



CapNMR™ Probe Manual

Table of Contents

Welcome!	2
Sample Management	2
Spectrometer Power and Gain Levels	3
Transmitter power	3
Receiver gain	4
Gradient Power Settings	4
Placing the Probe in the Magnet	5
Probe Positioning	5
Adjusting the probe height.....	5
Orienting the coil along the X axis of the shim set.....	6
Matching and Tuning	6
Locking	7
Shimming	7
Variable Temperature Control	8
Manual Injection	9
Calibration of Solvent Push Volume for Manual Flow Injection	13
Maintenance Procedures	16
Appendix 1: Dimensions for Probe Installation	19
Appendix 2: Performance Tests and Specifications	20
Appendix 3: Probe Storage and Shipping	21
Appendix 4: Miscibility and Viscosities of Common Solvents	22
Appendix 5: Estimating Pressures	23
Appendix 6: Chemical Shifts of Common Residual Solvents	24
Appendix 7: Cutting and Trimming Fused Silica Capillary	25
Appendix 8: Microfilter Assembly and Use	26
2-µm Mini MicroFilter (see diagram for assembly but use these part numbers)	26
Appendix 9: Standard Acquisition Parameters	27
Appendix 10: Contributors	28

©Copyright, Protasis/MRM Corporation, August 2005

Updates of this Manual, along with Technical Bulletins, are maintained at
www.microNMR.com

Welcome!

Welcome to the Protasis/MRM family of capillary-scale NMR detection products! Your new CapNMR™ probe combines recent scientific advances in capillary-scale fluidics and enhanced mass sensitivity to provide a highly sensitive, cost-effective, and robust platform for flow NMR analysis of microliter volumes and nanomole quantities.

The CapNMR™ probe is designed to be fully compatible with your existing NMR spectrometer. However, since “what’s inside the box” is quite different from that of other probes, it is very important that you read the short procedures described in this installation document. Lower RF transmitter power settings, higher receiver gain settings, and slower flow rates are several of the most pronounced differences from what is typical for non-capillary flow probes.

Attainment of repeatable and accurate NMR results with the CapNMR™ probe is inseparably tied to the fluidic management system used to introduce the sample. Protasis/MRM offers a variety of sample management options, including support of the Waters CapLC® system for chromatographic applications. Please contact us if you have any questions regarding fluidic management systems.

Thank you for your purchase of our product. The staff of Protasis/MRM are available to assist you with your technical questions. Our sales and marketing staff can be reached in the USA at (508) 481-4163, and our technical staff can be reached at (217) 351-4359.

Sample Management

Fluidic management of samples in a flow probe is of utmost importance. Careless procedures can result in bubbles, contamination, or loss of sample. For installation, the probe is most often completely filled with a standard sample such as 5% CHCl₃ in acetone-*d*₆, or 10 mM sucrose in 100% D₂O by using a syringe with a Luer lock capillary adapter.

The probe is shipped with an in-line microfilter (Upchurch M-135) on the “A” capillary segment and a capillary union (Upchurch P-779-01) on the “B” capillary segment. Begin by injecting fluid into the “A” side of the probe. Connections should be made at the free capillary ends, and *not* at the microfilter and union already in place. *The capillary segments between the base of the probe and the microfilter and union can never be replaced.* Consequently, those connections should only be remade infrequently, and only when necessary. Both the microfilter and union are fluidically invisible, in that they cause no significant peak broadening or back pressure.

The M-135 in-line microfilter contains a screw-in filter capsule, and several spares are provided. If the filter ever needs removal, be certain to replace it pointing in the same direction as the original direction of flow. If the filter capsule is replaced, it makes no difference in which direction a new filter capsule is put into place. A new microfilter should be rinsed at about 5 μL/min for 5 min before being connected to the probe.

If assembling a new M-542 filter (usually used with FEP tubing), take note that it requires a specific sequence of assembly which can be determined from inspection. If the filter capsule is placed into the housing, it hits a lip and stops. The capillary must be mounted from the same side the filter capsule

went into the housing, otherwise, the capsule will be pushed out. (This will seem complicated until you have actually used the microfilter.) See Appendix 8 for additional clarification.

The most important things to take notice of in a manual sample entry procedure are:

- 1) Samples can be loaded into an empty flowcell. Always precede sample loading by rinsing the probe with at least 50 μL of acetone- d_6 (use a 250- μL syringe and the syringe vise) and then remove this by pushing air through the probe with a large syringe (5-mL syringe preferred; use the syringe clamp). Only after this procedure should the sample be loaded. This procedure eliminates the possibility of incomplete sample rinsing especially in the case of immiscible solvents, such as CHCl_3 followed by D_2O .
- 2) When the probe is removed from the magnet for storage, it should be rinsed with acetone- d_6 (syringe vise) and then air (syringe clamp). If shipping the probe, rinse with acetone- d_6 and flush with air prior to returning the probe to its shipping case.
- 3) Flow probes with long capillary hanging below the level of the microcoil position are susceptible to siphoning. This can be a particular problem when syringes and syringe capillary adapters get old. The best way to eliminate this problem is to plug the output capillary with the Upchurch P-779-01 union and PEEK P-550 plug included with the probe.
- 4) Should the probe exhibit unstable NMR behavior, check for bubbles or fluid siphoning.
- 5) Typical samples for NMR probe evaluation are:
 - 5 % CHCl_3 in acetone- d_6 for line shape and line width
 - 10 mM sucrose in D_2O
 - 5 mM sucrose in D_2O
 - 1 mM sucrose in D_2O
 - 500 mM sucrose in D_2O (for indirect carbon experiments)
 - extra acetone- d_6

These samples should be kept refrigerated when not in use.

The CapNMR probe is designed to accommodate pressures up to 1000 psi. However, pressures in excess of 1000 psi can still be applied to an upstream fluidic system or flow path. This is different than the pressure in the probe itself, which is estimated using the Pressure Equation (Appendix 5), and knowledge of the length of capillary on the outlet of the probe (usually about 1 meter), where the pressure is always nearly zero. Under any reasonable operating conditions, the pressure applied to the probe itself will seldom exceed 1000 psi.

Spectrometer Power and Gain Levels

Due to the increased sensitivity of microcoil probes, spectrometer power levels and gain settings must be adjusted to prevent probe damage and maximize probe performance.

Transmitter power

Power levels 20-23 dB below the maximum on 50 W systems should result in proton pulse widths of 5-10 μs . Higher powers can be used when shorter pulse widths are desired. **However, for all pulses or pulse trains (presats, decoupling, spin locking, etc.) the power used should be at least 20 dB lower than that used for standard 5 mm probes. Failure to reduce transmitter power levels in these conditions will result in permanent damage to the microcoil. One should be able to make adjustments to the power level to attain the desired 90 degree pulse width.**

Receiver gain

The small sample volumes investigated using microcoil probes require that the gain be optimally maximized for a given sample. For the evaluation samples of 2 and 5% chloroform and 10 mM, 5 mM, and 1 mM sucrose, the spectrometer gain should be set to maximum. If one uses gain settings more typical of standard volume probes and standard concentration samples, sensitivity loss may occur due to digitization noise.

