A store in Rome—also, the Caltech NMR Facility

NMR time at Caltech is very Expensive!

- Our NMR charges are among the highest in the U.S. and probably 3x-4x what many public universities charge.
- Being proactive about experiment planning, and checking early results before starting longer experiments, can have a bigger $$$ payback here (depending on how you value your own time)
- It makes specialty and high quality NMR glassware a relative bargain (though treat expensive tubes carefully so they hold their value)

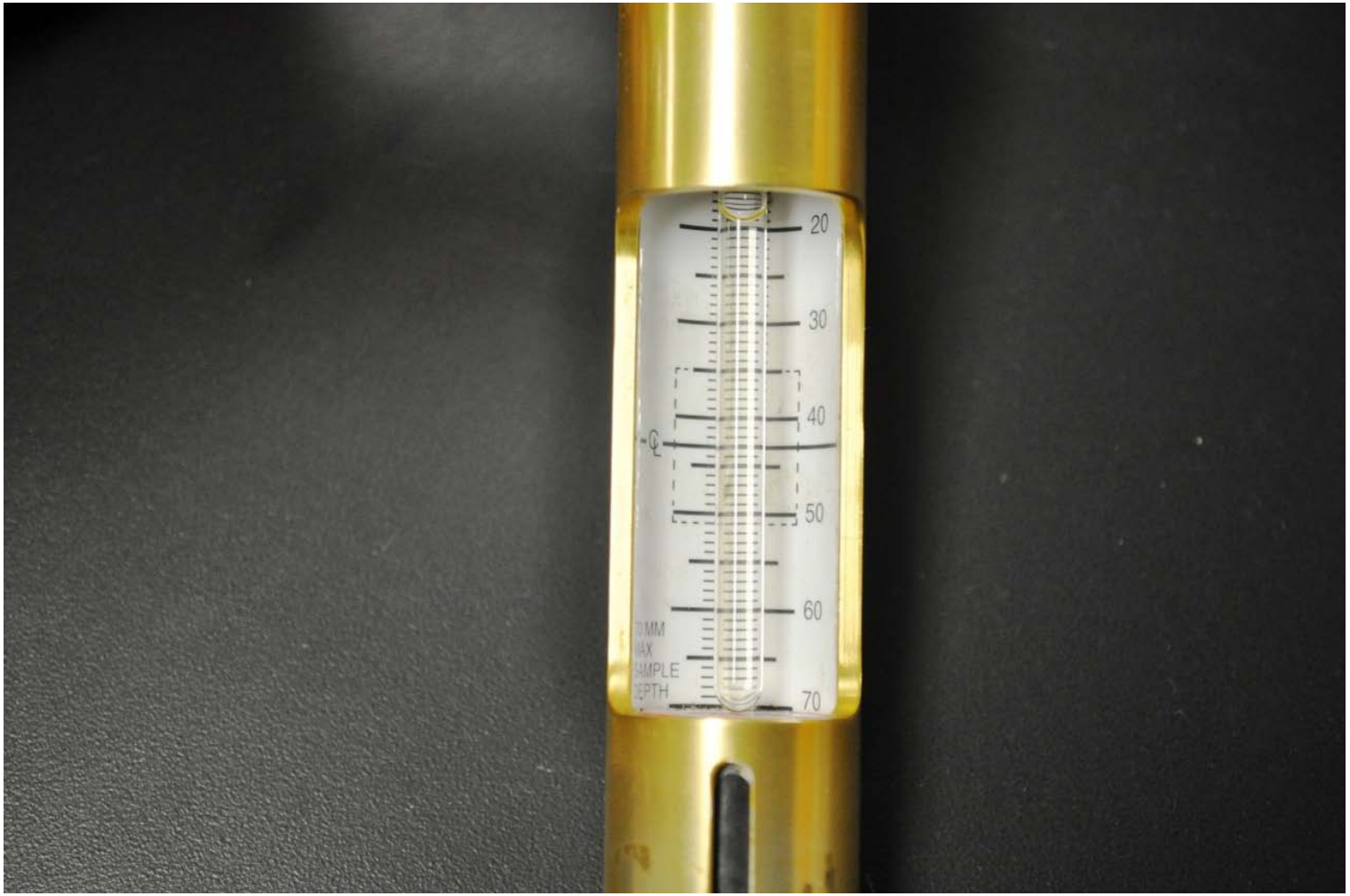
The instrument does many things for you, but you still have to make the sample

- How you do it should reflect your highest priority: quality of the results? Fastest turnaround? Quantitation? Least effort? Smallest NMR bill? Smallest solvent costs? Etc.
- Good NMR tubes generally give better spectra, especially at higher field. They cost less than the price of 1 hour of NMR time. IMHO, they only have to work a little bit better to justify the investment.

The rest of the talk in a nutshell …

- 1. For highest spectrum quality with the least effort: make samples full length in a high quality tube.
- 2. For highest mass sensitivity: use a Shigemi tube, load it by hand, customize shim setup, verify correct shimming.
- 3. For highest mass sensitivity if you don't or can't use a Shigemi tube: shorten the sample, but not too much, and verify correct shimming.
- 4. To limit use of expensive solvents, consider using a 3 mm tube. These shim well in a 5 mm probe using only 175 microliters of solvent. There is some loss of sensitivity that way; if you use them with the 3 mm probe on the 600, there is no down side.

Varian-supplied standard sample—liquid height is 5 cm, about 700 microliters, depth gauge set at 70 mm



The combination of full length (5 cm) samples and gradient shimming is usually very effective!

- Gradient shims go out to z^4 in organic solvents, to z^5 in water, and properly handle the interactions between the shims. This is much more rigorous than what most people do when shimming by hand.
- Full length samples should shim well, in a smaller number of shimming cycles, than short samples
- Gradient shimming is not a black box—you can fine tune it. If it does not work well, it may be quite easy to diagnose and correct the problem by looking at the gradient profiles and field map.

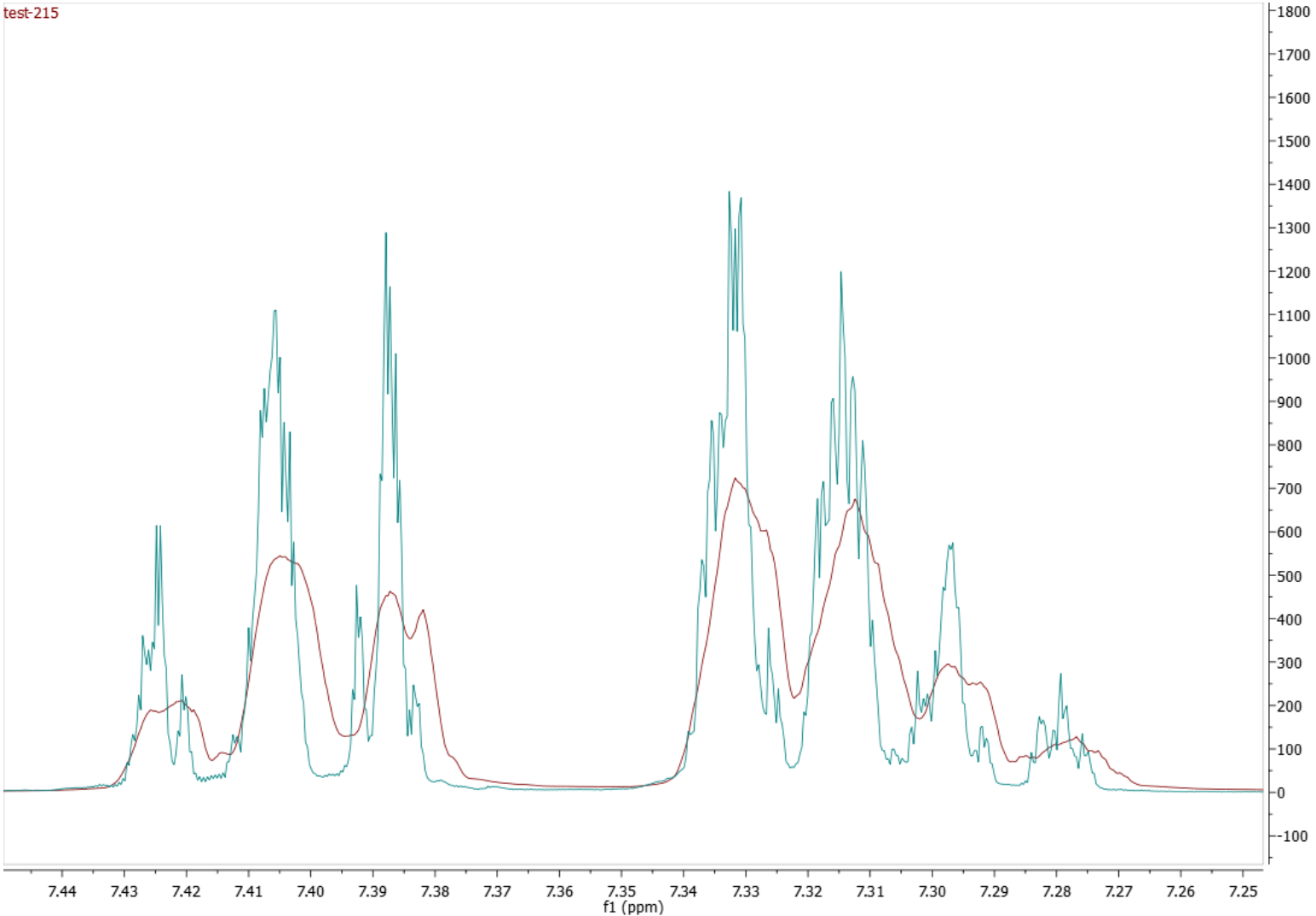
Full length samples are a winning strategy when …

