

Cryoprobe Training Course

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Cryoprobe checklist

1. Maximum sample depth = 21mm on all cryoprobes. Use the white or blue plastic spinners. Do not use ceramic spinners.
2. Don't use the black cap.
3. EDTE: air flow=670L/hour, heater should be on at all times.
4. BCU05 should be off, and switch on heat sink should be set to high unless low temperatures ($\leq 10^{\circ}\text{C}$) are required. 50°C is the maximum allowed temperature.
5. Do not exceed any of the maximum power levels. Generally, the power corresponding to $8\text{ }\mu\text{sec}$ (^1H), $15\text{ }\mu\text{sec}$ (^{13}C), and $40\text{ }\mu\text{sec}$ (^{15}N) 90° pulses is the maximum allowed power. Simultaneous ^{13}C and ^{15}N pulses requires that the power for each be dropped by 3dB (and pulse lengths recalculated).
6. Do not exceed the maximum average power. Acquisition time (aq) should not exceed 140msec if decoupling is used. Consult the *Typical pulses for 5mm CryoProbe* guides, located on the CryoCooling units.
7. Use only Z gradients. All cryoprobes have only Z gradients. 3D gradient shimming can be done on Bosch and Mulder, but not Bond.
8. **Monitor the sample temperature** when starting any experiment. The blue line (RF coil temp) in any of the graphs on the laptop should not move above the “nominal temp.” line. If the RF coil temp rises, stop the experiment and check all parameters.

What to do if the cryoprobe stops chirping

- 1) Stop the experiment, if one is running.
- 2) Push the “cooldown” button on the front of the cryoplatfrom.

If this is done within 20 minutes or so, it can be cooled back down without warming up.

- 3) Check the Neslab.

If the “cooling water” light is red, that means it isn’ t pumping. Push the “push to start” button. The “cooling water” light should now be green.

Check the water level in the reservoir. If the water level is too low it won’ t stay on. Fill the reservoir all the way to the top with deionized water (there are deionized water faucets in the main NMR lab, and in the MIC NMR lab - rm 1421).

If the floor around the Neslab is wet, one or more of the hose clamps in the Neslab is loose. Try to tighten all of them (you will need a 5/16” nut driver, or a screwdriver).

- 4) Read the rest of this manual.

