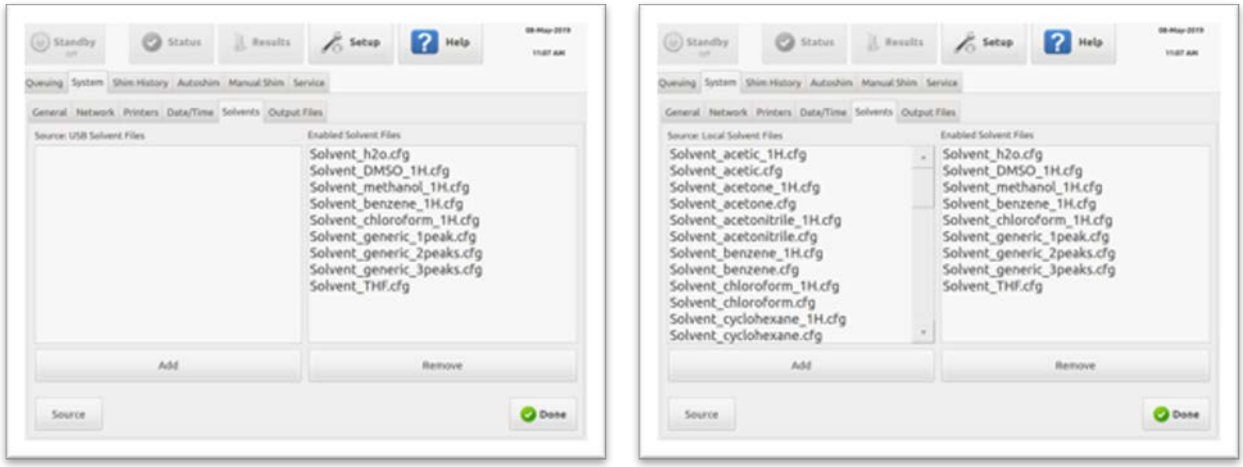
6.4.2.4 Date/Time

This tab enables the date and time of the instrument to be changed depending on the time zone you are in. If you are without a network and find that the time is not correct, it can be modified by hour, minute and second as is necessary.



6.4.2.5 Solvents

If a user requires augmentation of the solvent list on the main screen, the list can be modified in **Setup** ➔ *System* ➔ *Solvents*. Here the solvent list can be augmented from a file source on a USB or Local File. To toggle between, one can select **Source** in the bottom left-hand corner. One can scroll through the comprehensive list on the left-hand side, select the desired solvent and choose **Add**. Conversely, one can remove unwanted solvents from this list by selecting them under Enabled Solvent Files and selecting **Remove**. To ensure that this correctly adds to the solvent menu (section 3.2.1) the instrument must be restarted. To restart, click on the power button on the right-hand side of the 60MHz benchtop NMR, or the front of the 100 MHz, choose **Restart** and wait for the instrument to reboot itself. The instrumnt is defaulted to lock on deuterated solvents, although non-*deutero* options are also available.

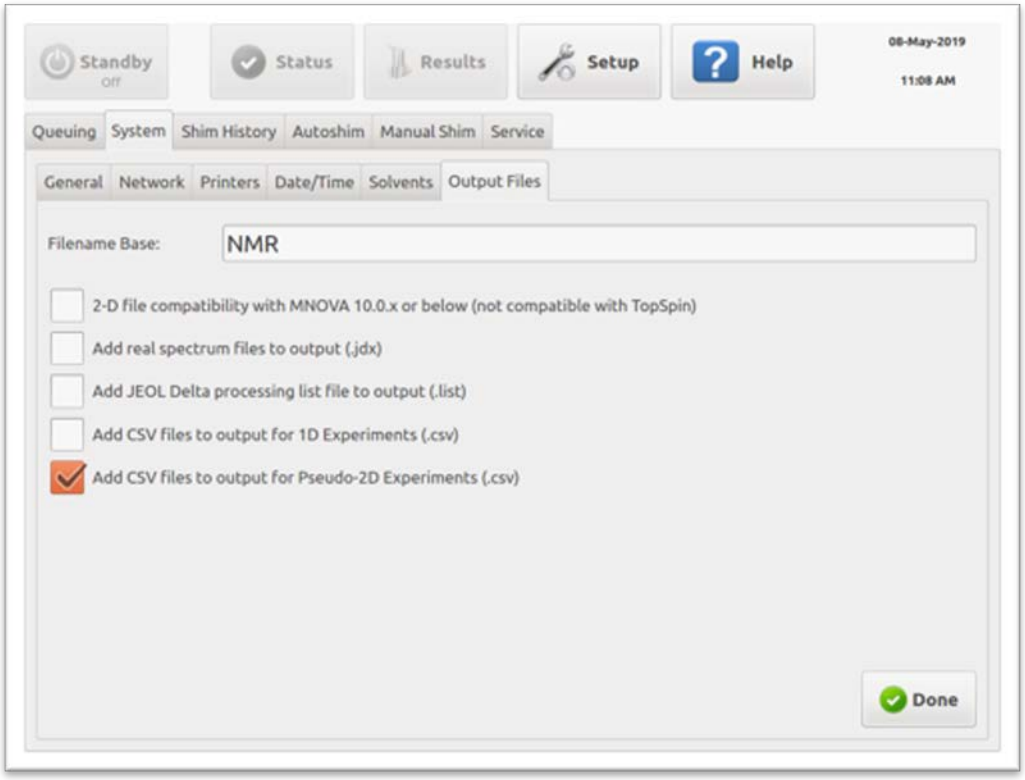


If you require no-D NMR or a *deutero*- or *proteo*- solvent that is not in the list, please contact your Nanalysis representative.

6.4.2.6 Output Files

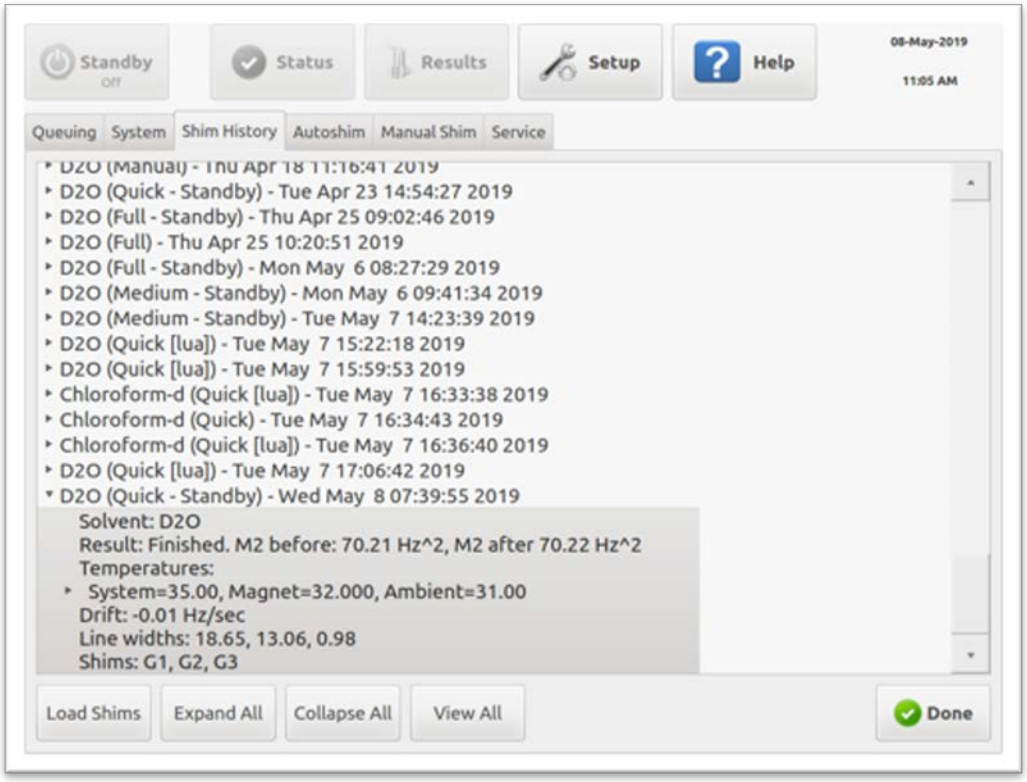
By selecting **Setup** ➔ *System* ➔ *Output Files*, the user can make amendments to how they would like the data exported, and/or which third party data processing files they would like their spectra to be compatible with.

Additionally, the Filename Base is the field that specifies the base filename that is automatically loaded in the data processing window when attempting to save spectral data (as either a dx or a png). If no base name is desired, this can be left blank and the file names will start with the date upon which the data was obtained.



6.4.3 Shim History Tab

The *Shim History* Tab displays a chronological list of the most recent shims that were applied to the instrument. Both auto and manual shims are included in this list. To re-apply an old shim set, select the desired entry in the list and touch the **Load Shims** button. It is rare that recalling a shim set from the shim history is required. However, in case of: (1) a bad manual shim; (2) difficulty shimming the instrument; or (3) leaving the instrument in standby (section 8.3) with the wrong reference sample, this is helpful to reset the system to known working values.

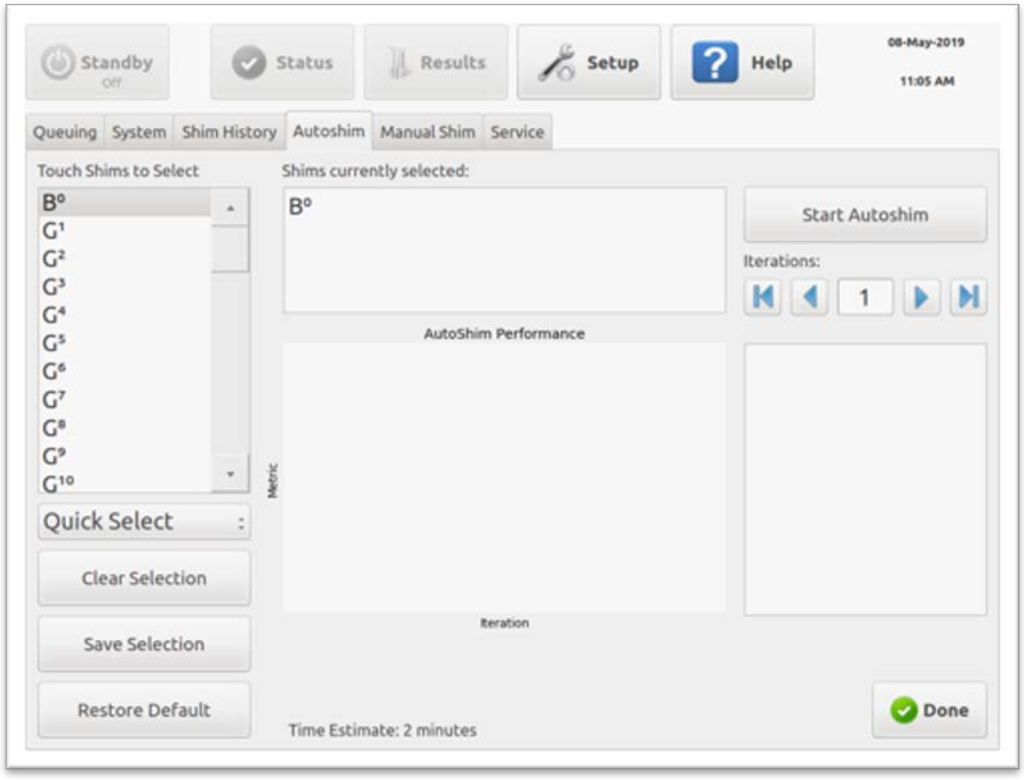


To get more information about each shim, the user can click on it and will be provided with a summary in a light grey box as is illustrated above. To get even more detailed information the **Expand All** button, and **Collapse All** hides the extra information. The instrument by default shows a month's worth of shim history, but more can be observed by selecting **View All**.

6.4.4 Autoshim Tab

The *Autoshim* tab allows advanced users to perform more carefully specified tune-ups to specified shim gradients. On the left-hand side, there is a column that shows a list of gradients that can be manipulated to shim the field. Users of high-field instrumentation, who are used to gradients that are named (*x*, *y*, *z*, *xy*, *etc.*) according to polynomial, spherical harmonic functions may find the names that are listed unfamiliar. Nanalysis benchtop NMR spectrometers use a set of gradient functions that are based on a principal-component analysis that exploits the lower symmetry of a Halbach-based magnet. This naming should not interfere with either automated or manual shimming in practice.