- You are using automation
- You are not sample limited
- The proton spectrum, or fast homonuclear proton 2D experiments, are all you are interested in

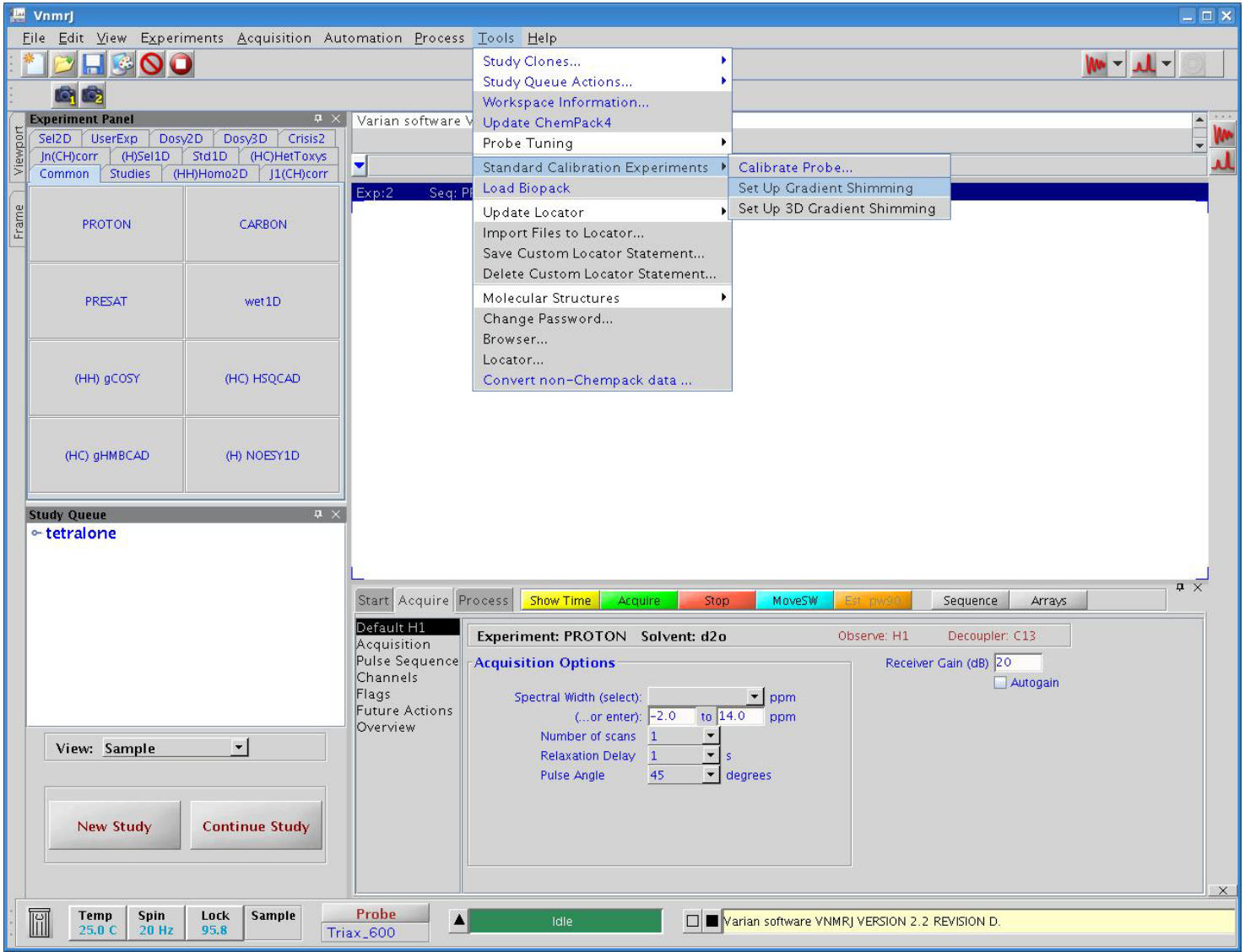
Sample to sample shim variations between full length samples are usually small

- They are least when you use high quality NMR tubes
- They are much bigger when you use short samples of varying height and position
- The standard shims are normally a very good starting point for full length samples
- The last person's shims are a random starting point for your sample—reload the standard shims early and often!