The Bruker CryoProbe system

The CryoProbe system consists of

The CryoProbe and

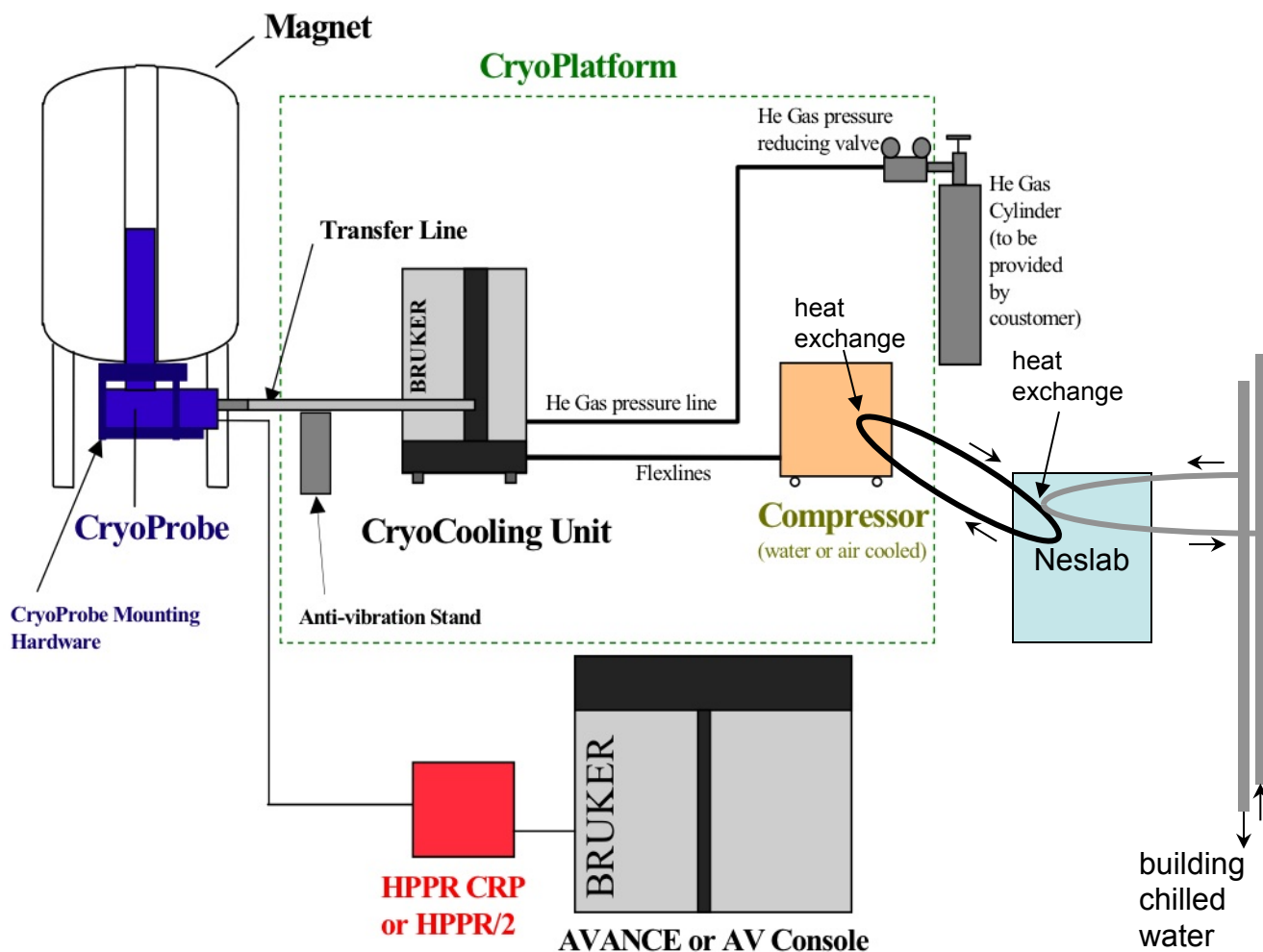
The CryoPlatform

The CryoCooling unit contains the coldhead.

The 99.9999% pure helium gas cylinder provides pure helium gas at ambient temperature.

The helium compressor compresses the helium and supplies it to the coldhead at ambient temperature. It also generates a large amount of heat. We also have a Neslab water to water heat exchanger to remove the heat.

Figure 2.1. The CryoProbe™ System



Cold RF coils (20-30K)

Cold ^1H (^{13}C) preamp (77K)



Magnet

He Regulator

He steel-cylinder

He Hose

Push buttons for warm-up / cool-down

He Compressor

CryoProbe

He Transferline

Vacuum bellow

Transferline Support

CryoCooling Unit



Principle of Operation

1. Pressure buildup.

With the displacer at the bottom of the cylinder and the outlet valve closed, the inlet valve is opened and the pressure in the system increases. High pressure gas enters through the inlet valve to fill the regenerator and the space above the displacer, volume 1.

2. Intake stroke

With the inlet valve still open, the displacer is moved from the bottom of the cylinder to the top. This displaces high pressure gas from the space above the displacer, volume 1, through the regenerator to the space below the displacer, volume 2. In passing through the regenerator, the gas cools down and contracts. Additional gas enters the system to maintain the maximum cycle pressure.

