SpinAligner User Manual

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POLARIZE

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Please report comments, errors, and omissions to:

Polarize
Asmussens Alle 1
1808 Frederiksberg C
Denmark
Tel: +45 40272775
E-mail: adm@polarize.dk
www.polarize.dk

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13. Personal Safety

This chapter contains information on the safe operation and maintenance of a SpinAligner.

It is assumed that those using and maintaining a SpinAligner have a basic level of cryogenic competence and understand the use of the basic equipment required to maintain the instrument. Supplied with this manual is a copy of “safety matters” that contains background information on cryogenic handling and magnetic fields.

Under normal operation there is no need to move the SpinAligner. If this is required, it should only be undertaken by qualified Polarize staff or those who have undergone appropriate training. Polarize accepts no responsibility for the consequences of magnet moves that are undertaken by those who are not employees of the company or who have not undergone appropriate training.

The magnet of a SpinAligner generates a very strong magnetic field (approximately 6.7 T at the magnet center). A magnet of this type has significant stray fields external to the magnet and these will attract ferromagnetic objects that are in its proximity. This can generate three types of potential problems that are either hazardous to the operator or can damage the instrument or other equipment:

- External equipment, such as sensitive electronic instruments and heart pacemakers, can be damaged by the stress forces produced by the strong magnetic attraction of ferromagnetic parts within it to the SpinAligner magnet. The stray field plot of the SpinAligner should be used to ensure that objects remain at a distance from the magnet so that these minimum magnetic field values are not exceeded.
- Placement of large ferromagnetic items (i.e. automobiles, forklift trucks) close to the SpinAligner magnet can lead to excessive internal stresses on the mechanical structure of the magnet that can lead to serious damage to the magnet.
- Small ferromagnetic items can be strongly attracted by the SpinAligner magnet and will become projectiles. Such items could be hazardous to an operator who happens to be between the flying object and the magnet.

This instrument contains several electrically powered items, many of which are running at mains voltage. Removal of parts of the outer casing of a SpinAligner is likely to expose the user to hazardous voltages. Disconnect the instrument from all voltage sources if it is deemed necessary to open any part of it apart from the access doors. In addition, the instrument also uses solvents for the sample dissolution process. The SpinAligner is a Safety Class 1 product, meaning that it is provided with a protective ground connection to earth. Any interruption of the ground connection may make the instrument dangerous.

The SpinAligner is designed so that the risk of solvent or moisture encountering parts of the equipment at high electrical voltages is minimized. Care should be taken to ensure that excess solvent or moisture does not ingress into the outer casing of the instrument accidentally and that no unauthorized modifications are made to the unit that will compromise the resistance of the electrical sub-systems to solvent ingress. In the event of a serious solvent or moisture ingress into the instrument return the instrument to the IDLE state and power down the electrical parts of the unit. Contact Polarize immediately for advice on how to deal with the problem.

The SpinAligner can only be serviced or modified by Polarize by personnel trained for the purpose. Do not attempt any servicing or modifications of the SpinAligner unless you are qualified to do so and have the permission from Polarize. Malfunction or failure of the system due to the user having modified or changed the installation, is without warranty or responsibility of Polarize. Any required service of the system will be at the cost of the owner. When in doubt about any operational or functional behavior of the SpinAligner, please consult Polarize.

As part of the sample dissolution process the solvent used for dissolution is heated up to approx. 200 °C and pressurized up to approx. 10 bars. This material is potentially hazardous, and care must be taken when handling it. Do not attempt to override the safety measures put in place to prevent access to the solvent heating and pressurization system during the heating and pressurizing of the solvent. Always wear personal protective equipment, safety glasses or face shield, lab coat and gloves.

14. Dissolution Dynamic Nuclear Polarization (dDNP)

The SpinAligner provides easy and cost-efficient access to highly polarized liquid samples by the dDNP method. The sample, consisting of radical (trityl) and the carbon-13 enriched chemical, most commonly [1-^{13}C]pyruvic acid (PA), is cooled to around 1.3 K. At this temperature and magnetic field, the electron spin of the radical is almost fully polarized. The polarization transfer from the electrons to the ^{13}C is mediated by microwaves and typically reaches a dynamic equilibrium of 60-70% ^{13}C polarization in less than an hour (^{13}C-pyruvic acid under optimized conditions). When steady state has been reached, the sample is lifted out of the liquid helium while remaining in the high magnetic field of the polarizer, and rapidly dissolved by hot solvent (typically buffer). In a few seconds, the sample is dissolved and provided for NMR or MRI experiments with enhancements of around 10^5 compared to thermal equilibrium for the measurement system.

The sample to be polarized is added to the *Sample Vial*, which is mounted to the *Fluid Path* and inserted by the *Insertion module* through the *Airlock*. The vial is automatically lowered into the *DNP Probe*, where the sample is irradiated by the *MW Source*, whereby it is polarized. The build-up is measured by a low flip angle NMR detection. The solvent is added to the *Dissolution Module*, and the polarized, dissolved sample is collected from the *Receiver*. The process is controlled from the SpinAligner software, which guides the user through the process.

15. Overview of the SpinAligner and Installation

This manual contains detailed instructions on how to correctly and safely operate a SpinAligner. The manual contains no information on the installation or decommissioning of a SpinAligner, as this should only be attempted by appropriately trained Polarize staff. The main components of the SpinAligner are described in Figure 1.

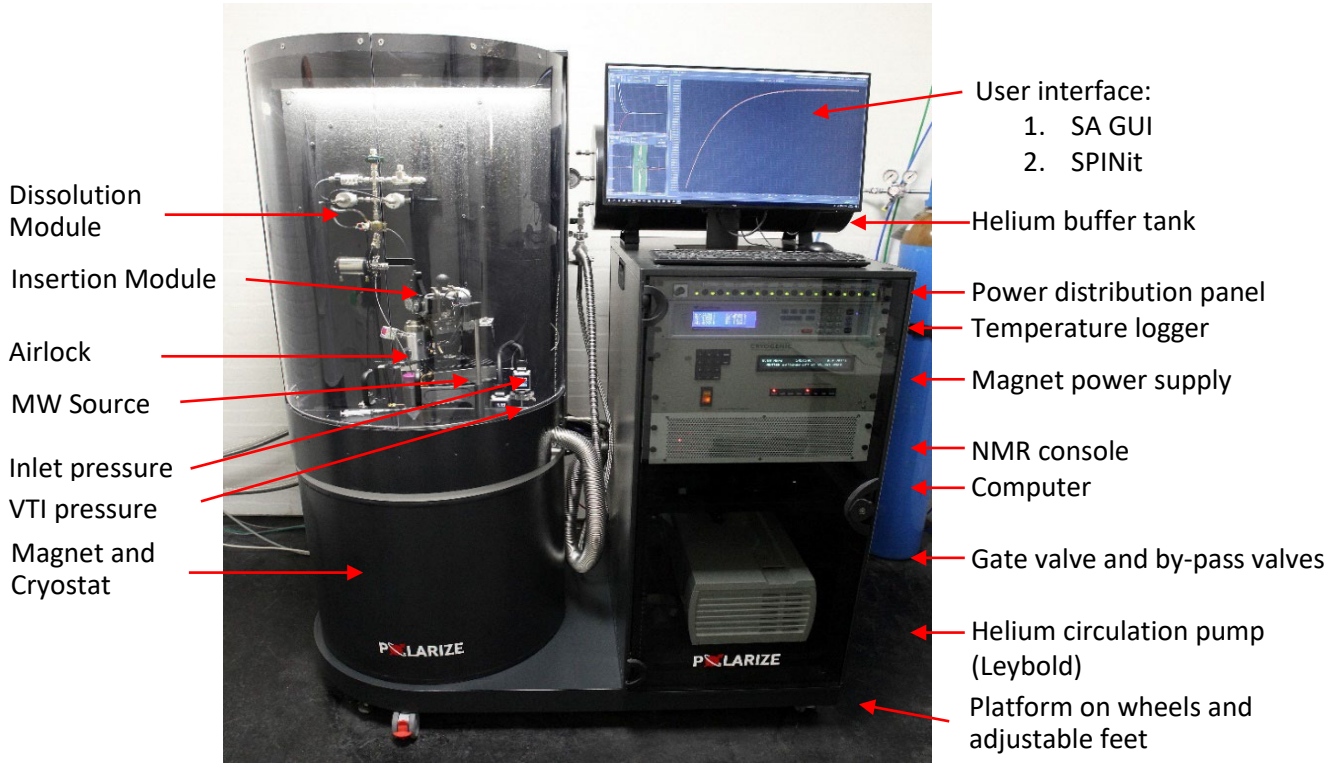


Figure 1: The SpinAligner, overview.

Nomenclature

- VTI – Variable Temperature Insert. The temperature-controlled volume used for sample preparation. The VTI is isolated from – but lies within - the superconducting magnet.
- SA GUI – SpinAligner Graphical User Interface. The operational software used for controlling, e.g., sample temperature, magnetic field, dissolution
- SPINit – NMR console software
- MW source – Microwave source
- Airlock – is the intermediate chamber between free air and the liquid He in the VTI
- Insertion Module – enables automatic loading of sample
- Dissolution Module – used for heating solvent liquid. Enables dissolution. Connects to sample vial and container for final hyperpolarized agent
- Helium circulation pump – the He circulation pump together with the Needle Valve enables the VTI to reach lower temperatures (typ. 1.4 K) than the 2nd cooling stage (typ. 3 K)
- Inlet Pressure – He buffer tank pressure
- VTI Pressure – He pressure in the VTI. During operation the VTI Pressure reflects the VTI temperature according to the He(l) vapor pressure curve
- Magnet and Cryostat – The magnet is inside the cryostat vacuum
- Helium buffer tank – is before startup and cooling at 1 bar with 99.999% He-gas

- Power Distribution Panel – Main power off (switch) and fuses for all electronics running at 230 Volts
- Temperature logger – logging all temperatures
- Magnet power supply – remotely controlled power supply for the superconducting persistent current magnet
- NMR console – controlled by SPINit
- Computer – Industry computer running Windows 10 Enterprise, SA GUI and SPINit
- Gate valve and bypass valves – used in conjunction with cleaning of He-circulation circuit
- Platform on wheels and adjustable feet – The SpinAligner can be rolled slowly on a smooth and bump-free floor. Care should be taken to avoid any bumps creating large accelerations. Wheels can be locked. When the SpinAligner is in place, the adjustable-height feet must be lowered and the SpinAligner wheels lifted off the floor. This is done by means of a wrench.

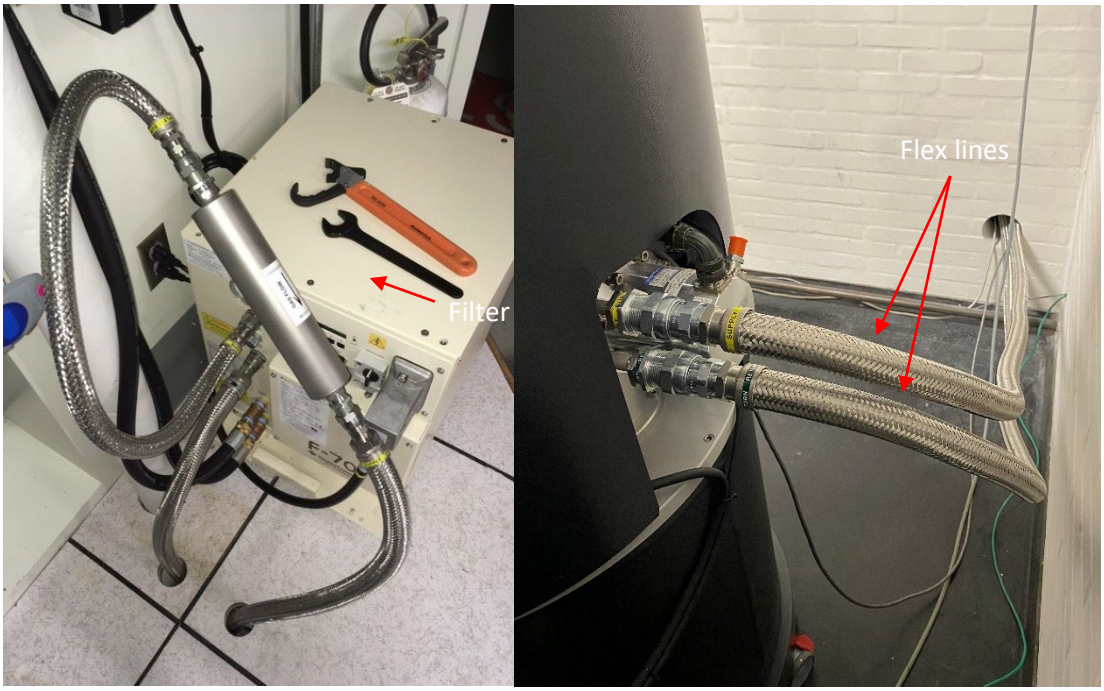


Figure 2: Left: F-70H compressor, flex lines and filter. Right: On the SpinAligner rear side the SRP082B cold head is connected to flex lines and a cold head control cable. The compressor is also connected to the SpinAligner by a USB serial communications cable.

The superconducting magnet is cooled by a Sumitomo SRP082B cold head that is connected to a Sumitomo F-70H compressor through 20 m of flex lines, Figure 2.

The compressor is normally placed in an adjacent equipment room since it is noisy and requires cooling water. The 20 m of flex lines from the compressor to the cold head of the SpinAligner provides flexibility in the installation of both.

The superconducting magnet must always be connected to the magnet power supply in case a magnet quench occurs. The magnet power supply can be switched off, when not in use. The superconducting magnet is a dry magnet, i.e., it is cooled only by the cold head and has no liquid cryogens inside. This,

in consequence, means that the magnet will quench (loose magnet field) in less than 5 to 8 min if the compressor turns off. The compressor will automatically turn off if it loses of power or cooling water. It is, however, designed to automatically turn on again. Repetitive power cut-offs within minutes may result in a compressor failure that requires manual restart.

The sample loading and dissolution is handled by the insertion module and the dissolution module. The dissolution module consists of a 10 mL boiler with adjustable pre-pressurization and final pressures. The dissolution is fully automated via a 6-port dissolution valve and a reusable fluid path. The user is guided through the entire process from sample mounting to hyperpolarized end-product and cleaning via the user interface.

Floor plan and magnetic stray field

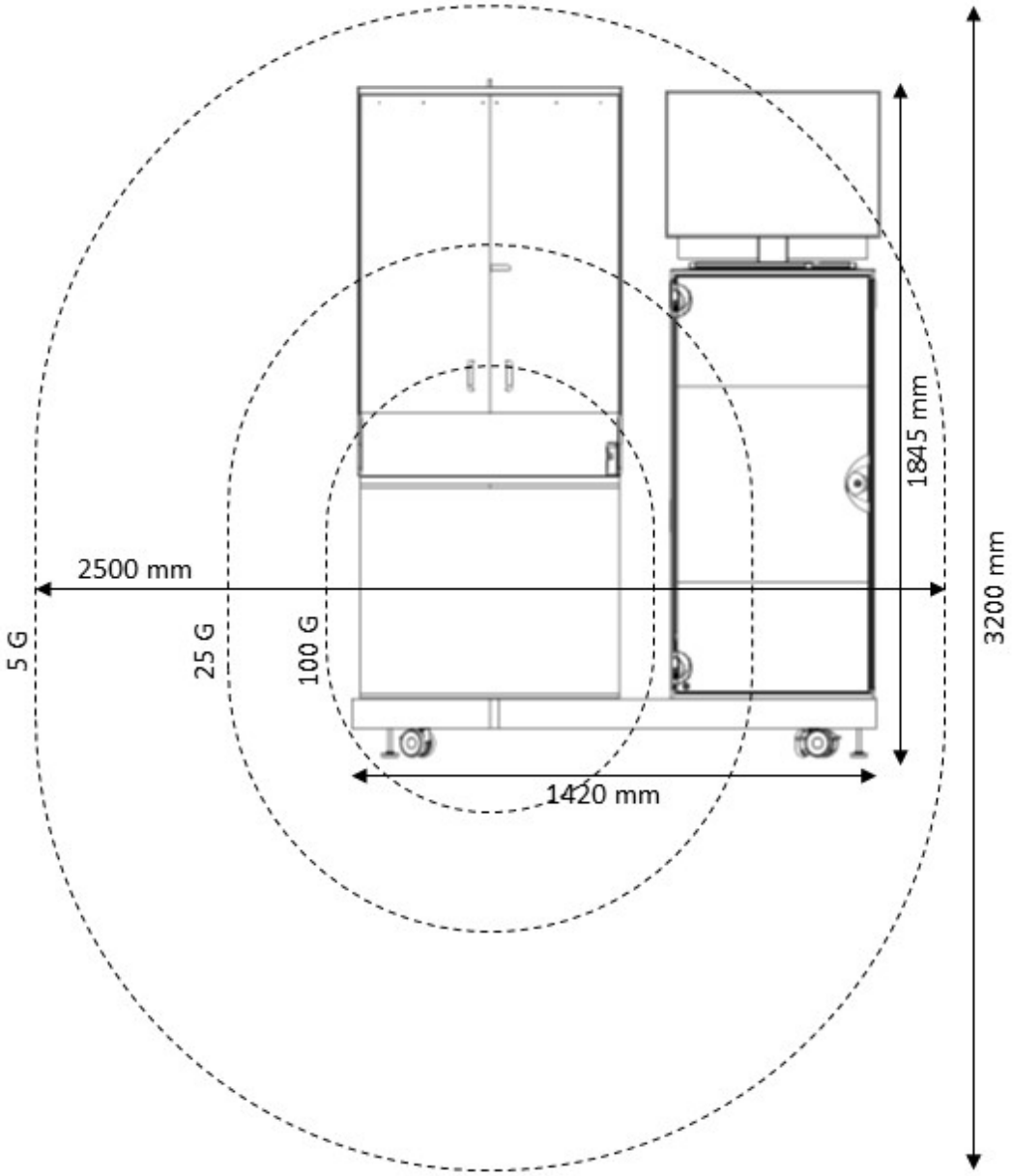


Figure 3: Front view of the SpinAligner with magnetic stray field contour lines.

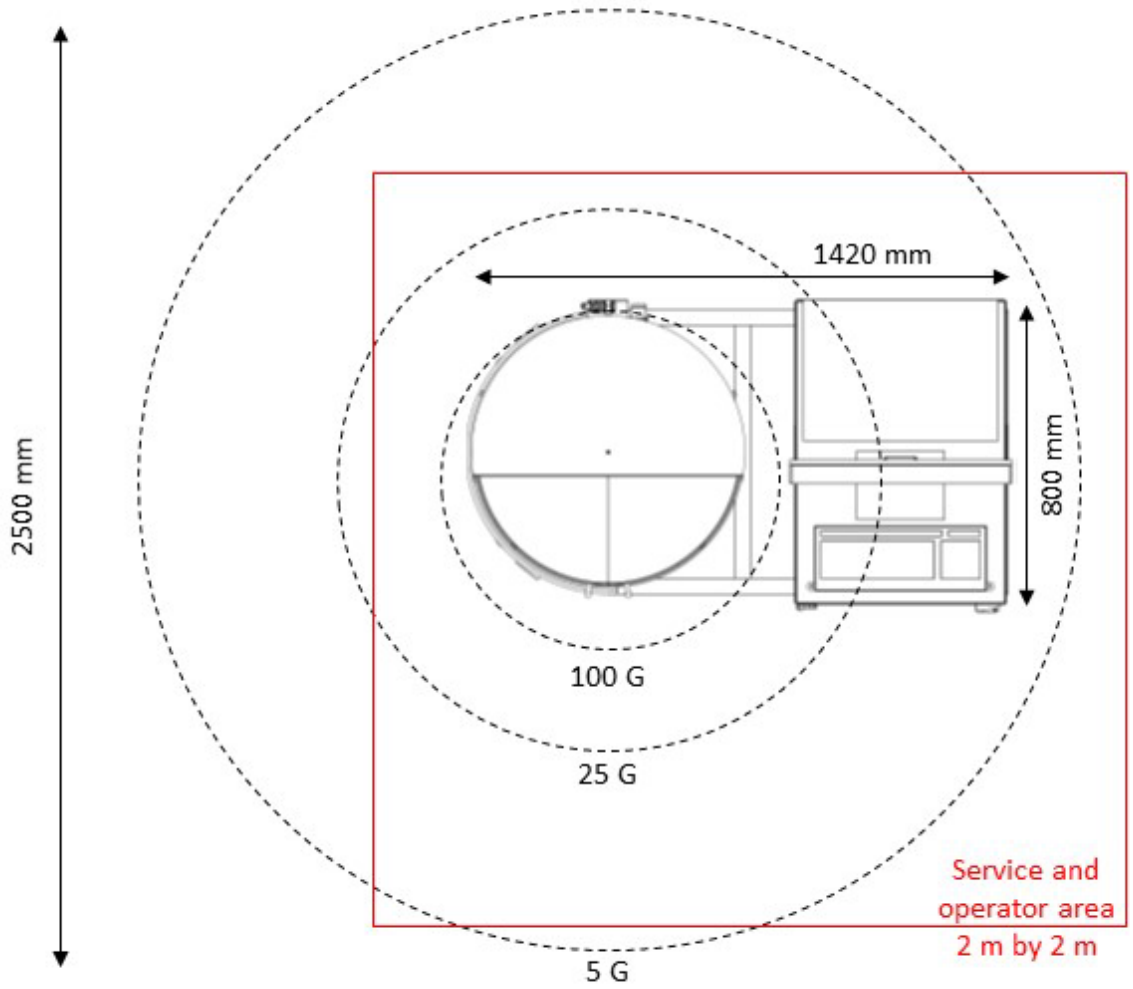


Figure 4: Top view of the SpinAligner with magnetic stray field contour lines.