Gradient Power Settings

The (z) gradient coil provided with the CapNMR™ flow probe is designed to have high gradient sensitivity, that is, a high value of gradient strength (G/cm) per unit current. The values of the spectrometer settings used to control the CapNMR probe gradient strength will typically be considerably less than the values used with conventional gradient coils and probes. The following general guidelines should be used. In general, the gradient duration can remain the same as with conventional probes, but in some cases may be modified slightly to compensate for “rounding errors” in the calculations described below. For gradient calibration purposes, the inner diameter of the flow cell is 800 μm.

Bruker. Pulse sequences on Bruker spectrometers typically specify gradient strength as a percentage of maximum gradient strength. **CapNMR probe users should begin by dividing the Bruker percentage values by a factor of 7.** For example, if a 20% z-gradient value is specified in a particular pulse sequence for a standard Bruker probe, the CapNMR probe value should be $20\% / 7$, which is about 3%. If this proves insufficient, change the correction factor from 7 to 6 or 5.

JEOL. Pulse sequences on JEOL spectrometers typically specify gradient strength as either a step value (older spectrometers, ranging from +15 to -15 in integer values) or as a percentage of maximum gradient strength. **CapNMR probe users should divide the JEOL step or percentage values by a factor of 13.** For example, if a 40% z-gradient value is specified in a particular pulse sequence for a standard JEOL probe, the CapNMR probe value should be $40\% / 13 \sim 3\%$. Similarly, if a step value of +/- 6 (out of +/-15 maximum) is specified in a particular pulse sequence for a standard JEOL probe, the CapNMR probe value should be $6/13 \sim 0.5$ (round to 1).

Varian. Pulse sequences on Varian spectrometers typically specify gradient strength in terms of DAC units. Varian accommodates four different models of z-gradient, as described in the table below. **CapNMR probe users should divide the Varian DAC number by the appropriate factor, corresponding to the appropriate gradient model in the table below, to obtain the appropriate CapNMR probe DAC number for the pulse sequence/experiment.** For example, a Performa ii user with a previous DAC value of 3000 for a particular sequence would now change that DAC value to $3000/6 = 500$.

Model	Factor
Performa i (z only)	21
Performa ii (z only)	6
Performa iii (z only)	0.75*
Performa x,y,z	6

*Performa iii customers should limit DAC values to +/- 16k. Use of gradient strengths beyond this DAC range should not be attempted without first consulting technical support at MRM.

Placing the Probe in the Magnet

Bruker users must install the adapter plate before placing the probe in the magnet. The circular ring fits into the recessed region in the shim stack and the brass mounting bolts should be tightened to their fully recessed positions using the supplied BeCu Allen wrench. After the adapter is in place, the probe may be mounted by installing it through the adapter plate and tightening the captive screws.

Probe Positioning

Positioning the MRM Micro-flow probe appropriately in the magnetic field simplifies shimming. There are two steps in probe placement:

- 1) adjusting the probe height
- 2) orienting the coil along the X axis of the shim set

Adjusting the probe height

The probe height should be adjusted so that the microcoil is placed in the center of the shims to within about 1 millimeter. The probe height can be coarsely adjusted using the diagram shown below and the procedure in Appendix 1. The distance X from the top of the connector box to the center of the shims is provided in Appendix 1. The distance Y from the bottom of the *shim* mounting plate to the center of the shims is provided by the shim manufacturer or the spectrometer vendor. The difference, Z (= X-Y), of these two numbers will yield the distance between the top of the probe connector box to the top of the *probe* mounting plate.

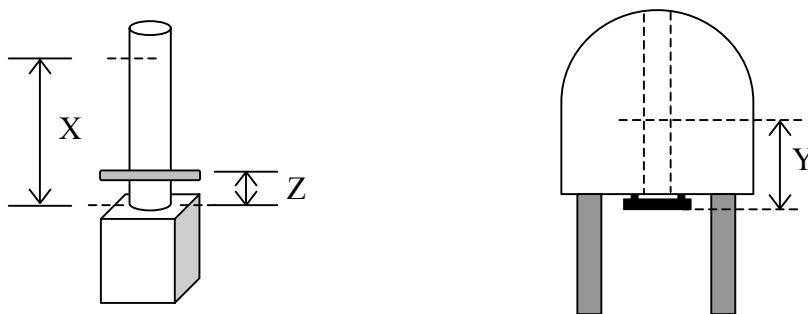


Figure 1

The probe height may be finely adjusted by loading a 5% CHCl_3 sample in acetone- d_6 and executing the following procedure.

1. Turn off the lock.
2. Acquire a spectrum.
3. Make a change in the Z1 shim of approximately 10% of its maximum value and acquire a spectrum. Note the difference in frequency for the spectrum obtained in step 2 as compared to that obtained in step 3 (including + or - direction).

If the coil is centered, then the change in Z1 will not produce a frequency shift. If changing Z1 does shift the frequency, then,

4. Move the probe up or down by a small amount (about 1 millimeter). Return Z1 to its initial value and repeat steps 1 through 3, being sure to make the same change in Z1.

If the movement in step 4 does not result in a condition in which the frequency difference is less than 1 Hz (indicating that the coil is centered) then repeat step 4 moving the probe up or down according to:

- if the frequency difference is greater, move the probe in the opposite direction
- if the difference is less move the probe in the same direction
- if the difference has changed signs, the center has been passed; so, go back

Repeat step 4 until the frequency difference is less than 1 Hz or until a movement of approximately 1 millimeter causes the frequency difference to change sign. At this point the coil will be within 1 millimeter of the center of field. It is useful to mark the probe at this point to indicate the position of the mounting plate for future reference.

Orienting the coil along the X axis of the shim set

The final step in probe placement is to orient the coil along the X axis of the shim stack. (An option is to orient the flow cell along the Y axis if this provides for convenient access to the capillary tubing; however, the following description is for X axis alignment.) This step can make shimming the solenoidal coil easier, since in these conditions the geometry of the coil along the X and Y shims is significantly different. The coil axis is parallel to the face of the connector box containing the fluid connectors. If you know the direction of the X shim in your magnet, this step can be achieved simply by rotating the probe so that the connector box face containing the fluid connectors is parallel to this direction. If the X or Y directions of your shim set are unknown, it will be necessary to orient the probe using the following procedure. (Note: It is often the case that the X or Y direction of the shim set is along the direction of the electrical connector for the shim currents.) This is a good assumption to use when establishing an initial orientation.

1. Touch up the X, Y and Z1 shims to give a reasonable initial line width (peak half-height of less than 5 Hz; line shape unimportant).
2. Increase the X shim by a value that is approximately 20% of its maximum possible value.
3. Take a spectrum and note the difference in half-width produced by the change in the X shim.
4. Return the X shim to its initial value, rotate the probe about 15 degrees and repeat steps 1 through 3.

If the half-width is reduced, this indicates that the probe is being rotated away from the X axis. If the half-width increases, the probe is being rotated closer to the X axis. Repeat steps 1 through 4 until a rotational orientation is found for which the prescribed change in the X shim produces a maximum change in half-width. At this point the coil will be oriented along the X axis.