Ethylbenzene hand shimmed vs. gradient shimmed—a wealth of fine detail revealed



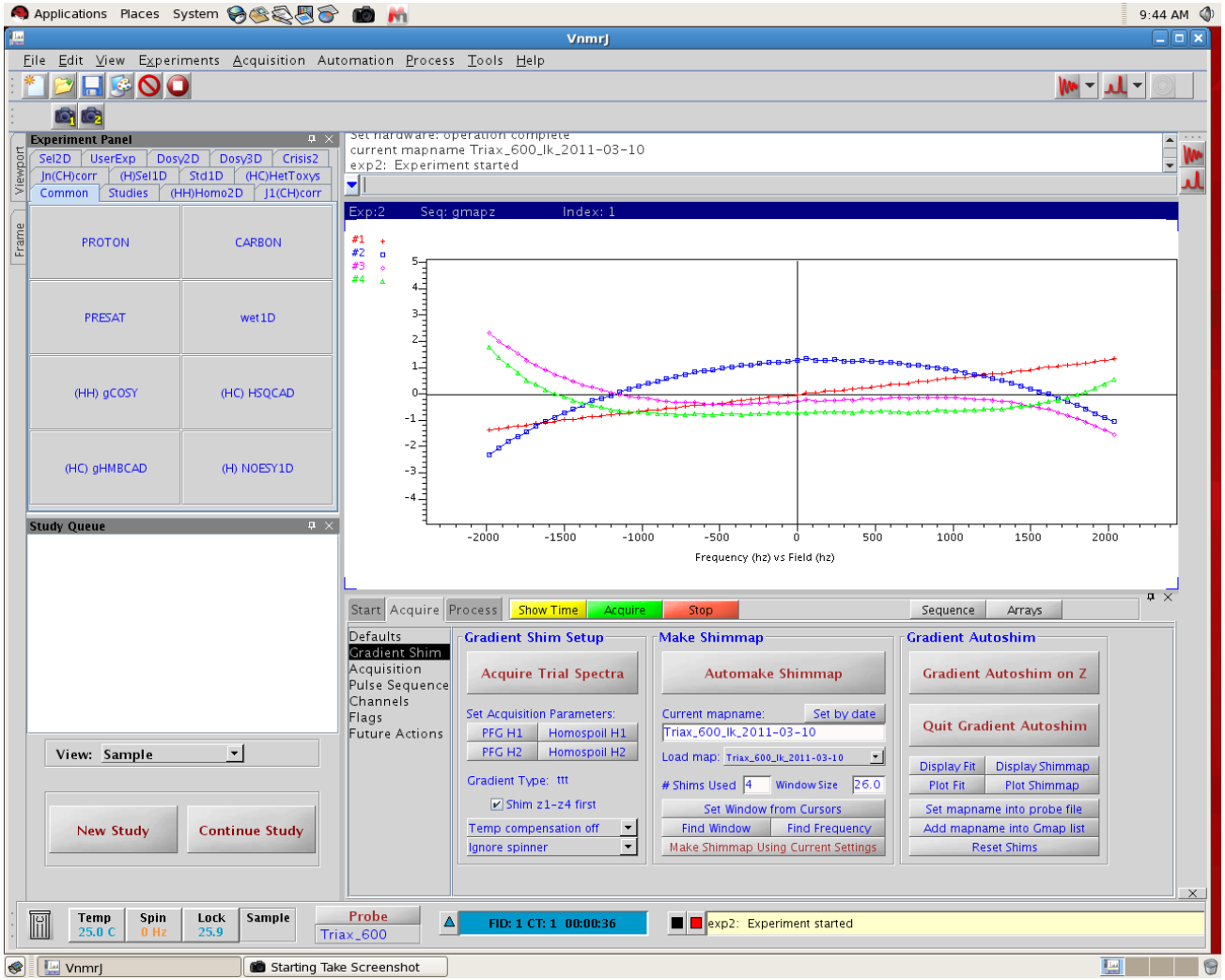
How to reach gradient shim controls



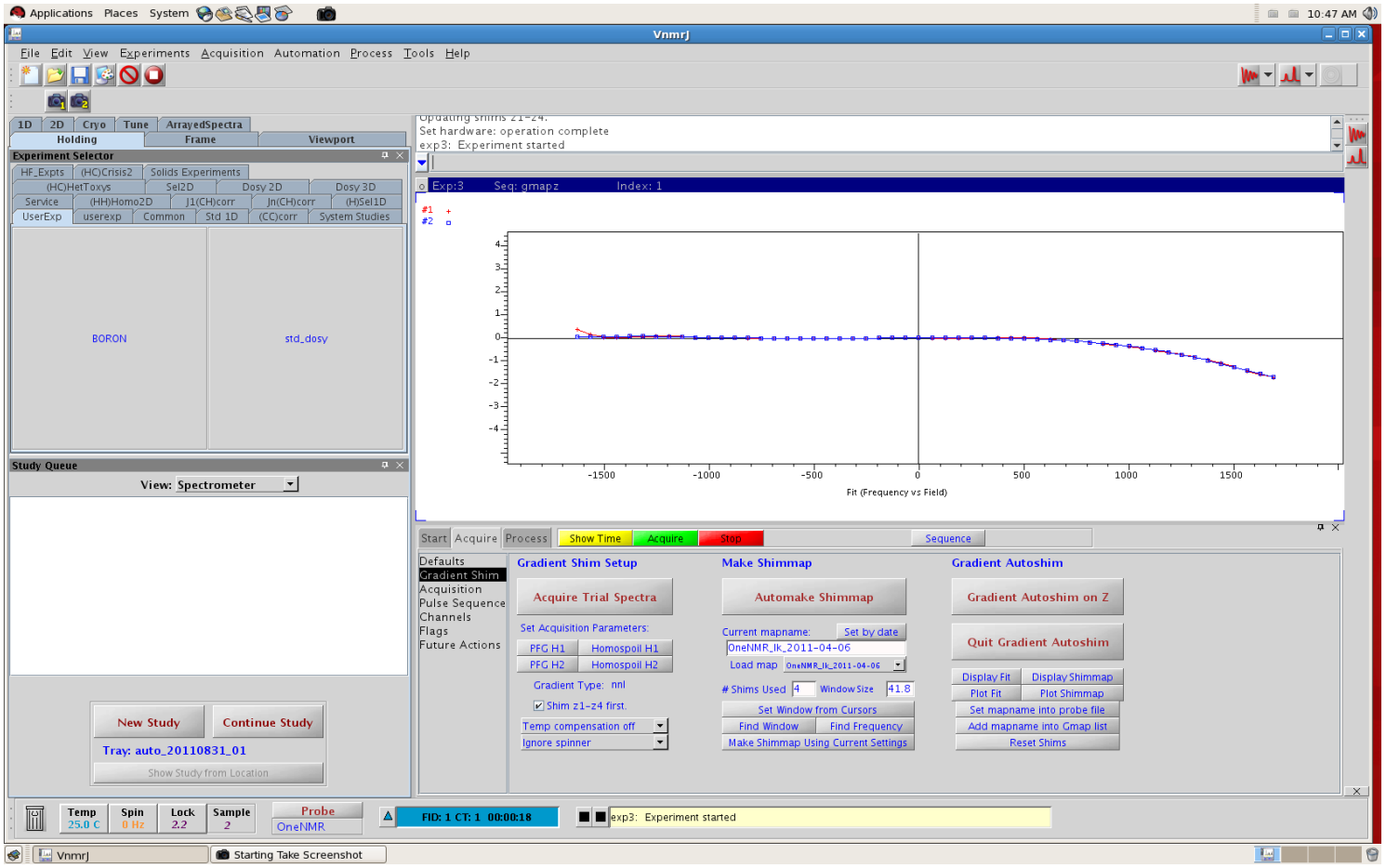
A typical shim map for z^1-z^4

Z^1 (linear ramp) and Z^2 (parabola) act throughout the sample

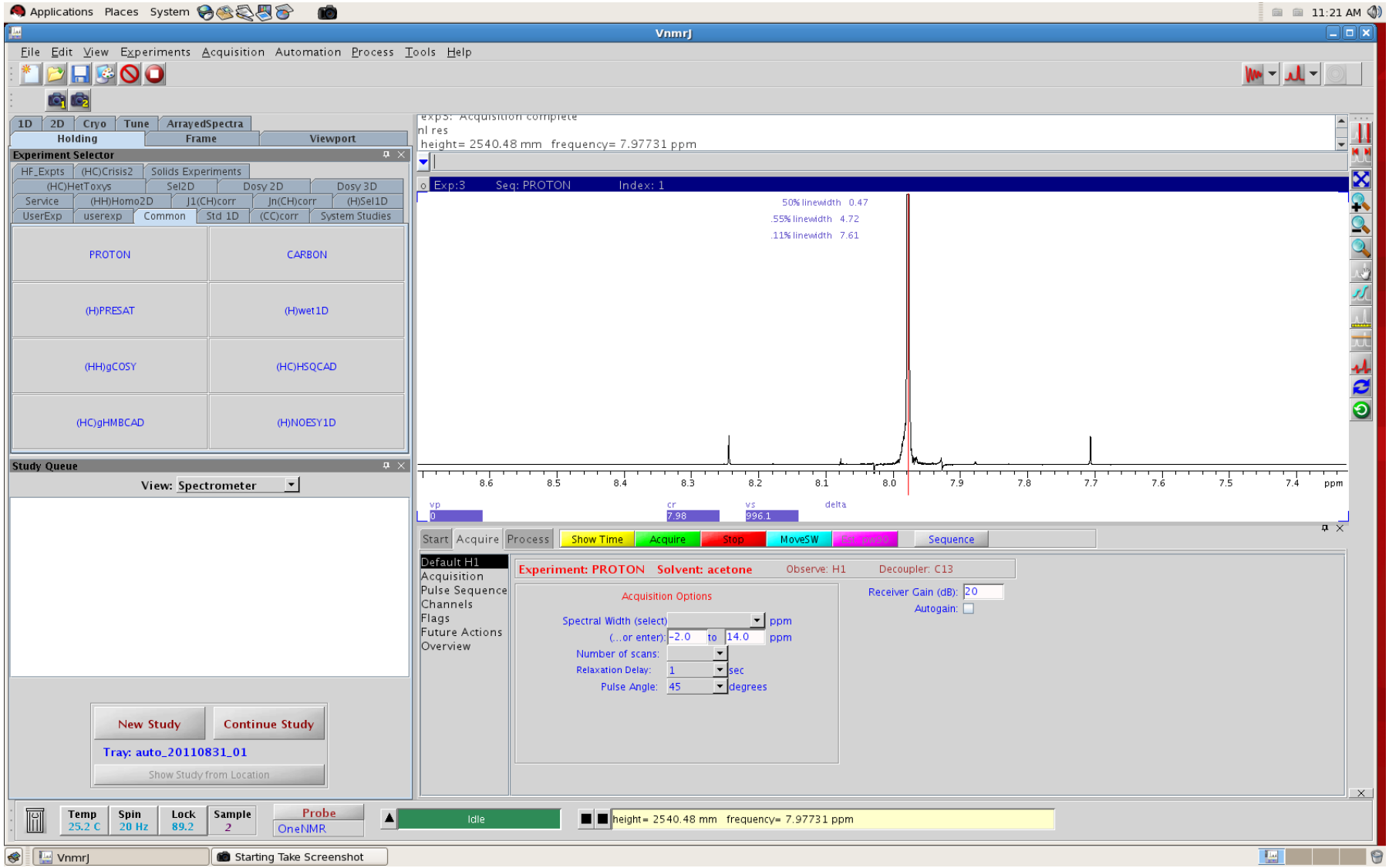
Z^3 (antisymmetric) and Z^4 (symmetric) act primarily at the edges of the sample.



First field map from full length sample starting from default shims—in this case, mostly z^3 and z^4 error, very little z^1 or z^2. Gradient shimming easily handles this.