Specifications

- 6.7 T (9.4 T) persistent, dry magnet with low drift rate and fixed leads (Cryogenics Ltd).
- Re-condensing, variable temperature insert (VTI) with base temperature of <1.4 K; temperature controllable to 300 K continuously.
- No liquid cryogens needed. Cooling provided by Sumitomo SRP082B cold head and F-70H compressor.
- Microwave frequency 188 ± 2.0 GHz / frequency modulation / pulse modulation / gating / 100 mW / 20 dB attenuation.
- NMR probe tuned for ^{13}C (72 MHz).
- Solid-state NMR spectrometer for monitoring polarization build-up (RS2D Cameleon 4, T/R switch, 300 W RF amplifier (5-310 MHz) and SPINit software).
- Fluid path for dissolution of samples optimized for pre-clinical/in-vitro applications.
- Fully automated software control.
- Polarizer footprint 1.42 x 0.80 m^2, height 1.85 m. Minimum floor space required for operation and service 4 m^2.
- Weight 400 kg.
- Heat dissipation: the SpinAligner dissipates an average of 1 kW to air. The compressor is water cooled, see Sumitomo F-70 manual for specification.

Shipment size (dimensions and weights)

- SpinAligner: One crate of 1.6x1.1x2.0 m^3. Weight 595 kg.
- F-70 compressor: One crate of 0.7x0.7x1.3 m^3. Weight 175 kg.

Consumables/accessories

A box is provided with the SpinAligner containing:

- 200 mg trityl.
- 3 vials.
- A bag with 100 O-rings.
- Wrench tool to tighten vial to fluid path.
- Solvent Syringe to load Dissolution Medium.
- Receiver.
- Plastic screwdriver to tune and match the NMR probe.
- Keys to electronics rack.

Manuals

Some manuals can be downloaded from the Polarize website: www.polarize.dk/documentation.

- SPINit Advanced User Guide (RS2D).
- SPINit SequenceDev User Guide (RS2D).
- Compressor F-70 (Sumitomo).
- Ecodry 60 plus manual (Leybold).
- Temperature controller, LS244 manual (Lakeshore).
- Magnet Power supply, SMS manual (Cryogenic).
- Magnet system manual (Cryogenic).

16. Installing the Sumitomo compressor and SpinAligner

Installation requirements

- F-70H compressor (see Sumitomo manual for additional specifications):
    - Cooling water: 7-9 L/min and 5-25 °C inlet (1/2'' MNPT).
    - F-70H power: 3-phase, 480 V/60 Hz or 400 V/50 Hz. The power cable comes unterminated and require local electrician to fit appropriate plug.
    - 2 ea. 20 m flex lines (gas lines) from compressor to SpinAligner (cold head). Flex lines cannot be shortened.
    - Filter Unit with 1 m flex line. Filter connects between F-70H and 20 m flex line
- Power to SpinAligner: 230 V / 10 A (50 Hz or 60 Hz) of high stability <10 ms power outages. A 3 kVA UPS is recommended. The power cable (5 m) comes unterminated and require local electrician to fit appropriate plug.
- Internet socket open to a VNC connection.
- Helium gas 5.0 (50 L/200 bar cylinder or equivalent) with regulator 1-25 bar and 6 mm Swagelok fitting.
- Compressed air: 5-10 bar, 6 mm push fitting.
    - Dew point <-40 °C (ISO 8573-3 class 2)
    - Oil content <0.1 mg/m^3 (ISO 8373-2 class 2)
    - Particles according to ISO 8373-4 class 2
    - Flow rate: 10 L/min

Compressor

The compressor is typically shipped in advance of the SpinAligner for installation by the local facilities staff. Please refer to manual: 'Sumitomo F-70H, F70-L and F70-LP Helium Compressors Operating Manual Rev. E' for correct installation of the compressor.

Our experiences

- When unpacking the compressor, keep all unpacked tools by the compressor. They are needed for the further installation.
- As the compressor is noisy, it is best to have it located in a room adjacent to the SpinAligner, such as an equipment or utility room.
- The power cable is supplied without plug. Have an electrician install a locally approved plug and connect the compressor power cable to the wall socket
- To route the flex lines from the compressor to the SpinAligner, our experience is that a hole of 10 cm minimum diameter is required in any wall between the compressor and the SpinAligner.

If in doubt, always refer to the Sumitomo manual.

Unpacking SpinAligner from shipping crate

The shipping crate is constructed with one side having the function of providing a slide when removed.

Procedure

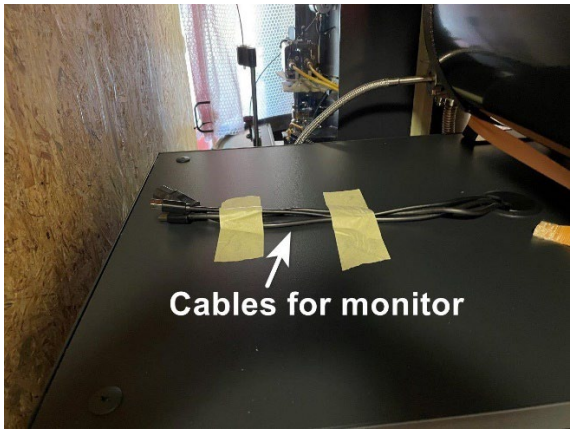
1. Unscrew all screws securing the end sides in the ‘Cryostat end’ and in the ‘Ramp end’
2. Unlatch the latches and remove both ends – 2 persons are needed
3. Place the ramp in the ramp area
4. Visually inspect from both sides for possible damage
5. Remove wooden ‘end stops’ at the floor of the box, both in Cryostat-end and Ramp-end
6. Increase the height of all 4 ‘Adjustable-height’ feet until you may extract both ‘Longitudinal Supports’
7. Lower the height of all 4 ‘Adjustable-height’ feet until the SpinAligner rests on all 4 wheels
8. Remove screws that hold the ‘Inside fixation bar’ in place
9. Roll out the SpinAligner from the box using the ramp – please be cautious – 2 persons are needed for this operation
10. Wheel the SpinAligner to its intended location. With a wrench lower the feet such that they support the system instead of the wheels (lifted from the ground to freely rotate)

Crate – cryostat end

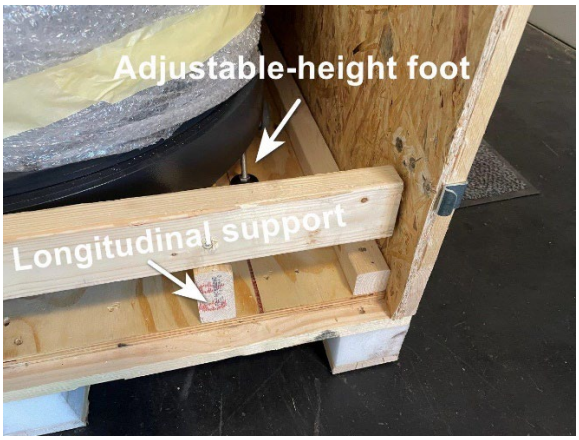
Cryostat end - opened

Ramp end

Ramp end - opened



Ramp side – opened



Ramp side – opened

Figure 5 Shipping crate

External connections to the SpinAligner

Helium gas supply (blue colored tube)

Provide a He gas cylinder of 99.999% (5.0) purity for permanent use with regulator 1-25 bar and 6 mm Swagelok fitting. Keep it securely fastened to the wall. Connect the regulator output with 6 mm tubing to the helium inlet (push fitting) on the back side of the SpinAligner and adjust the pressure on the regulator to 8 bar.

Compressed air supply (green colored tube)

Provide air pressure for permanent use capable of giving at least 5 bar pressure and with 6 mm push fitting. Connect the compressed air to the inlet (push fitting) on the backside of the SpinAligner with 6 mm tubing.

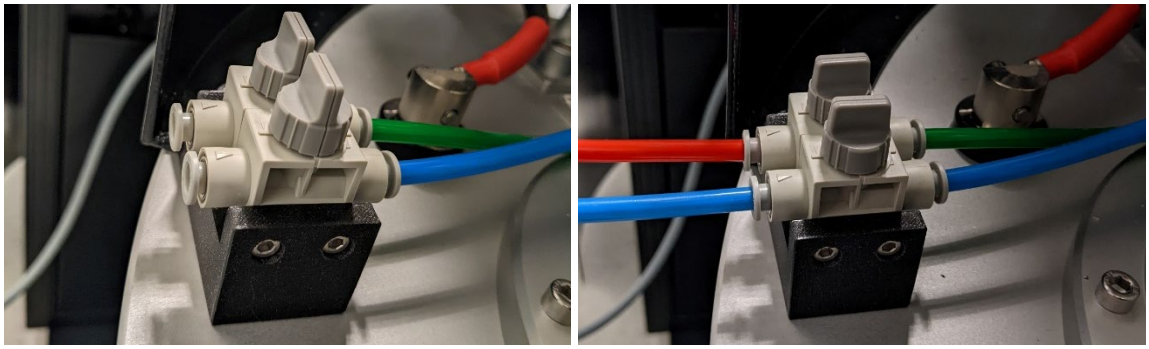


Figure 6: Valves on the back side of the SpinAligner for helium gas and compressed air supply with 6 mm push-fitting connectors. Left photo shows the valves in off position and the right photo has the valves opened after gas supplies have been established. The blue colored tube is for helium supply and the green colored tube is for compressed air.

Power Cable

A wall socket for 230 V should be available near the SpinAligner. A plug for the socket that meets local standards should be provided by the customer and mounted to the power cord by a certified electrician. The power cable is 5 m long and can be shortened at installation. The wire marked 1 should be connected to phase and the wire marked 2 should be connected to neutral. Green/yellow is connected to ground. Power up the SpinAligner by switching on the power distribution panel at the top of the rack.

Internet Access

Provide a LAN socket with internet connection. Ensure that the firewall allows a VNC to be made for installation, support, and maintenance. The SpinAligner has RealVNC installed.

Data connection between compressor and SpinAligner

An extended USB cable with RS232 to USB converter must be connected. Connect the DB9 RS232 converter to the Sumitomo Compressor (‘Serial Connector’) to the black USB hub on the rear side of the SpinAligner. The rear side cover of the cylindrical cryostat cover must be lifted off first

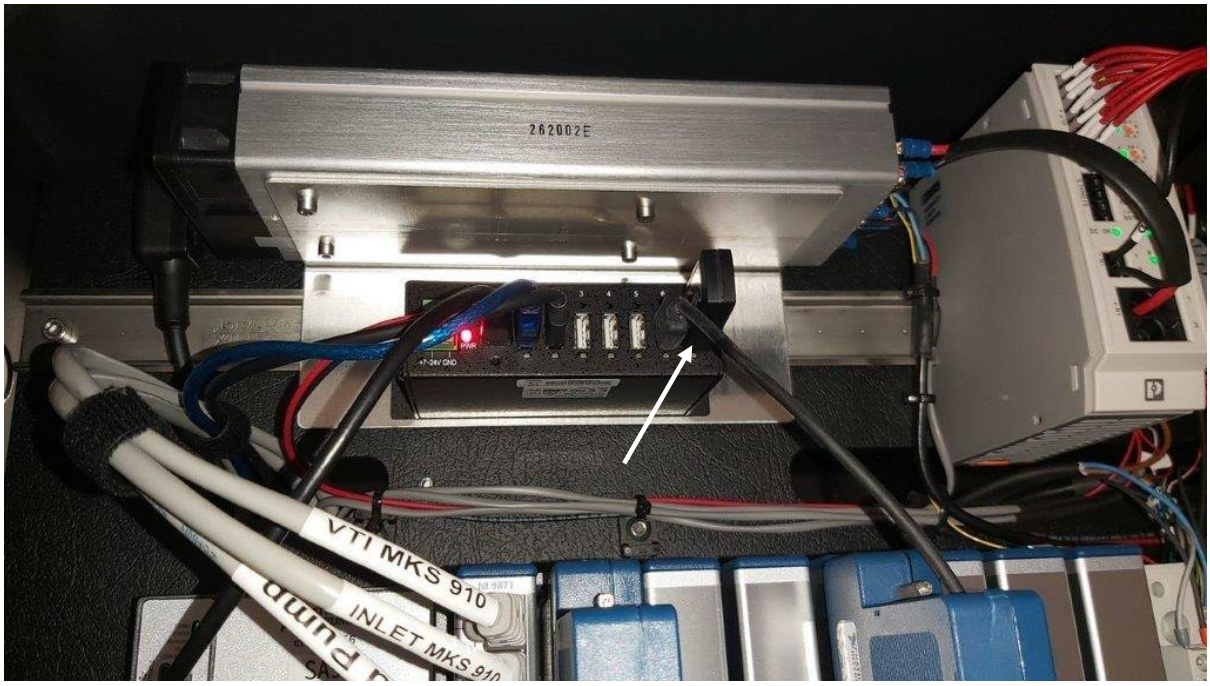


Figure 7: USB cable connection (to compressor) on the rear side of the SpinAligner. Black USB hub and cable indicated by white arrow.

Initial procedure

After the SpinAligner has been positioned, start by evacuating the cryostat, see chapter 23 “Pumping the enclosure vacuum – consult Polarize”.

17. Software and Control

In the case of a brief power failure the SpinAligner (PC and control electronics) will shut down in a safe mode. In the case of longer power failure or loss of cooling water to the compressor, the magnet may quench, causing a loss of the magnetic field. Once power or cooling water returns, the compressor should start automatically, and the magnet will begin to cool. In this event, contact Polarize for guidance on energizing the magnet and making necessary adjustments. See chapter 23.

The following assumes that the compressor is kept running and the magnetic field is on.

Starting up the SpinAligner

Step 1 – Turn on the SpinAligner on the "Power and Fuses" unit.

Log into the computer as user 'SpinAligner', password '1234'.

Step 2 – Start the SpinAligner Program called "SA GUI"

Step 3 – Cooldown: Press the "Cool Down" button in the SpinAligner software.

Wait for the cooling normally 2-3 hours. The system is now ready to polarize.

Step 4 – Start NMR program: SPINit

Double click the SPINit icon on the desktop and log in as 'SpinAligner' with password '1234'. Make sure all devices are on (Control Panel > Power). Check tuning (Control Panel>Main) - cold VTI required. Go to a Study and Sample to enable Acquisition. SPINit is now ready to acquire the desired experiment (see later chapters).

Shutting Down the SpinAligner

Step 1 – Press the "Goto Idle" button in the SpinAligner software.

Wait for the Cooldown button to turn off. The Leybold pump will stop and the VTI temperature and pressure will start to increase.

Step 2 – Close the SpinAligner software "Close button" and the SPINit software "X"

Step 3 – Shutdown the computer via Windows shutdown

Step 4 – Shut down the SpinAligner by turning of the power button on the "Power and Fuses" unit

Shutting Down or Restarting the PC only

Step 1 – Close the SpinAligner software "Close button" and the SPINit software "X"

Step 2 – Close all programs and shutdown or restart PC via Windows shutdown.

SpinAligner Software

The SpinAligner is controlled by a PC and a National Instruments cRIO system. The user interface runs on the Windows PC, while the real-time automation control runs on the FPGA under Linux.

The PC also has SPINit (RS2D) installed for NMR data acquisition and realVNC for remote access.

The important windows are the Main panel, Figure 8, the MW panel, Figure 9, and the Service panel Figure 10. The automated procedures for loading the vial, dissolution etc. are found on the main panel, while the MW is used for optimizing the microwaves for optimum polarization. The Service panel provides further maintenance controls. The tick box “Advanced” enables further controls in all panels and access to IM panel and Control panel for advanced users.

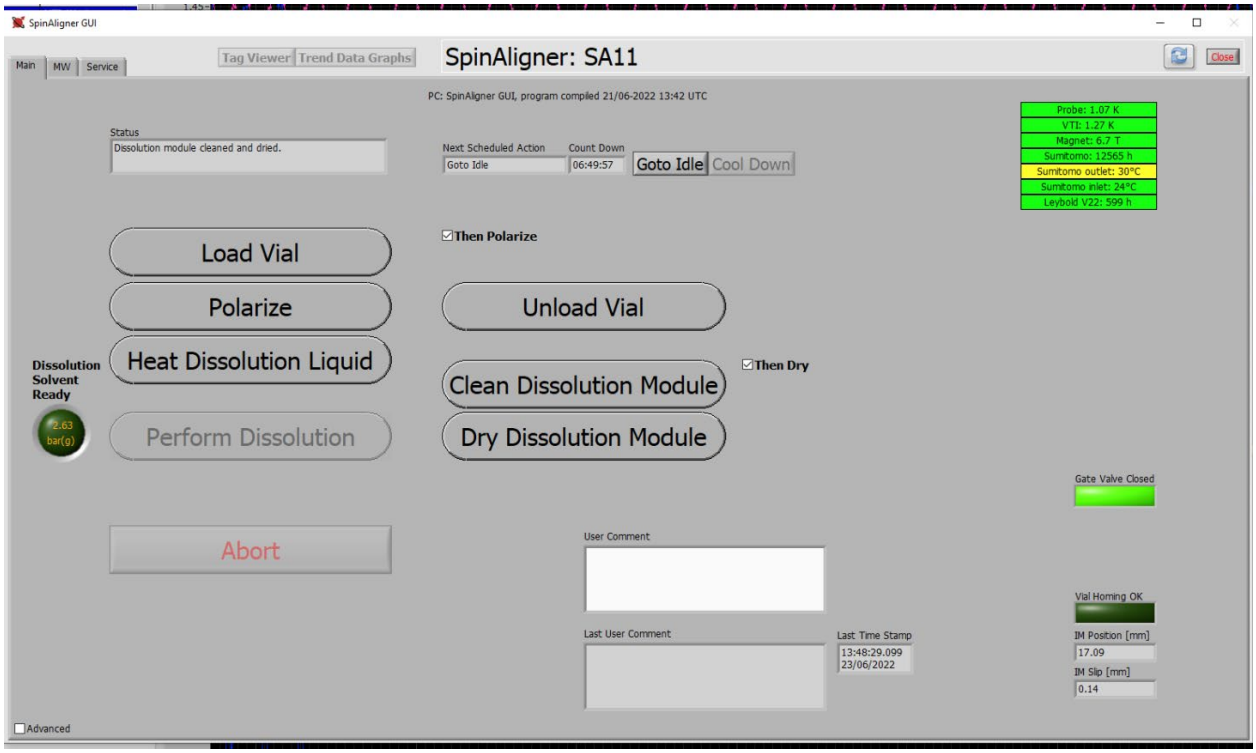


Figure 8: SpinAligner software, Main panel.

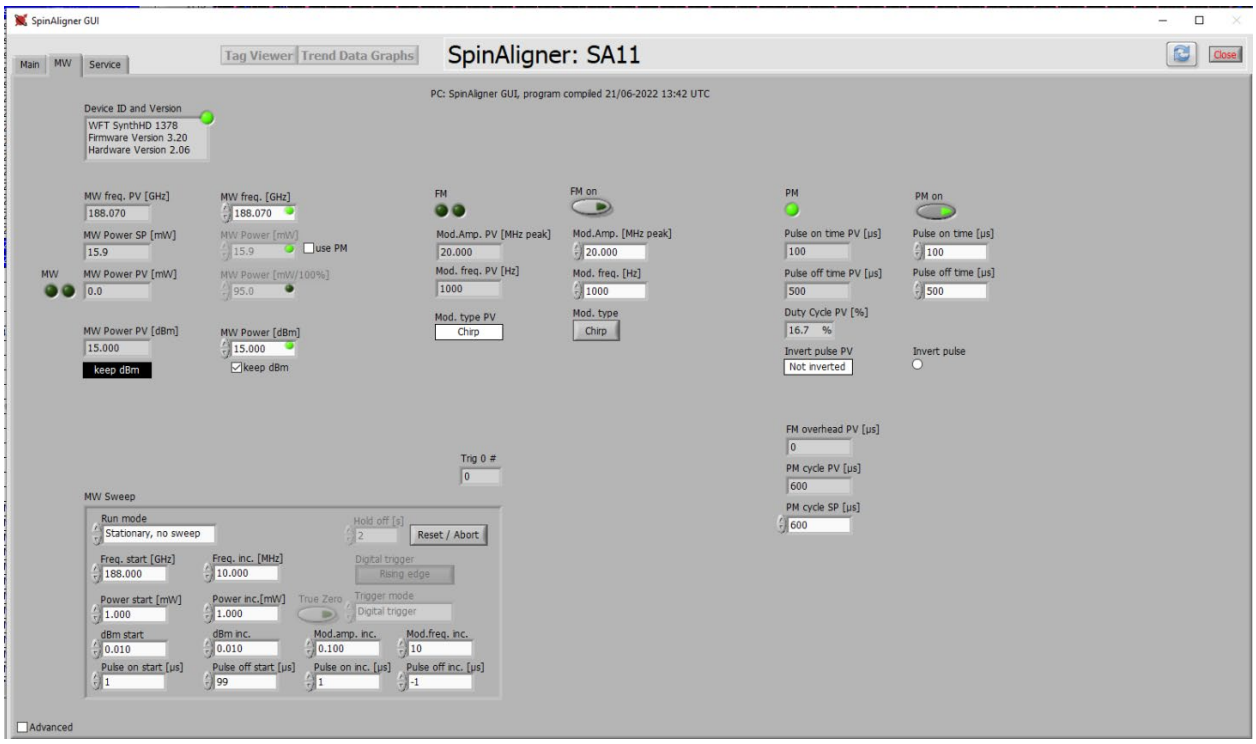


Figure 9: SpinAligner software, MW panel.