Once the probe height is adjusted and the coil is oriented along the X axis, the set screws in the mounting plate should be tightened using the BeCu allen wrench. (In Varian/Oxford systems, the spin stack should be gently lowered against the top of the probe.)

It is worth noting that some users have had success by orienting the microcoil along XY, that is, 45° from the X axis. It has been our experience that this is not necessarily the best place to begin.

Matching and Tuning

MRM CapNMR probes are adjusted at the factory to match the proton frequency of a customer-specified magnet. Probes are equipped with typical matching networks which are more finely adjusted with wands to match and tune for a particular spectrometer just before actual use.

The proton (observe) and carbon channels should be optimized for the given experimental conditions (solvent, temperature). The probe is matched and tuned in a conventional fashion with the tuning wands at the base of the probe. The wands for matching and tuning are spring loaded so that they each maintain contact with their capacitor. The wands are long rods with screwdriver tips which must be turned to an appropriate position to engage the slot in the variable capacitor. Users should be gentle with the wands, and keep in mind that the full adjustment range of each capacitor is 7 full turns. Also, take care to protect the wand handles from any bumps or abuse.

It should not be necessary to adjust the tuning for the lock channel. If the lock tuning is adjusted (a rarity), it should not be too highly optimized since the sensitivity of the CapNMR probe makes it susceptible to saturation of the lock signal (see Locking section).

Locking

Locking an MRM CapNMR probe is achieved in a manner similar to conventional NMR probes. Two things to keep in mind when trying to lock:

1. The increased sensitivity of the CapNMR probe makes it susceptible to saturation. In general,
 - a) Making sure that the lock channel is not very well-tuned (in general the factory tuning should be adequate).
 - b) Using low lock transmitter power settings
2. Since the solvent volume of the MRM probe is small, it is generally necessary to use larger values for the lock receiver gain compared to conventional probes.

It is sometimes necessary to use a lock attenuator of 1 or 2 dB to prevent saturation.

Shimming

When using an MRM CapNMR probe, a reasonable line width and line shape is generally obtained using just six shims. These are X, Y, Z, Z², XY and R² (also called X²-Y²). To simplify shimming, it is helpful to orient the probe in the magnet with the solenoidal coil at the shim center (see Probe Placement), and along the X direction (again, see Probe Placement). Once the probe is positioned correctly it may be shimmed with the following procedure. The small sample volume of the probe results in a lock signal that is smaller with more noise. This makes lock shimming less sensitive, particularly in the final stages of shimming. It is recommended that the line shape be examined in the final stages of shimming, and if necessary, FID shimming be considered at this point. Consider shimming first with a 2-sec acquisition time, then later switch to a 4-sec acquisition time for finer shimming. To verify that damping does not occur, a check of the line width with 2.5% CHCl₃ is recommended, at least initially. When checking the line shape as you shim, be certain the line broadening is set to zero.

1. The shims should initially be set either to all zeros or to a set of values used for a standard 5 mm probe already generated by the user.
2. Shim up X, Y, and Z. Since the probe has no sample spinner at the top, it often requires a Z shim value which is considerably different than that required by standard probes.

3. Make a change in Z^2 that is large enough to produce a change in its response of about 10%. An alternative to this is to try changing R^2 by an amount large enough to produce a change in its response of about 10%.
4. Optimize X, Y and Z. (Note that since Z^2 has components of X^2 and Y^2 in it, changing Z^2 can have a large affect on X, Y or Z.)
5. Shim XY in a manner similar to that in which Z^2 is shimmed. However, every time XY is changed R^2 should also be optimized.
6. Optimize XZ and YZ, and also attempt R^2 , X^3 , and Y^3 .
7. If R^2 , X^3 , or Y^3 make a significant difference, re-optimize X, Y, and Z.

If the shimming has improved, repeat steps 3 - 5 until Z^2 is optimized. If the FID is worse, repeat steps 3 - 5 this time changing Z^2 (or R^2) in the opposite direction.

At this point, the line width and line shape should be good. Additional line width improvements may be made by re-optimizing Z^2 or XY or by making changes in XZ and YZ.

Variable Temperature Control

The MRM CapNMR probe is designed to allow for temperature control in the ranges of 0° - 50°C. To reach 0°C, the air source must be at least -25°C. The air source should not be at a temperature less than -50°C. After making a temperature change of more than 5 degrees, although the controller may indicate an equilibrium condition, a waiting time of 15 minutes is advised to allow the sample environment to reach thermal equilibrium.

It may be necessary to adjust the air flow rate for effective temperature control. The flow rate should be high enough to provide stable control, but not so high as to vibrate the probe. To check for vibration, take repeated, single scans (use a long acquisition time), and examine the baseline region of a signal.

Manual Injection

Introduction

A user with a small quantity of analyte may choose to inject the sample manually into the CapNMR probe using a hand-held syringe. A total sample volume of a few microliters (1-3 μL) is a reasonable minimum and easily compatible with the skill level most users will readily attain. Since no direct interaction occurs between the sample handling device (a syringe) and the NMR spectrometer, the method is universal.

The Manual Injection Module (MIM) can be attached to the magnet leg, and it serves to hold the injection port and anchor the syringe. The MIM also provides a storage tray for small parts and a place to keep a small sample vial. It is intended to be portable. The syringe vise provided with the probe fits nicely into the rear portion of the MIM.

Description of Equipment and Supplies

The user needs only the proper syringe and fittings, and extra solvent (if desired), to pick up the sample from a container and inject it directly into the CapNMR probe.

Syringes. One gas-tight (Teflon-tipped plunger), 10- μL syringe, with 22s, 2", blunt-tip, removable needle, such as Hamilton or Alltech #80065; one 50- μL gas-tight syringe Hamilton or Alltech #80930, with 22s, 2", blunt-tip (point style 3), removable needle. The needle is ordered separately, Hamilton or Alltech #80464.

Fittings. The syringe needle is coupled to the capillary of the probe using a metal Valco union, ZBU1XC. The needle fits into a sleeve (Upchurch #F-247) which is mounted in the union using the Upchurch PEEK nut (#F-287) and ferrule (#F-192). The 360- μm o.d. capillary is similarly joined to the back of the union, but with a different sleeve, Upchurch #F-242. An additional union (P-779-01), nut and ferrule (Upchurch #F-331N), and plug (Upchurch #P-550) are recommended.

The P-779-01 union can show some internal wear and tear. If its through-hole is not clear when held up to a light and seen through the magnifying glass, replace it.

Filtration. A M-135, 2- μm , in-line microfilter is provided on leg A of the probe. It should always be in place and will not broaden samples entering the probe. The replacement filter capsule is part M-130. A cracked tip can result in a condition that appears to be a blockage. The capillary tips can be checked, recut if necessary, and remounted.

The capillary actually attached to the probe should be trimmed sparingly, because it is a permanent probe component.