Beneath the gradient list is a drop-down menu. If it says **Quick Select**, the auto-shim is performed by: (1) choosing the gradient to be tuned (*e.g.*, B^0), these will appear in the 'Shims currently selected' box; and (2) pressing **Start Autoshim**. The estimated time it will take to autoshim will appear next to this.



The Quick Select box has many shimming options including quick, medium and full shim set. If **Quick Shim Set** is selected, then the auto-shim window will automatically select the gradients that adjust the field to lowest order. To start this autoshim, **Start Autoshim** must be selected. Similarly, **Medium Shim Set** will load the gradients that shim up to quadratic order, and **Full Shim Set** will load all 25 gradients. The autoshim will be initiated by selecting **Start Autoshim**.

Once a shim has been initiated in this window it can be cancelled at any time by selecting **Stop**. When the shimming route is completed, a metric will be shown in reference to the success of the machine and it will say 'Finished'. To see the line width at half-height, select **Status** or the *Manual Shim* tab within the **Setup** menu.

6.4.5 Manual Shim Tab

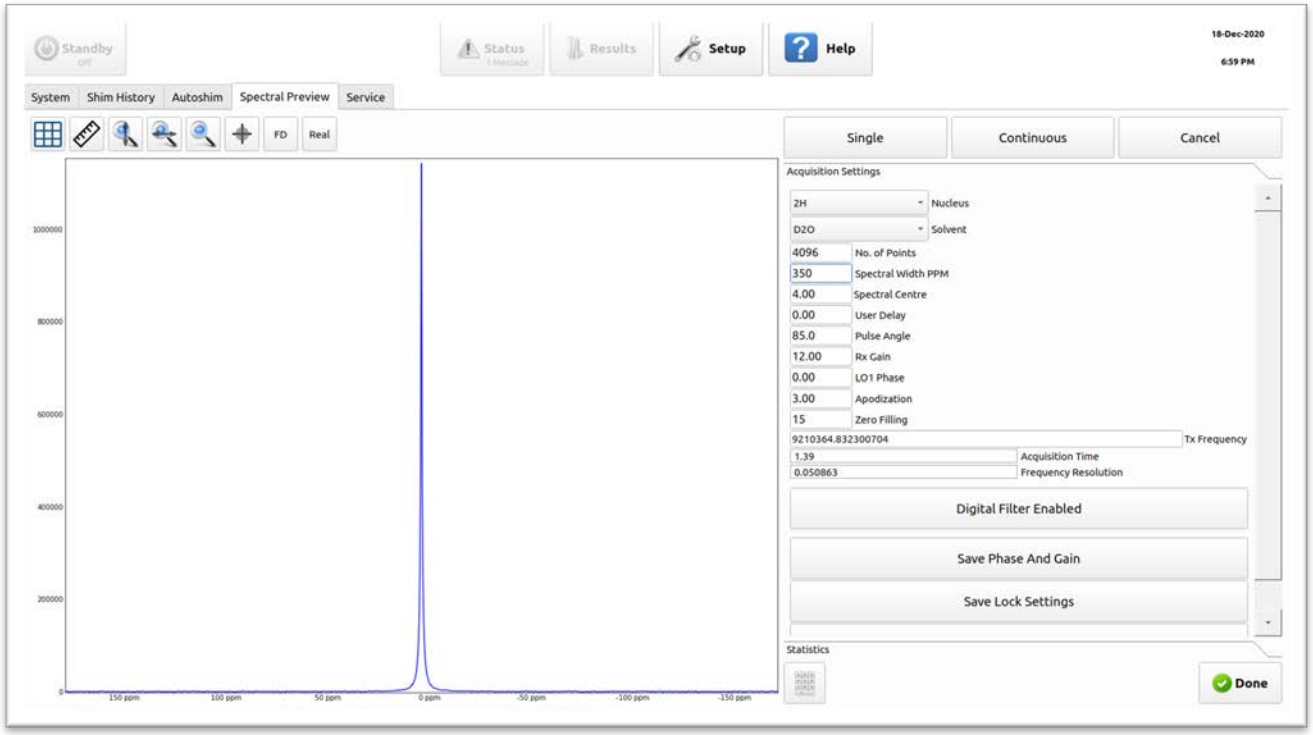
The *Manual Shim* tab screen is only on the 60 MHz benchtop NMR spectrometers. It allows manual manipulation of individual electronic gradients for each shim profile. Please note that manual shimming can be a complex procedure for those not familiar with routinely adjusting the field gradients and is not recommended for novice users. Consequently, the window loads with a warning to inform users that any changes made in this screen can be detrimental to the performance of the instrument and should be used with caution.

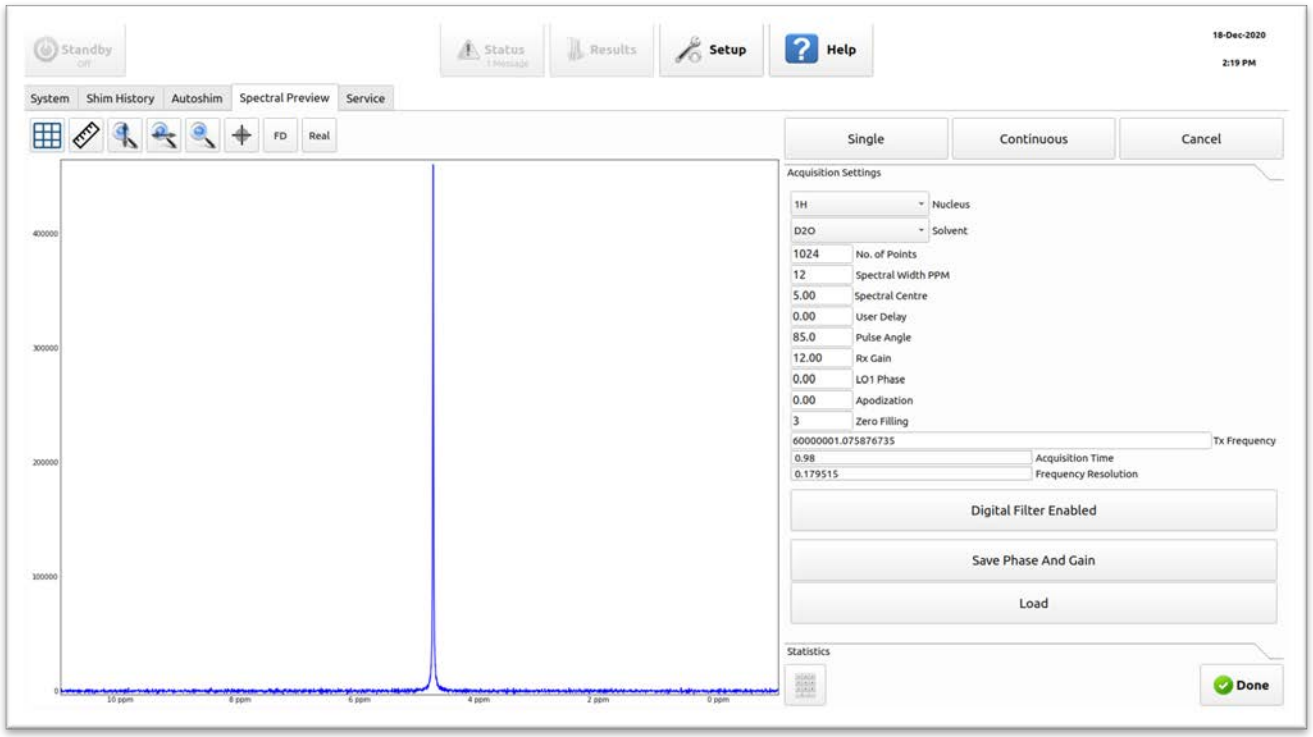
Manual shim adjustments can be made on the instrument by selecting: (1) the appropriate shim set; and then (2) a shim from that set. The active gradient can be altered in electronic field adjustments of 1 or 10. The effect that increasing or decreasing the shim value can be viewed actively on the inset spectrum image and from the resultant 0.55, 1, and 50% linewidths. Please remember that it is advisable that after any adjustment of higher order shims is complete a simple touch-up shim be performed. Once done select **Save**. If you are attempting a manual shim and are unable to produce an acceptable line shape, the system can be reverted to its most recently saved shims by selecting **Reload**.

It is recommended that the solvent reference sample be used for manual shimming. However, any expert confident on their manual-shimming prowess can shim directly on their sample.

6.4.6 Spectral Preview

In 100 MHz benchtop NMR spectrometers, the spectral preview window is meant to offer the user greater flexibility over both the deuterium and proton channels. This window allows the user to optimize acquisition parameters, and observe the effect in real time, either with one acquisition by pressing **Single** or in successive acquisitions by selecting **Continuous**. The Nuclei can be changed between deuterium *2H* or proton *1H* using the *Nucleus* dropdown menu. The no. of points, spectral width (ppm), spectral center, user delay, pulse angle, Rx gain, LO1 phase, apodization and zero filling. These parameters are discussed more in depth in section 3.2.2. The Tx Frequency can be modified as a means of changing the chemical shift reference, or this can be done by selecting the appropriate solvent from the dropdown menu. Depending on the number of points chosen and the spectral width the acquisition time and the frequency resolution will automatically be calculated.

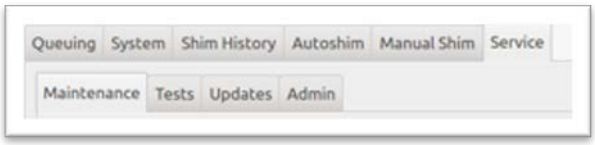




6.4.7 Service Tab

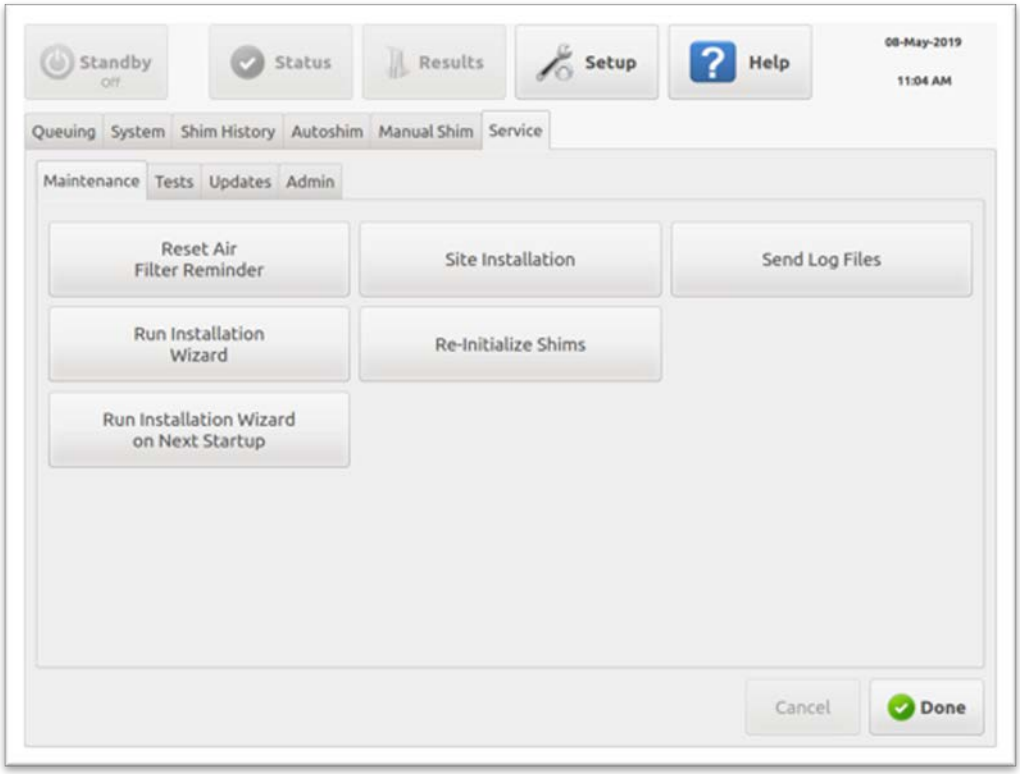
The *Service* tab contains very powerful operations that govern the configuration of your benchtop NMR spectrometer and is primarily intended for use by Nanalysis service personnel. It can be restricted by a

separate PIN access than the **Setup** panel to prevent unauthorized users from accessing this screen. There are four subtabs (1) *Maintenance*; (2) *Tests*; (3) *Updates*; and (4) *Admin*.