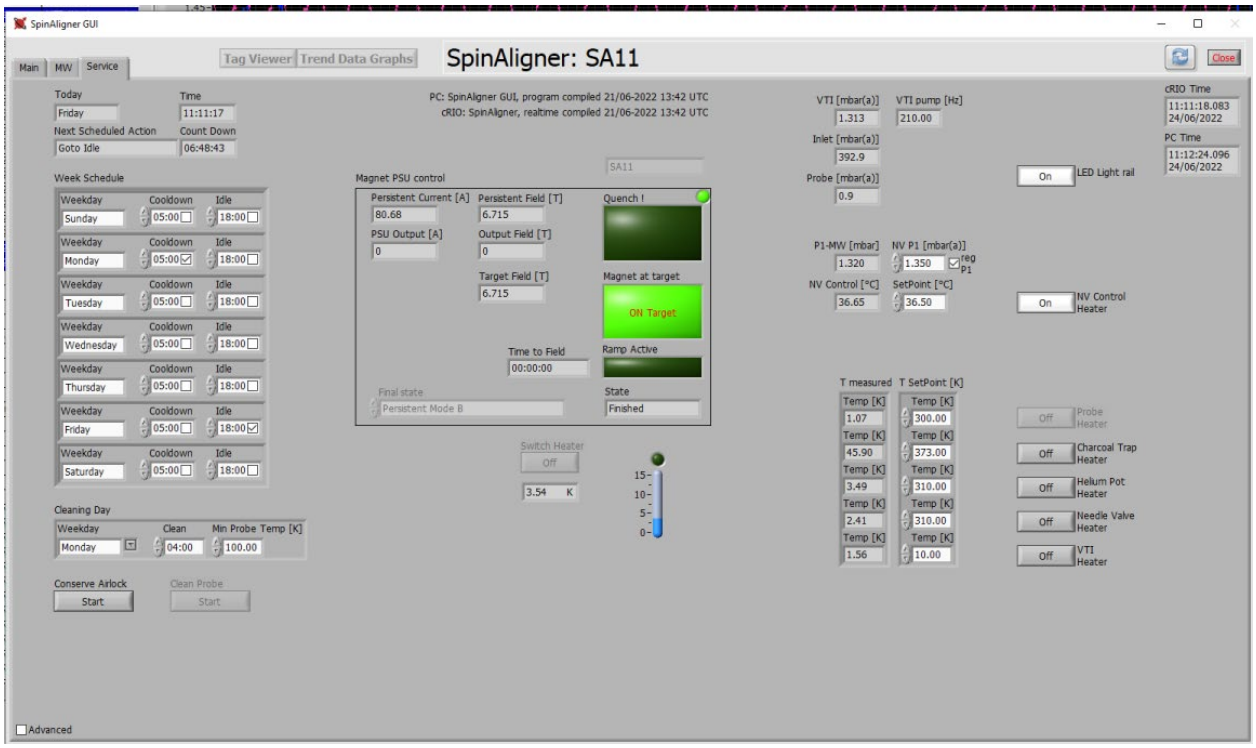


Figure 10 SpinAligner software, Service panel.

The Data Logger, Figure 11, provides convenient access to all logged data most useful for troubleshooting. It's opened by clicking the "Tags Data Graphs" button.

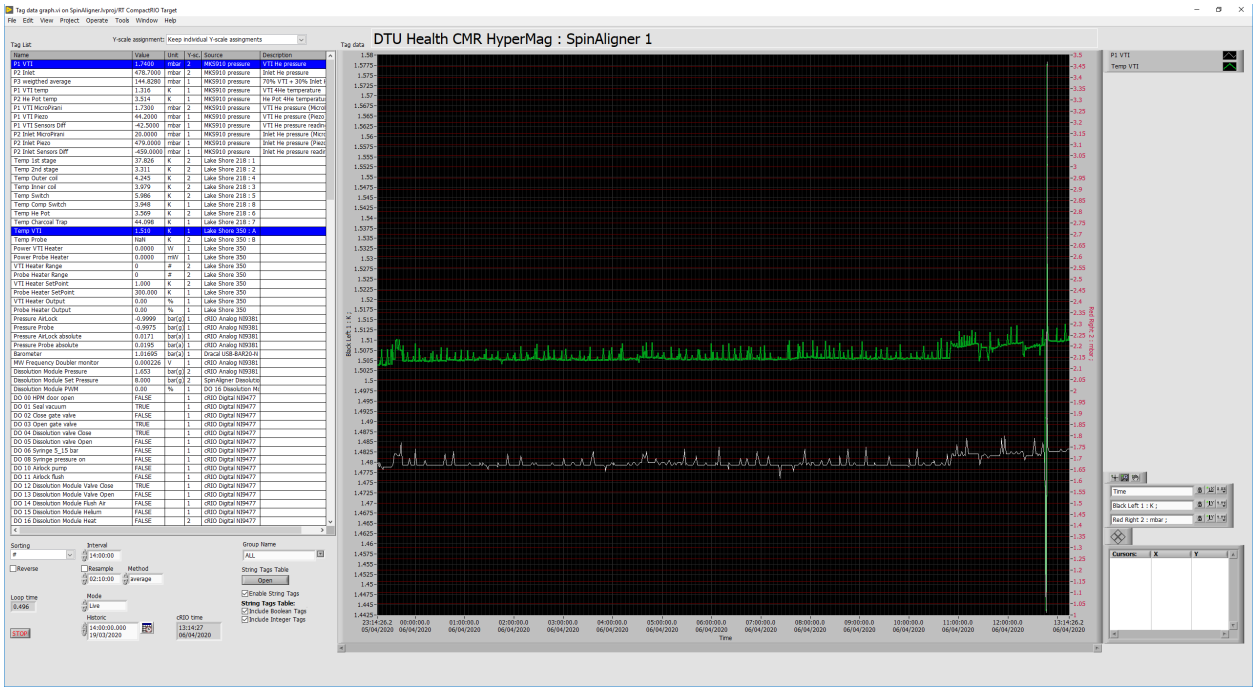


Figure 11: Data Logger.

Automatic Cooldown

On the service page, a weekly scheduler is found, to control the Idle and Cool Down periods of the SpinAligner. By putting the SpinAligner into Idle mode, the tear-and-wear of the Leybold pump is reduced and other power saving functions are activated. Furthermore, the probe cleaning procedure can be engaged only in Idle mode. It is not recommended to put the SpinAligner into Idle during the night but optionally during weekends and holiday periods. It can operate continuously.

Every day of the week has a check box to define whether Cool Down will happen on that day. The time of starting the Cool Down can be entered. The SpinAligner will be ready for operation 2-3 hours after the start of the Cool Down, Figure 12.

The SpinAligner can also be programmed to automatically clean the DNP probe during the idle period. This is a good precaution to ensure that accumulation of residual air in the probe from sample loadings is minimized. Below the weekly schedule, the preferred day of the week is chosen and the time of the cleaning. Typically, Monday morning is a good choice, about an hour before the Cool Down.

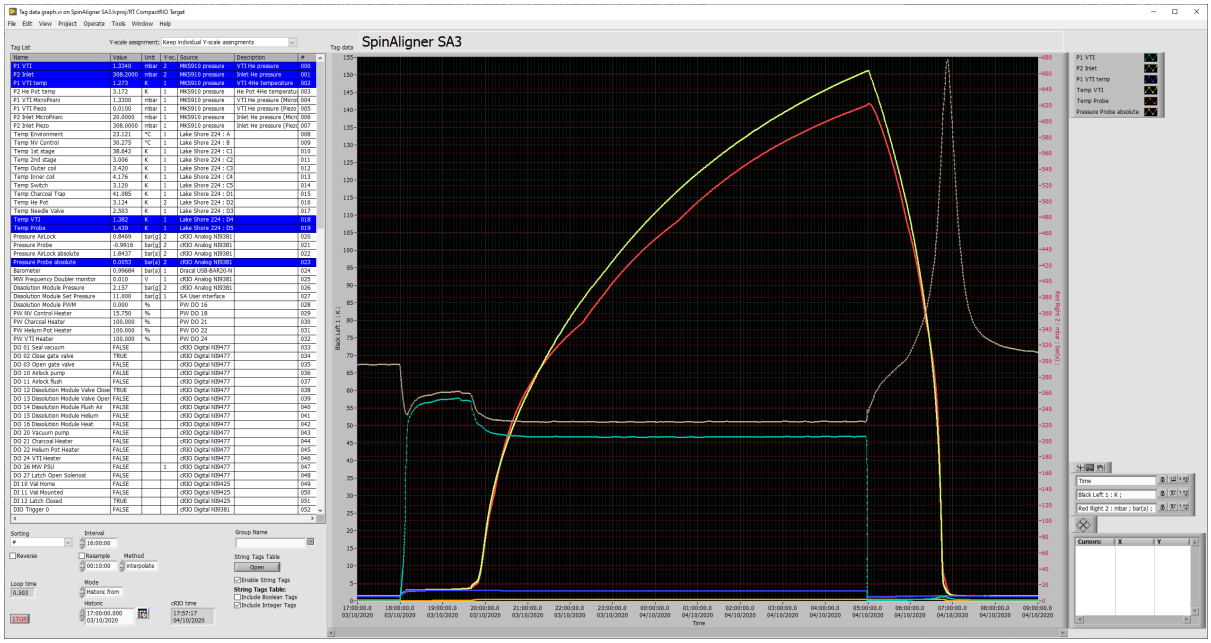


Figure 12: VTI and inlet pressures as well as VTI and probe temperatures are shown during the idle period as the pump is turned off at 18:00. The pump starts automatically at 5:00 to cool the VTI. Base temperature is reached approx. 7:30.

SPINit – the NMR Spectrometer Software

SPINit is started by clicking the SPINit icon on the desktop:  . Please consult the SPINit manual for expert guidance. The software is briefly described below.

In the Home panel, “SpinAligner” is chosen as sample and “SpinAligner” as study.

Then the Acquisition panel is enabled. Add a new experiment by using the  icon.

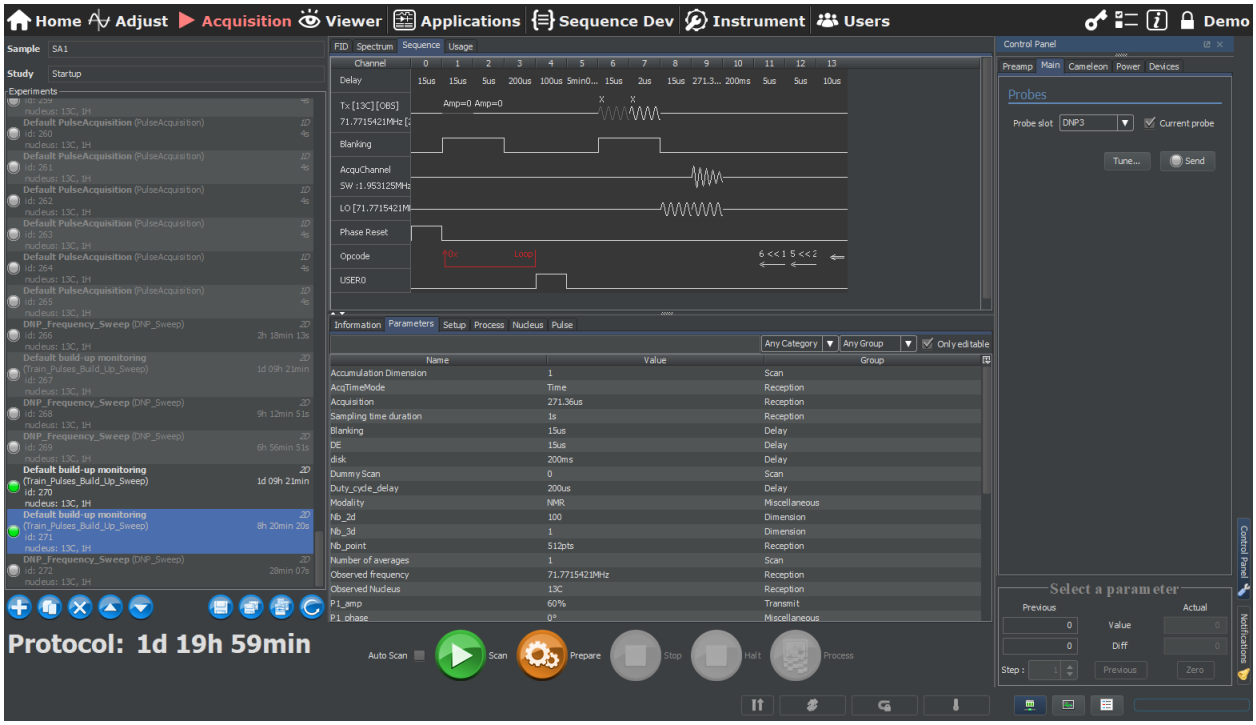


Figure 13: SPINit Acquisition tab.

Figure 13 shows the SPINit acquisition tab. All previous experiments are listed in the left column. The top middle provides data display for the FID, Spectrum, Sequence and Usage by selecting the correct tab. The bottom middle provides tabs for Information, Parameters, Setup, Process, Nucleus and Pulse. These tabs are used for setting up the experiment. For the standard experiments only the tabs Parameters, Setup and Nucleus are relevant.

The control panel is opened using the button at the right side of the window. Here the console can be powered up or down and the probe can be tuned and matched. The control panel, right column in Figure 13, provides tabs for Preamp, Main, Chameleon, Power and Devices. The power tab is used to turn on power to the spectrometer. Devices will indicate the state of the devices (green when everything correct). The main tab provides access to the Tune window to check proper tuning and matching of the DNP probe.

The standard experiments can be viewed in the Applications panel from the top line menu, Figure 14. These applications are invoked by clicking the  , and selecting the desired application. Previous experiments can also be cloned by clicking the  button.

Previous acquisitions can be studied in the Viewer tab. All experiments are stamped with an “id-number”, which serves as a unique identifier.

For further details please consult the SPINit user guides.

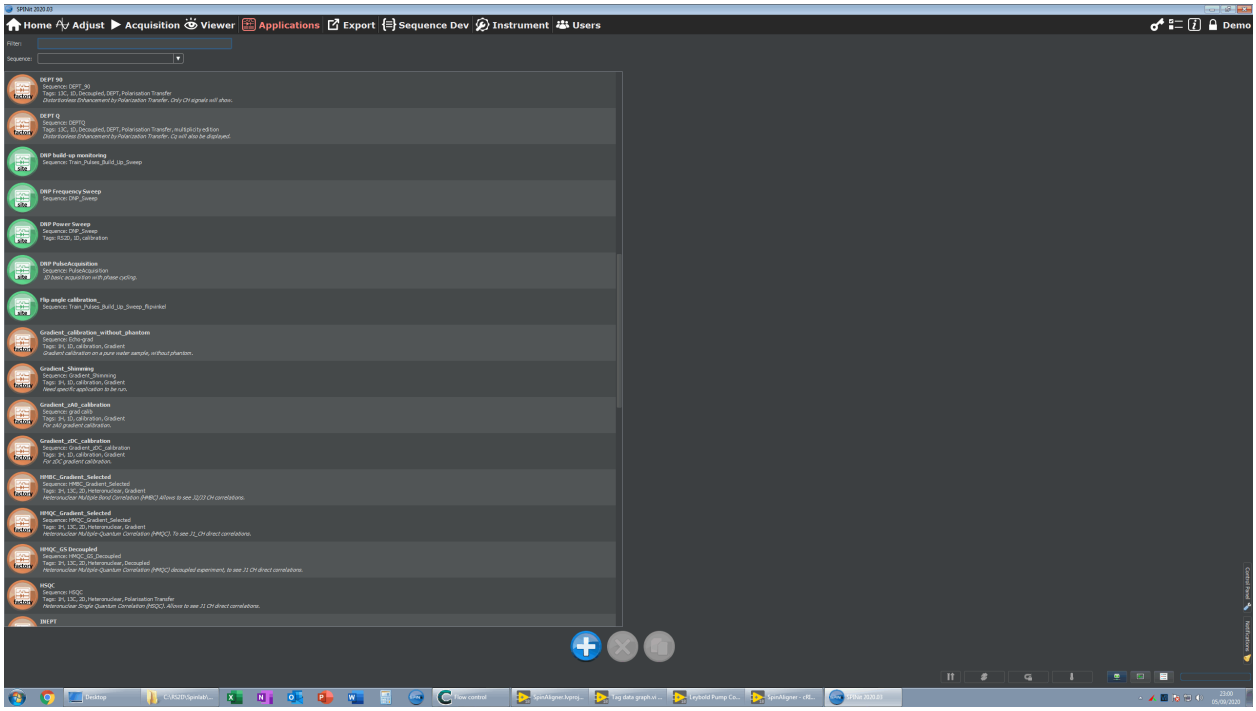


Figure 14: SPINit Applications tab. The orange applications are RS2D factory applications, and the green are the five applications used by the SpinAligner.

18. DNP Sample Preparation

This section describes the preparation of a standard DNP sample. A standard sample contains 18 uL of [1-^{13}C]pyruvic acid with 30 mM trityl. Figure 15 and Figure 16 show the chemical structures of the two ingredients.

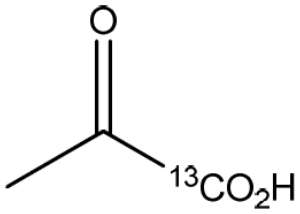
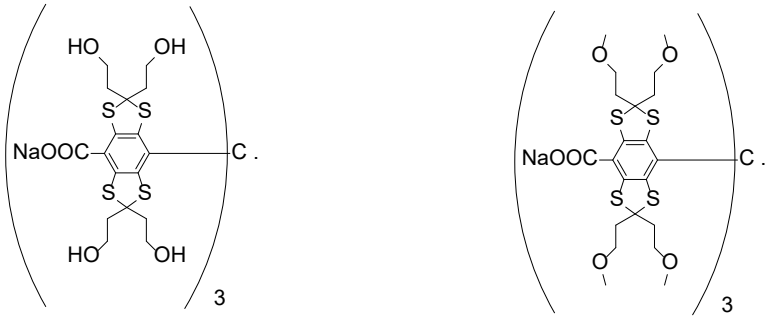


Figure 15: [1-^{13}C]pyruvic acid (density 1.27 g/mL, MW=89 g/mol).



OX063 **AH111501**

Figure 16: Trityl radicals OX063 (MW=1427 g/mol) and AH111501 (OX063Me) (MW= 1595 g/mol).

If the sample is dissolved in 3.2 mL of dissolution medium, a final pyruvate concentration of 80 mM should be obtained, which is a common recipe for pre-clinical imaging. The recipe ensures neutral pH, isotonicity and a temperature between room temperature and physiological temperature.

Preparation of PA mix (stock solution)

30 mM AH111501 in [1-^{13}C]pyruvic acid.

Materials

- 1-^{13}C labelled pyruvic acid (PA), M= 89.06 g/mol, ρ= 1.267 g/mL.
- Trityl (AH111501), M= 1595 g/mol. AH111501 is a dark green powder that is stored at -20 °C. Before handling, make sure it is warmed to room temperature as the powder is hygroscopic. If another trityl is used, apply correct molecular weight.
- Eppendorph vial.

Procedure (1 mL)

Weigh 47.9 mg of AH111501 (correct for purity). Use antistatic precautions. Add 1.267 g of PA or proportional volumes. The quantities are most conveniently weighed directly into an Eppendorph vial. First weigh the AH111501 as close to the desired amount as possible, calculate correct amount of PA by proportionality, and then weigh the correct amount of PA into the same vial.

The AH111501 readily dissolves in the pyruvic acid by vortex or ultrasound of the vial for a few minutes. The stock solution can be aliquot into smaller fractions, e.g. four Eppendorph vials of approx. 300 mg.

Keep the stock solution in the freezer and protected from light. The stock solution will be stable for at least several months under these conditions. The solution should not be left at room temperature and in light for extended periods. However, make sure to thaw the stock solution completely and vortex before dispensing into the sample cup.