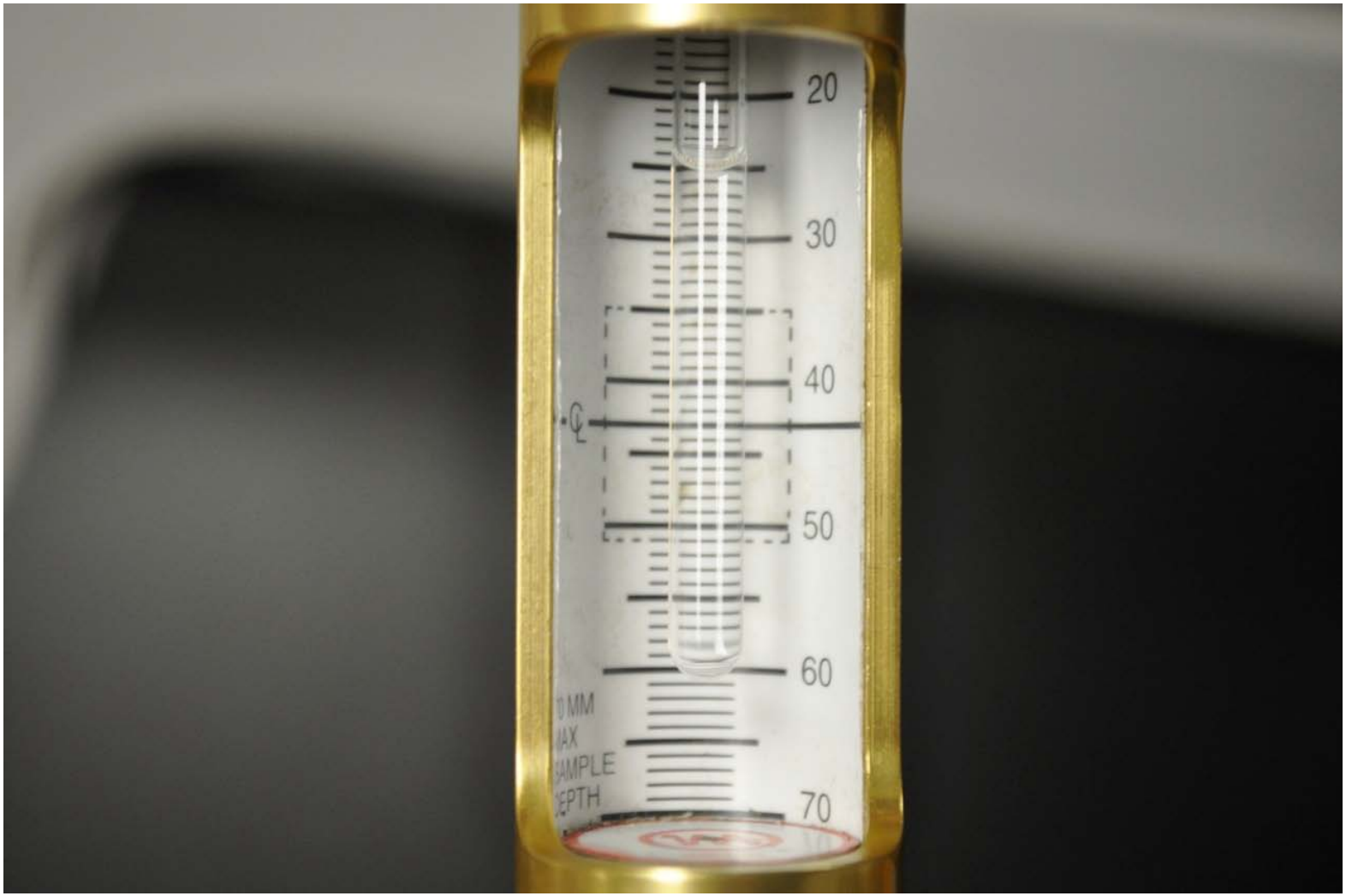
Representative good lineshape from test sample: less than 5 Hz wide at 0.55%, less than 8 Hz wide at 0.11%



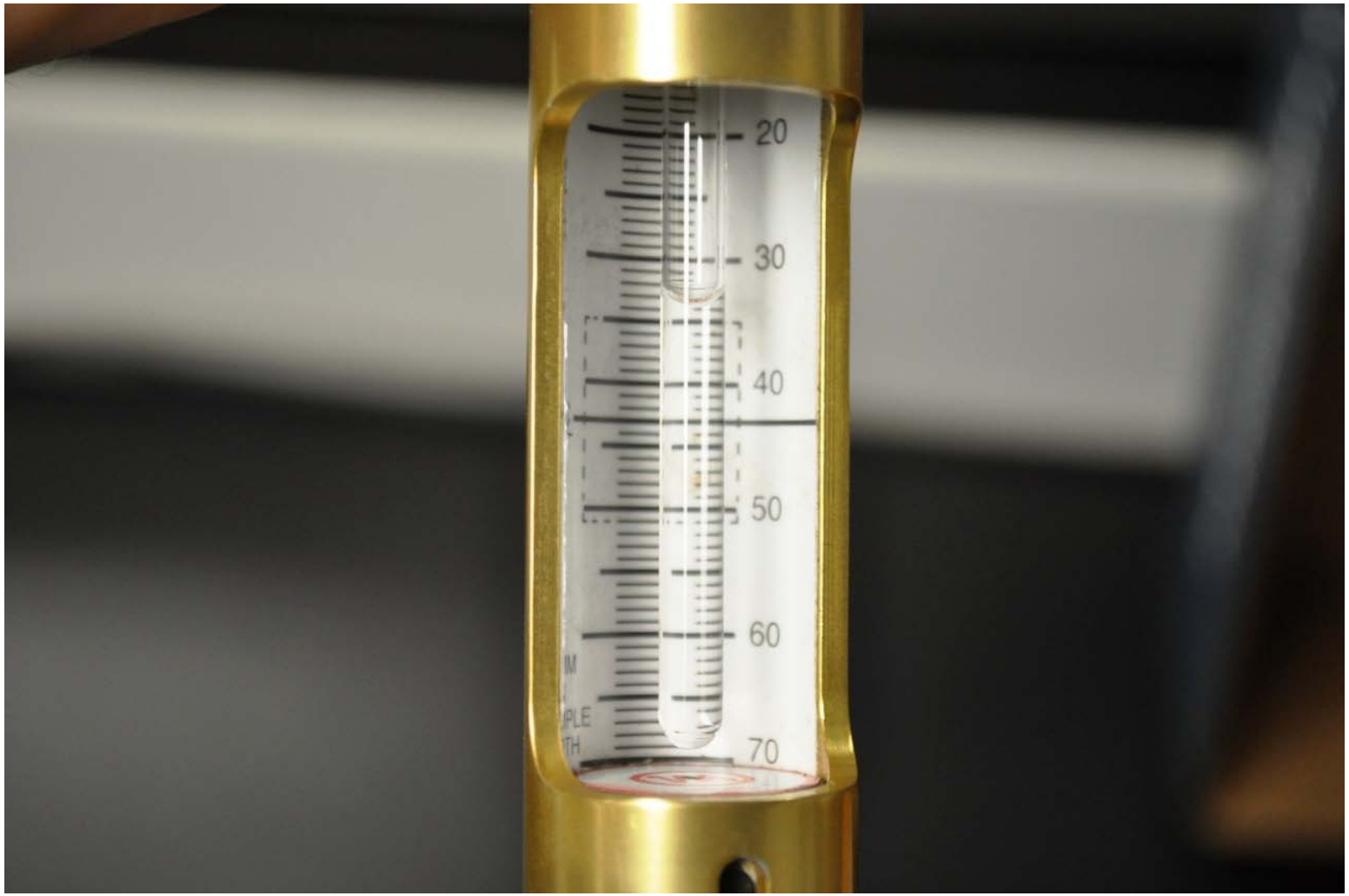
Check the performance of your shimming through the “res” command

- All it takes is a quick proton spectrum
- Particularly important before spending hundreds of dollars on a long experiment!
- If your sample or experiment setup doesn’t maximize spectrum quality, what are you trading it away for?
- If you aren’t satisfied with your first shim, reload defaults, change parameters and try again

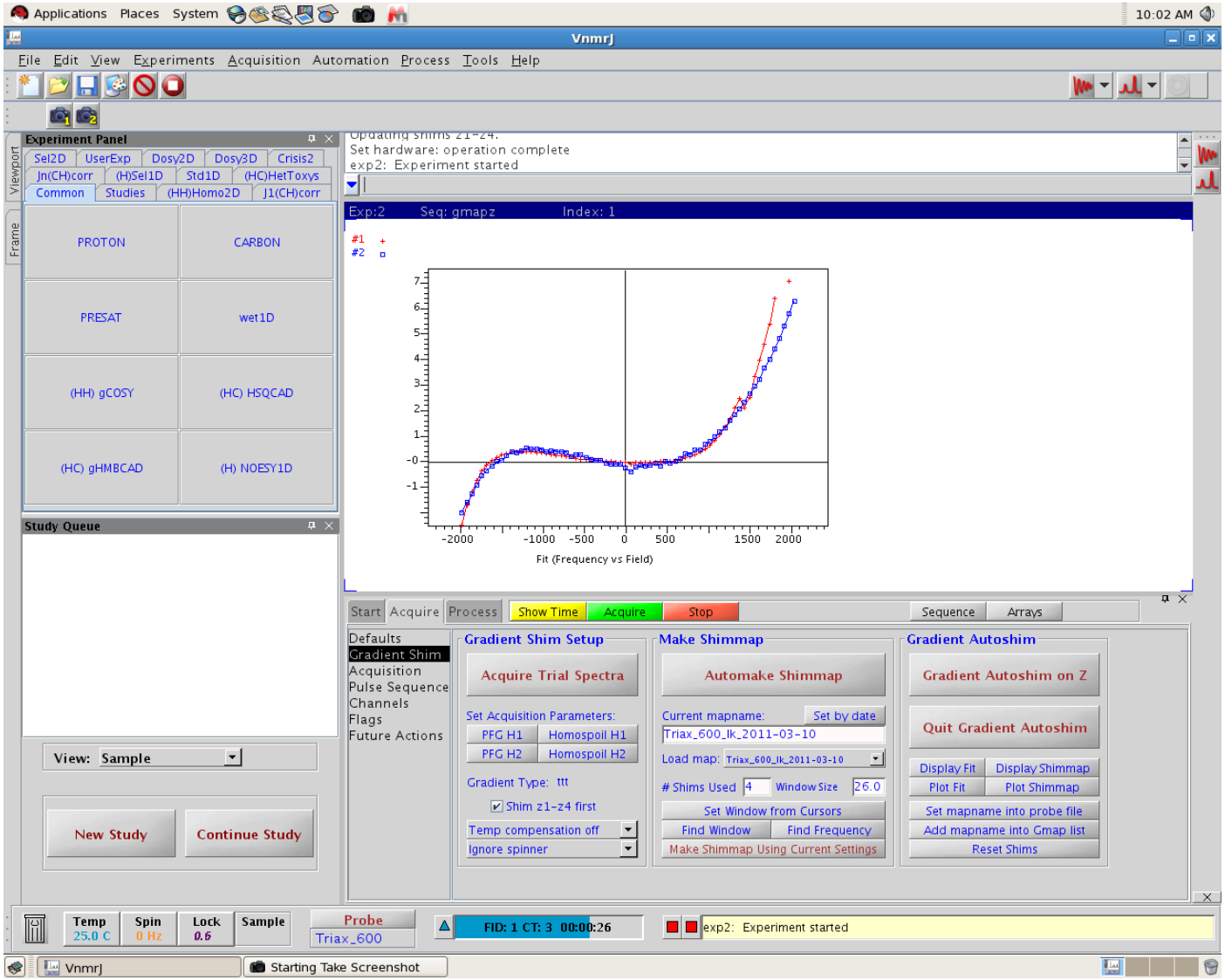
3.5 cm sample, centered: somewhat shimmable