6.4.7.1 Maintenance

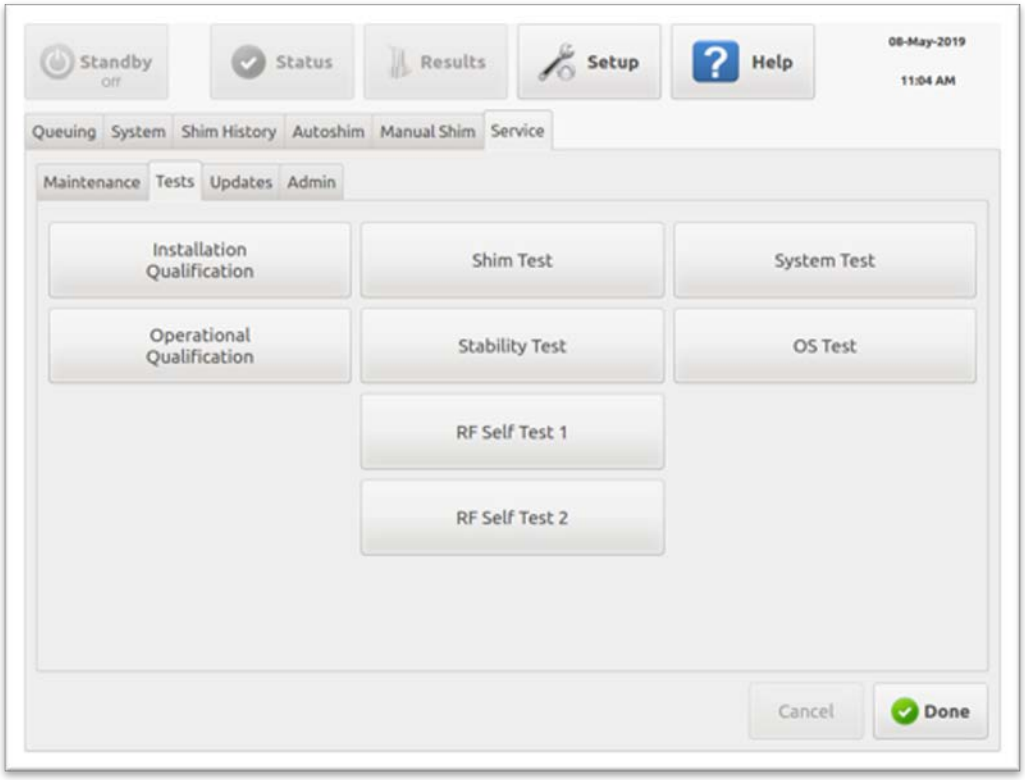
This sub-tab allows the user to perform advanced shimming protocols or air filter maintenance on the instrument. The most commonly accessed will involve the air filter and shimming routines. The **Reset Air Filter Reminder** will be discussed more extensively (section 9.2). The **Site Installation** and **Re-Initialize Shims** can help an instrument shim to a new location, either upon shipping or moving it to a new location to help it reach optimal performance. For more information about shimming see section 8.0 or talk to your Nanalysis Customer Service representative. They may instruct you to **Send Log Files**.



Run Installation Wizard and **Run Installation Wizard on Next Startup** can be useful to get an instrument acclimatized to a new site. Pressing the Run Installation Wizard will bring up

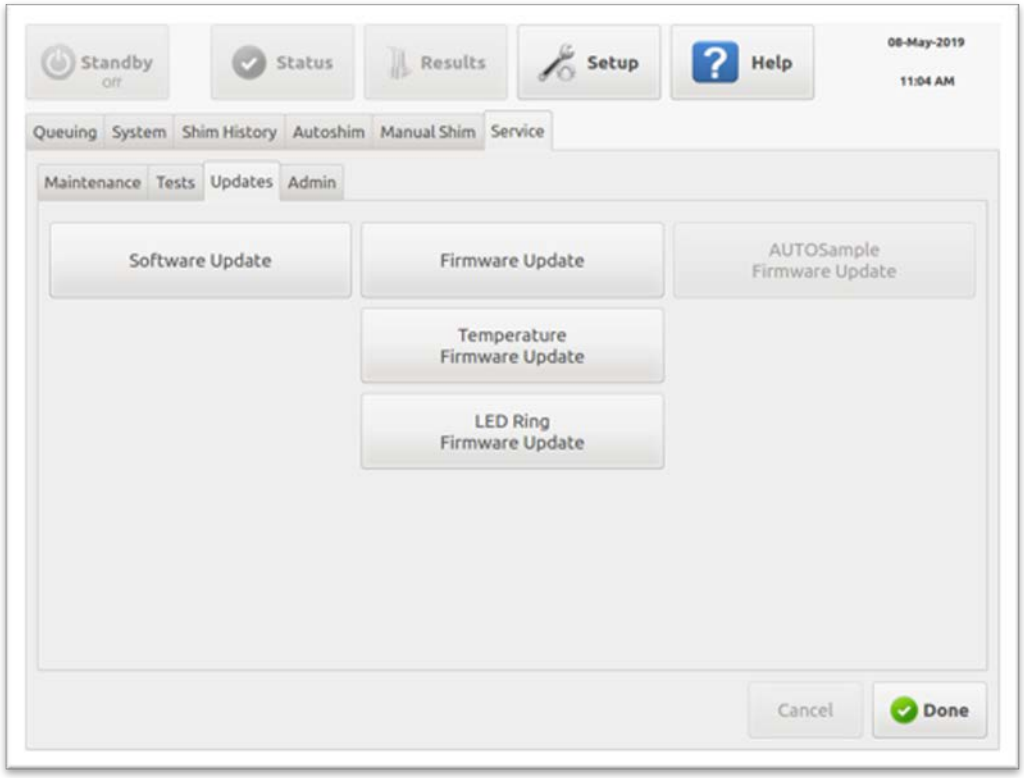
6.4.7.2 Tests

The *Tests* tab is meant primarily as a diagnostic tool for Nanalysis Customer Service representatives to assess the radiofrequency system, the instrument, and temperature performance over time. This allows them to perform diagnostic testing using the **Shim Test**, **Stability Test**, **RF Self Test 1**, **RF Self Test 2**, **System Test** and **OS Test**. These tests have also been optimized for routine customer use in form of the **Installation Qualification** and **Operation Qualification** (see section 7.2).



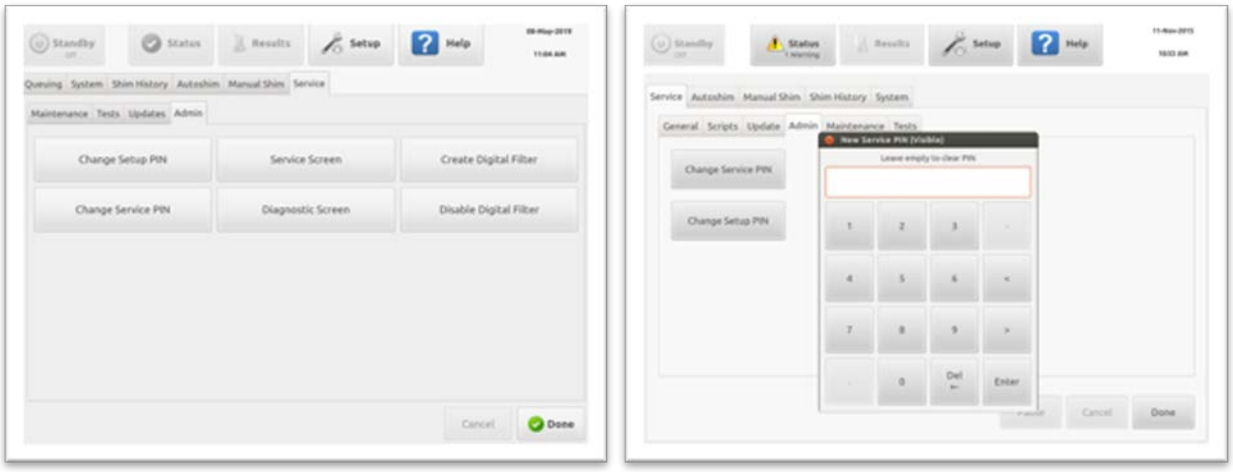
6.4.7.3 Updates

The *Updates* tab is meant to simplify software and firmware updates. Nanalysis will notify you when it is necessary to update the software. It can be done either through the Ethernet network connection, or by putting the updated files on a USB key. When a software update is selected, the software will automatically detect any versions that are higher than that being run on the spectrometer.



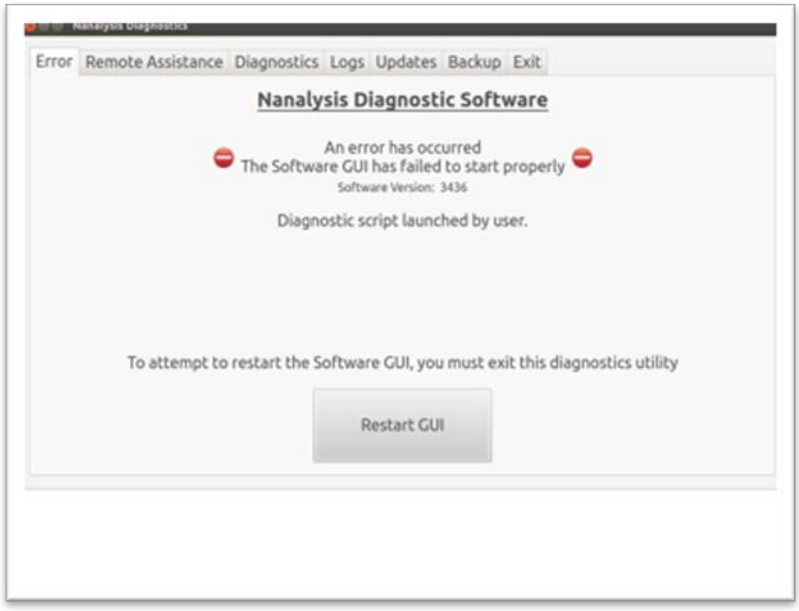
6.4.7.4 *Admin*

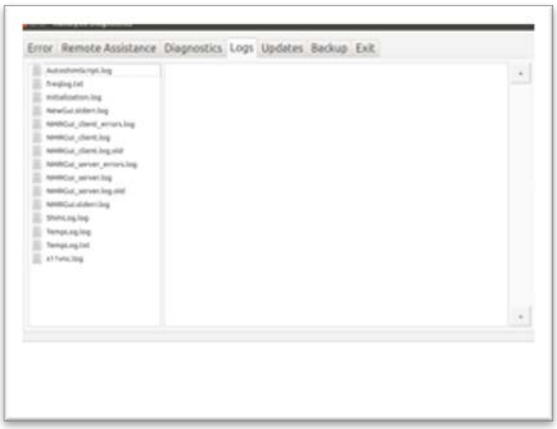
The *Admin* tab allows the user to password protect or change a pre-existing PIN for the **Setup** panel or *Service* tab. You can select the desired window to PIN protect and a keyboard will pop-up. This can be changed to whatever number the local administrator chooses or can be left blank to remove the PIN protection.



Additionally, one can access the **Service Screen** or alter **Digital Filters** but these are not recommended without guidance from a Customer Service Representative. Of note is the **Diagnostic Utility**. This utility is used for troubleshooting, backing up files, updating the instrument software and uploading

logs to Nanalysis. If selected, or if the instrument detects an error, the utility will be launched automatically, and the glow ring will turn red and enter the diagnostic screen.





These screens act as a backup in case the user cannot access the main software interface or needs to replace the computer or flash card. Using this screen, a trained operator can easily backup and restore data and the OS.