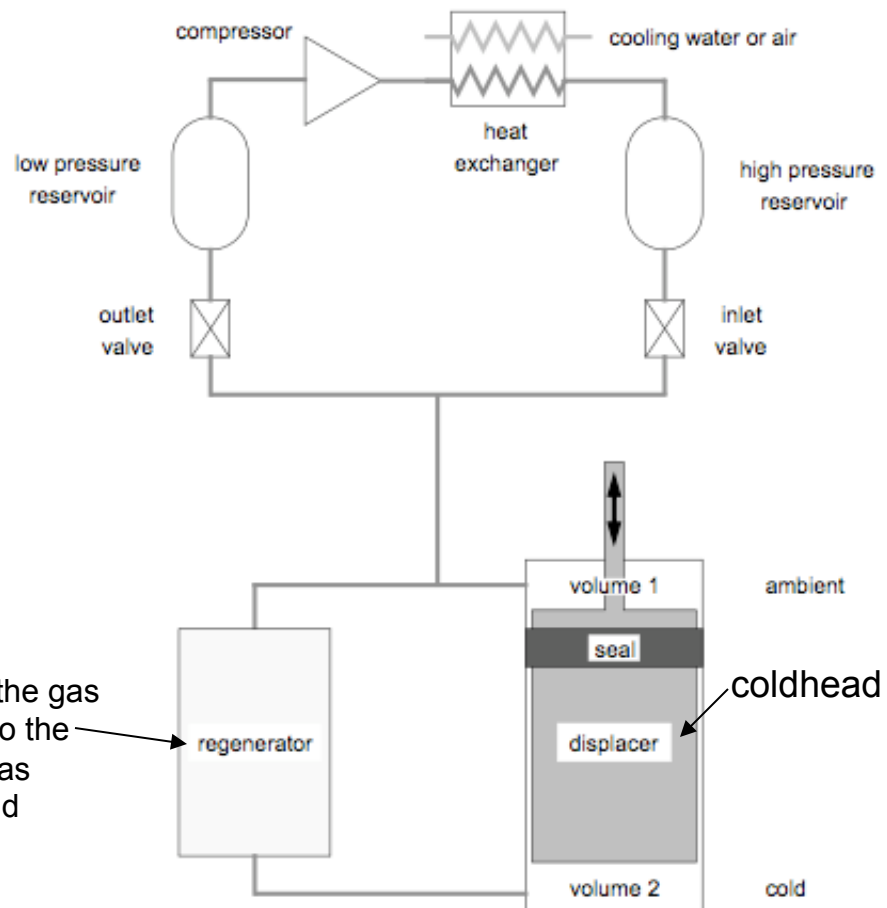
3. Pressure release and expansion

With the displacer at the top of the cylinder, the inlet valve closes and the outlet valve opens to the low pressure reservoir. Gas escapes and the pressure decreases, causing a drop in the gas temperature. The temperature decrease in volume 2 is the useful refrigeration step.

4. Exhaust stroke

With the outlet valve open, the displacer moves from the top to the bottom of the cylinder, displacing fluid from volume 2 to volume 1. As it flows through the regenerator, the fluid is heated by the matrix to near ambient temperature. This cools the regenerator ready for the gas entering in the next cycle.

Figure 3.7. Gifford-McMahon cycle refrigerator



Operating the CryoProbe safely

RF coil maximum power

The RF coil can easily be damaged by excessive pulse power. Fixed attenuators are placed on the amplifiers by the installation engineer so that it will be difficult or impossible to exceed the maximum allowed power. The maximum allowed power level on each channel has also been set by the installation engineer. This power appears in the edprosol table, and on the pulse calibration list. Here are representative maximum power levels and 90° pulse lengths:

^1H	8 μsec	-5dB	This means you should never exceed these power levels. For example, you should never use -6dB on the carbon channel.
^{13}C	15 μsec	-5.5dB	
^{15}N	40 μsec	-5.2dB	