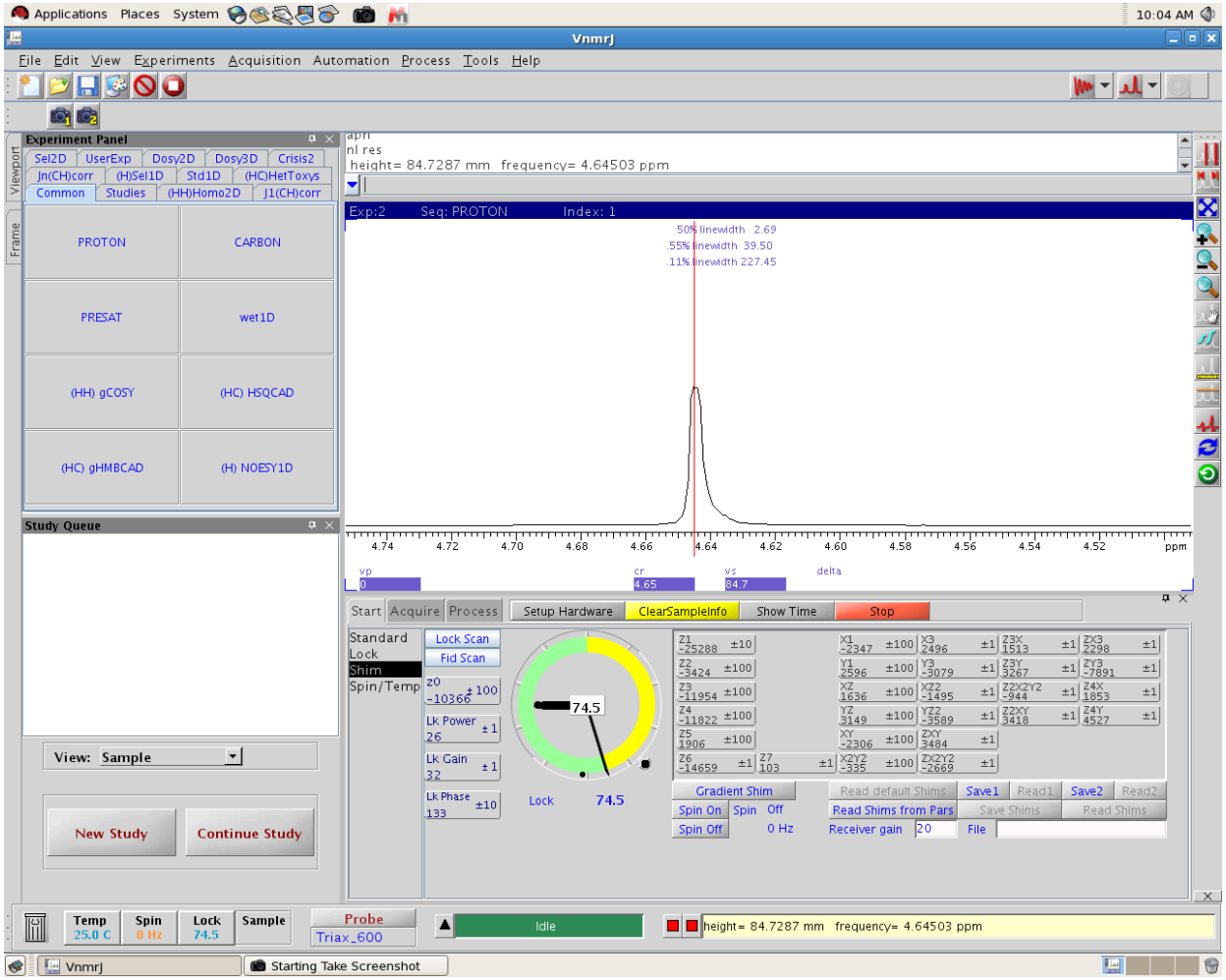
3.5 cm sample, bottomed out: all but impossible to shim



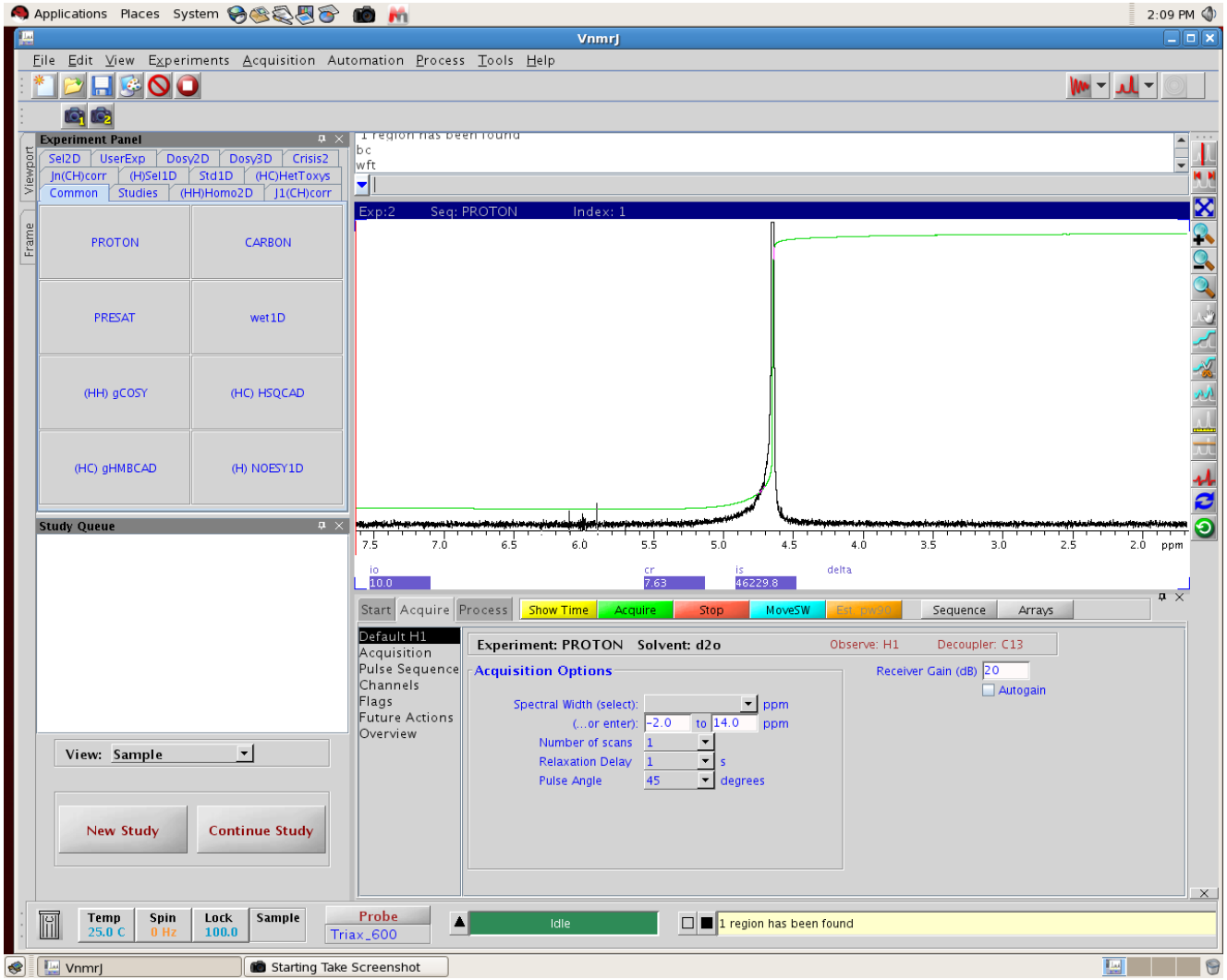
More complex profile from short, off center sample (lots of z^3 needed)



Lineshape from short, off center sample—broad at half height, but even worse at 0.55% (40 Hz) and 0.11% (225 Hz!)