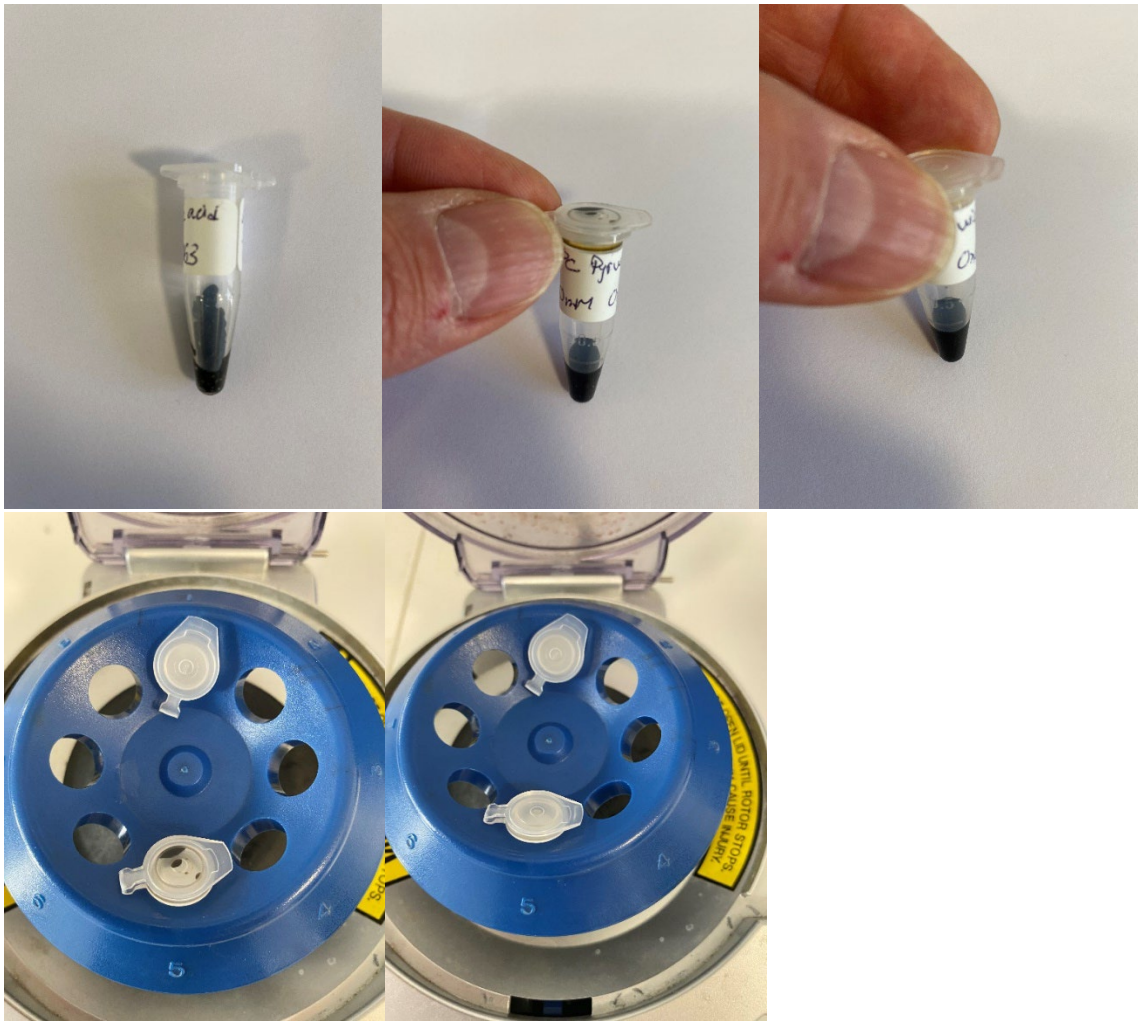


Figure 17: Top row: Eppendorph vial with sample stock solution. First photo shows the frozen sample, second photo shows the melted sample smeared out and last photo shows the sample spun down. Bottom row: Eppendorph vial in spinner before and after spinning.

Preparation of Dissolution Medium (stock solution)

40 mM Trizma PreSet Crystals pH 7.6, 50 mM sodium chloride, 0.27 mM disodium ethylenediaminetetraacetate dihydrate, and 50 mM sodium hydroxide.

Materials

- Sodium Hydroxide, NaOH(s), M=39.997 g/mol.
- Disodium ethylenediaminetetraacetate dihydrate, $Na_2EDTA.2H_2O$, M=372.24 g/mol.
- Sodium chloride, $NaCl_{(s)}$, 58.44 g/mol.
- Demineralized water.
- bottle.

Procedure (1 L)

100 mg of Disodium EDTA dihydrate, 5.96 g of Trizma PreSet Crystals pH 7.6 (Sigma-Aldrich T7943) and 2.92 g of Sodium Chloride (NaCl) to 1 L of distilled H_2O. Smaller quantities can be made by proportional scaling.

To neutralize 18 uL PA mix by 3.2 mL dissolution medium, 3.20 g of sodium hydroxide (NaOH) is added to the solution.

Alternatively, prepare a 10 M $NaOH_{(aq)}$ and place the correct amount of $NaOH_{(aq)}$ in the receiver prior to dissolving the polarized sample (see Section “19. Hyperpolarizing a Sample, Dissolving the Sample”).

Preparation of Cleaning Medium

0.27 mM disodium ethylenediaminetetraacetate dihydrate.

Materials

- Disodium ethylenediaminetetraacetate dihydrate, $Na_2EDTA.2H_2O$, M=372.24 g/mol.
- Demineralized water.
- Bottle.

Procedure (1 L)

100 mg of Disodium EDTA dihydrate to 1 L of distilled H_2O. Smaller quantities can be made by proportional scaling.

19. Hyperpolarizing a Sample

System checks

Check that the temperature of probe and the VTI are both below 1.5 K.

Check that the dissolution module and sample cup has been cleaned and dried.

Loading the Sample

Materials

- DNP sample.
- Liquid nitrogen (optional).
- SpinAligner vials.
- O-rings.
- Wrench tool.

The materials are seen in Figure 18.

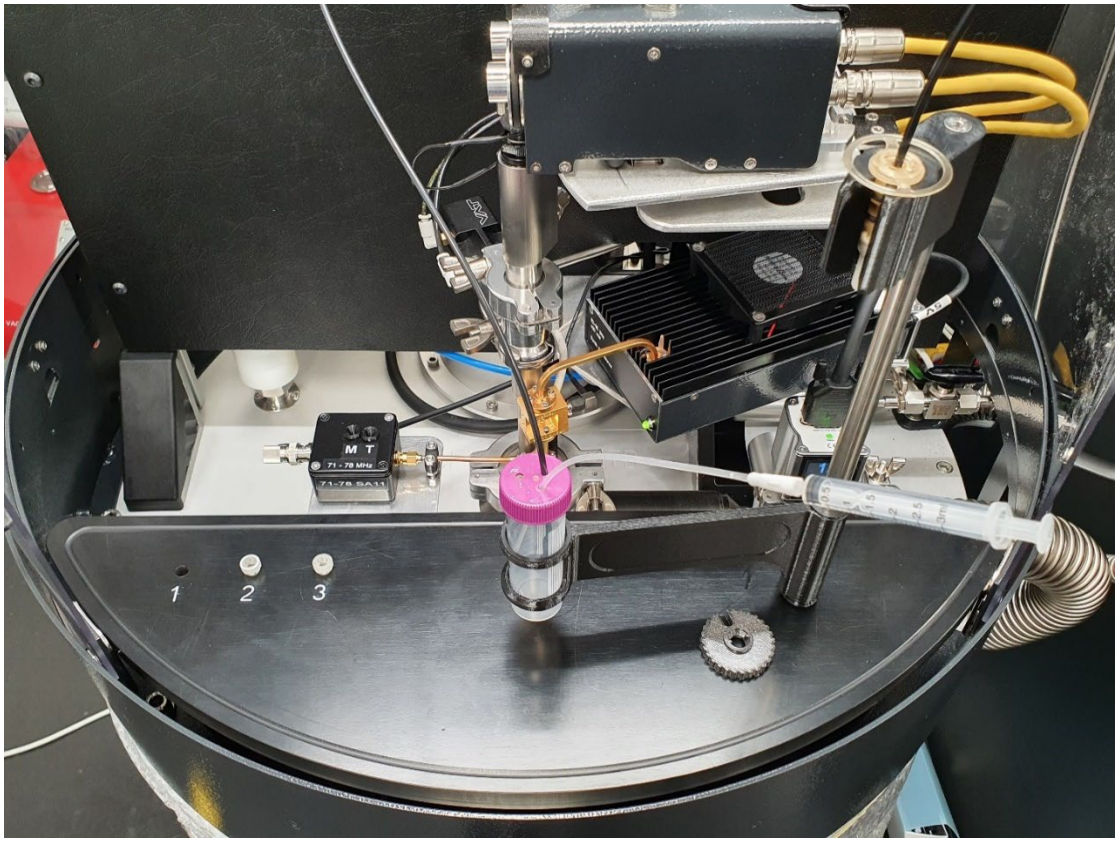


Figure 18: Photo of the SpinAligner tray with three vials placed in the holders, the receiver and wrench tool.

Always check that the vial is clean and without scratches, dents, or other damages. Do not remove the O-ring with a sharp tool. If it is not easily removable by hand with gentle tapping, compressed air is a convenient means to blow the used O-ring out of the grove. Compressed air is also used to dry the vial thoroughly after any cleaning.

Load up to 100 µL DNP sample into the SpinAligner vial (default pyruvic acid sample is 18 µL (approx. 22 mg)). Always supply a new O-ring to the vial groove when preparing a new sample.

The loading of the fluid path causes minimal heating of the DNP probe temperature, Figure 19.

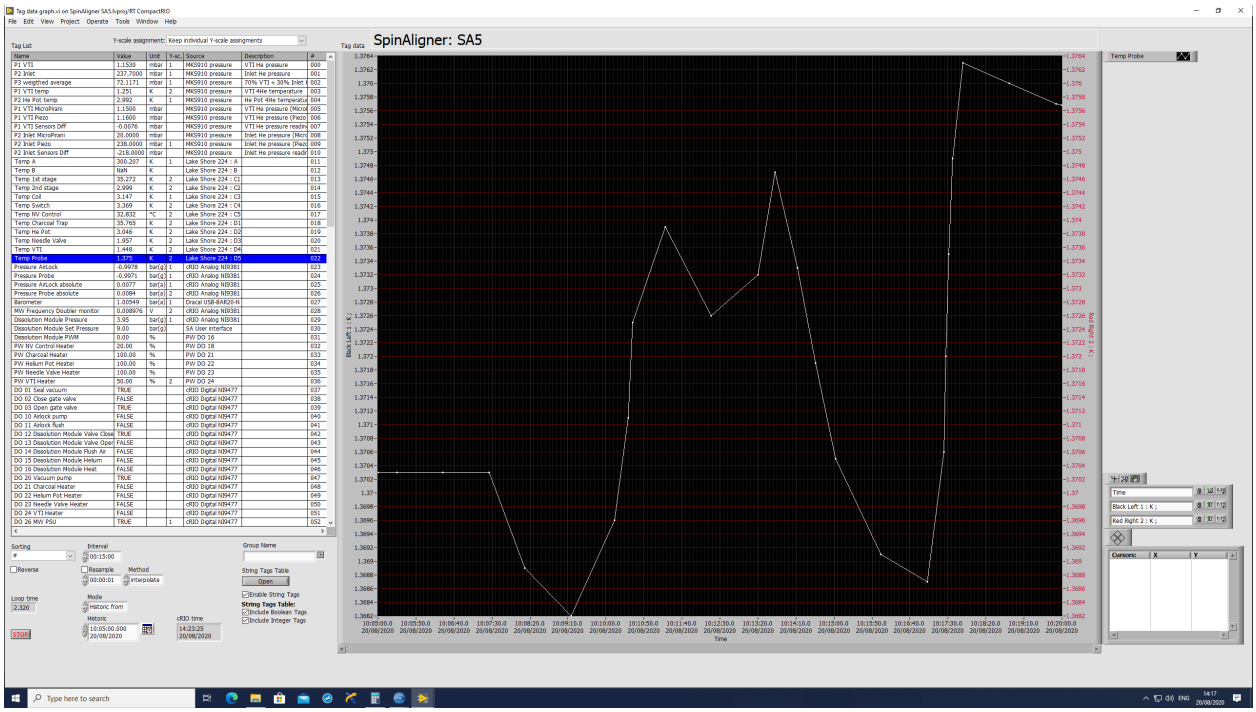


Figure 19: Temperature profile of DNP probe during sample insertion. The initial temperature is 1.370 K. When the gate valve opens, the temperature drops slightly before rising due to the introduction of the warm sample. The temperature rise is 10-20 mK due to the heat load of the warm sample.

Procedure

A convenient procedure for polarizing samples is to:

1. Take a clean sample vial and inspect for any debris. Use it only if the sample vial is clean and intact. Add your sample to the vial by pipetting the correct amount of sample into the vial, see Preparation of PA mix. Take precaution not to leave any material on the threading of the vial. To ensure that the sample is placed correctly at the bottom of the vial, the vial can be centrifuged. Place the vial in slot 1, 2 or 3 on the SpinAligner tray and supply a new O-ring to the groove.

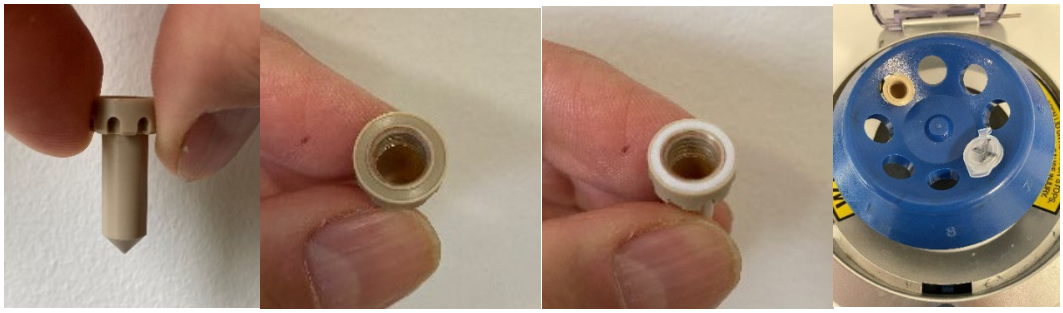
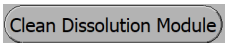


Figure 20: Sample vial. The grove should be clean and without dents and scratches. A new O-ring should be placed in the groove between each experiment.

2. Click the Load Vial button 

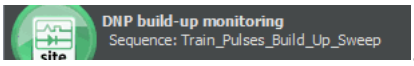
in the software (Figure 8 and follow the on-screen instructions. Typically, the fluid path is left with the previous sample vial attached, after a cleaning cycle*. Otherwise, attach an clean, dry vial (with a used O-ring). The software purges the fluid path with helium gas for 30 s.
3. Remove the empty vial (make sure that the old O-ring is also removed) and attach the new vial with your sample. When fastening the vial to the fluid path, tighten by hands, and then tighten further approx. ¼ of a turn using the wrench tool.
4. Insert the vial in the airlock. Close the airlock by pushing the dynamic seal in place while pulling the tube to keep the vial above the gate valve and click ok.
5. The insertion module will “home”, i.e. determine the position of the vial. The SpinAligner will then pump and flush the airlock with helium several times. Finally, the gate will open, and the fluid path is introduced into the cryostat and positioned within the NMR coil and microwave cavity.
6. When complete, the status field will say “Lowering vial to He done”.

**If in doubt about the condition of the fluid path, clean it by clicking* 

and following the on-screen instructions prior to mounting the vial containing a sample to be polarized. Either mount an empty sample vial (with a used O-ring) or leave the previous sample vial on the fluid path, while cleaning or drying the fluid path.

Polarizing the Sample

Procedure

The polarization is automatically initiated by the loading procedure if the box “Then Polarize” has been ticked. If not, click the “Polarize” button. Start observing the polarization build-up by switching to the SPINit software. Add  a new experiment: 

. Click  and click 

in the new window.

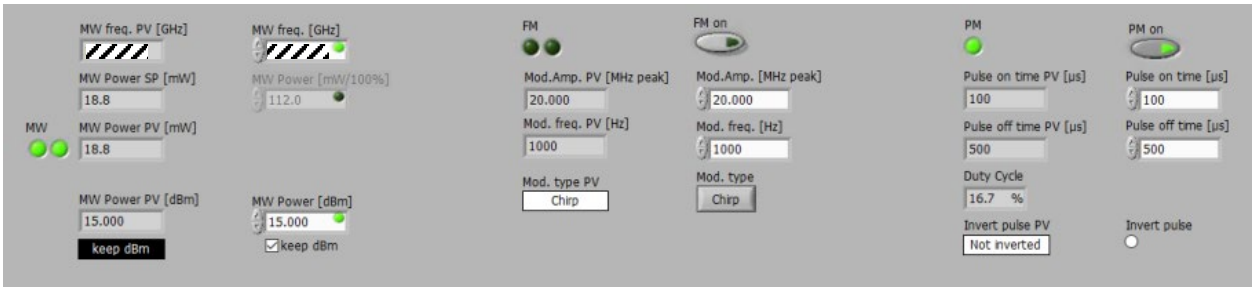


Figure 21: Default MW Source settings.

A DNP polarization build-up looks like the example in Figure 22.

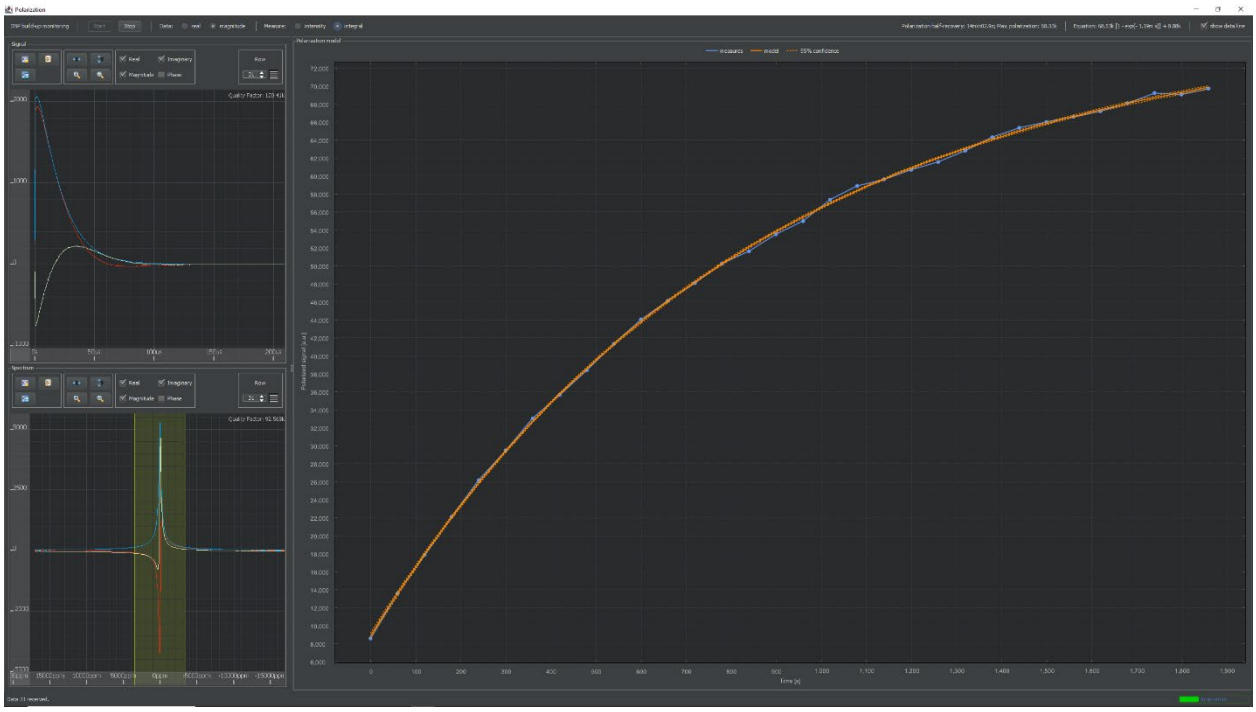


Figure 22: Example of DNP polarization build-up.

The experiment observes the polarization every minute (can be changed from the Parameters tab). The experiment can be terminated by clicking the "Stop" button and closing the pop-up window by clicking the X in the top-right corner.

The relaxation time constant, T_1, of the nuclear spins and the build-up rate constant of a DNP experiment is obtained by measuring the magnetization with a small constant flip angle, α<<90°, at equidistant timepoints, Δt. The magnetization at time *n+1* is related to the previous measurement as

$$M_{n+1} = M_n \cos\alpha \, e^{-\Delta t/\tau} + M_\infty\left(1 - e^{-\Delta t/\tau}\right)$$

By recursion

$$M_1 = M_i \cos\alpha\, e^{-\Delta t/\tau} + M_\infty\left(1 - e^{-\Delta t/\tau}\right)$$

$$M_2 = M_1 \cos\alpha\, e^{-\Delta t/\tau} + M_\infty\left(1 - e^{-\Delta t/\tau}\right)$$
$$= M_0 \cos^2\alpha\, e^{-2\Delta t/\tau} + M_\infty \cos\alpha e^{-\Delta t/\tau}\left(1 - e^{-\Delta t/\tau}\right) + M_\infty\left(1 - e^{-\Delta t/\tau}\right)$$

$$M_3 = M_2 \cos\alpha\, e^{-\Delta t/\tau} + M_\infty\left(1 - e^{-\Delta t/\tau}\right)$$
$$= M_i \cos^3\alpha\, e^{-3\Delta t/\tau} + M_\infty \cos^2\alpha\, e^{-2\Delta t/\tau}\left(1 - e^{-\Delta t/\tau}\right) + M_\infty \cos\alpha\, e^{-\Delta t/\tau}\left(1 - e^{-\Delta t/\tau}\right) + M_\infty\left(1 - e^{-\Delta t/\tau}\right)$$

and for M_n

$$M_n = M_i \cos^n\alpha\, e^{-n\Delta t/\tau} + M_\infty\left(1 - e^{-\Delta t/\tau}\right)\sum_{i=0}^{n-1} \cos^i\alpha\, e^{-i\Delta t/\tau}$$
$$= M_i \cos^n\alpha\, e^{-n\Delta t/\tau} + M_\infty\left(1 - e^{-\Delta t/\tau}\right)\frac{1 - \cos^n\alpha\, e^{-n\Delta t/\tau}}{1 - \cos\alpha\, e^{-\Delta t/\tau}}$$
$$= \frac{M_\infty\left(1 - e^{-\Delta t/\tau}\right)}{1 - \cos\alpha\, e^{-\Delta t/\tau}} + \left(\frac{M_\infty\left(1 - e^{-\Delta t/\tau}\right)}{1 - \cos\alpha\, e^{-\Delta t/\tau}} - M_i\right) e^{-n\Delta t\left(\tau^{-1} + ln\cos\alpha/\Delta t\right)}$$

Thus, the observed polarization build-up rate constant is increased as

$$\frac{1}{\tau_{observed}} = \frac{1}{\tau} + \frac{ln\cos\alpha}{\Delta t}$$

and the true build-up rate constant can be found by subtracting $\frac{ln\cos\alpha}{\Delta t}$ from the experimental value.