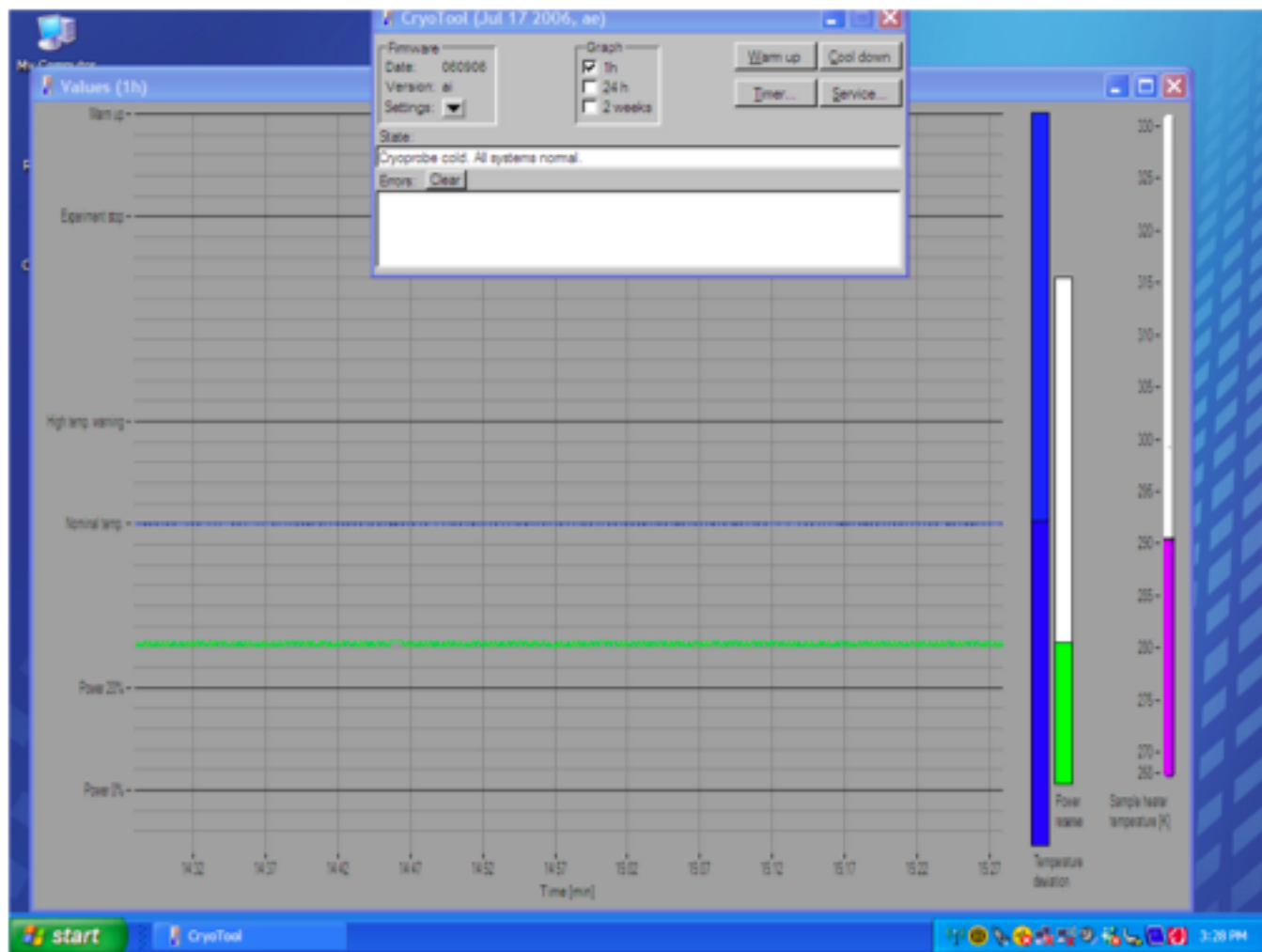
RF coil average power

In cases where the average RF power exceeds the cooling capacity of the system, the CryoProbe will warm up.

It's very important to monitor the coil temperature when you start any experiment.

Optimally the coil temperature shouldn't rise at all during your experiment. If it rises by 5° the 'cold' LED will start flashing and an error "slight rf coil overheat" will appear in the CryoTool window. It's possible to run in this condition, but it's better to stop your experiment and check the parameters. If it rises by 15° the 'error' LED will flash - stop your experiment immediately. If it rises by 20° the CryoController begins a warmup.

In the CryoTool window, there are three graphs normally available - 1 hour, 24 hours, and 2 weeks. In the 1 hour graph there is a blue line that indicates the current temperature of the coil. Ordinarily this will be on the 'optimal temperature' line. There are also lines at 5, 15, and 20 grid units above the 'optimal temperature' line called 'high temp. warning', 'experiment stop', and 'warm up'.



The maximum average power can easily be exceeded by long pulses or pulse trains. If running your experiment causes the coil temperature to rise at all, you should stop it and check all pulses and pulse trains.

If none of the powers levels exceeds the maximum allowed power level, check all pulse trains, such as TOCSY and CPD sequences. If any pulses (including shaped pulses) have long durations (msec range), check the power levels.

There is a small book labeled **Typical Pulses for the 5mm CryoProbe**, which contains typical allowed combinations of power levels and lengths. This book is kept on the CryoCooling unit. There is one of these for 500 and 600 MHz, and one for 800 MHz.

For each nucleus it contains information like this:

^{13}C

Hard pulse 15 μsec
(max length 360°)

CC spin lock 20 msec @ 25 μsec

GARP decoupling 140 msec @ 65 μsec

Here is what it means, taking the GARP decoupling as an example:

It's OK to apply GARP decoupling on ^{13}C for 140msec, using the power necessary for a 65 μsec 90° pulse. So if the power for a 65 μsec 90° pulse on ^{13}C is 8dB, then you could set pcpd2=65u, and pl12=8dB. Then if GARP decoupling was set during acquisition, your acquisition time could be up to 140msec long.

The **Typical Pulses** book gives examples of allowed power levels and durations that are meant as a guide. If you need different power levels and/or lengths than are listed there, you can interpolate to some extent (i.e. apply GARP decoupling for a longer time with less power/longer pulses). **Be very conservative if you need to use higher power levels than listed** (and of course never exceed the maximum power). If you're not sure, get help.

Miscellaneous:

Total recycle delay should be 1 second or longer if you're anywhere even close to any of the maximum or average power limits.

If you apply simultaneous ^{13}C and ^{15}N hard pulses, you must reduce the power of both by 3dB. Of course you must also adjust the pulse length. For example:

Max.	length	power	reduced	length	power
^{13}C	15 μsec	-5.5dB		21.2 μsec	-2.5dB
^{15}N	40 μsec	-5.2dB		56.5 μsec	-2.2dB

The same is true for simultaneous ^{13}C and ^{15}N decoupling (see next page).