Sensitivity loss from really bad lineshape—a lot of peak intensity lost in the long downfield tail, where it does you no good

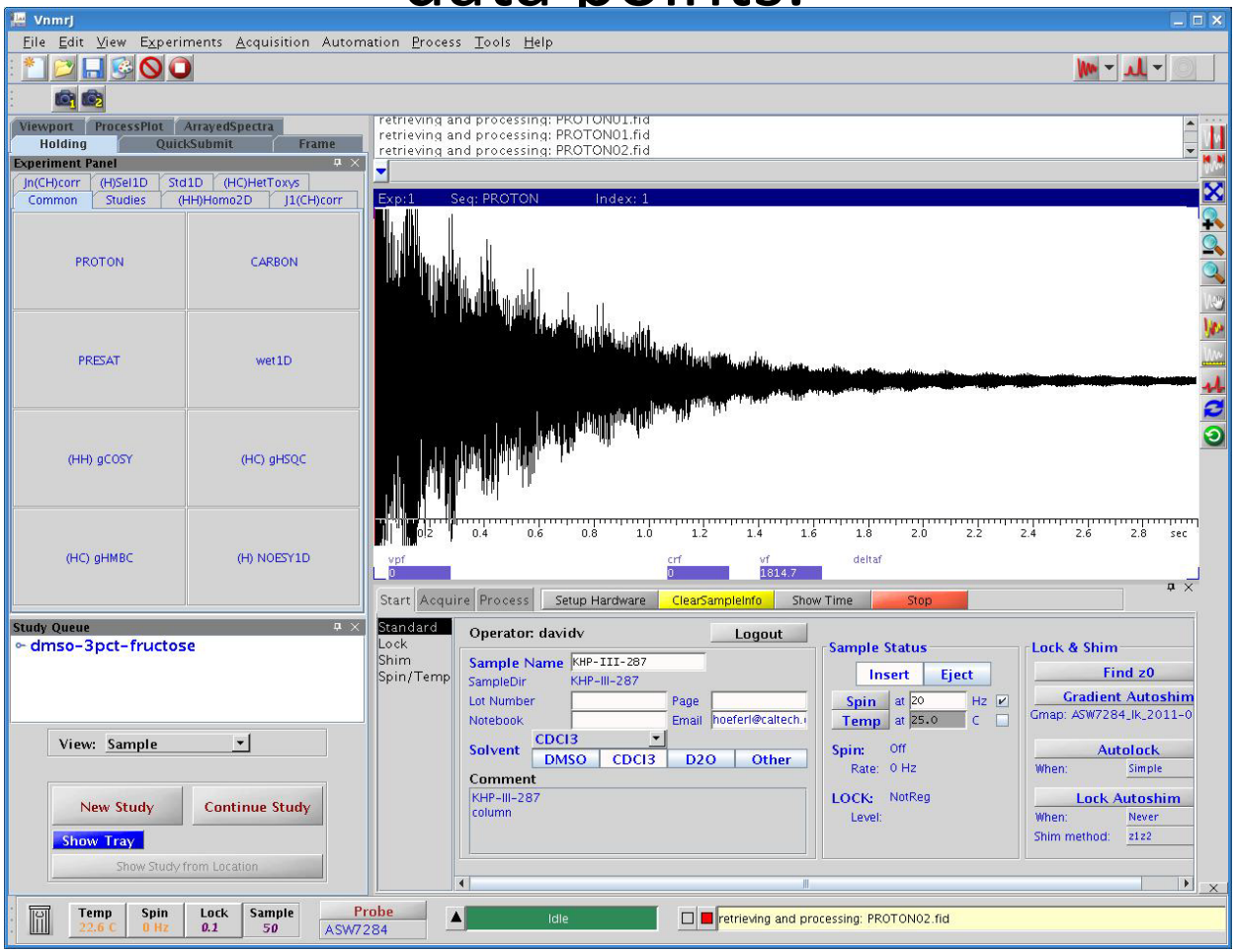


Short samples frequently won't be locked after gradient shimming

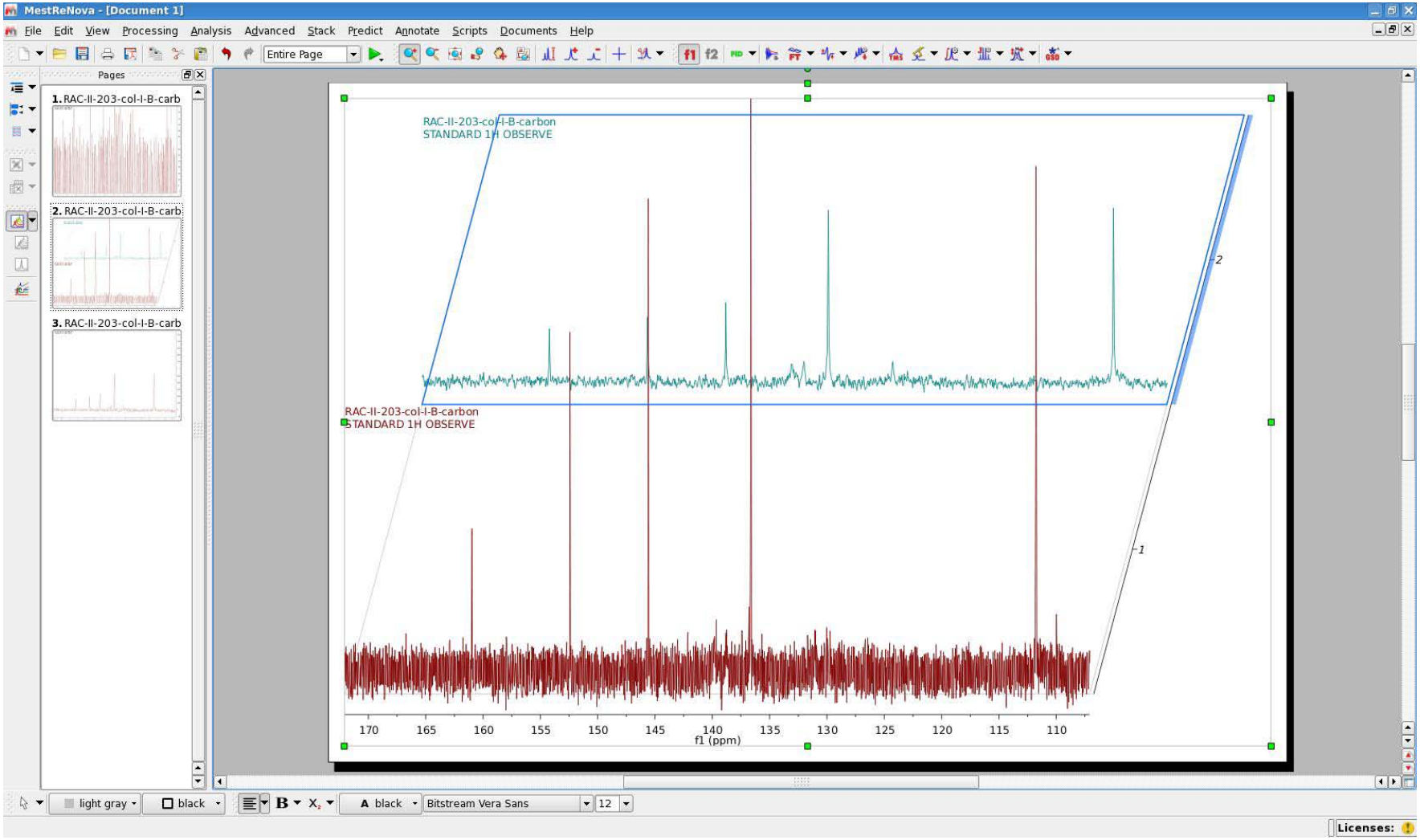
- This is a consequence (unavoidable?) of doing find z^0 first, then applying large z^2 and z^4 corrections, which contain a z^0 impurity
- If you are setting up the experiment manually, verify lock after shimming
- If the instrument is searching for lock during the acquisition, the results will be terrible

Adjust acquisition and processing parameters to maximize recovery of small signals and spectral detail

Tailor acquisition conditions (like “at”) to maximize spectrum quality—looking directly at the FID helps you decide how long to acquire data points.