Complete Parts List for Manual Injection

Here is a complete list of replacement parts for manual injection NMR:

- Hamilton/Alltech #80065 - gas-tight (Teflon-tipped plunger), 10- μ L syringe, with 22s, 2", blunt-tip, removable needle (needle dead volume is 0.9 μ L)
- Hamilton/Alltech #80965 - 50- μ L gas-tight syringe, with 22s, 2", blunt-tip (point style 3), removable needle (needle dead volume is 0.9 μ L)
- Hamilton/Alltech #80464 – removable needle for 50- μ L gas-tight syringe
- Hamilton/Alltech #81120 - 250-mL gas-tight syringe
- Hamilton/Alltech #81520 - 5-mL gas-tight syringe
- Valco #ZBU1XC – Metal injection port for MIM, syringe needle to capillary (Alternate PEEK port is P-779-01 Upchurch union with F-331N nut and F-242 and F-247 sleeves)
- Upchurch #F-247 – Injection sleeve for syringe to injection port, green, fits 22 gauge needle tips
- Upchurch #F-287 – PEEK nut holds sleeves (F-247, F-242) in metal injection port
- Upchurch #F-192 – Black PEEK ferrule to be used with F-287 nut and sleeve Valco metal injection port
- Upchurch #F-242 – capillary sleeve, green, to fit F-287 nut and F-192 ferrule
- Upchurch #1533 – PEEK tubing, green used for air push
- Upchurch #P-659 – Quick connect Luer adapter for connection of syringe to nut & ferrule
- Upchurch #F-331N – Nut and ferrule that connects P-659 Luer adapter to PEEK tubing
- Upchurch #F-334N – Nut and ferrule that connects PEEK tubing to Valco injection port
- Upchurch #FS-315 – Fused silica capillary diamond-wheel cutter
- Upchurch #FS-150 - Extra fused silica capillary; 50 μ m i.d., 360 μ m o.d.
- McMaster #1503T6 (at 1-630-833-0300, Chicago) – Magnifying glass

Usually received direct from MRM with CapNMR Probe:

- Syringe vise to mount and push 250- μ L syringe (MRM only source)
- Syringe clamp to mount and push 5-mL syringe (MRM only source)

Sampling Handling

The flow cell of the CapNMR probe has a volume of 5 μ L. For a given sample concentration, a reasonable choice of injected sample volume is 5 μ L. A sample volume less than this will yield a smaller S/N, with the rate of signal decrease becoming significant below 3 μ L. A sample larger than 5 μ L will yield somewhat higher S/N up to about 8 μ L, above which only a small improvement in S/N is seen because the flow cell is appreciably over-filled. Another consideration is that the capillary (50 μ m i.d.) connected to the probe has a nominal internal volume of 2 μ L/m, and a length of 2 m (4 μ L total). The two-inch, 22s syringe needle is an additional volume of 0.9 μ L. The 75 μ m i.d. capillary has a volume of 4.6 μ L/m.

Typically, the sample concentration should not exceed 25 μ g/ μ L, unless additional rinsing procedures are employed. Also, if a sample needs to be sonicated or heated to dissolve, avoid pushing that sample into the probe.

Parking the Sample in the CapNMR Probe

Begin with a probe that has been rinsed with deuterated acetone and dried with air. The very first, key step is to establish a bubble-free flow path through the probe from inlet to outlet. This is most effectively accomplished with a 250- μL syringe containing the solvent of interest. If you are using the MIM, connect the syringe and the MIM port using the green PEEK tubing. Otherwise, you can connect the syringe directly to the inlet capillary. Fill the 250- μL syringe with about 200 μL of solvent, and mount it in the syringe vise. Bear in mind that a Teflon-tipped plunger pulls back when released suddenly (such as by one's thumb), and air can be pulled into the probe via the outlet, especially with a large syringe. Using the syringe vise prevents this, as the applied pressure is reduced gradually by the spring built into the device. The use of a relatively large push volume and pressure helps compress bubbles and move them out of the probe. Turn the knob on the syringe vise several times, and flow should be seen from the probe outlet. You will notice that the lock signal and shimming will remain stable once bubbles are eliminated.

Then, connect the probe inlet capillary to the MIM (or the back of the P-779-01 PEEK union alone) using the nut, ferrule, and F-242 sleeve. Prepare the front end of the MIM port (or PEEK union) to accept the needle of the syringe with the F-247 sleeve.

Always use a microfilter in the flow path (M-135 containing a 2- μm , M-130 capsule). Even seemingly clean solvent can contain particulates.

Once a fluidically stable flow path has been established, fill the 50- μL syringe with about 45 μL of solvent, being sure to expel all bubbles, and set it aside. Rinse the 10- μL syringe with solvent, expelling all bubbles, and leave the plunger in the empty position, such that the needle is still filled with solvent.

Next:

- Pick up 5 μL of sample with the 10- μL syringe
- Insert the needle into the sleeve
- Inject all the contents of the syringe slowly at a rate of about 0.5 $\mu\text{L}/\text{sec}$
- Switching to the other syringe, inject an additional 4 μL of push solvent (or other appropriate push volume), and leave the syringe in place. Be aware that bubbles could enter the capillary, so inject carefully.

Proceed to acquire NMR data. The probe outlet may be plugged using the additional union and fittings (P-779-01 and F-331N), the F-242 sleeve, and the plug (P-550). This is especially recommended for data acquisition times of more than one hour.

After NMR data acquisition, remove the plug if necessary, and rinse the probe with at least 50 μL of solvent using the 50- μL syringe, and if desired, recover the sample at the capillary outlet. If a multi-scan, proton NMR spectrum shows that the flow cell is not clean, rinse again with another 50 μL of solvent, and check again with a proton spectrum that the CapNMR flow cell is clean. If concentrations higher than 25 $\mu\text{g}/\mu\text{L}$ are used, larger volumes of rinse solvent are usually needed.

Depending on the availability of sample, the user may elect to rinse the sample syringe with sample itself (instead of solvent) before picking up the 5 μL for injection. In addition, the use of push solvent itself can be eliminated if sufficient sample volume exists.

Pneumatic Connection

The 5-mL syringe can be connected to the Manual Injection Module injection port with the following components provided at installation:

- PEEK tubing, green, #1533 (60 cm)
- P-659 Quick Connect Luer Adapter
- F-331N Nut and Ferrule
- F-334N Nut and Ferrule

Calibration of Solvent Push Volume for Manual Flow Injection

This section describes how to determine the most appropriate manual push volume, V_{push} , to maximize S/N for a given sample injection volume, V_{inj} . The following procedure is best performed with a model compound before the analyte of interest is employed. A reasonable sample injection volume is 3-5 μL , with 3 μL preferred. The resultant V_{push} is dependent on the sample injection volume used, so for careful work, it is necessary to perform the following procedure for each V_{inj} you choose to use.