The most common way that the maximum average power is exceeded is by applying decoupling for too long. This happens because decoupling is applied during acquisition, and the acquisition time is too long.

In general, you should never decouple for longer than 140msec. Before starting your experiment, type aq. If it's longer than 140msec, you must decrease it. **To decrease the acquisition time, you must decrease TD.** Verify that aq is less than 140msec before starting the experiment.

*If you're decoupling on two channels at the same time, then you must reduce the decoupling power. Use pcpd2=100μsec (¹³C) and pcpd3=200μsec (¹⁵N), with appropriate power levels.

Another option to reduce the decoupling power is to use **adiabatic decoupling**. To use adiabatic decoupling on ¹³C, in eda set CPDPRG2 to "p5m4sp180". Then set pcpd2=1.5msec and set spnam15 to "CHIRP95". The power level for this decoupling sequence is sp15.

sp15 should be set to pl12+2dB. ← This means 2dB higher than the power normally used for GARP decoupling with pcpd2=65μsec.

Adiabatic decoupling has the dual advantages that it uses less power and it decouples a larger bandwidth.

If the coil overheats and a warmup cycle starts accidentally:

Immediately stop the experiment then push the "Cool down" button on the front of the CryoPlatform. If you do this quickly enough, it will abort the warm up cycle and cool back down.

Temperature

Always have EDTE on. Do not change parameters in the EDTE window, except for the set temperature.

The flow should always be 670L/hour

The heater should always be on

The CryoProbe has a limited temperature range.

Minimum temperature: 0°C^*

Maximum temperature: 60°C^{**}

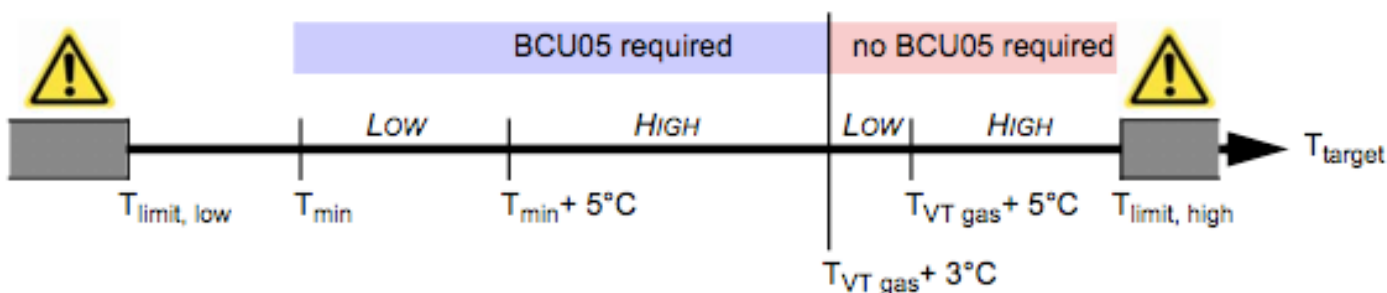
*With sample protection system. Otherwise, most probes can only get down to $\sim 10^{\circ}\text{C}$.

**The white and blue spinners can only go as high as 50°C . The ceramic spinners can go higher, but they may not be used with the cryoprobe. So the max. temp. is really 50°C .

If the vt air stops flowing for any reason, the sample temperature will quickly drop to around -20°C . If this happens, your sample could freeze and the NMR tube may break. The sample protection system ejects any sample if the air stops flowing. So don't use the black cap on top of the magnet.

Another common reason that the vt air stops is that the BCU05 (the cooling unit) freezes. The BCU05 on Mulder sometimes freezes although now it's running on pure nitrogen. For this reason, it's a good idea to only use it when you need it. Also, it's not always possible to reach higher temperatures with the BCU05 on.

Figure 5.1. The different ranges of the target temperature



"Low" and "High" refer to the setting of the VT Interface box.