Use line broadening or other apodization to best advantage: in this case, much greater LB than normal exposes broad, weak peaks

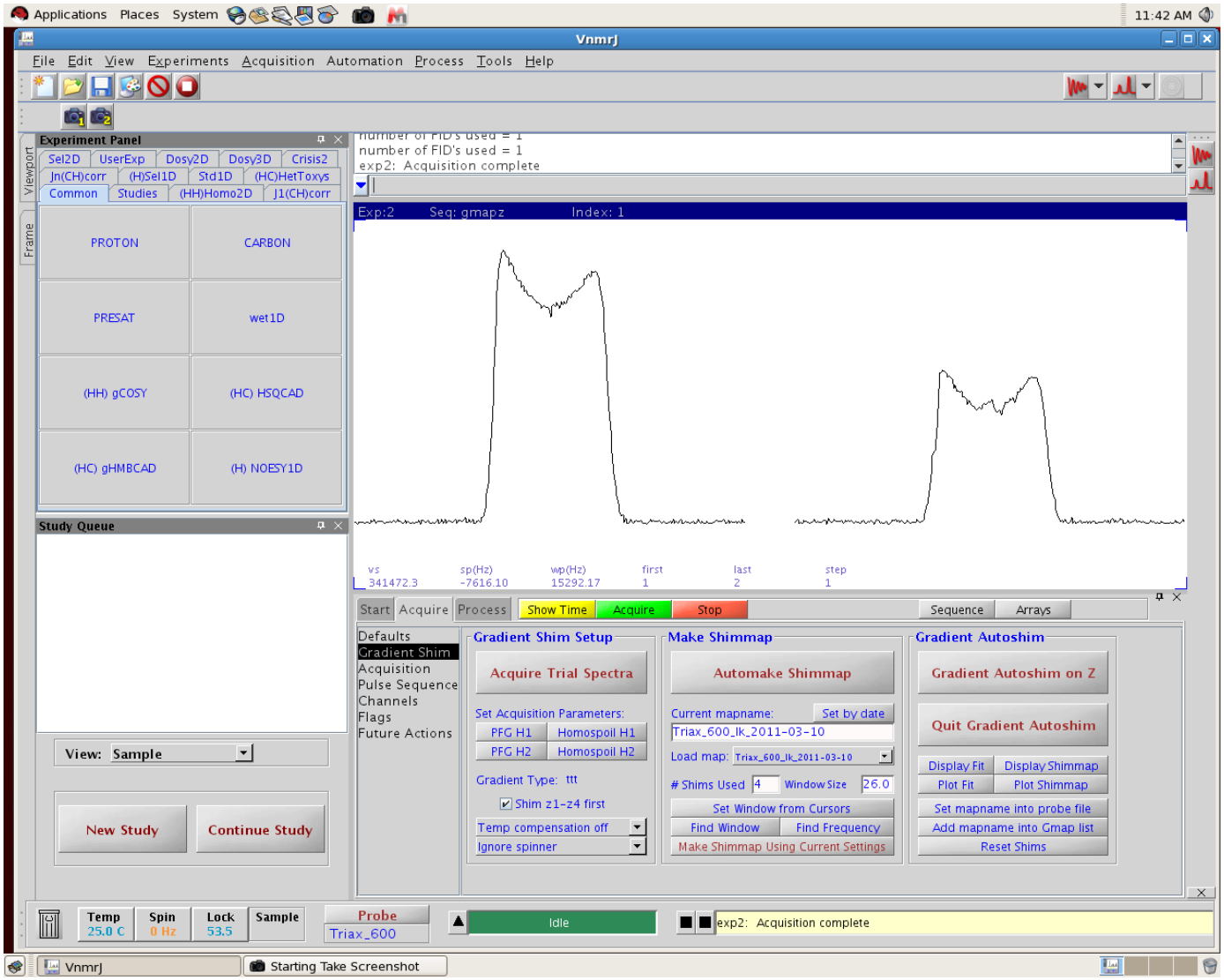


If you want the best quantitation

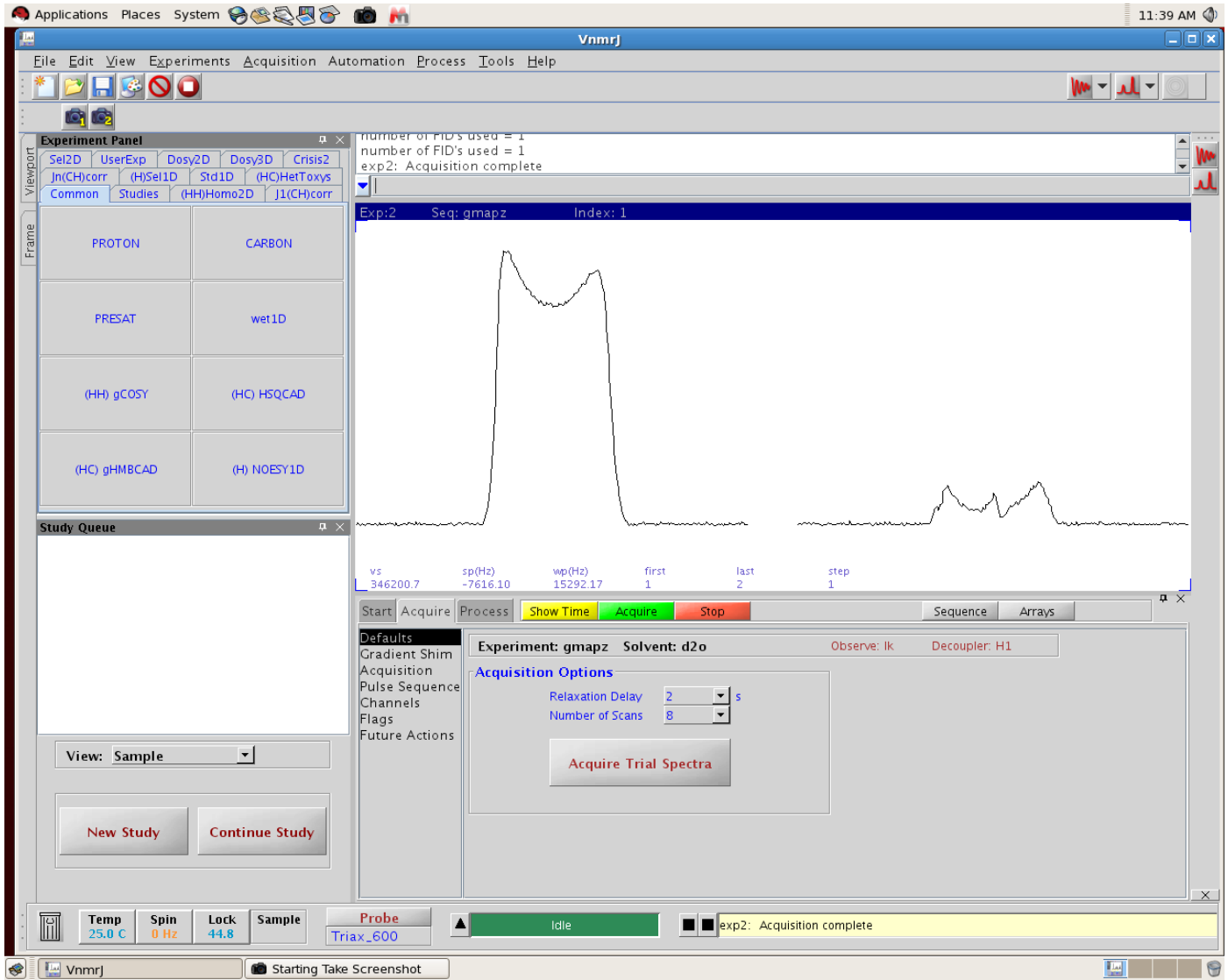
- The delay between scans (d1+at) will have to be long, at least $5*T_1$. T_1 is very molecular weight, solvent, and temperature dependent, and gets much longer in the absence of oxygen, for samples under an inert atmosphere.
- In order to minimize the number of scans, use a 90 degree flip angle (the default is 45 degrees)
- Use generous amounts of line broadening, as noise adversely affects integral accuracy

Troubleshooting problems with gradient shimming performance

Ratio of first/second profile in the normal range



Weak second profile, won't shim correctly: shorten d3, or for VT experiments, wait for temperature gradients to relax



Temperature problems

- Temperature gradients that lead to convection cells in the NMR tube cause many problems, particularly with any experiment using gradients
- Even without convection, temperature gradients cause strange lineshapes of any peak whose chemical shift is temperature dependent

Challenges with small samples/low sensitivity

Long acquisitions offer diminishing returns!