6.5 Help

The Help Panel is present in virtually every window of the instrument's interface. It can be used to offer assistance for proper operation of your spectrometer, as well as for diagnosis and fixing issues that may arise during routine use. Once this button is selected, the operator will note that there are two tabs: The *User Manual* and the *Customer Service Agent*.

User Manual is broken into two features: **Contents** and **FAQs**. The contents section is a content-specific inline user's guide. In other words, the information contained on this guide depends on the screen the operator is viewing when they select **Help** from the task bar. This is meant to help you diagnose problems and explain what the parameters mean to simplify use of the spectrometer.

The FAQ's section is a compilation of frequently asked questions to help get new users acclimatized to the benchtop spectrometer.

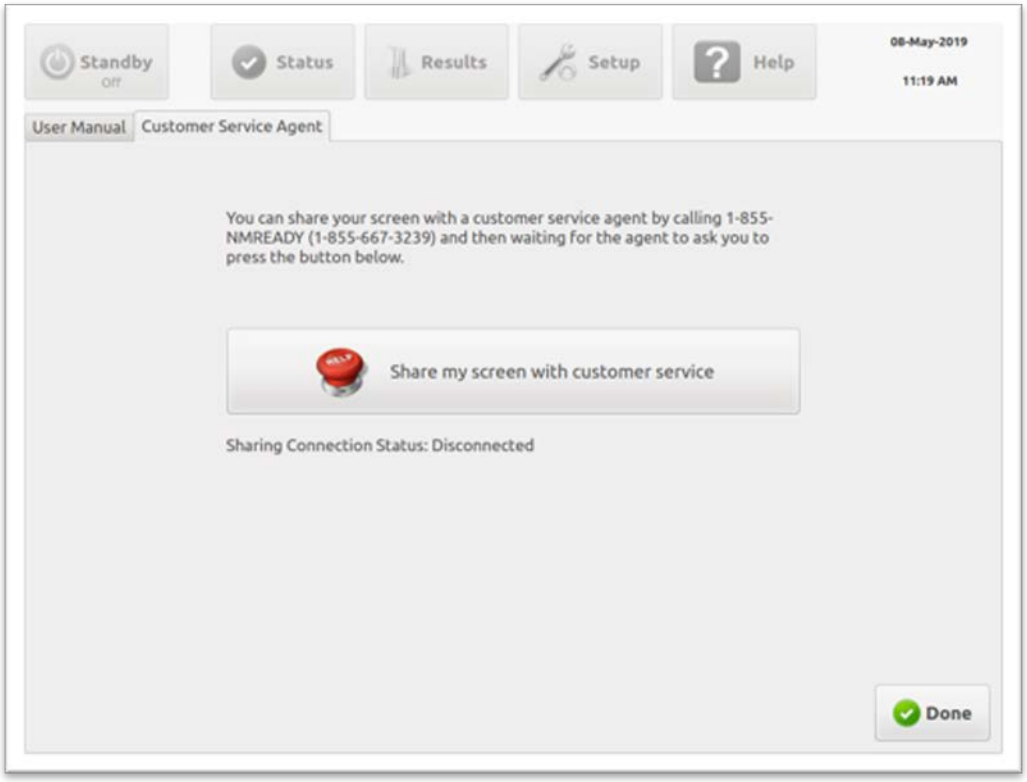
6.5.1 Customer Service Agent

If the instrument is connected to a network (section 2.3.4) as is highly recommended, a customer service agent can be requested. By selecting this button, the operator is giving access and control of the spectrometer directly to Nanalysis. This is useful for a speedy resolution of any typical software related issues, and if necessary, can serve as a basic training tool.

To effectively use this screen:

- Contact Nanalysis via phone at 1-855-NMREADY (1-855-667-3239) toll-free in the US and Canada or email service@nanalysis.com and request customer service.
- When instructed by Nanalysis, press **Share my screen with customer service**. This will provide Nanalysis with access to your NMReady. This icon will be present in the top right-hand corner of the screen.

For those concerned about privacy, please note that Nanalysis cannot access your spectrometer unless **Share my screen with customer service** is activated. The **Stop sharing** option will remain in the top right-hand corner of the screen for the entire duration in the spectrometer is connected to the Nanalysis service network. To terminate the connection at any time, simply press **Stop sharing**.



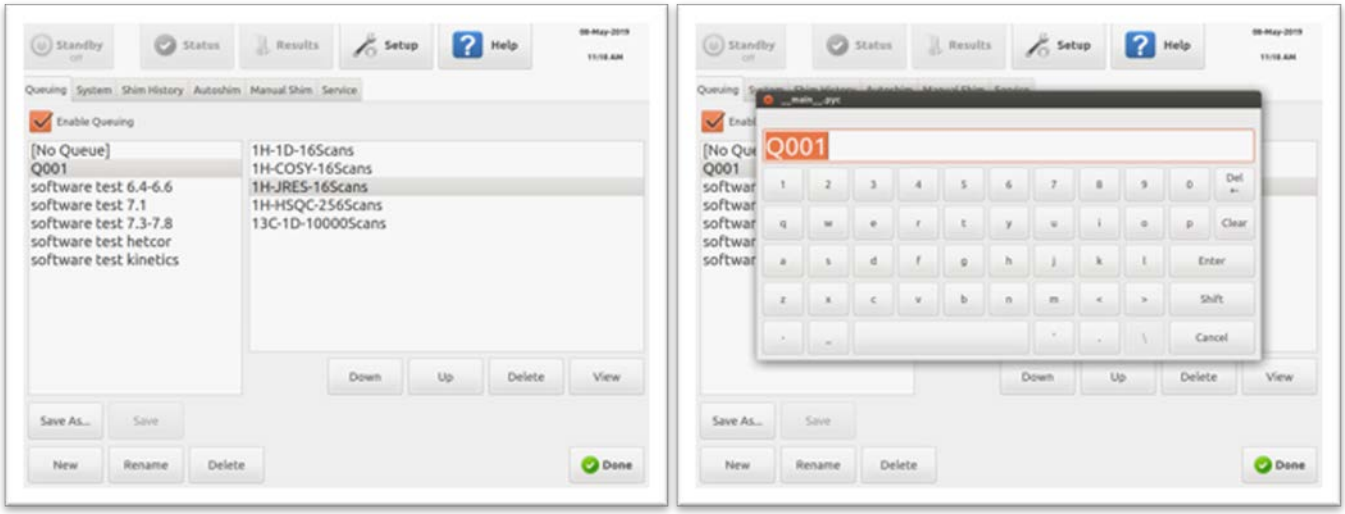
7.0 Optional Software Packages

7.1 Queuing Experiments

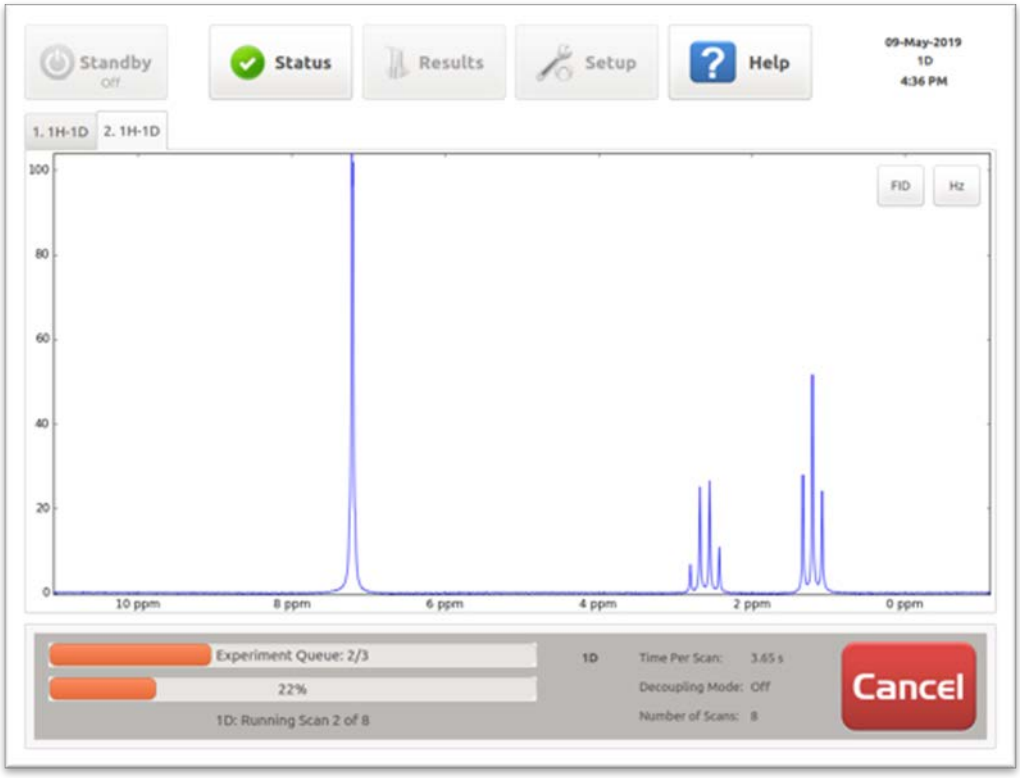
If Queuing is enabled on a spectrometer an orange '**+**' will appear in the right-hand corner of the Go button. To set-up a queue:

1. Select the desired **Observe Nucleus** (choices depend on which model you have) **Lock Nucleus** (^{1}H or ^{2}H lock), **Solvent**, and **Experiment** on the front screen.
2. Select the **Experiment Settings** if you wish to modify the acquisition parameters (*e.g.*, sweep width, relaxation delay, number of scans etc.).
3. After the desired experiment is configured, press **+**. This adds the experiment to the queue. This is visualized by text on the **Go** button that say how many experiments are currently in the queue.
4. Repeat steps 1-3 to add desired experiments
5. To view or modify this queue select **Setup** and the *Queuing* tab.
6. After all experiments are added to the queue, press **Go** to start the experiment queue.

For the example queue, the default name is Q001, and proton detected 1D, COSY, JRES and HSQC experiments have been configured in addition to a ^{13}C{^{1}H} experiment. To rename the queue, select the default name, and select **Rename**. Additionally, the order of the experiments can be modified by moving them **Down** or **Up** in the queue or removing them. By selecting **View**, the user can see the experimental settings for each experiment before or after running the queue.



Now when '**Go**' is selected from the main screen, the experiment queues will start to run, and as each experiment completes and the next one starts a new tab will be created in the acquisition and processing window so one can view their experiments as they are completed. Data saved from this window will be entered into the **Results** window in a folder under the queue name. By selecting the arrow on the left-hand side of the name, you can expand and access each spectrum.