Parts and Apparatus

- 250- μL syringe
- 5-mL syringe
- 10- μL syringe (needle dead volume is 0.9 μL)
- 25- μL syringe (needle dead volume is 0.9 μL)
- Injection port connected to probe:

Manual Injection with MIM or HPMI

- Upchurch F-287 black nut and ferrule
- F-247 sleeve, green; for FEP tubing
- F-242 sleeve, green; for fused silica capillary sleeves

Manual Inject

- Upchurch P-779-01 union
- F-331N nut and ferrule
- F-242 (for capillary) and F-247 (for needle)

For a summary on how to assemble the fittings, see the following tech bulletin at www.microNMR.com:
For FEP tubing, see Tech Bulletin G0113 - Fused Silica Capillary Fluidic Connections
For Fused Silica Capillary, see Tech Bulletin G0310 - FEP Tubing Fluidic Tutorial

Procedures

Starting with a Solvent-Filled Probe (Recommended for $V_{inj} \leq 5 \mu\text{L}$)

- Rinse the probe with solvent alone (250- μL syringe and syringe vise; no port); leave solvent in probe.
- Rinse the 25- μL syringe with solvent.
- Connect syringe to injection port, and inject 25 μL of solvent into probe. Leave syringe in port.
- Rinse another 25- μL syringe with solvent, and empty the syringe barrel leaving its needle filled with solvent.
- Draw sample (model compound) up into the second 25- μL syringe; be sure bubbles are eliminated.
- Inject sample into probe (slowly push plunger all the way down to zero).
- Acquire a spectrum, preferably a 1-scan spectrum. This spectrum should be blank, or indicate very little sample.
- Remove the sample 25- μL syringe and replace it with the first 25- μL syringe, filled with solvent to the 20 μL mark.
- Measure the amount of tubing/capillary from the base of the probe to your injection port. At 4.5 $\mu\text{L}/\text{meter}$ for 75 μm tubing/capillary, calculate how much volume there is between your injection port at the base of the probe. This volume (volume=X) will ensure you are before the maximum by only pushing the sample to the base of the probe.

- Slowly inject volume X; leave syringe in port.
- Acquire under the same conditions and measure S/N as before. Measure S/N of a distinct spectral feature under well-relaxed conditions.
- Take the 25- μ L syringe out and refill with solvent.
- Draw the same exact volume of sample (model compound) up into the second 25- μ L syringe; be sure bubbles are eliminated.
- Mount 25- μ L syringe in port by placing the fitting onto the syringe before placing the syringe tip into the injection port and tightening the fitting.
- Inject sample into probe (slowly push plunger all the way down to zero).
- Remove the sample 25- μ L syringe and replace it with the solvent 25- μ L syringe being careful to place the fitting onto the syringe before placing the syringe tip into the injection port to prevent any air bubbles.
- Slowly inject volume (X + 1 μ L); leave syringe in port. This additional 1 μ L will position the sample 1 μ L (4.5 meters) closer to the flowcell.
- Acquire under the same conditions and measure S/N as before.
- Repeat these steps (wash out probe with solvent, inject sample, inject previous push volume of solvent plus incrementally adding an microliter to the push volume until the S/N value goes through a distinct maximum.
- Repeat this entire exercise using 0.5- μ L increments around the S/N maximum to more carefully determine the V_{push} that yields the largest S/N.

Use the V_{push} determined above to push the sample volume of interest into the flow cell to yield the maximum S/N.

Starting with an Empty Probe (Recommended for $V_{inj} > 5 \mu\text{L}$)

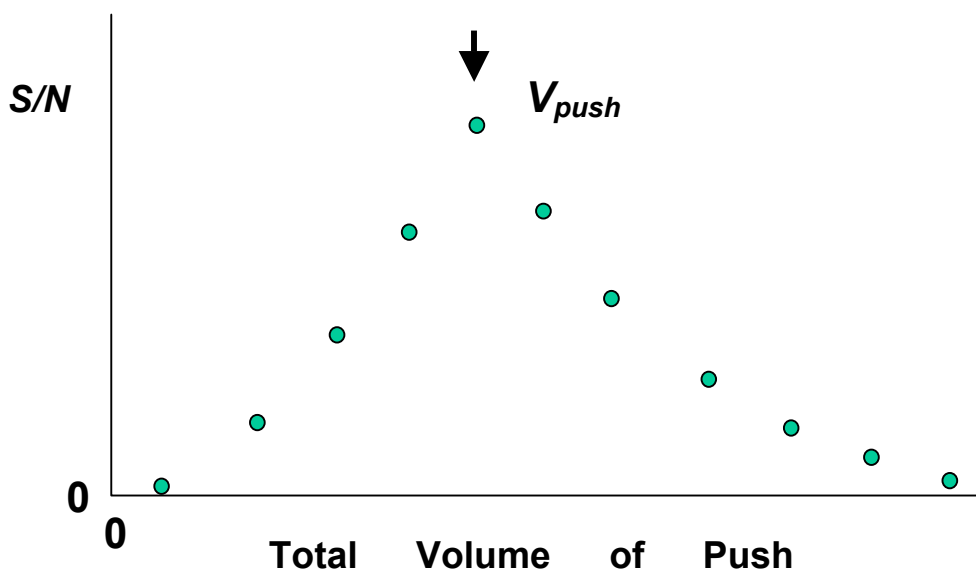
- Rinse the probe with solvent alone (250- μ L syringe and syringe vise; no port).
- Blow all the solvent from the probe with air (5-mL syringe and syringe clamp; no port).
- Rinse 25- μ L syringe with solvent, and empty the syringe barrel leaving its needle filled with solvent.
- Draw sample up into the 25- μ L syringe; be sure bubbles are eliminated.
- Attach syringe to injection port and inject sample into probe (push plunger all the way down to zero).
- Acquire a spectrum, preferably a 1-scan spectrum. This spectrum be blank, or indicate very little sample. If the fluid has not slightly overflowed the flow cell, the condition may be unshimmable. If so, proceed to the next step.
- Measure the amount of tubing/capillary from the base of the probe to your injection port. At 4.5 $\mu\text{L}/\text{meter}$ for 75 μm tubing/capillary, calculate how much volume there is between your injection port at the base of the probe. This volume (volume=X) will ensure you are before the maximum by only pushing the sample to the base of the probe.
- Slowly inject volume X; leave syringe in port.
- Acquire under the same conditions and measure S/N as before. Measure S/N of a distinct spectral feature under well-relaxed conditions.

- Using the solvent syringe, rinse the probe entirely with solvent 25-50 μL .
- Blow all the solvent from the probe with air (5-mL syringe and syringe clamp; no port).
- Draw sample up into the 25- μL syringe; be sure bubbles are eliminated.
- Attach syringe to injection port and inject sample into probe (push plunger all the way down to zero).
- Slowly inject volume ($X + 1\mu\text{L}$); leave syringe in port. This additional 1 μL will position the sample 1 μL (4.5 meters) closer to the flowcell.
- Repeat these steps (rinse, inject sample, inject push volume plus additional microliter) until the S/N value goes through a distinct maximum.
- Repeat this entire exercise using 0.5- μL increments around the S/N maximum to more carefully determine the V_{push} that yields the largest S/N.
- Use the V_{push} determined above to push the sample volume of interest into the flow cell to yield the maximum S/N.