Tuning/Matching

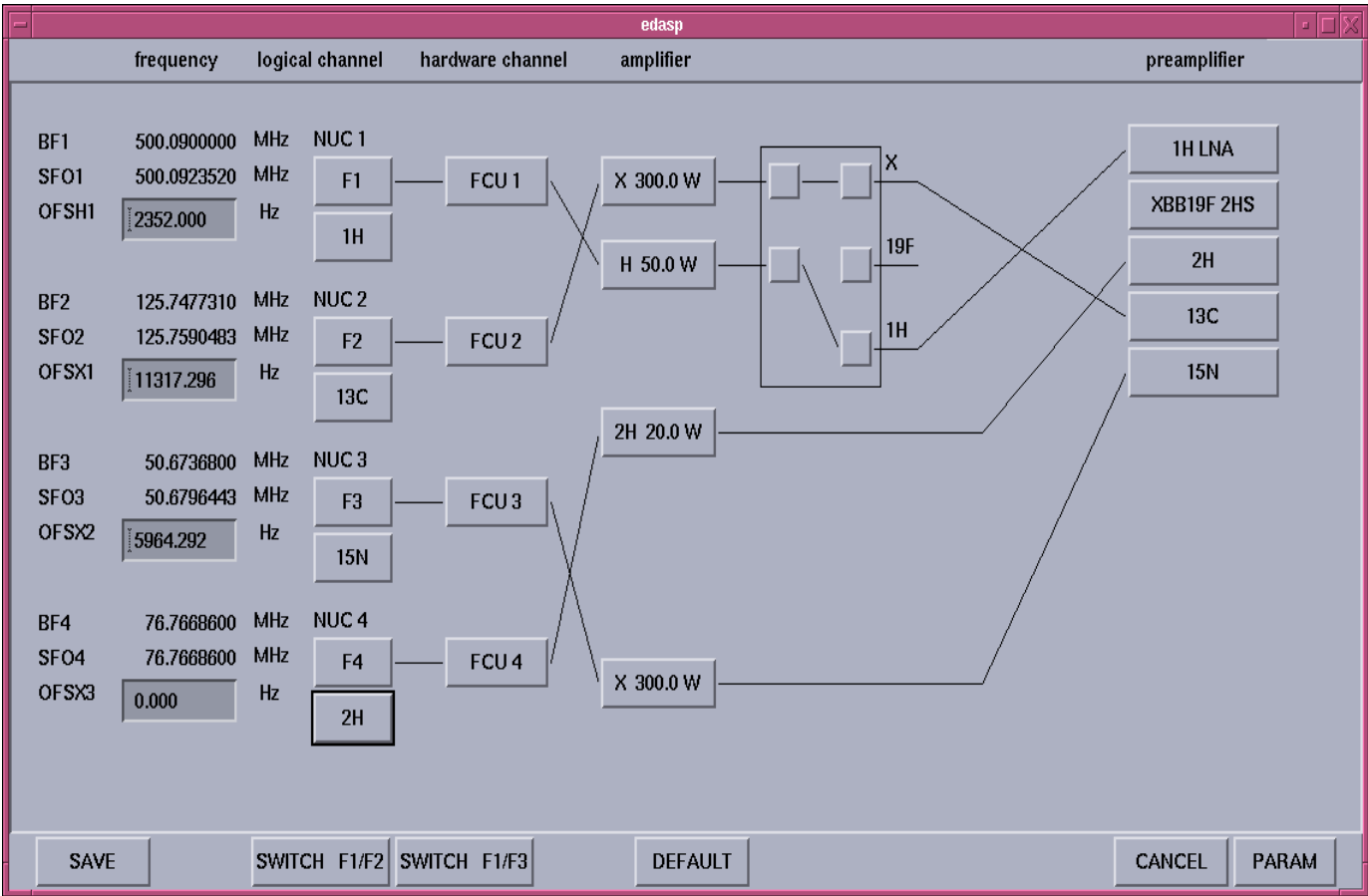
Do not try to tune the probe when it's warm.

Use the blue tuning wand.

Don't force the tuning/matching screwdrivers. The screws could be improperly seated, you might be turning the wrong one, or the rf routing (edasp) could be wrong.

RF Routing

The routing should look something like this:



If ¹³C and/or ¹⁵N are used, they should always be in channels 2 and 3, respectively.

If ²H isn't used, channel F4 can be turned off. If neither ¹³C nor ¹⁵N are used, channels F2 and F3 can be turned off.

Miscellaneous

The maximum sample depth is 21mm on all cryoprobes.

The command loopadj can be helpful in stabilizing the lock signal.

The CryoProbes are only equipped with Z gradients. Be sure to only use Z gradients in your experiments. If you use X or Y gradients, there won't be any error messages - the gradients just won't work. Also Mulder and Bosch, but not Bond, can perform 3D gradshim (Mulder and Bosch have rcb boards which enables them to use the X and Y shims as gradients in 3D gradshim).

CryoPlatform

There is an emergency off switch on the front of the CryoPlatform. In the event of an emergency, this switch can be turned to the off position. This will return all valves to their default position and initiate a passive warmup.

*If the switch is accidentally turned to the off position (which can happen if you just brush against it), just turn it back to the on position.

Software Tools

CryoTool is the software running on the laptop that controls the cryoplatform.