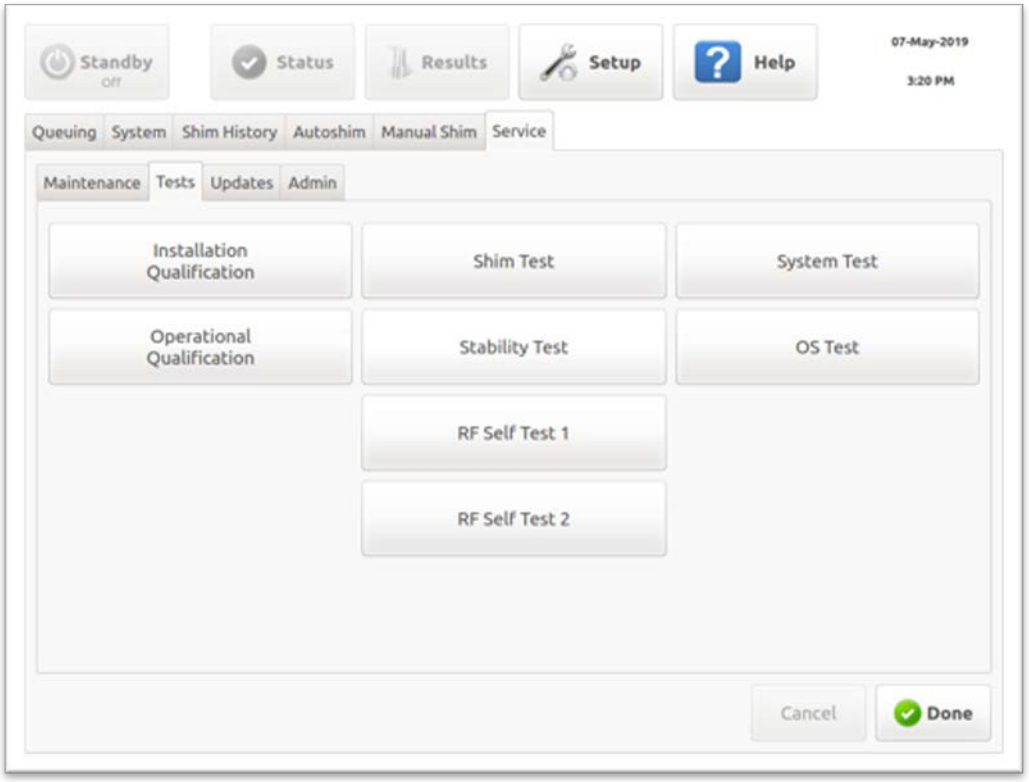


7.2 Installation Qualification and Operational Qualification (IQ/OQ)

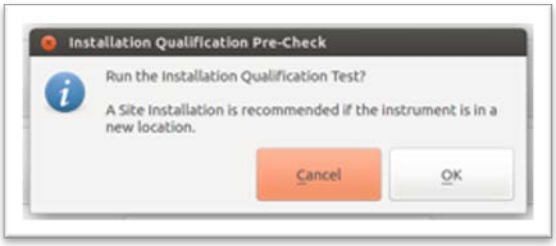
Adherence to regulatory compliance is critical in many workplaces, especially with contract research and manufacturing organizations (CRO and CMO). Nanalysis offers an optional software package for Installation Qualification (IQ) and Operational Qualification (OQ). These packages test the instrument's performance on initial siting, as well as in routine workflow to help validate result integrity over time. This automated procedure is meant to increase efficiency, minimize downtime while still providing users with a detailed report of their instrument's performance.

To perform these procedures, press the **Setup** button and select the *Service* tab. This will provide access to the *Tests* tab. The *Tests* tab offers a number of experiments that can be performed to assess the instruments health, and if necessary, are often used for diagnostic purposes by our customer service.

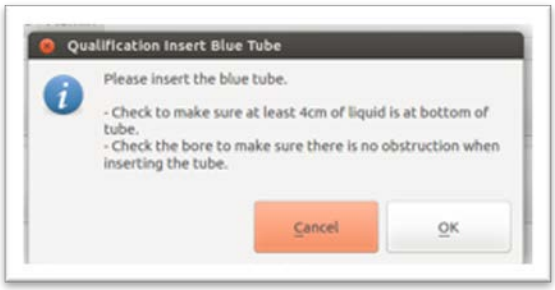
The **Installation Qualification** performs a series of tests to assess the instrument's health and performance to ensure that it has been installed or moved to a new location successful without compromising the performance of the instrument.



Once the **Installation Qualification** button is pressed a pop-up appear, prompting the user to confirm that they intent to run this procedure. It will take approximately 40 minutes.



If **OK** is chosen, another pop-up will appear prompting the user to ensure that the reference sample is intact, there is sufficient liquid to perform the tests and that the tube is properly inserted into the probe. Once this is manually completed by the user, and **OK** is selected and the procedure will begin to immediately perform the series of tests.



The IQ procedure performs the series of tests detailed in the table below.

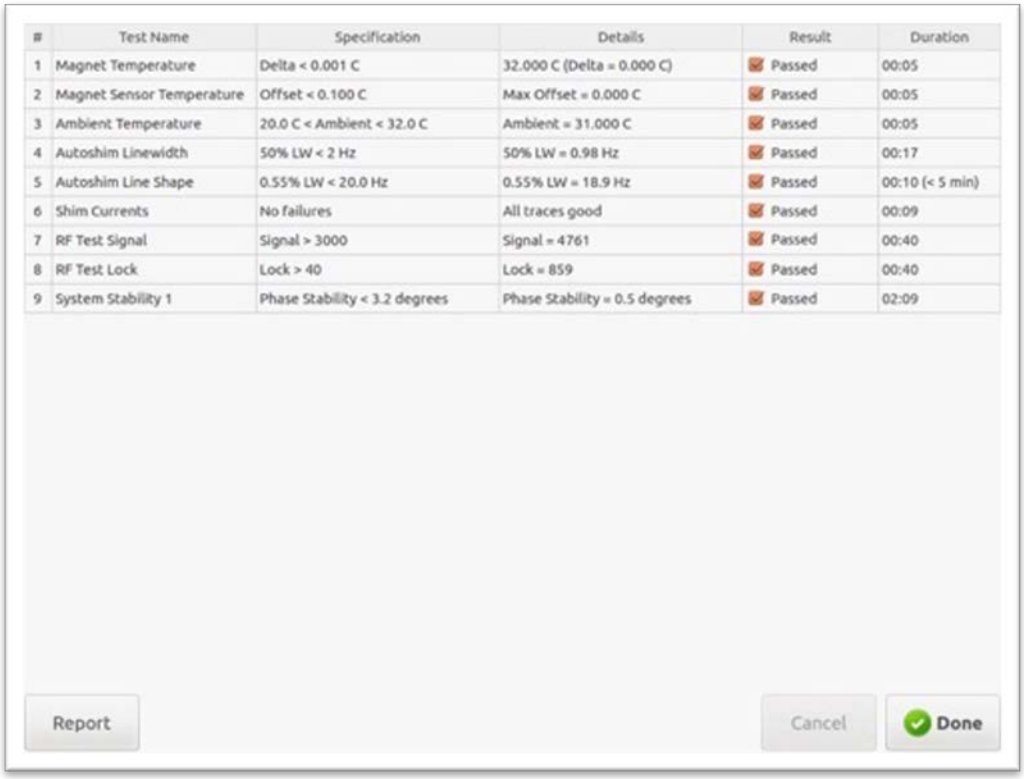
Test Name	Brief Description
Magnet Temperature	Checks the current magnet temperature to ensure it is being held consistently at set point.
Magnet Sensor Temperature	Checks each internal magnet temperature setpoint to ensure that each sensor is providing accurate reads relative to their set point temperature.
Magnet Frequency	Compares the current magnet frequency to the factory frequency to assess magnetic field strength and determine if there is any deterioration over time.
Ambient Temperature	Ensures that the instrument has been placed in an environment as specified for optimal data collection.
Autoshim Linewidth	Ensures that the 50% linewidth is within specification after a quick shim.
Autoshim Line Shape	Ensures that the 0.55% linewidth is within specification after the quick shim.
Shim Currents	Ensures that the shim panels are working properly, and current is being passed through each trace properly and within specification.
RF Test Signal	Determines that the SNR of the observe channel to ensure that the radio frequency boards, transmit and receive are functioning properly.
RF Test Lock	Determines that the SNR of the lock channel to ensure that the radio frequency boards, transmit and receive are functioning properly.
System Stability	Assess that signal has phase and amplitude stability over a specified window.
Network Test LAN	If there is no network connection this test is skipped, but it checks for IP address as well as ensures that the necessary ports are open.

As each test is completed, it will receive a pass or fail based upon the specified parameters. Example progress parameters are shown below.

Once completed, the **Report** button in the bottom left hand corner of the page will become activated. When pressed, it allows the user to see a report, instrument name and a time stamp of when the procedure was performed.

The Operational Qualification (OQ) works similarly and can be run as often is desired by local administrators. It provides a series of tests similar to the IQ, but in a more abbreviated form. When the **Operational Qualification** button is selected, the user is promoted to ensure that the blue reference sample is in good repair, and properly inserted into the bore.

Once the User selects **OK**, the instrument rapidly begins a series of tests as specified in IQ, excluding the magnet frequency test and the network connection and also performing a shortened stability test, such that the procedure takes approximately 5 minutes to complete. When completed the **Report** button becomes active and a report can be generated.



#	Test Name	Specification	Details	Result	Duration
1	Magnet Temperature	Delta < 0.001 C	32.000 C (Delta = 0.000 C)	Passed	00:05
2	Magnet Sensor Temperature	Offset < 0.100 C	Max Offset = 0.000 C	Passed	00:05
3	Ambient Temperature	20.0 C < Ambient < 32.0 C	Ambient = 31.000 C	Passed	00:05
4	Autoshim Linewidth	50% LW < 2 Hz	50% LW = 0.98 Hz	Passed	00:17
5	Autoshim Line Shape	0.55% LW < 20.0 Hz	0.55% LW = 18.9 Hz	Passed	00:10 (< 5 min)
6	Shim Currents	No failures	All traces good	Passed	00:09
7	RF Test Signal	Signal > 3000	Signal = 4761	Passed	00:40
8	RF Test Lock	Lock > 40	Lock = 859	Passed	00:40
9	System Stability 1	Phase Stability < 3.2 degrees	Phase Stability = 0.5 degrees	Passed	02:09

7.3 Experiment Designer

For more information on the experiment designer and customizing your own pulse programs, please contact your Nanalysis customer service representative.

8.0 Shimming on the NMReady

8.1 Solvent specific reference samples

Instead of shimming on every sample, Nanalysis provides specially prepared solvent-specific reference samples, which aid in the high throughput during normal operation. These samples include a solute whose signal is well characterized and suitable for efficient shimming. The reference sample is used to calibrate the instrument in a timely fashion. Depending on your chosen nuclide configuration, typically the instrument will be shipped with a blue reference sample – this is H_2O in D_2O. It is used to lock and provide evidence of B_0 homogeneity and instrument performance.

The instrument is preset with basic shimming protocols (*e.g.*, quick, medium and full, see section 6.2.8 and more advanced auto-shim (section 6.4.4) and manual shim (section 6.4.5) options.

8.2 Saving Shims

The system shims are saved after each medium and full auto-shim whether performed manually or through the Standby shim functions.

8.3 Standby Shimming

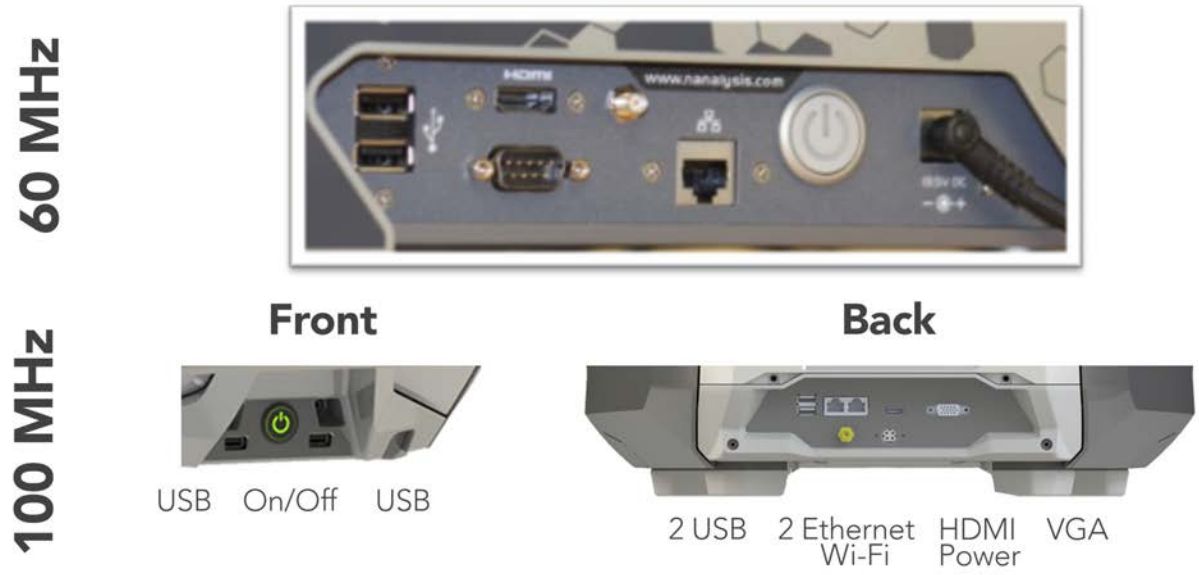
Your Nanalysis' benchtop NMR has a standby shimming protocol that can be used to maintain optimal line shape through times when the instrument is not being used. The **Standby** Shimming button is in the upper left-hand corner of the main screen (). Pushing the button will prompt the user to ensure that the appropriate solvent reference standard is in the instrument. It is vital that the correct shim standard be placed in the instrument. Auto-shimming on another reference standard (or on a non-standard sample) when a particular sample is requested by the instrument can result in degradation of performance, including poor line widths and severely poorly calibrated chemical shift values. This protocol simply follows the recommended intervals for each shim (*i.e.*, quick every 3–4 hours, medium every 8–10 hours, full every 12 hours). When **Standby** is engaged, the button will read **On** instead of **Off** as is shown above.