Use the V_{push} determined above to push the sample volume of interest into the flow cell to yield the maximum S/N.

Sample Data

See the data set below as an example of how data may appear.



Maintenance Procedures

Basic Maintenance

Filters. Always be sure that any fluid going into the probe is filtered. The probes with capillary tubing are installed with an in-line microfilter from Upchurch Scientific, part #M-135, which contains a stainless steel, 2- μm filter. The BioFlow probes with FEP tubing are installed with an in-line microfilter from Upchurch Scientific, part #M-542, which contains a stainless steel, 2- μm filter. You should become familiar with the filter and change the capsule within it on a regular basis, and keep a log of such changes. A probe that seems clogged should be fitted with a new filter capsule. Don't bypass the filter. Replace the capsule (Upchurch M-130 for capillary; Upchurch M-132 for FEP tubing). Also, check the capillary tips connecting to the filter. They can be crack-prone and cause a blockage. Re-cutting the tips will restore good flow.

Rinsing Devices. Every user is supplied with a syringe vise (black-anodized aluminum) at installation. We recommend that the vise be fitted with a 250- μL syringe for rinsing of solvents through the probe. The syringe clamp (black plastic) is to be used with a 5-mL syringe to allow air, solvent, or sample to be pushed through the probe.

A basic rinsing and storage procedure after an experiment is:

- Push solvent through the probe to remove the last sample
- Push air through the probe to remove the solvent
- Push acetone- d_6 through the probe
- Push air through the probe to dry it for storage

Never let dissolved sample dry out inside the probe. If this occurs, try rinsing additional pure solvent through the probe, or cut about 1 cm off each capillary tip, and attempt rinsing.

Solvent Miscibility. Some thought needs to be given to solvent miscibility, such as when chloroform in the probe (even 5% chloroform in acetone- d_6) is followed by water. Exposure of the probe to such combinations should include a transition solvent, such as acetone. Any doubts about the suitability of solvent compatibility can usually be tested by simple mixing experiments on the benchtop.

Loss of Good Shimming. A probe that needs rinsing or cleaning is usually indicated by a loss of symmetrical and narrow line shape. We recommend an initial rinse with the sequence of air-acetone-air, followed by the solvent of interest. This very often restores performance. If not, try the bleach rinse described below.

Acid Rinsing Solution

If an acetone rinse does not restore good shimming, try this acid rinse solution.

First, make a solution of 0.2 M HCl by diluting the appropriate volume of concentrated HCl from the stock bottle (~ 12 M). *Be sure to pour the acid into the water.*

Next, combine in a 10-mL volumetric flask:

5 mL 0.2 M HCl in H_2O
5 mL ACN

The result is:

0.1 M HCl
50% H₂O
50% ACN

Hydrogen Peroxide (H₂O₂) 10% Strength

Another rinsing option is hydrogen peroxide, which is a very effective rinse for urine, plasma, and biological samples.

Hydrogen peroxide can be purchased in two strengths; 3% and 30%. A commercial grade strength that is available in most drug stores is 3%. A reagent grade available from chemical supplies is generally 30%, which is a strong oxidizer, and proper precautions should be used.

Dilute 3 mL of the reagent grade (30%) H₂O₂ with 6 mL of water (H₂O).

This will yield 9 mL of 10% strength hydrogen peroxide.

After 50-100 µL of this mixture is pushed through an initially dry probe, the user can elect to allow the probe to soak, or proceed to push the remaining cleaning solution out of the probe with air, then rinse the probe with about 100 µL of D₂O, then some acetone-d₆, then air. The probe can then be reloaded with the solvent or sample of interest.

70% Formic Acid + 30% Acetonitrile

A final rinsing option for any kind of biological, lipid, or protein sample is 70% Formic Acid 30% Acetonitrile. This has been found to greatly improve lineshape after a variety of samples have struck in the flowcell. This solution can safely be left in the probe and tubing for extended periods of time (i.e. over the weekend) without harm. Protonated solvents are fine to use when rinsed out appropriate volumes of deuterated solvent.

After 50-100 µL of this mixture is pushed through an initially dry probe, the user can elect to allow the probe to soak, or proceed to push the remaining cleaning solution out of the probe with air, then rinse the probe with about 100 µL of ACN, then some acetone-d₆, then air. The probe can then be reloaded with the solvent or sample of interest.

Gadolinium Chloride

We have learned that GdCl₃ is very effectively rinsed from the probe with the HCl acid rinsing solution.

Other Rinsing Solutions

A similar concentration of HNO₃ can be substituted for the HCl in the acid rinsing solution, especially if metal contamination is suspected. However, Gd³⁺ forms a low-solubility complex with NO₃⁻ so nitric acid should not be used to rinse out gadolinium chloride.

Do not attempt to clean the CapNMR probe with HF, as the fused silica in the flow path will be damaged.

O-ring

The top end of the probe is fitted with an o-ring to ensure a snug fit into the magnet bore. It should be inspected and cleaned as necessary with a towel wetted with water or alcohol.

Summary

Basic Cleaning Procedure

- Rinse with solvent that the sample is typically dissolved in
- Rinse with air
- Rinse with acetone-d₆
- Rinse with air
- Probe performance can be evaluated at this point

Optional Acid Cleaning Procedure

- Rinse with acid solution (see recipes above)
- Rinse with D₂O
- Rinse with acetone-d₆
- Rinse with air
- Re-evaluate probe performance

Optional Hydrogen Peroxide Cleaning Procedure

- Rinse with 200 μL of 10% H₂O₂ for about 20 min
- Rinse with 200 μL of purified H₂O for about 20 min
- Rinse with acetone-d₆
- Rinse with air
- Re-evaluate probe performance

Appendix 1: Dimensions for Probe Installation

Follow the procedure below to determine the value of X for your probe (for “Probe Positioning” on page 3). This determination will enable coarse positioning of the NMR microcoil in the center-of-field of the shims.

Step 1) Measure the length (L) of your probe tube from the top of the connector box to the top of the probe as shown below.

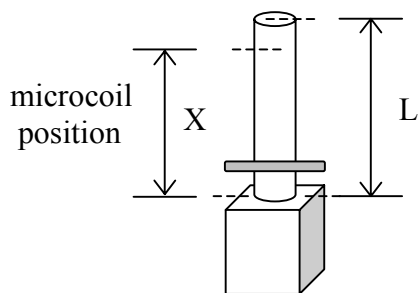


Figure 2

Step 2) For the value of L determined (left column), the value of X (right column; see Figures 1 and 2) is then:

L (length of probe from top of connector box to top of probe)	X (distance from top of connector box to microcoil)
493 mm	456 mm
538 mm	501 mm

Appendix 2: Performance Tests and Specifications

- 1) **Resolution and Line shape.** 5% CHCl₃ in acetone-d₆, stopped flow, probe completely filled with solvent. No line broadening (LB = 0); single scan. Line widths at 50%, 0.55%, and 0.11%. The 5% CHCl₃ in acetone-d₆ can become wet with repeated exposure to the atmosphere, so replace it occasionally.
- 2) **Static S/N.** 10 mM sucrose in 100% D₂O (0.1 mM NaN₃), quantity sufficient to fill flowcell and feed capillary; 15 nmol (5.1 μg) in 1.5 μL observe volume. Anomeric proton. LB = 0.7 Hz. Measure S/N using a spectral width of 1500 Hz, a noise window of 100 Hz, and 4500 points, and a region of 5 – 8 ppm.
- 3) **On-flow S/N.** 3 μL injection of 5 mM sucrose (in 100% D₂O; 0.1 mM NaN₃) at 5 μL/min for total of 15 nmol (5.1 μg) injected. LB = 0.7 Hz. Anomeric proton S/N, as above.
- 4) **Stopped-Flow S/N.** 3 μL injection of 1 mM sucrose (in 100% D₂O; 0.1 mM NaN₃) at 5 μL/min for a total of 3 nmol or 1 μg injected. LB = 0.7 Hz. Anomeric proton S/N, as above.