The steady-state polarization at infinite time is suppressed by the measurement according to

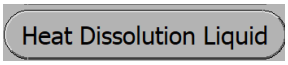
$$\frac{1 - e^{-\Delta t/\tau}}{1 - \cos\alpha\, e^{-\Delta t/\tau}}$$

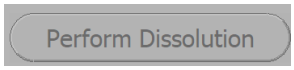
If a 1° flip angle is applied every minute for a time constant of twenty minutes, the polarization would be suppressed by 0.3%. However, if the flip angle is increased to 5°, it would be suppressed by 7.3%. If the time constant is sixty minutes for a 5° flip angle, the polarization would be suppressed by 18.5%.

Dissolving the Sample

When the sample is polarized, the buffer is added to the Dissolution Module:

1. Load the Solvent Syringe (Figure 23) with the correct amount of Dissolution Medium (3.4 mL (because of loss of 0.2 mL in tubing) for 18 uL PA mix).
2. Open the manual valve on top of the Dissolution Module.
3. Lower the tube to the bottom of the module (a mark on the tube indicates correct position) and dispense the dissolution medium, Figure 23.
4. It is important that the dissolution medium is dispensed in the solvent vessel. Do not dispense if the tube does not enter correctly.
5. Withdraw tube and close valve.

6. Push the 

bottom, Figure 8.
7. The Receiver is placed on the SpinAligner table. A small syringe (used for transferring the polarized sample to the NMR spectrometer/MR scanner) is connected to the Receiver retraction syringe to withdraw the hyperpolarized product. An example of the correct positioning of the receiver, fluid path and syringe can be seen in Figure 24.
8. When the dissolution module has reached the correct pressure, the "Dissolution solvent ready" light will turn green and the 

bottom will be enabled. Push the bottom and a dialogue box will ask if you are ready to perform the dissolution. Click OK when ready and the polarized sample will arrive at the Receiver within 5-10 s.
9. The polarized sample/solution is collected in the Receiver and transferred using the small syringe to the NMR spectrometer/MR scanner. Remember to mix the solution by overfilling the small syringe and pushing the liquid back again until the desired volume is left in the small syringe.
10. The fluid path is automatically removed from the DNP probe, into the airlock. The software will ask the operator to remove the fluid path from the airlock and into the cleaning and sample loading holder.

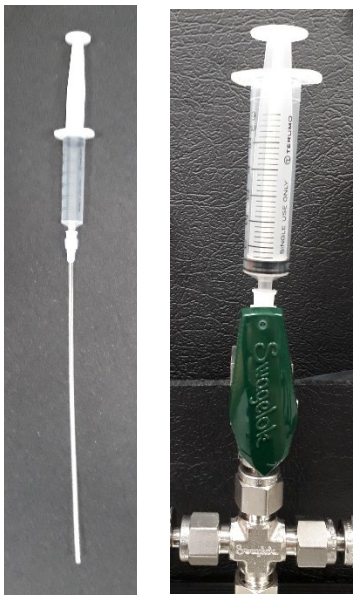


Figure 23: Syringe for loading dissolution medium.

Figure 24: Receiver with the exit tube inserted and a withdrawal tube attached.

Alternative to a neutralizing dissolution: It is possible to dissolve the pyruvic acid with a dissolution medium containing only EDTA and no base. In that case the neutralization must happen in the Receiver. An amount of base, corresponding to the amount of pyruvic acid in the vial, is added to the Receiver.

Corresponding amount of base: $V_{base}=V_{PA}*1.4$ ($V_{base}=V_{PA}*14$ mol/L / 10 mol/L)
V_{PA}: volume of pyruvic acid (sample in vial), V_{base}: volume of base to be added to receiver.

During the dissolution the temperature of the VTI rises briefly, Figure 25.

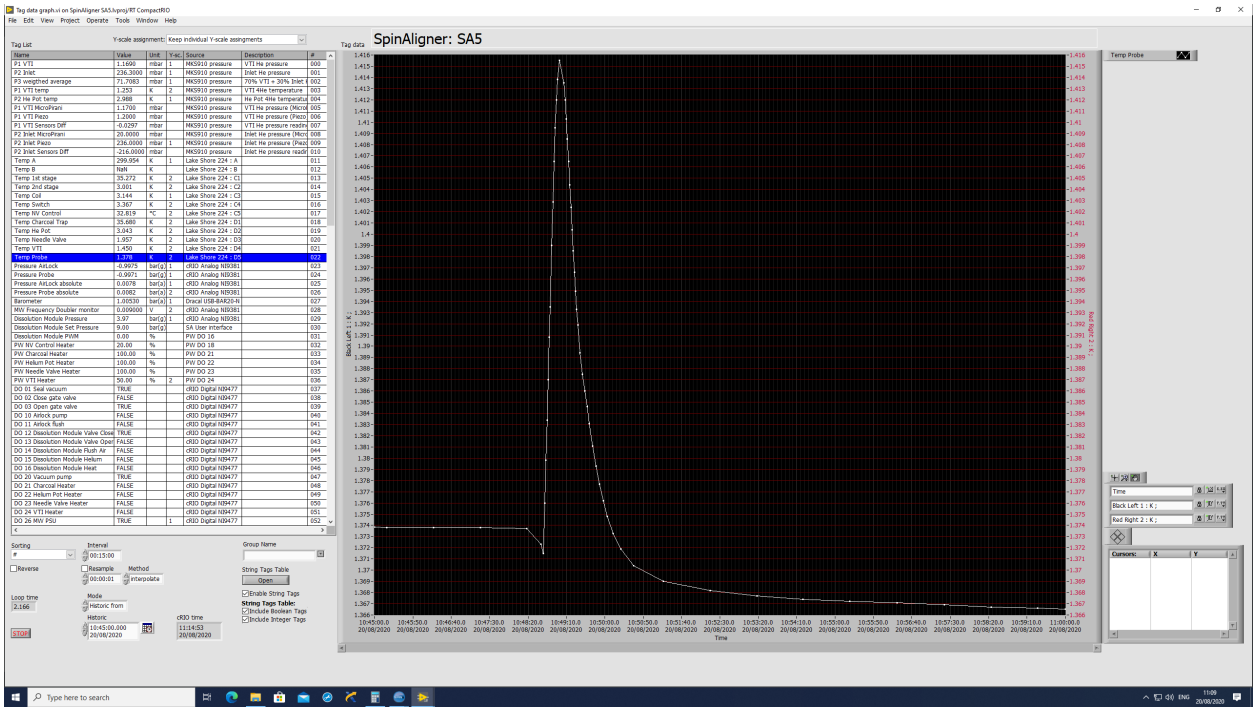


Figure 25: DNP probe temperature rise during the dissolution. The sample is raised approx. 10 cm before the injection of the hot solvent is initiated, and the fluid path is then immediately retracted to the air lock. The temperature rise due to the heat load of the injected hot solvent is approx. 40-50 mK.

Cleaning and Drying the Fluid Path

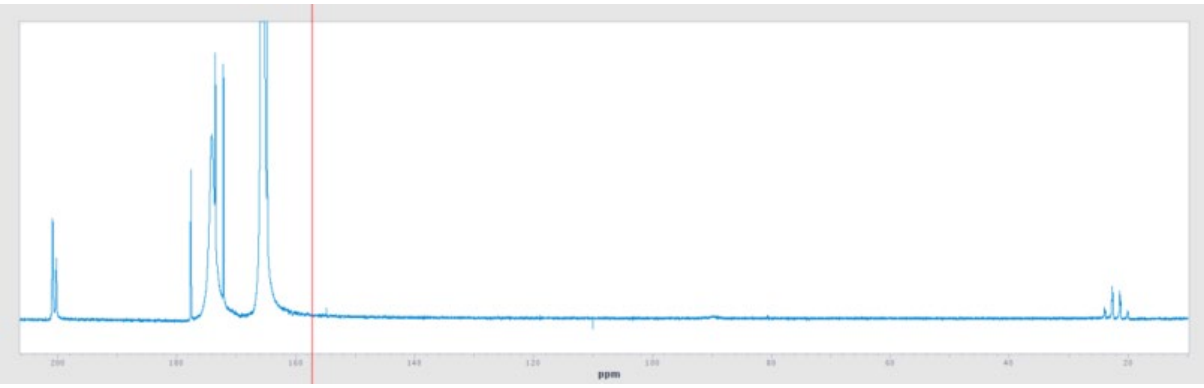
With the Sample Vial parked in the Vial Holder, the vial and fluid path should be cleaned and dried by clicking Clean Dissolution Module and following the on-screen instructions. For cleaning, use approx. 6 mL of de-ionized water added 0.1 g/L EDTA. The Exit Tube is inserted into the Receiver to collect the waste. When the cleaning and drying is completed, ensure that the Receiver is emptied and wiped with paper.

The drying time should be set to four minutes for a thorough drying. Drying can be repeated if the SpinAligner has been unused for some time (days) to ensure a completely dry Fluid Path. Likewise, the inlet port of the dissolution module can be opened during drying to remove droplets from the upper part of the dissolution module.

Quick guide to hyperpolarizing a sample

1. Initial setup:
    a. An empty vial is mounted to the fluid path.
    b. New vial containing sample is prepared.
2. Load vial:
    a. Click “load vial”.
    b. Remove empty vial (ensure that the O-ring is removed) and attach the new vial.
    c. Transfer vial to airlock and close insertion module.
3. Polarization:
    a. Click “Polarize” (if it’s not set to start automatically).
    b. Start acquisition of the build-up in SPINit.
4. Dissolution:
    a. Add dissolution medium to Dissolution Module.
    b. Heat dissolution medium.
    c. Remove stopper and mount exit tube to a clean and dry receiver.
    d. Mount syringe to withdrawal tube on receiver.
    e. Perform dissolution by clicking the button.
5. Cleaning
    a. Remove the vial from the airlock and place airlock plug.
    b. Clean and dry the fluid path.
    c. Now the system is back at the “initial setup” and ready for another sample.

20. Measuring the liquid state polarization



The polarization is quantified by measuring a series of pulse-acquire spectra with a low flip angle. Typically 3 s in between acquisitions and flip angle of 5°. If the thermal signal is too low, a 20° flip angle can be used, but the calculation of the polarization must take this into account. Before the thermal signal is measured, the sample is doped with approx. 15 uL of a Gadolinium contrast agent in the 600 uL NMR tube to reduce the T_1 of the ^{13}C.

The polarization is determined by comparing the integral of the signal of the first experiment in the series of hyperpolarized experiments with the thermal signal:

$$P = P_0 \times Enhancement = P_0 \frac{Integral\ of\ hyperpolarized\ signal}{Integral\ of\ thermal\ signal}$$

The polarization level at the time of dissolution can be obtained by extrapolating the time series of hyperpolarized experiments (the rate depends on the magnetic field, so this is only an estimate):

$$S(t) = S_0 e^{-t/T_1}$$

S_0 and T_1 being fitting parameters, t the time and S the NMR peak integral.

$$P_0 = \tanh\left(\frac{\hbar\gamma_{13C}B_0}{2k_BT}\right) \cong \frac{\hbar\gamma_{13C}B_0}{2k_BT} = \frac{h\nu}{2k_BT}$$

Magnetic field B_0 [T]	Frequency ν [Hz]	Temperature T [K]	Thermal polarization P_0 [ppm]
1.0	$11.02 \cdot 10^6$	301 (28 °C)	0.878
9.4	$100 \cdot 10^6$	298 (25 °C)	8.05
11.75	$125 \cdot 10^6$	298 (25 °C)	10.1
k_B=$1.381 \cdot 10^{-23}$ J/K, h=$2\pi\hbar$ =$6.626 \cdot 10^{-34}$ Js, γ_{13C}=2π 10.7084 MHz/T			

Polarization Measurement on a Magritek SpinSolve

Introduction

The SpinSolve bench-top NMR comes equipped with two software packages, Spinsolve and Spinsolve Expert. This document will describe how to shim the magnet (thereby also setting the spectrometer

reference frequency) in the SpinSolve software, and how to acquire a T_1 decay and make a polarization measurement of a hyperpolarized sample using the SpinSolve Expert and the MNova NMR software.

This will include the steps:

1. Shimming the magnet/checking the shims using SpinSolve software
2. Create a map for storing the experimental data
3. Choose type of experiment in the Spinsolve Expert software
4. Modifying a script to set the desired parameters for the experiment
5. Running the script (and the experiment)
6. Import to MNova
7. Calculating the polarization by comparing to a thermal reference

Shimming the magnet (once every experimental day)

1. Place the standard sample containing D_2O with 10% H_2O in the SpinSolve magnet
2. Open the SpinSolve software
3. Click on the tag “System” and choose “Shim”
4. Choose “Checkshim” and wait for the result
5. If shims are ok, then proceed to the next part. If not, run the recommended shimming procedure
6. When shimming is completed (line width is < 1 Hz) click on Ok
7. Close the SpinSolve software

Measuring a ^{13}C T_1 decay and using the first spectrum to calculate the polarization

1. Create a user map (*e.g.* with your name) in the Magritek Data folder on the Desktop
2. Open the “Spinsolve Expert“ software
3. In the Scripts menu choose the entry “PolarizationMonitoring_v1.2.2”. This is a GUI that allows setting up standard Polarization/T_1 measurements for ^{1}H or ^{13}C.
4. In the window that opens you should set the parameters as follows:
    a. Number of spectra: 1.
    b. Flip angle: 5 (degrees).
    c. Sampling time: 3 (number of seconds between pulses).
    d. Receiver gain: 40 (dB).
    e. Nucleus: 13C.

    The parameters you have set in the script will over-ride the pulse program parameters you see in the parameters box in the main window
5. You can set automatic integration limits if you want to follow the signal development in the Spinsolve expert software (not necessary). For this, you can also set processing parameters such as shown spectral width and line broadening function
6. In the Data Path box navigate to your project folder and give your experiment a sample name

After placing your hyperpolarized sample in the Spinsolve, start the NMR acquisition by choosing “Start” from the GUI. When the experiment has completed you will find the saved experiment in your project map. You will need to copy this map to a USB-stick, if the SpinSolve is not connected to a network drive.

Import to MNova

1. In the MNova "Stack" menu choose "Directory Spectra Stack"
2. In the pop-up menu window entry "Import Directory" navigate to the map that contains all your individual spectrum maps
3. Choose "Select Folder" (it will appear empty in the "Select Directory" window)
4. By pressing "Ok" the import of the spectra starts. By default, they are all collected into a stack after completed import
5. This stack can now be treated as any stack of spectra in MNova

Measuring a thermal equilibrium reference

This part assumes that you add 15 µL of gadolinium contrast agent to each 600 µL of sample, to reduce the T_1.

1. Close the Polarization Measurements GUI
2. In the "Experiments to run" box delete all entries by selecting each entry and pressing X on the panel
3. In the Experiments to add box choose **1Pulse-C**
4. The default pulse length (62 µs) corresponds to a 90° pulse. If you want to run your reference the same way as you HP experiment set the pulse length to 3.8 µs (5°). *A better SNR is obtained by running at 20° (15.2 µs) and recalculate the obtained signal integral value by multiplying with sin(5°)/sin(20°)=0.255*
5. Make sure the receiver gain is set to a value of 40 (the same as the hyperpolarization experiment)
6. Set the repetition time to something sensible with respect to relaxation. A value of 1.5 s should be fine for samples with gadolinium added
7. Adjust the number of scans to e.g. 3,600 (1h45min scan time). How many scans you need will depend on the chosen pulse angle and the concentration of the sample
8. Start the experiment by pressing the "Run" button

After importing spectra into MNova the integral scale of the spectrum must be changed to be able to read the integral value. To do this perform the following:

1. Choose "Arithmetic" from the "Advanced" menu. In the "Formula" column click on "B" and change it to 10,000*A. Click "Ok". This will produce a new spectrum page with the scaling enhanced by a factor of 10,000
2. Remember to divide the obtained value with 10,000 before calculating enhancements
3. Remember that the SpinSolve software performs an internal averaging of the integral to the number of scans

The polarization enhancement is calculated as the ratio of the integrals of the hyperpolarized spectrum (first) and the thermal equilibrium reference. The polarization can then be calculated from the equation above.

Analysis using external reference

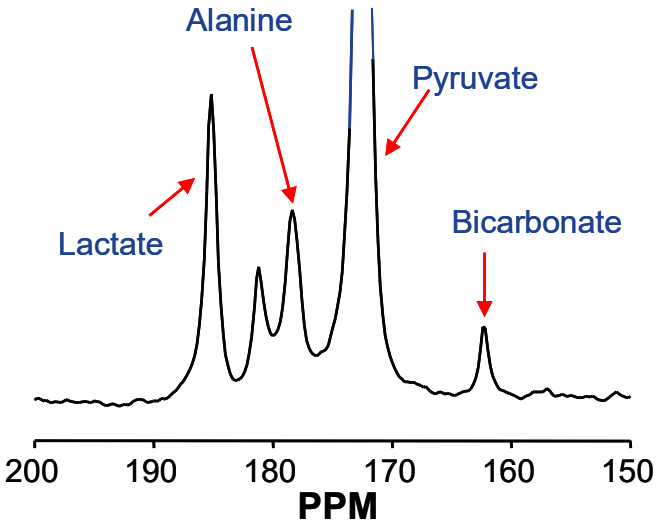
External polarization reference: a sealed NMR tube with ^{13}C urea (1960 mM) in H_2O / D_2O with addition of gadolinium contrast agent. The T_1 of this reference sample was measured to 30 ms on the Varian 400 MHz (9.4 T, 303 K).

This reference is sufficiently concentrated to acquire a reference signal using the typical HP settings (5° flip angle and 40 dB receiver gain) and 256 scans. Since the SpinSolve software performs an internal averaging of the integral to the number of scans, only the concentration will need to be corrected for. For a rough estimation of enhancement, a pre-recorded reference integral value may be used together with an estimated concentration of the hyperpolarized sample. The urea integral (PW 5, RG 40) was measured to 2.2E-6 area units / mM ^{13}C.

*To use this method simply multiply this factor with the estimated sample concentration. This results in the rough thermal value. Example: If the sample concentration is 50 mM and the recorded integral of the hyperpolarized sample is 15 the thermal factor will be 50 * 2.2E-6 and the enhancement will be 15 / (50 * 2.2E-6). The polarization will then be 15 / (50 * 2.2E-6) * 0.878E-6 = 12.0%.*

For more careful determination of the enhancement the reference should be run in close connection to the HP experiment, and a careful concentration analysis of the HP sample will have to be performed, e.g. by UV-Vis (knowing the ratio of radical / ^{13}C) or by high-field NMR at a later occasion.

21. Analyzing the data (post processing)



For kinetic modelling of in vivo data (e.g. acquisition of a series of spectra or chemical shift imaging) the data can be modelled in jMRU in order to quantify metabolites. For that purpose, the following parameters can be a good starting point.

The following prior knowledge can be used (32.131 MHz/3.0 T):

Peak #1 (pyruvate):

Frequency – estimated

Amplitude – estimated

Shape – fixed – Gaussian

Phase – fixed relative – 0.0

Line width – constrained – 0.0-1.0

Peak #2 (lactate):

Frequency – fixed shift – peak#1+392.0 (chemical shift difference of 12.2 ppm)

Amplitude – estimated

Shape – fixed – Gaussian

Phase – fixed relative – 0.0

Line width – fixed ratio – peak#1*1.0

Peak #3 (pyruvate hydrate):

Frequency – fixed shift – peak#1+270.0 (chemical shift difference of 8.4 ppm)

Amplitude – estimated

Shape – fixed – Gaussian

Phase – fixed relative – 0.0

Line width – fixed ratio – peak#1*1.0

Peak #4 (alanine):

Frequency – fixed shift – peak#1+180.0 (chemical shift difference of 5.6 ppm)

Amplitude – estimated

Shape – fixed – Gaussian

Phase – fixed relative – 0.0

Line width – fixed ratio – peak#1*1.0

Peak #5 (bicarbonate):

Frequency – fixed shift – peak#1+321.3 (chemical shift difference of -10.0 ppm)

Amplitude – estimated

Shape – fixed – Gaussian

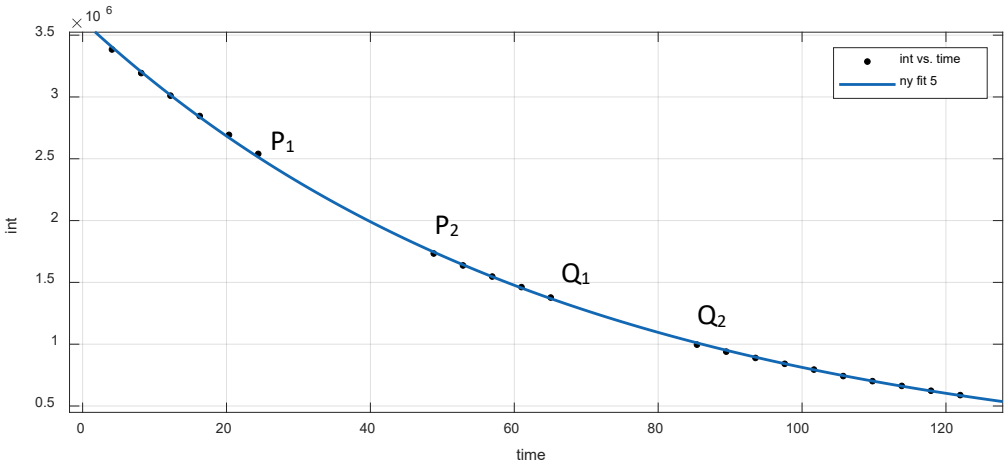
Phase – fixed relative – 0.0

Line width – fixed ratio – peak#1*1.0

22. dDNP data

Liquid state T_1 for [1-^{13}C]pyruvate

The earth field T1 has been determined by observing the decay of the hyperpolarized magnetization by lifting the sample out of the SpinSolve magnet into the earth field for two periods of approx. 15 s.