Suggested times for using **Standby** shimming are lunchtime, overnight, weekends, during holidays and through work periods when the NMReady will not be required for an extended period of time. Once standby shim is engaged it can be exited at any time by simply pushing the 'stand-by' button a second time.

While this is engaged a "quartet" shimming graphic rolls across the main screen. When the light ring is green, the instrument is ready for use, whereas when it is blue a shim is in progress. It is recommended that you wait until the ring is green before taking it out of standby to ensure that the instrument is properly shimmed and ready for use.

8.4 Hardware Description

The benchtop NMR spectrometer is made up of a temperature controlled permanent magnet, an electronic shimming system, a fully digital radio-frequency transmitter/receiver subsystem, digital data acquisition and signal processing, a suite of pre-programmed pulse sequences, a touchscreen interface, a light ring, and ports for USB, Ethernet and VGA/HDMI connections. The basic connectivity is shown below.



8.4.1 Magnet

The external magnetic field is generated from precisely engineered and patented neodymium-iron-boron (NdFeB) based permanent magnets fitted into a proprietary Halbach-type array. The resultant spectrometer is 60 MHz, which equates to a 1.4 T magnetic field, or 100 MHz, which equates to a 2.35 T magnetic field.

8.4.2 RF and Digital Data Acquisition Electronics

The instrument uses state of the art digital electronics and proprietary designs and methods to achieve the most compact, all-in-one compact NMR spectrometer in its class.

8.4.3 Lock System

The NMReady uses a frequency-agile deuterium lock system. There are other options available, please contact your Nanalysis service provider for more information.

8.4.4 Shim System

Nanalysis' benchtop NMR spectrometers use a patented electronic shim system that shims the field to fourth order in spatial coordinates.

8.4.5 Progress Ring

This has been introduced to the instrument so the user has an easy visual identification of the progress of an experiment and the status of the instrument. The process ring can be disabled in the **Setup** ➔ *System* ➔ *General* tab by selecting the LED Ring toggle so **Off** is displayed.

The colour blue indicates that the instrument is still in use, whether that means it is shimming or collecting data. A green light ring means that it is ready for use. A red-light ring means there was an error and the spectrometer requires some diagnostic maintenance.

8.5 Specifications, Recommendations and Guidelines

60 MHz Specifications

Frequency	60 MHz	**Resolution**	50%: <1.0 Hz,
Magnet	Permanent, cryogen free	**Power**	100-240 VAC, 50-60 Hz
Stray field	<2G outside enclosure	**Weight**	55 lbs/25 kg
Nuclei	Dual mode: ^{1}H, ^{7}Li, ^{11}B, ^{13}C,^{19}F, ^{31}P Inquire about others.	**Dimensions**	11.8 x 11.0 x 19.2″ 30 x 28 x 49 cm
Lock	^{1}H or ^{2}H	**User Interface**	Built-in Touchscreen
Sample	Standard 5 mm NMR tubes	**File Format**	JCAMP-DX,
Sensitivity	100:1 1% Ethylbenzene, single scan	**API**	Microsoft .NET & JSON

Recommendations and Guidelines

Operating Temperature	18-26°C	**Additional Processing Software**	MNova or ACD/Labs
Temperature Fluctuations	+/- 1.5 °C per hour	**Power**	Dedicated outlet
Location	Avoid vibrations and moving motors		

100 MHz Specifications

Frequency	100 MHz	**Resolution**	50%: <1.0 Hz,
Magnet	Permanent, cryogen free	**Power**	100-240 VAC, 50-60 Hz
Stray field	<2G outside enclosure	**Weight**	240 lbs/108 kg
Nuclei	Dual mode: ^{1}H, ^{13}C,^{19}F, ^{31}P Inquire about others.	**Dimensions**	14.6 x 16.3 x 25.8″ 37.1 x 41.4 x 65.4 cm
Lock	^{1}H or ^{2}H	**User Interface**	Built-in, ergonomic Touchscreen
Sample	Standard 5 mm NMR tubes	**File Format**	JCAMP-DX,
Sensitivity	220:1 1% Ethylbenzene, single scan	**API**	Microsoft .NET & JSON

Recommendations and Guidelines

Operating Temperature	18-26°C	**Additional Processing Software**	MNova or ACD/Labs
Temperature Fluctuations	+/- 1.5 °C per hour	**Power**	Dedicated outlet
Location	Avoid vibrations and moving motors		

9.0 Maintenance and Troubleshooting

9.1 Cleaning

With normal operation of the spectrometer, there is no routine cleaning is required. However, if a tube is broken or tube contents are spilled, then it's recommended that all broken tube parts be removed, and the spill properly cleaned before continuing to operate the spectrometer. Note that a broken NMR tube can be sharp and spilled contents can contain dangerous materials. Please exercise caution when cleaning spilled material and use appropriate tools and/or personal protection equipment. Appropriate decontamination should be used if any hazardous material is spilled from the tubes. It's recommended to reference the "Laboratory Biosafety Manual" for more information on decontaminants, published by the World Health Organization.

9.1.1 Removing a broken NMR tube in 60 MHz benchtop NMR

If a tube is broken in the 60 MHz benchtop NMR spectrometer, there are measures in place to simplify this procedure. Please note that it should not be removed by grasping the broken end and extracting it:

1) Unscrew the NMR tube guide. This may expose a sufficient length of tube that is intact and can be used for tube extraction. In this event, grasp the tube end (with tweezers if too jagged to use your hand) and pull up carefully in a linear motion to remove the tube completely.

2) If a sufficient length of the tube is not exposed for top site extraction, then a tube removal tool can be used. In this case, power down the NMReady. Carefully, using two people if necessary, tip the instrument onto its back to expose the bottom. Snap out the black plug to expose the hole. Use a sharp small knife to cut a slit in the plastic sheet and the insulating layer. Carefully insert the removal tool (see images below) and push the tube out the top, taking care to minimize solvent spillage inside the magnet.

3) If the bottom of the tube has shattered and the inside of the magnet has been covered with the sample, turn off the CPU using the button on the right-hand side and flush the instrument with minimal amounts of *iso*-propanol. You can also use a long cotton tip to clean the probe from the top.

Please note that the broken NMR tube can be sharp and could hold remnants of dangerous materials. Please exercise caution when removing broken NMR tubes by using appropriate tools and/or personal protection equipment.

9.2 Changing the Air Filter in 60 MHz

The air filter can be changed in 3 easy steps.

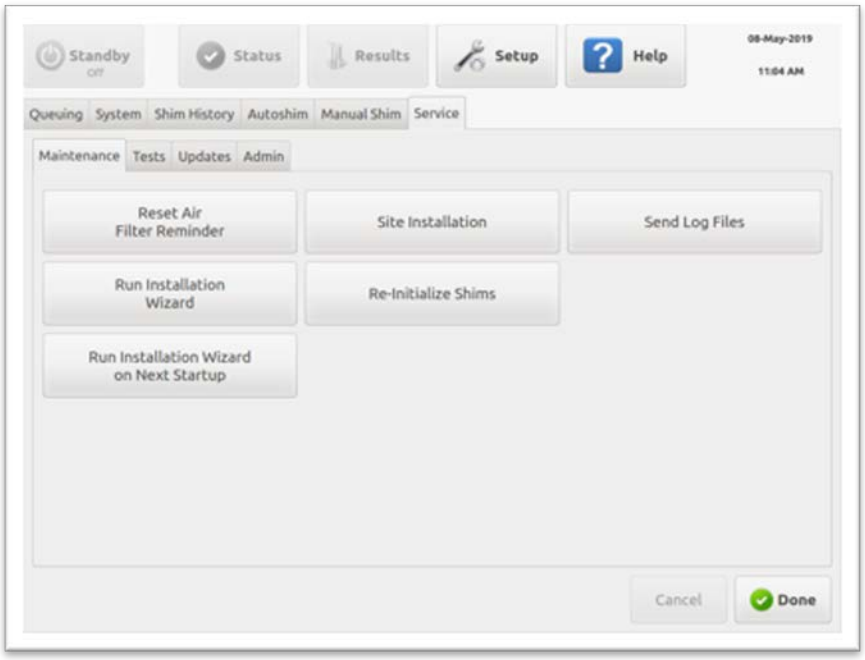
1) Remove the air filter, preferably with the instrument turned off but at least with no tube inserted and no experiments/shimming active. Carefully tilt the instrument back to expose the air filter between the two front feet. Take a small flat head screwdriver and very carefully pry off the two plastic tabs. This should be done with 2 people to ensure the instrument does not fall and the plastic tabs are not lost.

2) Clean the air filter by removing any captured dust. This can be done by rinsing with water and then drying in air and/or by scraping with a fingernail.

3) Replace the air filter. With one person holding the instrument, have the second person secure the filter by pushing the plastic tabs back into their holes.

4) Reset the air filter reminder by pushing the **Reset Air Filter Reminder** button, which is found

on the *Maintenance* tab under **Setup** ➔ *Service*.



9.3 Service

Service of Nanalysis benchtop spectrometers should only be performed by Nanalysis Corp. service technicians. Nanalysis Corp. is not responsible for damage and/or injury caused by misuse of the products covered in this manual, including improper handling, unauthorized access/modification of the instrument, and use in violation of any laws. More information on service packages can be obtained from your local customer service or salesperson.

10.0 AUTOsample-60

10.1 Introduction

The AUTOsample-60 is designed as an accessory to the NMReady-60 family of benchtop spectrometers, to improve workflow and allow for automated sample tube changing. The AUTOsample-60 can help automate NMR spectrometer experiment workflow when more than one sample is used. The AUTOsample-60 has the following features:

- ☑ interchangeable 25 tube capacity carousel;
- ☑ temperature controlled samples;
- ☑ easy connection to the NMReady software

10.2 Operation

The AUTOsample-60 uses 3 motorized stages to manipulate a sample carousel, sample gripper, and z-axis stage. The combined operation of these stages allows the unit to automatically move tubes between the carousel, integrated sample warmer, and NMReady spectrometer. This allows the instrument to be fully utilized and automatically run serial experiments

10.3 Using this Manual

This document is a resource to facilitate the incorporation of the AUTOsample-60 into routine laboratory use. This manual contains instructions for unpacking, installing, and using the AUTOsample-60.

10.4 What's in the Box?

Each AUTOsample-60 package includes:

- ☑ 1 AUTOsample-60 assembly
- ☑ 1 power cable
- ☑ 2 sample carousels
- ☑ 1 USB cable

10.5 Unpacking the AUTOsample-60

While the AUTOsample-60 is mechanically robust, it is a precision instrument and must be handled accordingly. To ensure that these systems are not damaged upon shipment, the AUTOsample-60 is transported in a pre-formed foam packing material.

When opening the box, you will find 4 smaller white boxes. These contain:

- 2 carousels
- 1 USB cable
- 1 24V external power supply

Removing the middle foam insert will expose the AUTOsample-60, which can be removed by grasping the unit from its sides.

10.6 Installing the AUTOsample-60

To facilitate optimum performance of the AUTOsample-60, please follow the guidelines detailed below. If you have additional questions, please do not hesitate to contact our customer service for more guidance (phone: 1-855-NMREADY or email: service@nanalysis.com).

10.6.1 Prepare the NMeady-60

The NMReady-60 should first be installed and operating as per the NMReady-60 Quick Start Guide. The spectrometer should be placed in the location where it will be used before mounting the AUTOsample-60. The Installation Wizard should be run before mounting the AUTOsample-60.

10.6.2 Mounting the AUTOsample-60

To install the AUTOsample-60 onto your NMReady, first remove the sample tube from the NMReady, grasp the AUTOsample-60 by its sides, and place it onto the round top cover of the NMReady-60. The top cover of the NMReady-60 mates to the bottom of the AUTOsample-60 and locates the two instrument pieces together. Place the AUTOsample-60 so that the Nanalysis logo and carousel holder are facing towards the front. It is recommended to allow the NMReady-60 to equilibrate with the AUTOsample-60 for 1 hour before use.