Starting CryoTool:

- 1) Double click the icon for CryoTool
- 2) Select the proper COM port
- 3) Click the 1 hour, 24 hour, or 2 week box

If the laptop crashes:

Don't worry, it won't affect the CryoPlatform. Just restart the laptop and CryoTool.

	<u>username</u>	<u>password</u>	<u>com port</u>
Mulder	none	none	6
Bond	Administrator	password	4
Bosch	Administrator	password	1

To determine the com port: right-click My Computer and click manage. Then click System tools, open device manager, and look under ports.

Cryoprobe Problems

Any problems that occur will generate an error message in the cryotool window on the laptop. The cryotool software is very good and it will automatically handle any problems so as to prevent damage to the system.

The overall system status can be seen by looking at the lights on the front of the cryocool. If there's any type of error at all, the red "error" light will be on. If the system is cold, the green "cold" light will be lit, and if it's warm, the "warm" and "unplug" lights will both be on. If the system is warming up or cooling down, the "warm" or "cold" light will be flashing.

To initiate a warmup, press the "Warm up" button on the front of the cryocool. To initiate a cooldown, press the "Cool down" button. You can also click "warm up" or "cool down" in the cryotool window.

There are many errors that can occur. Many of these are listed in the troubleshooting section of the CryoProbe System User Manual (chapter 7).

Some of the errors are inconsequential ("pressure drop in system" for instance). If there is an error but the system is still cold, and you can't find the error in the manual, you should call Bruker and ask them what to do.

Keeping the system cold

If the system begins warming up, you can often prevent it from warming up all the way. If you press “Cool down” within 20 or 30 minutes, it will stop the warmup cycle and cool back down again.

If the probe is accidentally overheated by application of too much RF power, it will go into a warmup cycle. In this case, you can just stop the experiment and press “Cool down”.

However, in most cases you will have to figure out what caused it to begin warming up and resolve that problem before attempting a cooldown.

Neslab problems

The most common thing that causes a warmup is that the Neslab (water to water heat exchanger) turns off and the He compressor overheats. This usually results in the error message “Trouble with compressor”.

One common reason for the Neslabs to fail is that the water reservoir gets too low. Check this and just refill it with deionized water if it's low. Then press the “push to start” button.

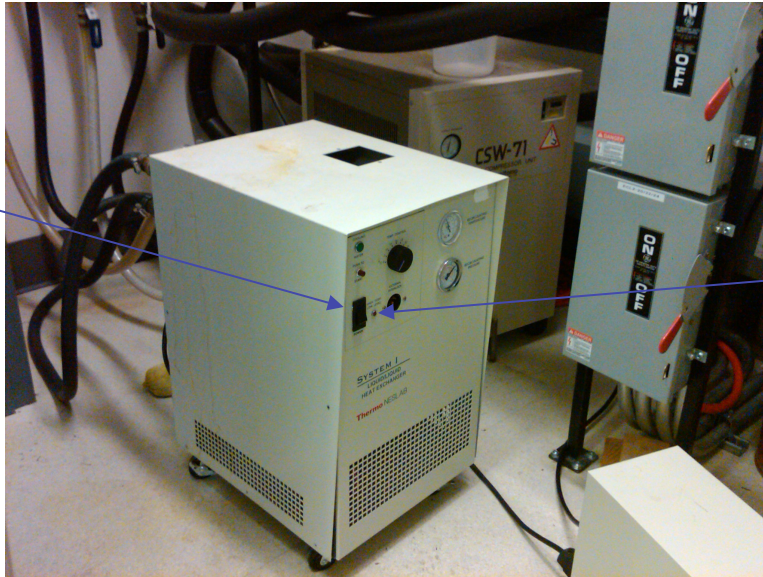
Another reason that this can happen is power outages*. Even short power outages (1 second) cause the Neslabs to stop circulating water. If this happens, the “cooling water” light on the front of the Neslab will be red (in normal operation it's green). But if it does happen, you can simply press the red “push to start” button and it will start again.

*Not so common since putting the Neslabs on UPSs

Often, the cryoprobe will start warming up for some reason unrelated to the Neslab, and then the water will leak out of the reservoir. In this case, the reservoir must be refilled, and the leak must be found and fixed. The hose clamps can be tightened with a 5/16” nut driver, or a screwdriver.