- Signal increases linearly with “nt”
- Noise also increases, with the square root of nt
- Signal to noise increases only with the square root of nt
- Concentrating the sample in a smaller volume may speed up acquisition a lot … *everything else being equal … especially lineshape*

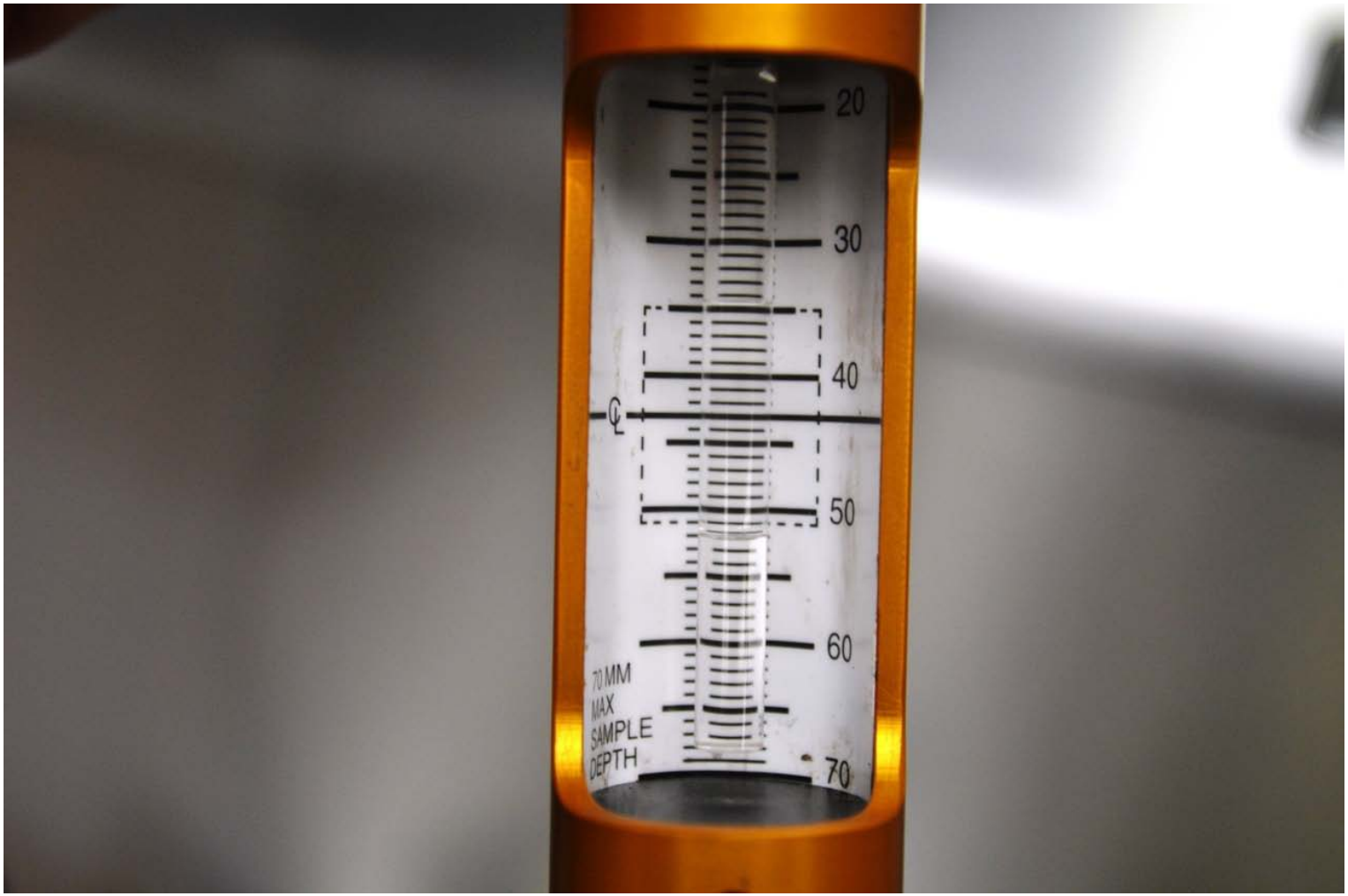
Hardware approaches to small samples

- Cryoprobes—wish we had one
- Micro flow probes (Protasis)—ditto
- Narrower tubes (3 mm, 2.5 mm, 1 mm)
- Shigemi tubes (no special probe required)

Shigemi tubes should be the first choice for small samples, 3mm tubes second

- Shigemi tubes available for D_2O, $CDCl_3$, DMSO, methanol
- We have a 3 mm inverse broadband probe for the 600, and a 3 mm spinner; you can put a 3 mm tube in any 5 mm probe if you want to economize on expensive solvents
- Shigemi tubes improve both ^1H-detected and X-nucleus sensitivity; in our 3 mm inverse probe, we get the benefit just on ^1H-detected experiments
- 3 mm tubes are a special order; 3 mm J. Young tubes are available; so are 3 mm Shigemis

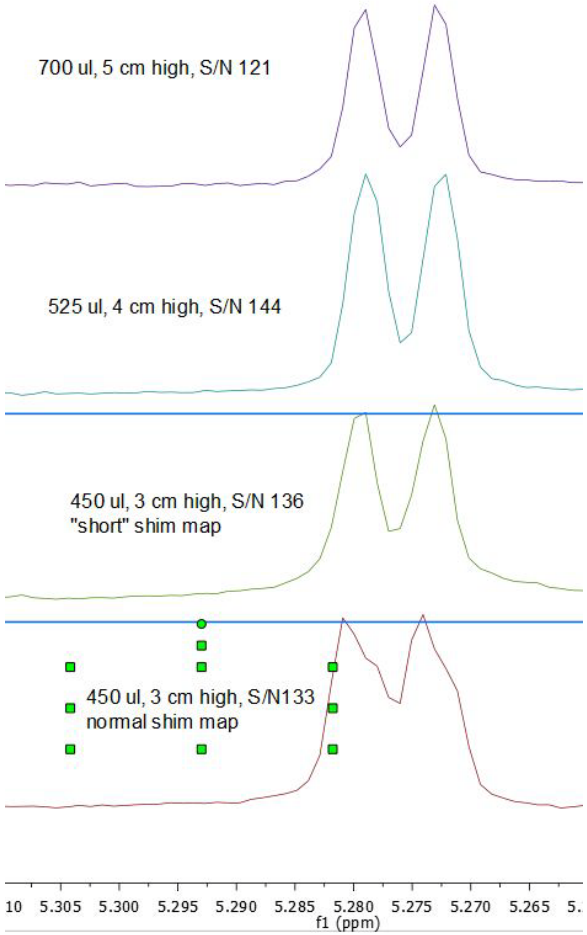
Shigemi tube adjusted for best fit to the observe coil

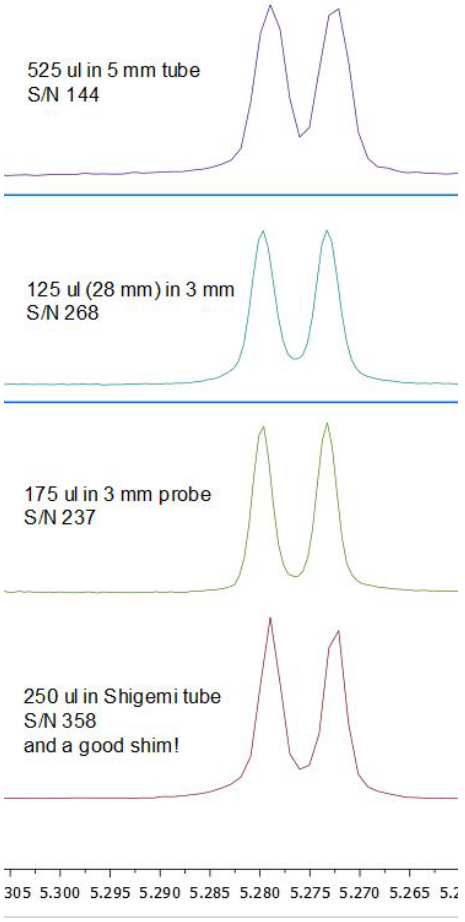


A practical sensitivity/shimming test: the ground rules

- 1 mg of sucrose per sample, in D2O
- Best available quality NMR tubes
- Sucrose is a good water-based sensitivity/ shimming/water suppression test sample (e.g. 2 mM sucrose in 90% H_2O/10% D_2O)
- The anomeric proton is a doublet with J ~4 Hz and the shimming goal is to split it as far down toward the baseline as possible

Anomeric proton from 1 mg sucrose in different sample geometries





Results of the sensitivity/shimming test

- Shigemi tubes win! Virtually all of the sample is inside the observe coil.
- Very short conventional 5 mm samples, although more concentrated solutions, are less than optimal because they cannot be shimmed as well.
- There is an S/N penalty for making the sample full length, but the spectrum quality continues to improve.
- 3 mm tubes shim really well! Also, there is less of a penalty for reducing sample height with a 3 mm tube.

The money slide!

- Assume that you could get a satisfactory ^{13}C spectrum in the Shigemi tube (^{1}H S/N 358:1) in 2 hours on Daytona, for $36
- If the ^{13}C S/N scales the same way as the ^{1}H, then for the optimal 4 cm sample in the 5 mm tube, the same ^{13}C S/N would take $(358/144)^2$ times longer, or 6.18 times longer; 12.4 hours, for an NMR bill of $223
- Shigemi tubes cost $95, **but they can earn you more than they cost back on their first use**, and you can re-use them many times!

Notes about the “Window Size” parameter in the gradient shim controls

- Using the full window size (normally 30-50%) in a shim map made on a long sample for a short sample will lead to large z^3 and z^4 corrections being applied. This may make lineshape better, but linewidth worse.
- If you reduce the window size used, gradient shimming will optimize the center of the tube, where homogeneity is best. Linewidth may be better and lineshape worse (see the comparison on slide 35).
- On a regular 5 mm tube, this is mainly a cosmetic improvement, as the “short shim map” spectrum may look nicer, but the S/N is not better. Peak intensity is being lost in the broad peak tails, instead of in distorted lines.
- A shorter window size is preferred for Shigemi tubes—adjust downward until there isn’t any noise at the edges of the field map. Z^3 and Z^4 are less useful with those tubes, because there isn’t any liquid sample out where those shims have the greatest effect—the liquid has been replaced by glass, so there is no signal originating out there, only noise.