10.6.3 Power and USB connections

Once the AUTOsample-60 is mounted onto the NMReady, the cable connections should be made. When facing the spectrometer, the power panel can be found on the back of the autosampler with your right hand. First plug the 24V power adapter into an AC supply, then into the rear barrel connection of the AUTOsample-60 and then the supplied USB cable from AUTOsample-60 to the USB directly into a USB port of the NMReady-60. It is ***not*** recommended to use a USB hub for the AUTOsample-60 USB cable.

AUTOsample USB

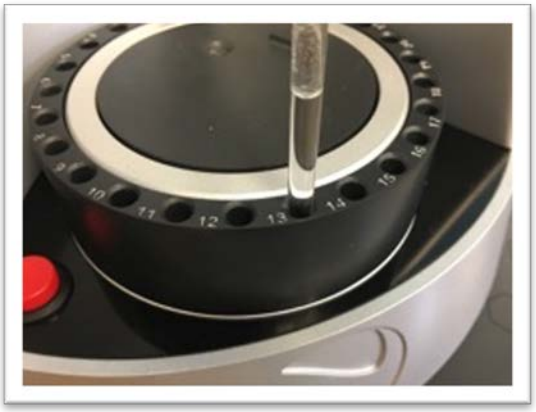
10.6.4 Moving the AUTOsample-60

If you choose to move the NMReady, it is recommended that the autosampler be removed prior to relocation. Unplug the AUTOsample-60, remove the carousel, then remove the AUTOsample-60 before moving the NMReady-60.

10.7 Using the AUTOsample-60

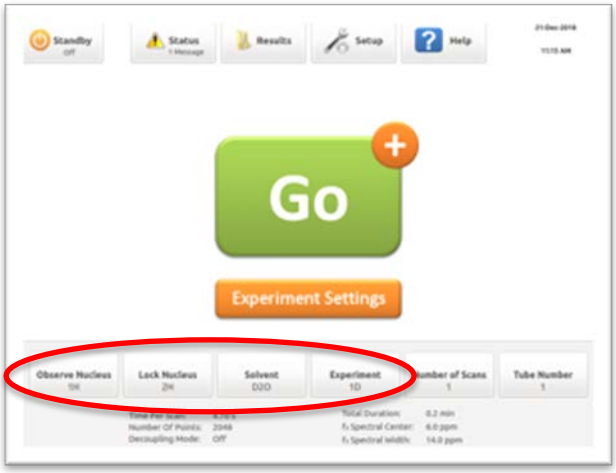
Once the AUTOsample-60 is mounted and power and USB cables are connected, the power switch at the rear of the AUTOsample-60 must be turned to the ON ("**I**") position. The NMReady software will automatically detect the presence of the AUTOsample-60 and use it to change to the desired tube.

Note that the NMReady-60 must have the AUTOsample-60 feature enabled in its license. When the AUTOsample-60 feature is enabled on the NMReady, the front screen will have a button for "**Tube Number**". The AUTOsample-60 will use this tube number position in the carousel for the selected experiment.

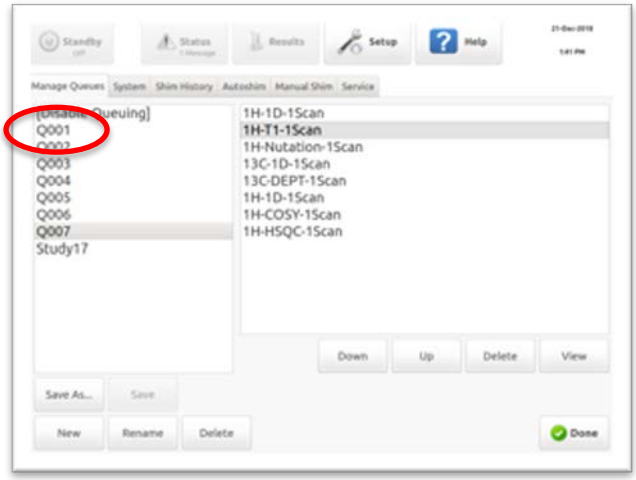


To queue experiments using different tubes, the procedure is similar to queuing (section 7.1), but the user must also specify the **Tube Number**:

1. Select the desired **Observe Nucleus** (choices depend on which NMReady-60 model you have) **Lock Nucleus** (^{1}H or ^{2}H lock), **Solvent**, and **Experiment** on the front screen
2. Select the **Tube Number** for which the experiment should be run.
3. Select the **Experiment Settings** if you wish to modify the acquisition parameters (*e.g.*, sweep width, relaxation delay, number of scans etc.).
4. Press **+** to add the experiment to the queue.
5. Repeat steps 1-4 to add experiments to the same tube or different tubes.
6. After all experiments are added to the queue, press **Go** to start the experiment queue.



Experiment Queues can be edited in the *Queuing* tab of the **Setup** button located at the top of the screen. Queue names can be changed, and queued experiments can be manipulated in this screen. Queuing can also be disabled in this screen.



10.8 AUTOsample-60 workflow

The AUTOsample-60 has the ability to detect if tubes are present in 3 locations:

1. The spectrometer
2. Each carousel position
3. The AUTOsample-60 warmer

Before starring any experiment, the condition of the AUTOsample-60 and NMReady-60 must be the following:

- No tubes should be in the spectrometer
- Not tubes should be in the AUTOsample-60 warmer

- Tubes requested by experiments should be present in the carousel.

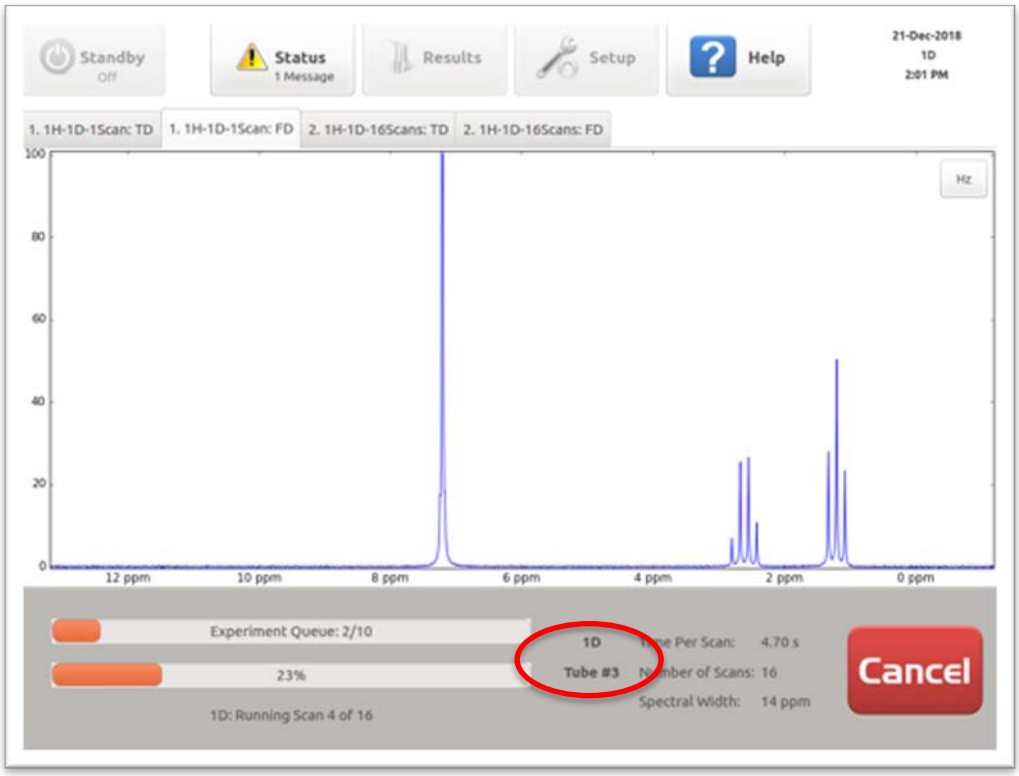
If any of these conditions are not true, the autosampler will detect this and will not perform the experiment.

To initiate a queue of experiments:

1. The **Go** button is pressed initiate the queue of experiments detailed above.
2. The AUTOsample-60 will take the first sample from the carousel and place it directly in the spectrometer.
3. If there is a subsequent experiment requested next in the experiment Queue, then the second tube will be taken from the carousel and placed into the AUTOsample-60 tube warmer
4. The NMReady-60 will perform the current experiment(s) on the first NMR sample
5. After the current experiment is completed, the sample is moved back to its original location in the carousel
6. If there is a subsequent experiment requested, the next tube is moved from the sample warmer into the spectrometer, and the workflow continues to step 2.

Do not add or remove tubes after starting the experiment or experiment queue. The system will detect what tubes are present in the carousel, and stop the experiment if tubes are not in their expected locations.

When running a queue of experiments, the experiment data is displayed in tabs and information about the current experiment and tube used is shown.



10.9 Standby Shimming

The NMReady-60 has a standby shimming protocol that can be used to maintain optimal line shape through times when the instrument is not being used. The **Standby** Shimming button is in the upper left-hand corner of the main screen (

). Refer to the NMReady-60 User Manual for more information about Standby Shimming.

When using the AUTOsample-60, Standby mode will confirm that the reference tube is placed in carousel position 25. Make sure that the correct reference standard is in this position.

10.10 Cancelling Experiments

Cancelling an experiment using the **Cancel** button in the user interface will cancel the current experiment and all experiments in the queue. This cancel takes place AFTER the current AUTOsample-60 motor operation completes.

10.11 Emergency Stop

The red emergency stop button should be used when the AUTOsample-60 is not operating correctly. Pressing the emergency stop will toggle the emergency stop button in the "down" position. This will immediately stop all motor function, cancel the experiment, and cancel the experiment queue. After the emergency stop button is pressed, a post-emergency procedure must be completed.

The post-emergency procedure confirms that all tubes are removed, so that the AUTOsample-60 software logic can reset the state of the instrument. The software will inform the user of the procedure, to ensure that the instrument is safe to use. All tubes must be removed from the spectrometer and AUTOsample-60 before using the AUTOsample-60 after an emergency stop.

To use the instrument after an emergency stop, the emergency stop button must toggled to the "up" position. This is accomplished by depressing the emergency stop button after it's in the "down" position.

10.12 Operation Guidelines

The following guidelines should be followed for proper operation of the AUTOsample-60:

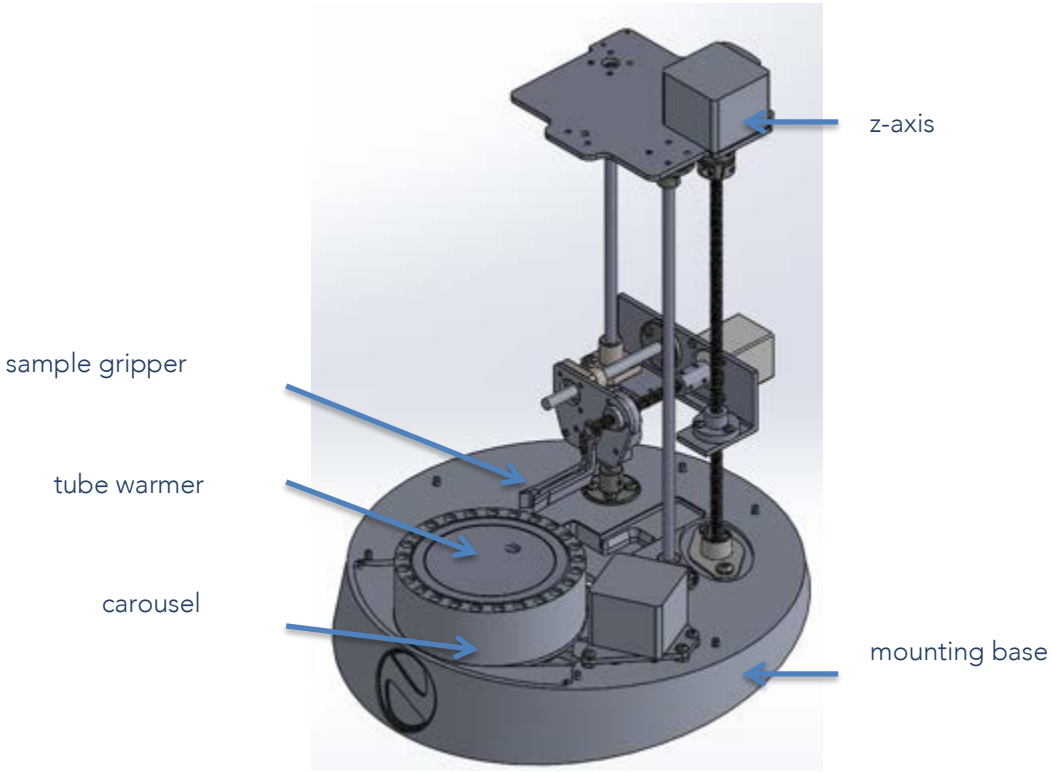
- ☑ Use 7" to 8" long standard, constricted, or J-Young NMR tubes
- ☑ Avoid using labels on the tubes
- ☑ Avoid unplugging the AUTOsample-60 USB cable while using the instrument

10.13 Hardware

10.13.1 Hardware Description

The AUTOsample-60 consists of the following hardware components:

- AUTOsample-60 mounting base
- Carousel, carousel holder, and carousel motor
- AUTOsample-60 warmer
- Sample gripper and motor
- Sample z-axis and motor



The motors have been factory calibrated and require no on-site calibration or maintenance.