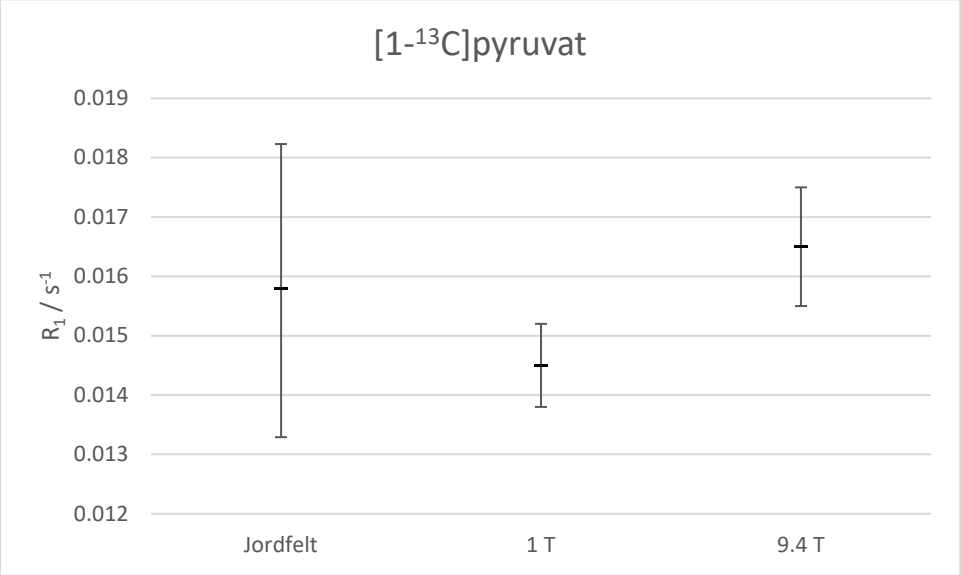


It is seen that R_1 (earth field) > R_1 (1 T). The overall R_1 is 0.0149 s^{-1} (67 s).

Q_2 can be corrected by the factor P_2/Q_1 and the three points can be fitted to an exponential earth field T_1: B_{PQ} = 0.0158 s^{-1} (0.01329, 0.01823).

The average of a series of measurements at 1 T and 9.4 T can be summarized as:

Magnetic field	R_1 / s^{-1}	T_1 / s
Earth field	0.0158 (0.01329, 0.01823)	63
1 T	0.0145 +/- 0.0007 (n=12)	69
9.4 T	0.0165 +/- 0.0010 (n=42)	61



The uncertainty for the earth field T_1 is large since the time resolution is only 4 s.

23. Calibrations, Adjustments and Optimizations

Adjusting the ^{13}C NMR frequency and checking the tune and match of the DNP probe

Over time, the magnetic field may drift, and the user should pay attention to this and perform calibration of the MW frequency regularly (1 kHz drift of the ^{13}C resonance frequency would correspond to a 2.7 MHz shift of the DNP optimal microwave frequency), see Optimizing the Microwave Frequency. The NMR frequency only needs to be adjusted if it has drifted more than approx. 50 kHz off-resonance.

The ^{13}C NMR signal can be acquired in a standard pulse-acquire experiment or determined from the latest experiment. The frequency offset can be measured by clicking the SNR button when viewing the NMR spectrum and then clinking the NMR signal peak. The offset will then be reported below the spectrum, and the transmitter frequency can be changed until on resonance. Once the ^{13}C resonance frequency has been determined the correct ^{1}H frequency and microwave frequency can be calculated from the Excel sheet on the computer desktop (SpinAligner Frequency Calculation Tool.xls) or calculated from the equation

$$f_{1H} = 3.97691 \cdot f_{13C}$$

The ^{1}H frequency is then entered in SPINit by going to the Instrument tab and then the magnet tab. Double click in the field to get a window where the ^{1}H frequency can be entered. This will ensure that SPINit calculates the correct ^{13}C frequency (for ^{13}C-pyruvic acid). The calculated microwave frequency from the Excel sheet is entered into the SpinAligner software as a starting point for the calibration of the microwave frequency. The optimal MW frequency (for trityl) is calculated from the equation

$$f_{MW}(GHz) = 2.6177 \cdot f_{13C}(MHz)$$

The tuning and matching of the DNP probe should also be checked at this occasion. Checking the tuning and matching of the DNP probe is also sensible if the signal is lost or reduced for unknown reasons. The tune and match box (Figure 26) is used to tune the coil to the frequency of ^{13}C (approx. 71.85 MHz). Go to the Control tab and Tune function in SPINit, chose ^{13}C and start the scan to see the NMR probe resonance while tuning.

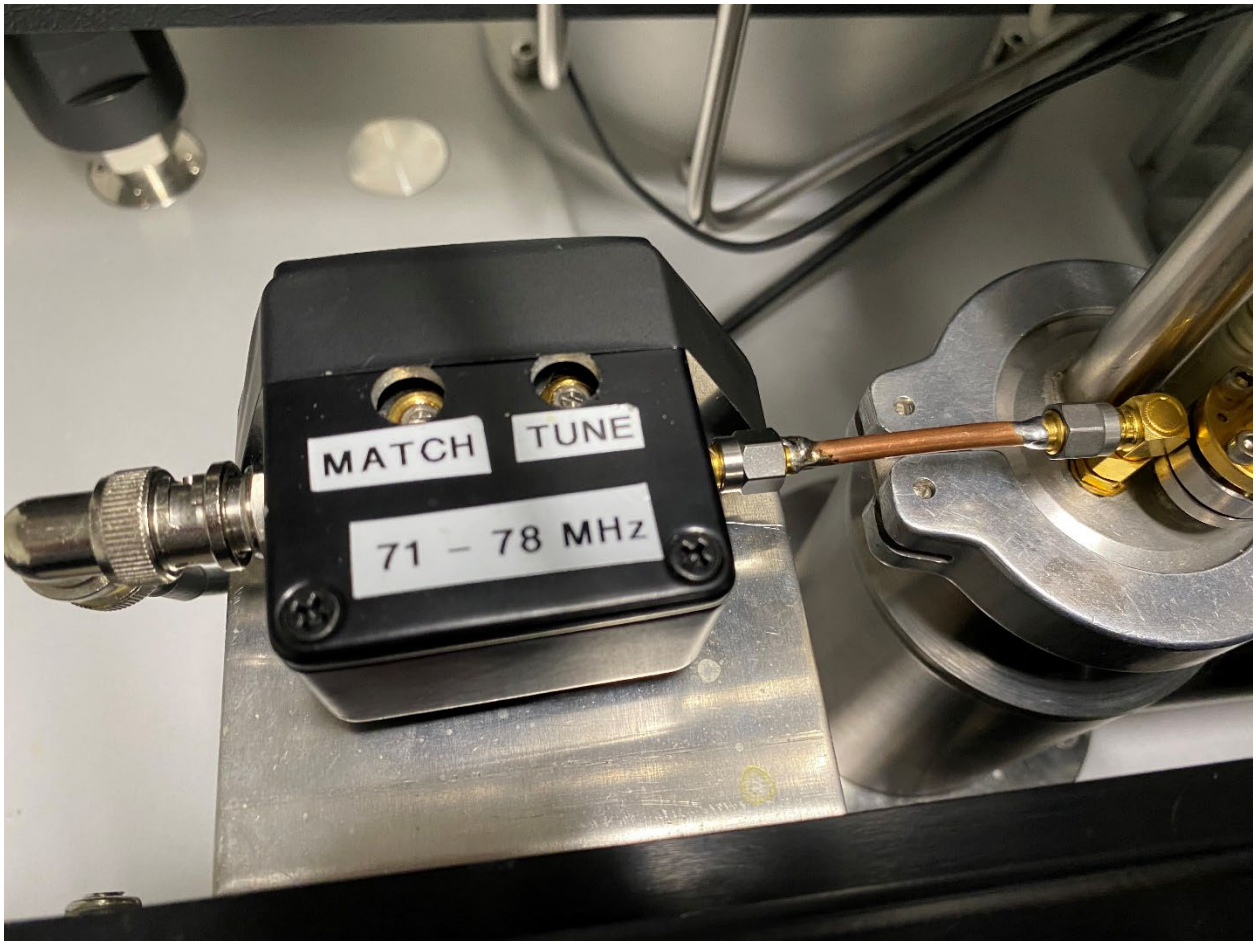


Figure 26: Tune and Match box.

Optimizing the Microwave Frequency

The microwave frequency is optimized by stepping through the frequency range that is expected to give DNP. At each microwave frequency, the ^{13}C polarization is saturated before it will apply microwaves for the period entered in SPINit. In the SpinAligner software Control panel, select the run mode “Frequency sweep” in the Sweep field on the Control Panel tab. Ensure that the MW PSU is “on”. Enter the desired MW frequency start value in GHz and the step size (Freq. inc.) in MHz. The MW frequency start value should be smaller than the expected optimal MW frequency e.g. the calculated theoretical optimal frequency (from the Excel sheet)

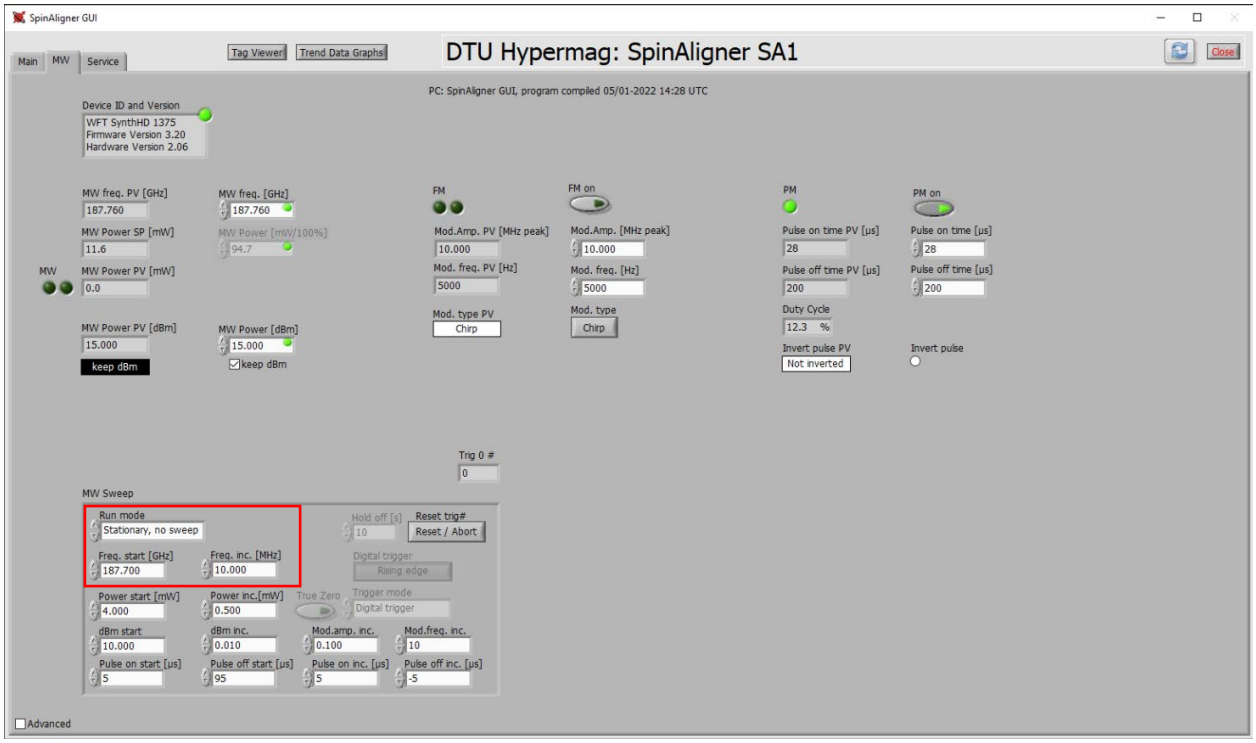


Figure 27: SpinAligner software Control panel. The red box indicates the dropdown menu to select microwave frequency sweep, the start value and increment.

In SPINit, either clone a previous microwave sweep experiment or load a new experiment (click the + button and select  "DNP Frequency Sweep"). You may want to adjust the number of microwave frequency steps (Nb_2d) and the time (Polarization_Growth_Delay) for polarization between each frequency increment. More points will cover a wider sweep and longer polarization growth delay will increase the signal. Next, you must adjust the initial frequency and frequency step to be consistent with the SpinAligner software. Click the Scan button,  , to open a new window. In the new window, click the start button. A typical wide DNP sweep is seen in Figure 28. The MW frequency of the first (positive DNP) of the two maxima is noted and entered as MW frequency in the SpinAligner software. At the end of the experiment, the pop-up window is closed by clicking the X in the top-right corner.

During the MW frequency sweep, the temperature of the DNP probe is affected by both the saturating NMR pulses and the MW power, Figure 29. The train of saturating NMR pulses provides a temperature rise of approx. 28 mK in the example and the MW power increases the temperature by approx. 24 mK for 50 mW output.

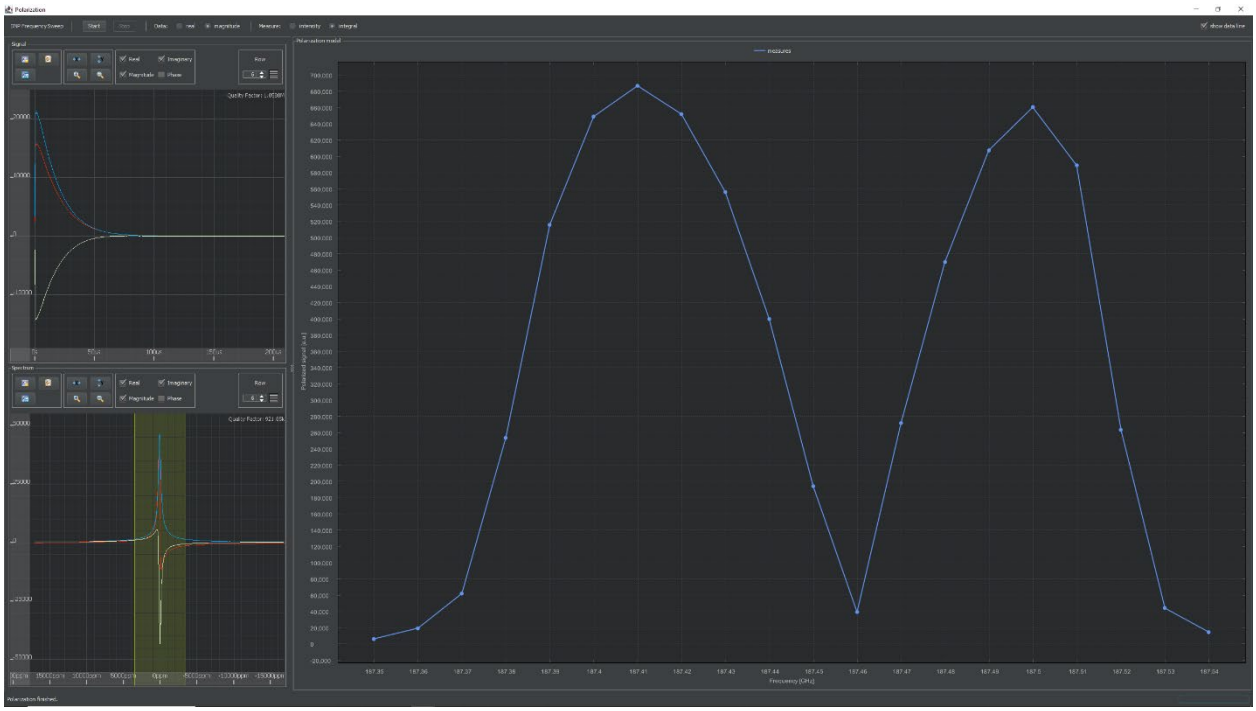


Figure 28: Example of microwave frequency optimization. Determine the microwave frequency of the first of the two maxima and enter the value in the microwave frequency field in the SpinAligner software.

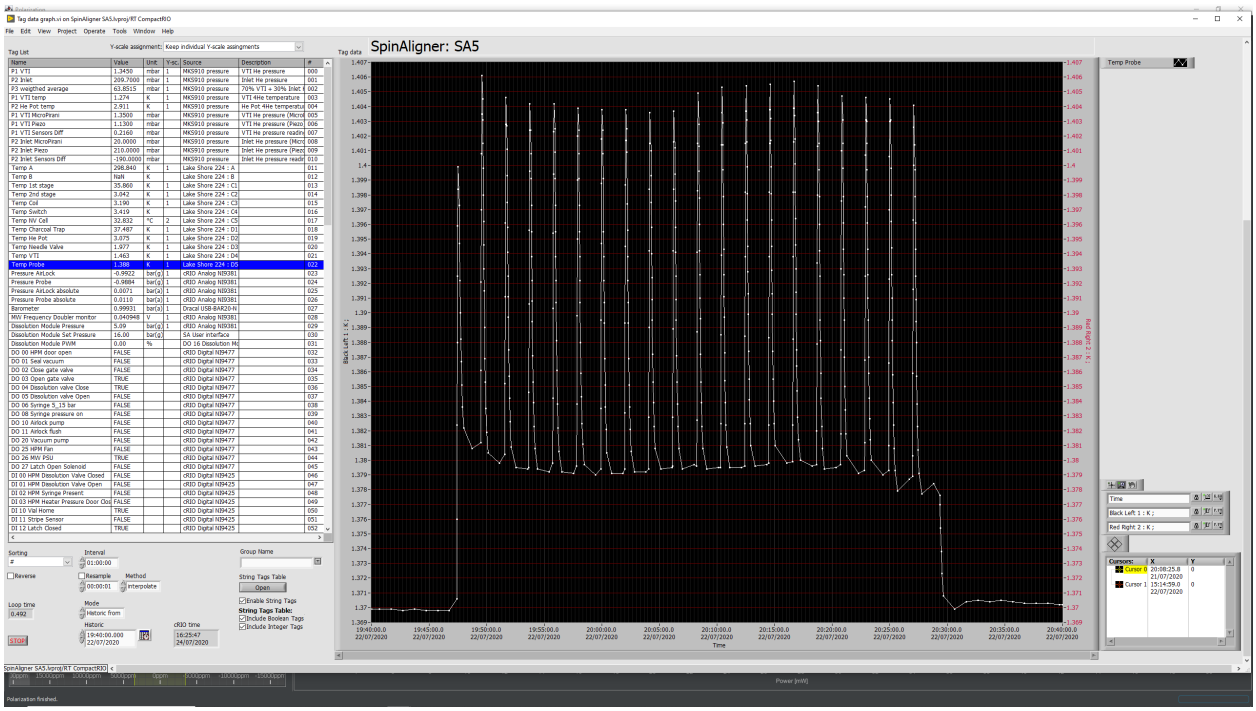


Figure 29: DNP probe temperature during a MW frequency sweep with 20 increments. The spikes are due to the saturating train of NMR pulses (approx. 25 mK increase), and the baseline shift is due to the MW power (total of approx. 10 mK).

Optimizing the Microwave Power

Optimizing the microwave power is performed in a similar manner as the microwave frequency. Select Power sweep in the run mode in the SpinAligner software. You may want to change the power start value (mW) and increment (mW) depending on sample properties or previous calibrations. In SPINit, either clone a previous microwave sweep experiment or load a new experiment (click the + button and select  "DNP Power Sweep"). You may want to adjust the number of microwave power steps (Nb_2d) and the time (Polarization_Growth_Delay) for polarization between each power increment. More points will cover a wider sweep and longer polarization growth delay will increase the signal. Next, you must adjust the initial power and power step in SPINit (the two parameters appear in the Setup tab) to be consistent with what you entered in the SpinAligner software. Click the Scan button to open a new window. In the new window, click the start button. A successful optimization is seen in Figure 30. You should select the power that provides the maximum signal by entering it into the SpinAligner software in the "MW power" field. At the end of the experiment, the pop-up window is closed by clicking the X in the top-right corner.

During the MW power sweep, the temperature of the DNP probe is affected by both the saturating NMR pulses and the MW power, as illustrated in Figure 31. The train of saturating NMR pulses provides a temperature rise of approx. 28 mK in the example and the MW power increases the temperature by approx. 24 mK for 50 mW output.

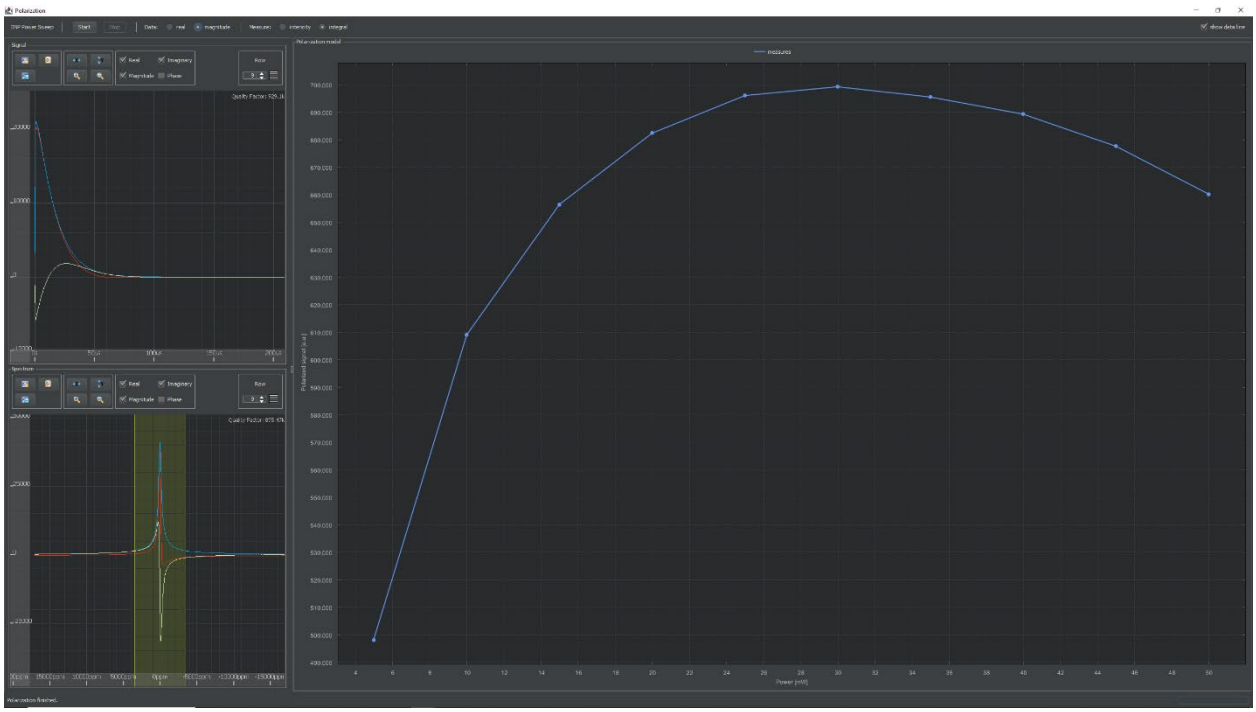


Figure 30: Example of microwave power optimization. Determine the power level that gives highest DNP and enter the value in the microwave power field in the SpinAligner software.