The quote for your probe defines the performance limits acceptable for these tests. When your probe is installed, you will be provided with a specifications report summarizing the performance of your particular probe in the described tests.

Appendix 3: Probe Storage and Shipping

As mentioned earlier, never store your probe for an extended period with solute-containing fluid in the probe. Dissolved sample can dry and plug the capillary tips. If this occurs, the tips of both ends of the capillary need to be trimmed (about 1 cm).

If you are shipping your probe, first rinse it thoroughly with acetone- d_6 , then push air through it with a 5-mL Luer lock syringe and fitting until you are sure all solvent has been cleared. Remove or safely secure the capillary, and ship it in the case you received it in.

Appendix 4: Miscibility and Viscosities of Common Solvents

Solvent Miscibility and Viscosity (cP)

Adapted from The HPLC Solvent Guide by Paul C. Sadek; Wiley-Interscience

Acetone (0.32)									
Acetonitrile (ACN) (0.37)									
Benzene (0.65)									
Butanol (0.73)					Miscible (viscosity)				
Carbon tetrachloride (0.97)					Immiscible (viscosity)				
Chloroform (0.57)									
Cyclohexane (1.00)									
1,2- Dichloroethane (0.79)									
Dichloromethane (0.44)									
Dimethyl formamide (DMF) (0.92)									
Dimethyl sulfoxide (DMSO) (2.24)									
Dioxane (1.54)									
Ethanol (1.20)									
Ethyl acetate (0.45)									
Ethyl ether (0.32)									
Heptane (0.39)									
Hexane (0.33)									
Iso-octane (0.54)									
Isopropyl alcohol (2.30)									
Methanol (0.60)									
Methyl- <i>t</i> -butyl ether (0.27)									
Methyl ethyl ketone (0.45)									
Pentane (0.23)									
THF (0.55)									
Toluene (0.59)									
Water (1.0)									

Sulfuric acid, reagent grade, has a viscosity of 19.1, and is miscible with water.

Appendix 5: Estimating Pressures

The pressure equation below can be used to estimate the pressure required to achieve a given flow rate under certain specified conditions. Note that the pressure applied to achieve a given flow rate is directly proportional to the fluid viscosity. The flow rate is not achieved instantaneously, and it can take several minutes for pressure to build up to achieve a certain flow rate setting, depending on the total volume of fluid being compressed. The larger the volume, the longer the compression time required.

To estimate the pressure for a certain flow rate, capillary length, capillary i.d., and fluid viscosity, use the following equation:

$$P = 26 \cdot F \cdot L \cdot \left(\frac{\eta}{1.0} \right) \cdot \left(\frac{50}{d} \right)^4$$

P	=	Pressure in psi
F	=	Flow in $\mu\text{L}/\text{min}$
L	=	Length of capillary in meters
η	=	Viscosity in centipoise
d	=	Capillary inner diameter in μm

For instance, for a flow rate of 5 $\mu\text{L}/\text{min}$ through 3 meters of capillary, a fluid viscosity of 1.0, and a capillary with an inner diameter of 50 μm , the pressure exerted is 390 psi. However, a change of capillary i.d. to 75 μm will decrease the pressure by a factor of five to 78 psi.

Appendix 6: Chemical Shifts of Common Residual Solvents

We found a very informative article which lists the chemical shift of residual protonated solvents in deuterated solvents. The proton and carbon shifts of many common laboratory solvent residual signals are documented. Below is an excerpt.

NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities

Journal of Organic Chemistry Volume 62, Number 21, 1997, pp. 7512-7515.

Hugo E. Gottlieb, Vadim Kotlyar, and Abraham Nudelman

Department of Chemistry, Bar-Ilan University,

Ramat-Gan 52900, Israel

	<u>Proton</u>	<u>Multiplet</u>	<u>CDCl₃</u>	<u>(CD₃)₂CO</u>	<u>(CD₃)₂SO</u>	<u>C₆D₆</u>	<u>CD₃CN</u>	<u>CD₃OD</u>	<u>D₂O</u>
Solvent residual peak			7.26	2.05	2.50	7.16	1.94	3.31	4.79
H ₂ O		s	1.56	2.84	3.33	0.40	2.13	4.87	
Acetic acid	CH ₃	s	2.10	1.96	1.91	1.55	1.96	1.99	2.08
Acetone	CH ₃	s	2.17	2.09	2.09	1.55	2.08	2.15	2.22
Acetonitrile	CH ₃	s	2.10	2.05	2.07	1.55	1.96	2.03	2.06
Benzene	CH	s	7.36	7.36	7.37	7.15	7.37	7.33	
Chloroform	CH	s	7.26	8.02	8.32	6.15	7.58	7.90	
1,2-dichloroethane	CH ₂	s	3.73	3.87	3.90	2.90	3.81	3.78	
Dichloromethane	CH ₂	s	5.30	5.63	5.76	4.27	5.44	5.49	
Dimethylformamide	CH	s	8.02	7.96	7.95	7.63	7.92	7.97	7.92
	CH ₃	s	2.96	2.94	2.89	2.36	2.89	2.99	3.01
	CH ₃	s	2.88	2.78	2.73	1.86	2.77	2.86	2.85
Dimethyl sulfoxide	CH ₃	s	2.62	2.52	2.54	1.68	2.50	2.65	2.71
Ethanol	CH ₃	t, 7	1.25	1.12	1.06	0.96	1.12	1.19	1.17
	CH ₂	q, 7	3.72	3.57	3.44	3.34	3.54	3.60	3.65
	OH	s	1.32	3.39	4.63		2.47		
Ethyl acetate	CH ₃ CO	s	2.05	1.97	1.99	1.65	1.97	2.01	2.07
	CH ₂ CH ₃	q, 7	4.12	4.05	4.03	3.89	4.06	4.09	4.14
	CH ₂ CH ₃	t, 7	1.26	1.20	1.17	0.92	1.20	1.24	1.24
Ethylene glycol	CH	s	3.76	3.28	3.34	3.41	3.51	3.59	3.65
<i>n</i> -Hexane	CH ₃	t	0.88	0.88	0.86	0.89	0.89	0.90	
	CH ₂	m	1.26	1.28	1.25	1.24	1.28	1.29	
Methanol	CH ₃	s	3.49	3.31	3.16	3.07	3.28	3.34	3.34
	OH	s	1.09	3.12	4.01		2.16		
2-Propanol	CH ₃	d, 6	1.22	1.10	1.04	0.95	1.09	1.50	1.17
	CH	sep, 6	4.04	3.90	3.78	3.67	3.87	3.92	4.02
Pyridine	CH ₍₂₎	m	8.62	8.58	8.58	8.53	8.57	8.53	8.52
	CH ₍₃₎	m	7.29	7.35	7.39	6.66	7.33	7.44	7.45
	CH ₍₄₎	m	7.68	7.76	7.79	6.98	7.73	7.85	7.87
Tetrahydrofuran	CH ₂	m	1.85	1.79	1.76	1.40	1.80	1.87	1.88
	CH ₂ O	m	3.76	3.63	3.60	3.57	3.64	3.71	3.74
Toluene	CH ₃	s	2.36	2.32	2.30	2.11	2.33	2.32	
	CH(<i>o/p</i>)	m	7.17	7.1-7.2	7.18	7.02	7.1-7.3	7.16	
	CH(<i>m</i>)	m	7.25	7.1-7.2	7.25	7.13	7.1-7.3	7.16	