10.13.2 Specifications

Power	24 V DC via power adapter	**Weight**	10 lbs / 4.5 kg
Connectivity	USB	**Height***	12.5 inches / 32 cm

*Above the top of the NMReady-60

11.0 Frequently Asked Questions

11.1 General Queries

11.1.1 Haven't benchtop NMR spectrometers been around for years?

Benchtop NMR *relaxometers* have been around for years, but high-resolution *spectrometers* for the benchtop are brand new. In a proton NMR spectrum of molecules in solution, it is very often desirable to resolve signal peaks that may only be separated by a few Hz (less than 1 ppm at an operating frequency of 60 or 100 MHz), and this is not possible with the magnets that are used in commercial low-resolution spectrometers and relaxometers.

11.1.2 Is the NMReady-60 spectrometer really a true benchtop NMR spectrometer?

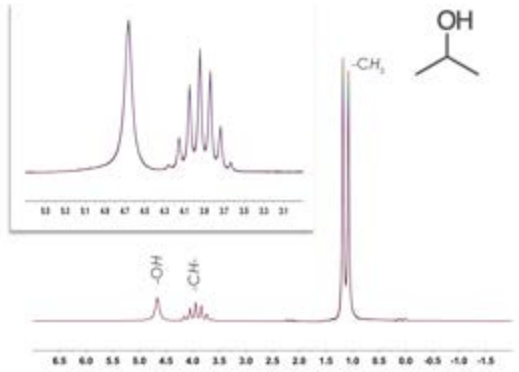
Yes. The instruments resolution is better than 23 ppb (parts per billion). That's less than 1.0 Hz at a 60 MHz operating frequency. This allows the benchtop NMR to resolve structural features of the sample at analysis as typified by typical three-bond scalar couplings ($^3J_{H\text{-}H}$) in organic molecules, an absolutely essential requirement for use in many applications.

11.1.3 How does it work?

The benchtop NMR is, for the most part, a conventional Fourier-transform NMR spectrometer. It contains all of the usual NMR spectrometer components, including a magnet, shim system, pulse sequencer, RF transmitter and coil, low-noise digital receiver, digital data acquisition, data processing and display system, and deuterium field lock. So, what is different exactly? It is just that everything is smaller and packed into a single enclosure. Instead of a superconducting magnet, we use a compact, ambient temperature permanent magnet. The digital systems signal generation, receiving, and processing are miniaturized, and we use a touchscreen computer for control and data display and management. All of the components are packaged in a single enclosure.

11.1.4 How stable is the NMReady-60? Can I use signal averaging to improve the signal-to-noise ratio?

The instrument uses a deuterium (or proton) internal lock to stabilize the transmitter position in the magnetic field. In addition, the magnet system is stabilized by a series of mechanical and electronic temperature controllers and through sophisticated software. The result of this coupled system is the ability to signal average for as long as is required. The below figure contains 24 overlaid spectra taken once an hour for 24 hours and superimposed.



11.1.5 Can I look at flowing Samples?

The basic design of the NMReady magnet is compatible with custom sample-flow systems! We offer a flow accessory, please inquire with your local Nanalysis representative about the NMReady-flow to use your NMReady as on online NMR detector.

11.1.6 Should I power down the spectrometer when I go home at night?

For best performance it is recommended to leave the spectrometer powered on at all times. Leaving the unit powered on keeps the NMR magnet at an optimal temperature, which is very important for its operation. If the unit is turned off overnight the magnet will cool down and possibly take hours the next day to heat up and shim to the target resolution / linewidth. We recommend leaving the instrument in standby shim mode.

11.1.7 What is the stray field of the instrument? Is it safe?

The technology used to engineer the magnet affords a strong magnet with a minimized external field. Regardless, there is a slight axial stray field (above and below the magnet) but the 5 Gauss line is completely contained within the sides

11.1.8 Does Nuclear Magnetic Resonance produce dangerous nuclear radiation?

No! The term nuclear refers to the involvement of atomic nuclei. There is NO relation to the radioactive decay of unstable nuclei (which can release high-energy subatomic particles and radiation). No radioactivity of any kind is produced or used during an NMR experiment.

11.1.9 What are in the solvent reference tubes?

At this time, we have chosen not to disclose the exact recipe of our shim standards. The blue shim standard contains H_2O in D_2O with some paramagnetic relaxation agent to facilitate faster shimming. Depending on the configuration of your spectrometer will come with additional coloured tubes (*e.g.*, $^{1}H/^{13}C$ will also contain a black reference tube). These additional tubes will help us diagnose instrument performance.

11.1.10 When do I use the calibrate solvent button?

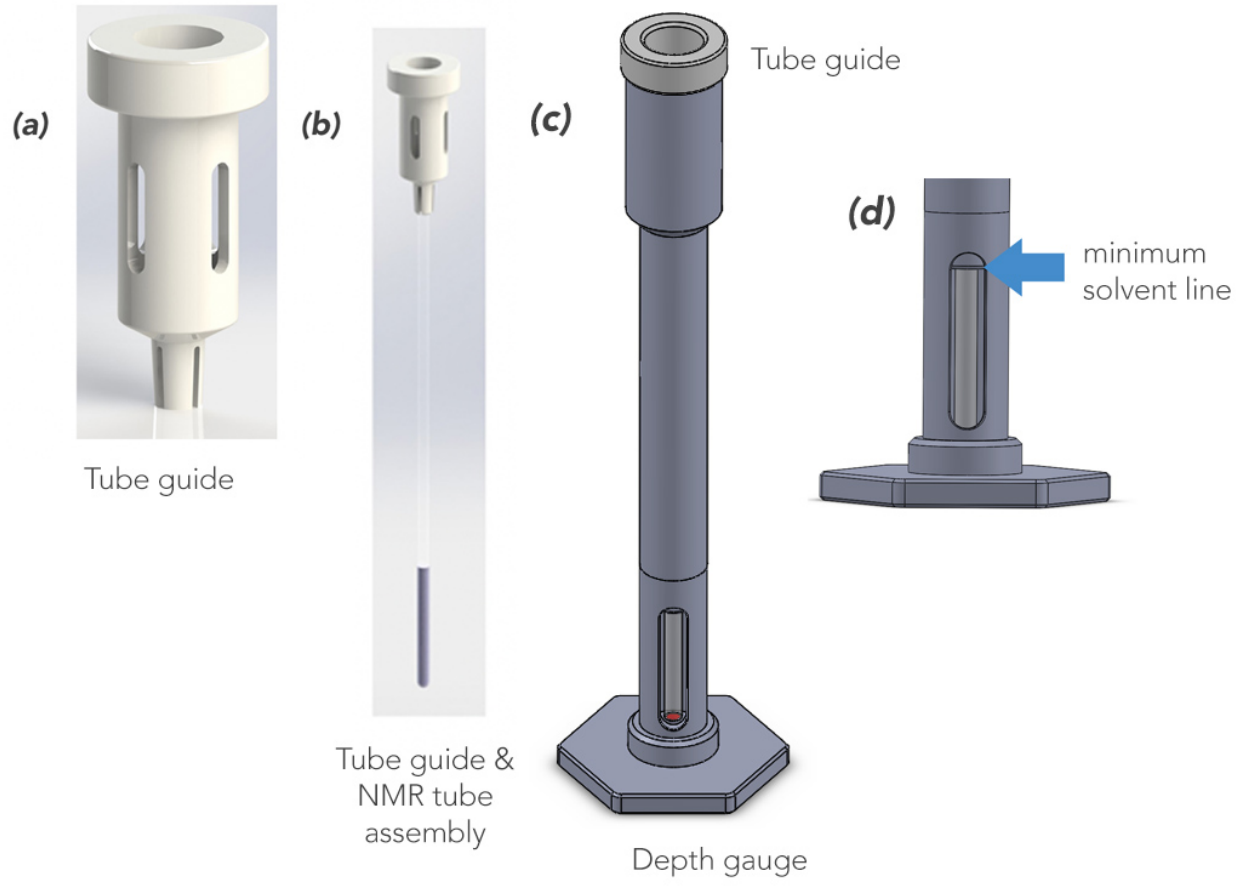
You should not need to use the **Calibrate Solvent** button located in the quick select solvent button unless you observe a lost lock warning. This very rarely is observed in routine use but may be required occasional with flow applications.

11.2 Sample Handling and Data Acquisition

11.2.1 How do I put a standard sample in the instrument?

The 60 MHz instrument uses standard 5 mm NMR tubes (3 mm tubes are also available upon request). There is no air, no sample spinners, and no additional sampling requirements, so the tube glides directly into the magnet bore until it is located in the coil, and then the operator will feel resistance.

The 100 MHz has a longer magnet, so to ensure the NMR tube is correctly located in the coil, it comes with a guide. Insert the NMR tube into the top of the tube guide (a) and pull the NMR tube down until you feel some resistance (b). Place the tube guide/NMR tube into the sample depth gauge carefully until the bottom of the tube. Push the NMR tube to the bottom of the depth gauge and ensure the tube guide is flush against to the top of it (c). The tube guide has a window, the solvent line in the NMR tube should be located at or above this window (d). Once completed, pick up the tube guide/NMR tube assembly from the top and put the whole assembly into the top of the benchtop NMR. Remove the assembly by grabbing the tube guide, being careful to lift straight up until NMR tube is cleared of the spectrometer.



11.2.2 What sample volume should I use?

The instrument is compatible with standard sample volumes. We typically recommend between 0.5 and 0.7 mL of sample in a tube.

11.2.3 Is it necessary to use the sample warmer?

The short answer to this question is no, it is not necessary. It is, however, strongly recommended for optimal performance. The magnet can be affected by temperature, so to prevent line broadening due to short-lived temperature gradients, we recommend that the sample equilibrate to the same temperature as the magnet for 1–2 minutes prior to introduction into the magnet OR that it sit for 2 minutes in the magnet before acquiring NMR data on the sample.

11.2.4 What experiments can I do?

All the instruments are configured with a standard one-pulse 1D experiment, a nutation experiment for pulse calibrations, standard T_1 and T_2 measurements, homonuclear 2D COSY and JRES experiments. Depending on the configuration of your instrument additional experiments will be available. For instance, a $^{1}H/^{13}C$ 60PRO or 100PRO will also have the DEPT-trio and heteronuclear 2D HSQC and HMBC.

11.2.5 What happens if I break a tube?

See section 9.1

11.2.6 How do I start an experiment?

See section 3.0

11.2.7 What concentration should I make my samples?

Because of the inherently lower sensitivity of a low field instrument, we recommend making sample concentrations somewhat higher than you might for a higher-field instrument. Typically, we recommend 0.15 M for good SNR ^{1}H NMR of small molecule work (< 400 Da or so) if a spectrum is required in a few scans (< 30 sec). If you are sample limited but not time limited, lower concentrations are fine, but they will require more signal averaging.