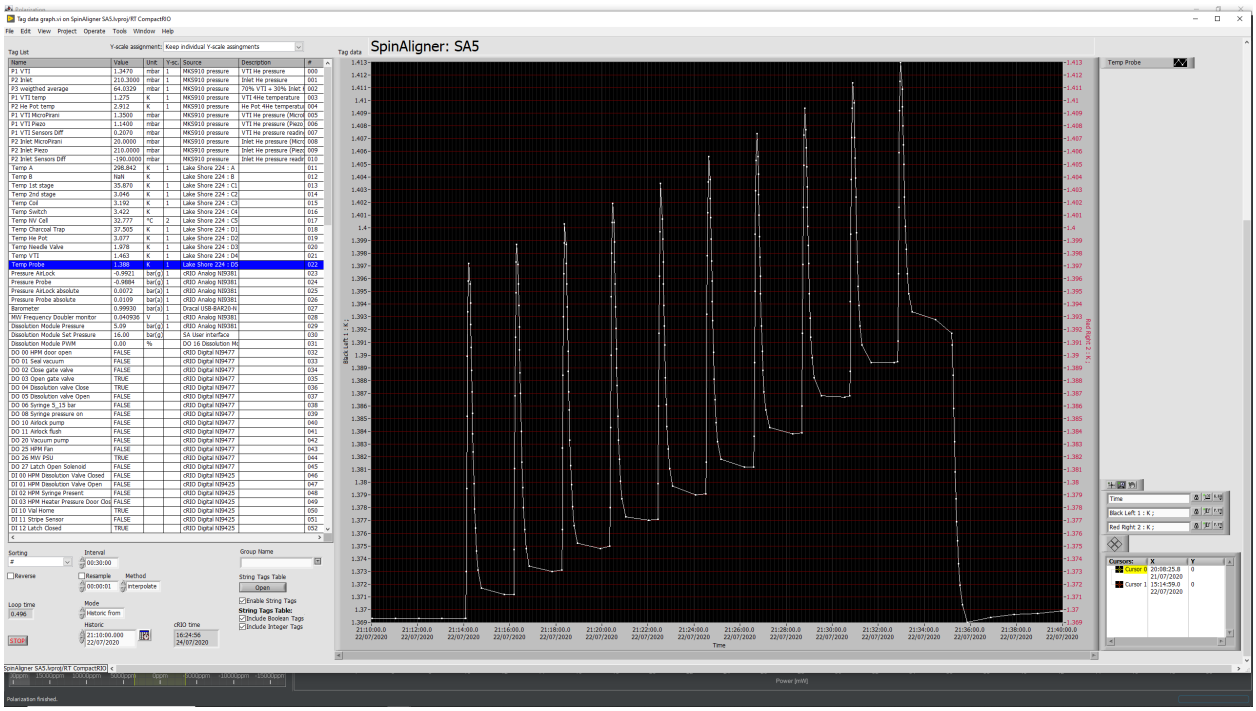


Figure 31: DNP probe temperature during a MW power sweep with 10 increments. The spikes are due to the saturating train of NMR pulses (approx. 28 mK increase), and the baseline shift is due to the increasing MW power (total of approx. 24 mK).

Calibrating the flip angle

The Experiment 

can be used for flip-angle calibration by the saturation method. The relevant parameters are encircled in Figure 32. The decaying signal integral or intensity is fitted to an exponential decay: $Ae^{-t/\tau} + B$, where A, B and τ are fitted parameters.

The flip-angle, α, and B_1 are calculated using the relations:

$\alpha = \cos^{-1} e^{-(\tau N)^{-1}}$, where N is the number of pulses per second, and $gB_1 = \alpha/\tau_p$ (in Hz), where τ_p is the pulse width.

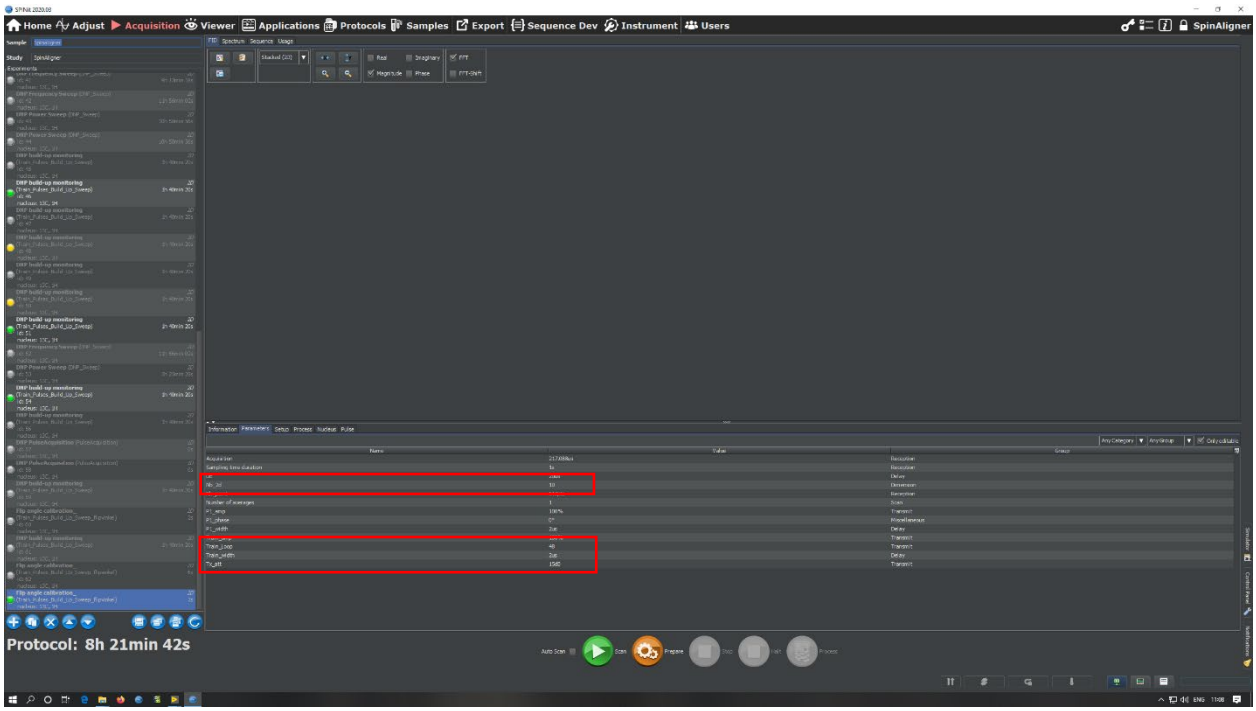


Figure 32 Flip-angle calibration. The important parameters have been highlighted in red. Train_loop is the number of pulses between each acquisition pulse minus 1. This setup is for calibration of 2 us pulses at 100% amplitude and 15 dB transmitter attenuation. 49 pulses are applied before each acquisition and 10 NMR signals are acquired.

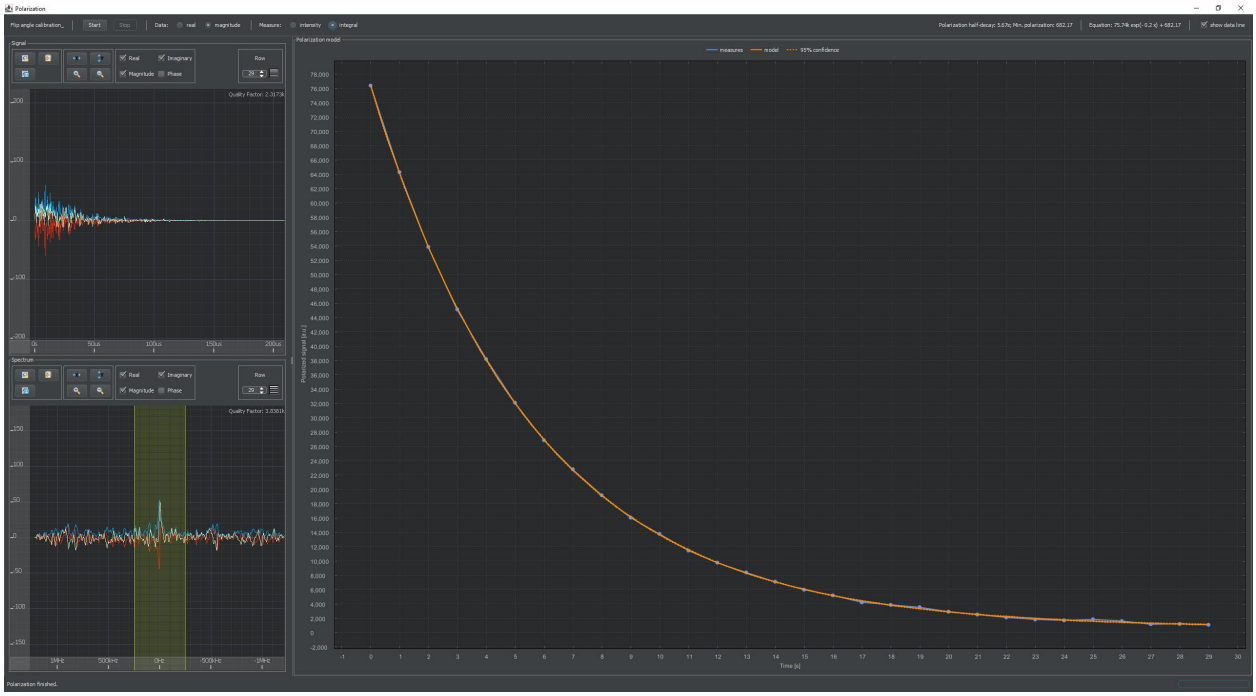


Figure 33: Example of flip-angle calibration. The fitted time constant is 5.67 s for 50 pulses of 2 us every second at 15 dB attenuation. $\alpha = cos^{-1}\, e^{-1s/(50 \cdot 5.67s)} = 4.81°$, *i.e.* $\gamma B_1 = 4.81\,°/(360\,° \cdot 2\,us) = 2\pi \cdot 1.1\,kHz$.

An example of a flip angle calibration is shown in Figure 33.

Adjusting the magnetic field – consult Polarize

When the SpinAligner is installed, energized after a quench or experience an extraordinary field change, the magnetic field has to set correctly. This is typically done from the ^{63}Cu signal from the probe (no sample; just the metallic copper). The magnetic field (^{1}H resonance frequency) should be set in SPINit to ensure that SPINit calculates the correct ^{13}C resonance frequency. This ensures that the ^{13}C NMR signal is always close to on-resonance (within a few kHz) and the MW frequency is close to optimal.

The NMR signal is acquired in a standard pulse-acquire experiment. SPINit has an application, which can be used to determine the ^{63}Cu NMR frequency. The tune and match box (Figure 26) is used to tune the coil to the frequency of ^{63}Cu (approx. 75.9 MHz). Go to the Control tab and Tune function in SPINit, chose ^{63}Cu and start the scan to see the NMR probe resonance while tuning. Once tuned, the ^{63}Cu signal can be searched by changing the NMR frequency in steps of e.g. 100 kHz until the signal is found. If the ^{63}Cu signal is far away (>100 kHz) from 75.9 MHz, it should be considered to adjust the magnet current to get the magnetic field closer to this resonance condition.

The frequency offset can be measured by clicking the SNR button when viewing the NMR spectrum and then clinking the NMR signal peak. The offset will then be reported below the spectrum, and the transmitter frequency can be changed until on resonance. Once the ^{63}Cu frequency has been determined the correct ^{1}H frequency and microwave frequency can be calculated from the Excel sheet on the computer desktop (SpinAligner Frequency Calculation Tool.xls). The ^{1}H frequency is then entered in SPINit by going to the Instrument tab and then the magnet tab. Double click in the field to get a window where the ^{1}H frequency can be entered. This will ensure that SPINit calculates the correct ^{13}C frequency (for ^{13}C-pyruvic acid).

The equation for calculating the ^{1}H resonance frequency from the ^{63}Cu resonance is

$$f_{1H} = 3.76446 \cdot f_{63Cu}$$

Remember to tune the DNP probe back to ^{13}C.

The magnet power supply is typically switched off in the rack but is turned on from the button on the front panel.

The power supply is controlled from the SpinAligner software and the Magnet PSU panel. If the magnet is at field and needs adjustment, the Target Current and Persistent Current should be the same. Choose Persistent Mode B and push the Ramp Start button. The power supply will ramp the current to the target and then turn on the switch heater. When the State turns to Finished, the Target Current can be adjusted, and the NMR signal observed to reach the magnetic field that is desired.

If the magnet has quenched, simply press the start button to make the power supply turn on the switch heater and the ramp to the Target Current. The software will turn off the switch heater when the target field has been reached and return current to zero.

Switch off the power supply in the rack again from the front panel switch.

Calibrating the SMC pressure gauges (airlock and DNP probe) – consult Polarize

If the airlock and DNP probe pressures seem unreasonable, contact Polarize for advice. Calibration can be performed by this procedure:

1. Cooldown should be disabled (Leybold turned off) and the VTI and DNP probe temperatures should be above 100 K.
2. Clean the airlock with pump-flush procedure ("Conserve AirLock" button on the Control panel). It takes approx. one minute).
3. Then open the gate valve and regulate the probe pressure to about 0 mbar(g) by pumping out helium (and adding again if necessary).
4. Adjust the offset of the Probe Pressure until the Probe and AirLock Pressure agrees.
5. Close the gate valve.
6. Run the cleaning procedure for the Probe, that will top up the helium pressure and close the gate valve.
7. Cool the probe by turning on the Leybold Pump.
8. When the probe is cold with super fluid helium condensed in the Probe, the scale factor of the Probe Pressure must be adjusted until the absolute Probe pressure matches the vapor pressure of helium as measured by the MKS sensor.
9. Conserve the AirLock again, and when evacuation is finished open the gate valve.
10. Adjust the scale factor of the relative AirLock Pressure, until absolute and relative pressure of the AirLock matches those of the Probe.

The two sensors are now calibrated.

Calibration of Needle Valve – consult Polarize

The needle valve (NV) is controlled electronically from the software. However, it also has a mechanical actuation in series. The NV control hardware is seen in Figure . The NV control heater is limited in software to 40 °C. Fully closed should have been adjusted to 38 °C. The procedure below is performed on a cold system.

1. Turn off the Leybold pump and let the VTI and inlet pressures settle, typically in the range 200-300 mbar.
2. Open the mechanical adjustment knob fully (by turning anti-clockwise several turns).
3. Set the NV control heater to 38 °C. If in doubt, check that the mechanical adjustment is fully open as the temperature of the NV control heater increases.
4. Turn the mechanical NV adjustment knob clockwise to closed position (firm by hand).
5. Turn on Leybold pump to verify that NV is fully closed. The VTI pressure should drop to almost zero (<0.01 mbar).

6. Turn off NV control heater and observe carefully the VTI pressure. As it starts to rise and reaches approx. 1 mbar, note the temperature of the NV control, and turn the NV control heater on at this temperature. The regulation is expected to happen a few degrees below 38 °C, and the VTI cool down begin. If satisfactory response of the NV is observed and the regulation temperature is as expected, the calibration is complete.

The automatic cool down can now be attempted and verified by going to the Main panel and pushing the Cooldown button.

Figure 34: The NV control hardware.

24. Maintenance

Cleaning the probe – consult Polarize

The SpinAligner can be programmed to clean the probe partially (due to accumulation of residual air from sample loadings) on a weekly basis, see Automatic Cooldown. However, in idle mode the probe temperature only reaches 150-200 K, which will only remove the nitrogen and oxygen. If water has accumulated, a more serious cleaning is required. This should only be performed after consulting Polarize.

Step 1 – Make sure that the sample has been removed from the probe.

If the sample will not move, then leave it until it becomes possible to do so as the temperature of the probe and VTI increases in step 4. Stop the Leybold pump from the 'Control' panel by pushing the Run button. Monitor that the inlet pressure P2 does not increase above about 700 mbar, and that the coil temperature always stays below 5 K.

Step 2 – Warm the Probe and VTI to 300 K

Increase the VTI High Limit Temperature to 5 K. Then set the VTI High Limit Temperature to 10 K. Then 50 K, and set the VTI heater power now to 10000, or 10 %. Then 100 K, and set the VTI heater power now to 20000, or 20 %. Then 150 K, and set the VTI heater power now to 50000, or 50 %. Continue in 50 K steps until reaching 300 K. Wait until the probe temperature is also close to 300 K.

If the ice blockage is due to air (as opposed to water ice), it should be possible to remove the ice at a temperature above approx. 70 K (100 K in step 4). In that case it is not necessary to increase the temperature further to 300 K. Instead, skip step 3 and go to step 4 and do Pump-Flush.

Step 3 – Clean Probe for Ice

When a temperature of 300 K has been reached, remove the stopper from the airlock. Use, e.g., a borescope to look for ice inside the probe. Blow with warm Helium gas to clean for ice. Close the Airlock with the stopper. If residual water is present, you may use a stick with a piece of cloth that is firmly taped or glued to the stick end to do a final cleaning. In case of severe contamination, alcohol can be used to dry and clean the probe in conjunction with the stick. Always end the process with Helium warm He gas flushing.

Step 4 – Pump-Flush 3 times

Insert stopper on top of the Insertion module. In the 'Control' panel; Press 'Gate valve' so it closes. In the 'Service' panel; Press 'Clean Probe'. The automated procedure takes approx. 30 minutes. This will leave the probe and airlock filled with 2 bar of helium gas.

Step 5 – Cool the Probe and VTI to base temperature

Turn off the VTI Heater. Start Leybold pump. In the cooling process, monitor that the charcoal temperature does not increase above about 80 K, that P2 stays below 700 mbar. If necessary, decrease the pump frequency to 80 Hz until the various pressures and temperatures stabilize.

Cleaning the char coal trap – consult Polarize

If the inlet pressure starts to increase significantly (due to blocking of the char coal trap) from the normal or the helium flow is insufficient to cool the VTI and probe, the system must be warmed to clean the char coal trap.

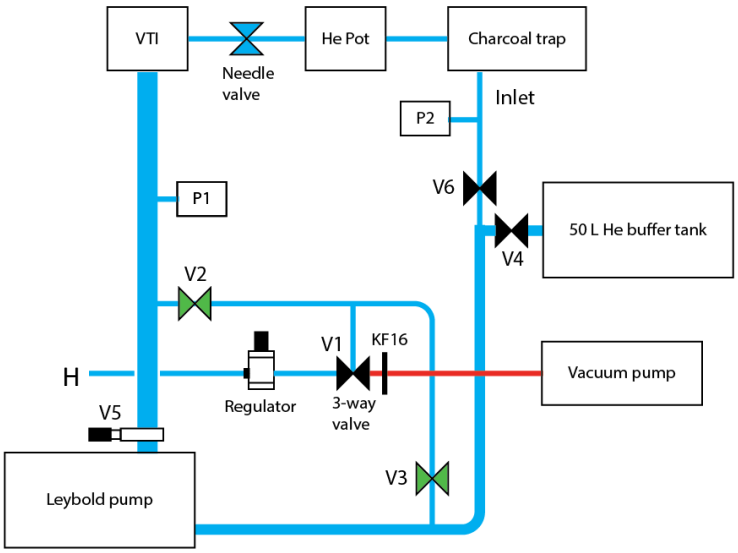


Figure 34: Schematic of the VTI circuitry including the by-pass. Valves, V1, V2 and V3, are normally closed. V1, V2 and V3 are closed when perpendicular to the tubing. Gate valve V5 and inlet valve V6 are normally open. P1 is the VTI pressure and P2 is the inlet pressure. Approx. 50 L of STP helium gas is present in the circuit. The blue lines are integral to the SpinAligner, and the red lne (KF16 flex line) and vacuum pump is attached to the system when needed. The regulator reduces the helium pressure to approx. 1000 mbar for filling of the circuit.

Setting up for pump-and-flush of the helium circulation system

Warming of the cryostat is needed. The principles of the pump-flush circuit are shown in Figure 34. In Figure 35 you can see the location of the valves and the connection to the external vacuum pump.