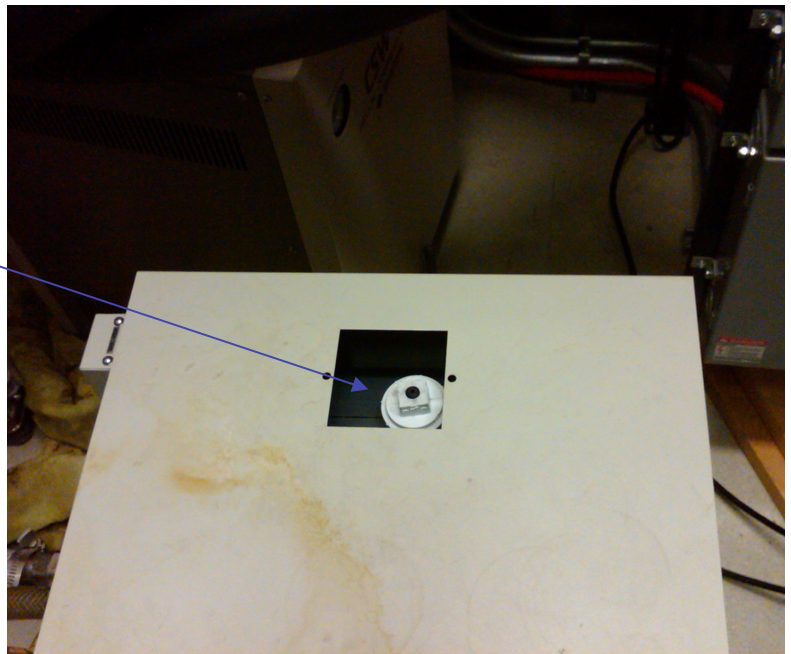
The Neslab water to water heat exchanger:

To turn it on,
you first have to
turn on the
power.

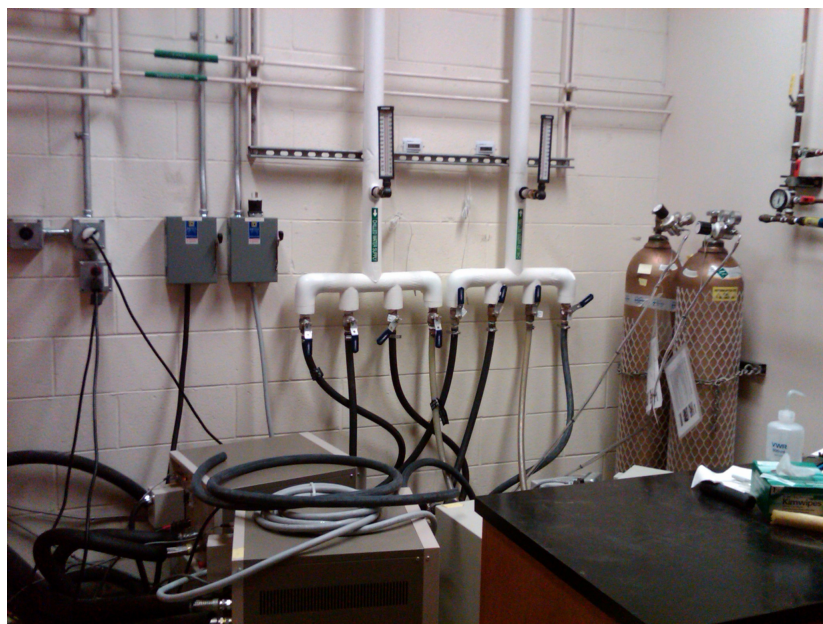


Then push the
red button
marked “push to
start”.

Refill water reservoir here:



More recently, the most common reason for the Neslabs shutting down has been the building chilled water heating up.



The temperature of the building chilled water can be monitored from the thermometer attached to the “Chilled Water Supply” pipe in MSB1425 (the room right next to Mulder).

Since the building heat exchanger was replaced and new electronic controls were installed, this thermometer always reads 52° F.

If this temperature is too high (above ~65° F), the water to the compressors will be too hot and they won't stay on. *This is the only way the cryoprobes can be shut down by a power outage.

So if the chilled water is hot, don't attempt the cool the cryoprobes.

Phone numbers of Bruker people:

(in the order in which I would call them)

Steve Grimaldi: (978)973-1454 (cell). Steve.Grimaldi@bruker-biospin.com
He's our local cryo engineer, and he's done most of the recent work on our systems

Bob Lord: (510)683-4300. Robert.Lord@bruker-biospin.com
West Coast service coordinator. He can find someone to help you.

Walter Osgood: (978)667-9580 ext 5451 (office) (978)204-4922 (cell)
Walter.Osgood@bruker-biospin.com
Cryo engineer. He's also done a lot of work on our systems.

Pavel Kostikin: (978)667-9580 ext 5360 (office) (978)204-8063 (cell)
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National cryoprobe service manager.

Bruker Center: (978)667-9580. #1 for NMR. #2 for service.
center@bruker-biospin.com
Bruker's main call center in the US. This is also the number for Bruker's hotline.