Appendix 7: Cutting and Trimming Fused Silica Capillary

You should have received a brief demonstration of how to handle fused silica capillary during your installation. Cut capillary to the approximate required length using a ceramic cutting stone. Then, trim the last centimeter or so of the tip with the Upchurch Precision Fused Silica Capillary Cutter, part #FS-315.

As a review, the sequence for trimming is:

- Push in the button on trimmer with your thumb and hold it down
- Position capillary within cutting wheel
- Tighten gently the gold wheel which holds the capillary in place with an o-ring
- Release the button
- Rotate the cutting wheel about one-half way around
- Push in the button
- Loosen the gold wheel which holds the capillary
- Slip the capillary out of place
- Run your index finger and thumb lengthwise along the capillary and crack the capillary
- Flick the tip with your finger, and check the tip with a magnifying glass

From time to time, clean the inside of the trimmer with water or alcohol. If the blade is damaged or indicates wear, replace it with Upchurch part #FS-315-02.

Appendix 8: Microfilter Assembly and Use

The Upchurch M-135 microfilter assembles and functions in a straightforward manner. We generally supply it with a probe that is used manually, or in combination with an automation platform.

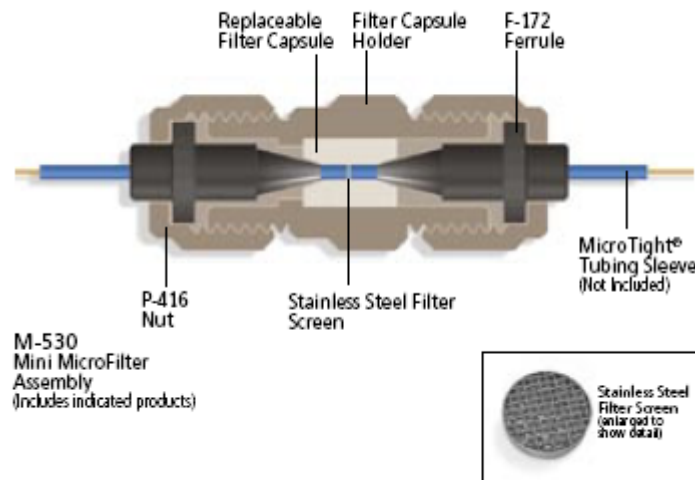
In certain instances, the Upchurch M-542 filter is employed, as it connects directly to 1/32" o.d. FEP tubing, and can be used with fused silica capillary by employing a sleeve. The Upchurch M-542 filter must be assembled in a certain sequence. The filter housing is asymmetric and the filter capsule rests against a lip. If you gather the nut, green sleeve (Upchurch #F-376x), ferrule (Upchurch F-112x), and capillary and push them together from the wrong side of the housing, the filter capsule will just slip out of place. So, push against the capsule so that it can't slip out of the opposite side, that is, push the capsule against the inside lip. (Note: If you are using 1/32" o.d. FEP tubing, you will not need the sleeve).

The filter capsule has a groove on one side of it for identification purposes, and may be two different colors. Nonetheless, it is functionally symmetric, and makes no difference which direction it points in the filter housing. There is no specified direction for flow.

As with any filter, it is a good idea to rinse it when it is first used. Also, if a filter is disassembled, the directionality of it needs to be recorded so it is re-mounted in the same direction. If the directionality of a filter is in doubt, replaced it with a new one. The replacement microfilter capsule for the M-542 filter is Upchurch part #M-132.

2- μ m Mini MicroFilter (see diagram for assembly but use these part numbers)

- Connects directly to 1/32" o.d. FEP; connects to fused silica capillary with a sleeve
- M-542 Filter body, filter capsule, filter ferrules
- M-132 Extra filter capsules (2-um screen)
- F-112 Extra filter ferrules
- F-376 Small green sleeves for fused silica only



Appendix 9: Standard Acquisition Parameters

Below are typical parameter values for the CapNMR probe. Note that the CapNMR probe requires significantly less power than conventional probes. Your solvent of choice affects how much lock power and gain is needed. A common mistake is to saturate the lock channel by using too much lock power.

The CapNMR probe also requires less transmitter power than traditional probes. For related information, please review the section in this manual titled *Spectrometer Power and Gain Levels* (see page 3).

Parameter	Varian Range	Bruker Range
Lock power	1 to 8	-55 to -45
Lock gain	34 to 48	110 to 130
Transmitter power level (dB)	40 to 45	15 to 20
90° Transmitter pulse width (μsec)	2.5 to 5	2.5 to 5
90° Decoupling pulse width (μsec)	7 to 12	7 to 12

For Varian spectrometers, the *tpwr* parameter defines transmitter power so that the larger the *tpwr* parameter, the more power goes into the probe. In other words, *tpwr* is proportional to transmitter power.

For Bruker systems, the *PL#* parameter specifies attenuation of the transmitter power, so that the larger the value of *PL#*, the less power goes into the probe. So, *PL#* is inversely related to transmitter power.

Appendix 10: Contributors

James Norcross of Protasis/MRM, Mark O'Neil-Johnson of Sequoia Sciences, Dave Detlefsen of Novatia Corporation, and Paul Molitor of the University of Illinois contributed significantly to this Manual based on their personal experience. We are also grateful to our many users for their helpful remarks and feedback.

Everyone is welcome to comment on the Manual by contacting the editor:

Dean Olson

dolson@microNMR.com

Protasis/MRM Corporation

101 Tomaras Avenue

Savoy, Illinois 61874 USA

Phone: (217) 351-4359

Fax: (217) 352-6655

This edition of the CapNMR Probe Manual was revised 24 August 2005. Updates of the Manual, along with Technical Bulletins, are maintained at www.microNMR.com.

©Copyright Protasis/MRM Corporation, August 2005