1. Start ramping the magnetic coil field to zero.
   'Service' tab, enable 'Advanced', Final state -> 'Zero Field', 'Ramp' 'Start'.
2. Turn off the Leybold pump.
   'Advanced' must be enabled, 'Control' tab, press 'Run' button.
3. Turn off NV Control heater so it will leave the Needle Valve fully open.
   'Service' tab, press button 'NV Control Heater'.
4. Wait until magnetic field has reached zero, then proceed to step 5.
5. Turn off the Sumitomo compressor to allow the system to warm.
6. Meanwhile, connect a vacuum pump (oil-free or with an oil-mist fore-line filter) to the KF16 flange at V1. The base pressure of the pump should be less than 10^{-2} mbar.

7. Make sure that ‘V1’ (the three-way valve) is in the closed position. V1, V2 and V3 are closed when perpendicular to the tubing.
8. Wait until most of the liquid Helium has evaporated (Figure 36) from Helium Pot before proceeding to the next step. We recommend leaving V4 open so all Helium is pumped/flushed, but you may choose to close V4 and preserve most of the Helium.
9. Turn the vacuum pump on. If using a turbo pump, do *not* start it! A sudden pressure increase in the next steps may damage a fully spinning turbo pump.
10. Turn V1 towards the vacuum pump and wait 10 s.
11. Open V3. If using a turbo pump, it must be started simultaneously with the opening of V3.
12. Wait until ‘P2 Inlet’ < 10 mbar.
13. Turn off the control <Max T 2nd stage> in the Leybold module. Turn on Leybold pump.
14. Set ‘Charcoal Trap heater’ to 373 K and turn it on.
    Set ‘VTI heater’ to 300 K and turn it on.
    Set ‘Helium Pot Heater’ to 300 K and turn it on.
    Set ‘Needle Valve Heater’ to 300 K and turn it on.
15. Wait until ‘P2 Inlet’ < 0.06 mbar.
16. Turn off the Charcoal Trap heater.
17. Turn off Leybold pump.
18. Close V3.
19. Open V4 in case it was closed in step 8.
20. If ‘Regulator’ (Figure 34) position is unknown, turn the 'Regulator’ *fully anticlockwise* to ensure that Helium flush pressure is reduced to below 1 bar.
21. Turn V1 back and forth between helium and vacuum three times and leave it in the helium position. In case you are using a turbo pump at full rpm, this step is not recommended.
22. Open V2 and V3.
23. Adjust ‘P1 VTI’ and ‘P2 Inlet’ to 950 mbar - 1000 mbar using the ‘Regulator’. Avoid pressures above 1000 mbar which can harm the Leybold pump.
24. Close V1, V2, and V3.
25. Turn off and disconnect the vacuum pump.
26. Put back the protective cap on the KF16 flange.
27. Start the Sumitomo compressor.
28. When ‘Temp 2nd Stage’ has reached steady-state below 4 K, turn on ‘NV Heater’.
29. The system is now ready for automated cool down and magnetic field ramping.

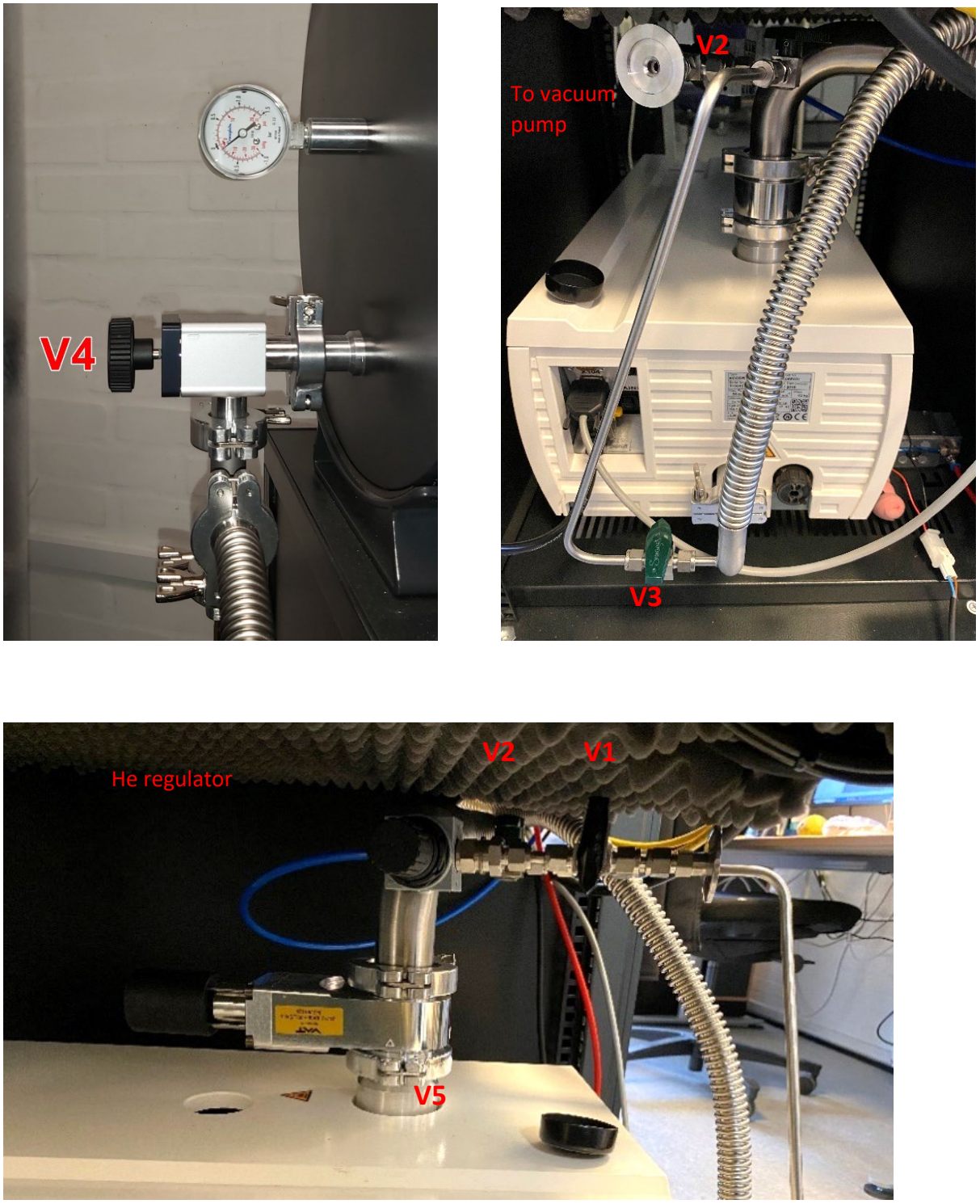


Figure 35: Photo of the back side of the Leybold pump when the sides of the rack has been removed. The KF16 for connection of a vacuum pump is marked as well as valves V1, V2, V3 and V4.

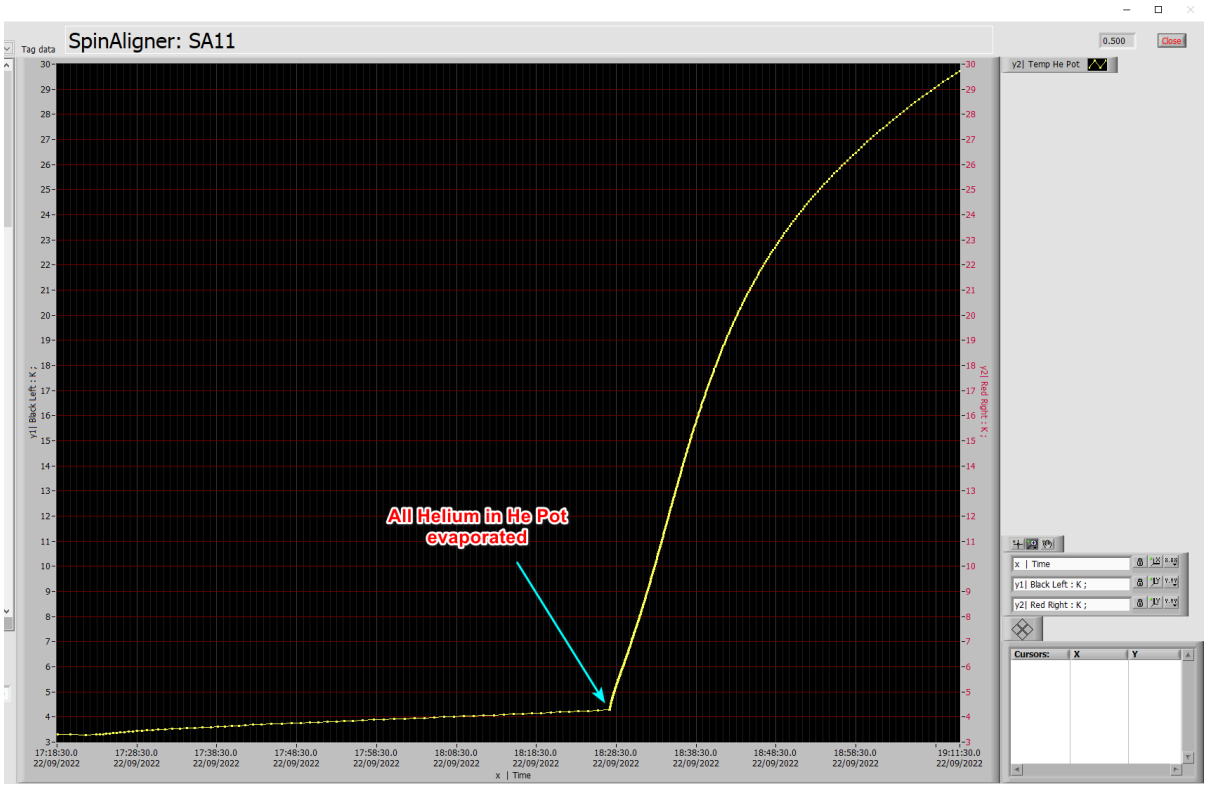


Figure 36 Helium Pot temperature after Sumitomo Compressor has been turned off. Time span is approximately 2 hours.

Pumping the enclosure vacuum – consult Polarize

The vacuum on the cryostat (enclosure) may become "soft" after a quench or a warm-up. In that case the enclosure needs to be pumped down again with a turbo-pump. A turbopump with pressure gauge that can reach $<10^{-5}$ mbar is required. The turbo pump is connected to the KF16 pumping port on the back of the SpinAligner (behind the cover), see Figure 37. Start the turbopump and let is reach a pressure below 10^{-5} mbar. Then gently open the valve, Figure 37. Watch the pressure rise on the turbo pump as the valve is opened and close if a fast pressure rise is observed. Otherwise, continue to open fully and allow the enclosure to pump down to $<10^{-5}$ mbar. This may take hours to days depending on the temperature of the cryostat and the level of contamination.

Figure 37: Enclosure KF16 pumping port.

Changing the DNP probe – consult Polarize

1. Place the flight case with the replacement DNP probe on a desk in proximity of the SpinAligner. Check that the new probe is intact and as expected. Locate the KF40 plastic cover that is needed for blinding the VTI between removing the old probe and the new probe.

2. Remove the coaxial cable and T&M box from the old DNP probe in the SpinAligner.

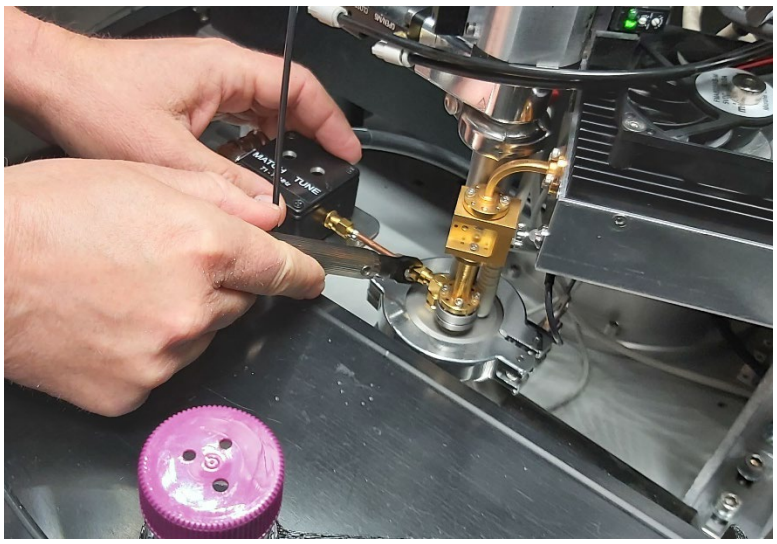
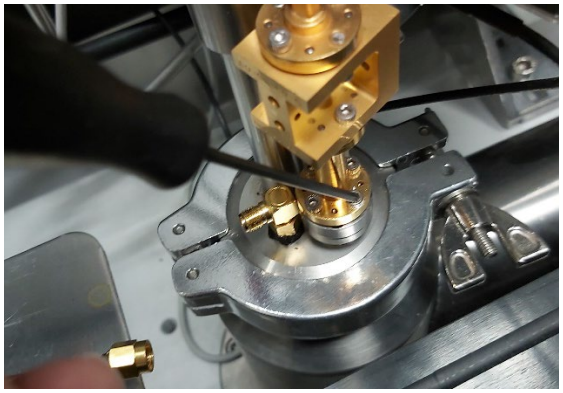


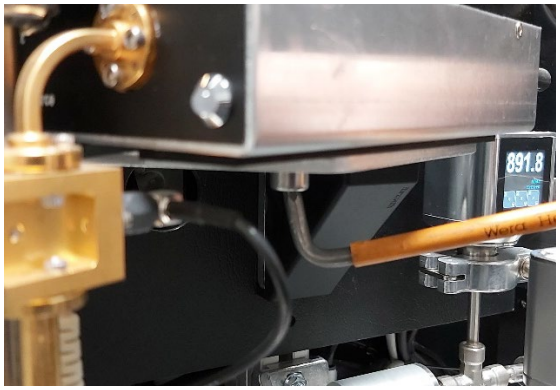
Figure 38 Unscrew Tuner & Match Box Coaxial cable

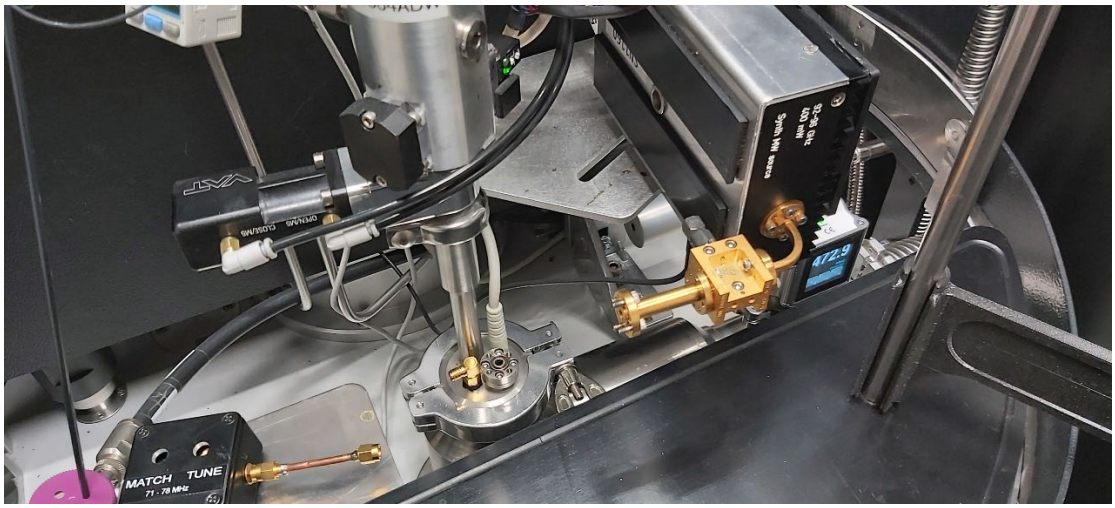
3. Remove the ODU connector from the old DNP probe.

Figure 39 Disconnect probe temperature monitoring cable

4. Loosen the screws between the microwave doubler and the transition on the old DNP probe and slide the microwave source away from the DNP probe (the microwave source is secured with a screw at the bottom of the base plate).





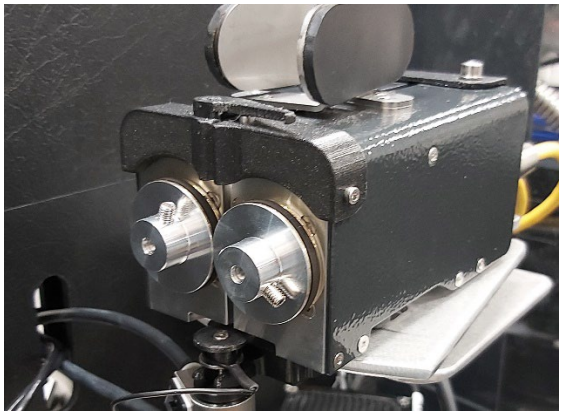


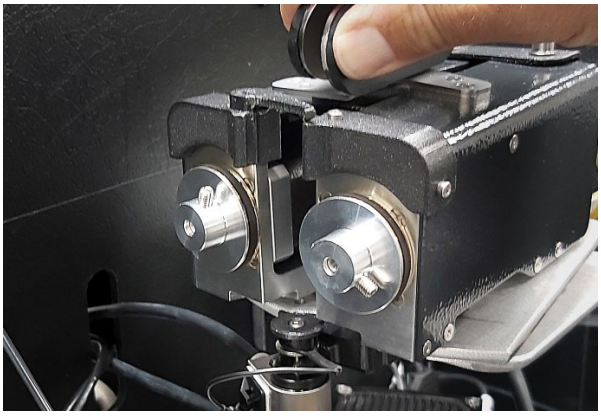
5. Loosen the screw that fixes the Insertion Module to its base plate (rear side).
6. Stop the compressor.

7. Set Helium Pot heater to 5 K in the software and turn on the heater. Wait for the inlet (P2) to reach approx. 900-1000 mbar and steady state before turning off the Helium Pot heater again. Let the VTI pressure (P1) reach steady state as well.
8. Close the Needle Valve in the software.
9. Switch V1 towards helium.
10. Close V4 (helium tank).
11. Open V2.
12. Adjust the regulator to a pressure of 1100 mbar (P1).
13. Remove the KF16 clamp that holds the Insertion Module (IM) to the DNP probe and rotate the IM to the side such that the DNP probe can pass when lifted out of the VTI.
14. Remove the KF40 clamp that holds the DNP probe to the VTI.
15. Take the new DNP probe from the flight case and have it ready for insertion immediately as the old probe is remove.
16. Lift the old probe out of the VTI and insert the new probe in a swift and continuous operation. Minimize the time that the VTI is open to atmosphere. There should be a continuous bleed of helium throughout the procedure. If the removal of the old probe and insertion of the new probe cannot be done in one swift operation, it is best to cap the VTI with the plastic cover in between inserting the new probe.
17. Attach and fasten the KF40 clamp between the new DNP probe and VTI.
18. Attach and fasten the KF16 clamp between the Insertion Module and the DNP probe.
19. Secure the Insertion Module to its base plate by firmly tightening the screw (rear side).
20. Connect the microwave source and tighten the screws between the doubler and the transition.
21. Connect the ODU cable.
22. Close V2.
23. Switch V1 to its middle, closed position.
24. Open V4 (helium tank).
25. Place the old probe in the flight case.
26. Start the compressor.

25. Instrumentation


SA14





26. Troubleshooting

Observation	Possible cause	Action
No DNP signal	Magnet has quenched	Check with slightly magnetic tool whether the magnetic field is still present. Check data logger for any sign of quench, e.g. elevated magnet or coldhead temperature. Contact Polarize.
Magnet does not cool again after quench	Compressor pressure is inadequate.	The compressor pressure should be adjusted to be between 21-22 bar (needle oscillating between the two values) when cold. See Sumitomo manual for instructions.
	Enclosure vacuum is “soft”.	Connect turbopump to enclosure and evacuate. The SpinAligner may have to be warmed at some point to have the enclosure properly evacuated.
Vial gets stuck while being lowered in the SpinAligner	Ice in the probe	1. Abort insertion 2. Click Unload Vial (Figure 8) 3. Clean probe for ice (Advanced User).
No polarization build-up	No carbon NMR signal. Wrong frequency settings.	Check that the integral region is correct (Left bottom in Figure 22). Check that the carbon NMR frequency is correct.
	Sample position incorrect.	Move sample manually to the bottom and lift it approx. 15 mm.
	Incorrect microwave frequency.	Determine/optimize the microwave frequency.
	Insufficient microwave power.	Set the MW power to 10 mW and optimize the other parameters before making a microwave power sweep to determine optimum.
	Incorrect microwave settings.	Default microwave settings: See Figure 21
Low polarization	Non-optimal microwave frequency	Optimize microwave frequency by performing a microwave frequency sweep.
	Non-optimal microwave power	Optimize microwave power by performing a microwave power sweep.
	Bad sample composition or	Prepare new sample, or resort to reference (known good sample preparation) sample.

	deteriorated sample	
The sample does not dissolve	Blocked fluid path. Sample position incorrect during dissolution.	First: wait until sample reaches receiver. If it does not at all, lift vial to Insertion module by clicking  (Figure 8) and wait again. Preventive steps: - Thorough cleaning of fluid path. - Check that vial is adequately fastened. - Inspect fluid path for leaks: with an empty vial tightened but outside SpinAligner, seal outlet using stopper, open dissolution valve, open He valve and let the pressure stabilize, close He valve, observe pressure drop and inspect for leaks by spray soap solution on suspected parts of fluid path.
Dissolution module does not reach required pressure	No solvent added to Dissolution Module	Stop heating dissolution liquid, wait until the module has cooled down, add solvent and restart heating.
Red light at fuse in the Power Distribution Panel	Fuse blown	Contact Polarize New fuse to be inserted. Check text on Power Distribution Panel for correct value.
Blinking red light in 24 V fuse box	Current limitation exceeded	Contact Polarize. Fuse is reset by a short press on the blinking button.