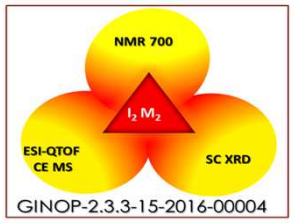


NMR Operator Training

TTKBL0004_EN

The procurement of the 700 MHz NMR spectrometer system was made in the framework of the GINOP-2.3.3-15-2016-00004 project supported by the EU and co-financed by the European Regional Development Fund.

Safety

- Personal safety
  - Cryogens: do not lean on or push magnets
  - Cryoprobes: avoid contact with transfer line
  - Magnetic and RF hazards

- Instrument safety
  - Know the limits of instruments
  - Probe limits: avoid excessive long decoupling and long hard pulses or their equivalents
  - Be conservative
  - Double check pulse program and parameters for any non-standard new experiment

- Data Safety
  - Back up data promptly and regularly
  - Data processing or manipulation has no impact on the raw (FID) data
  - Do not change parameters after data are acquired

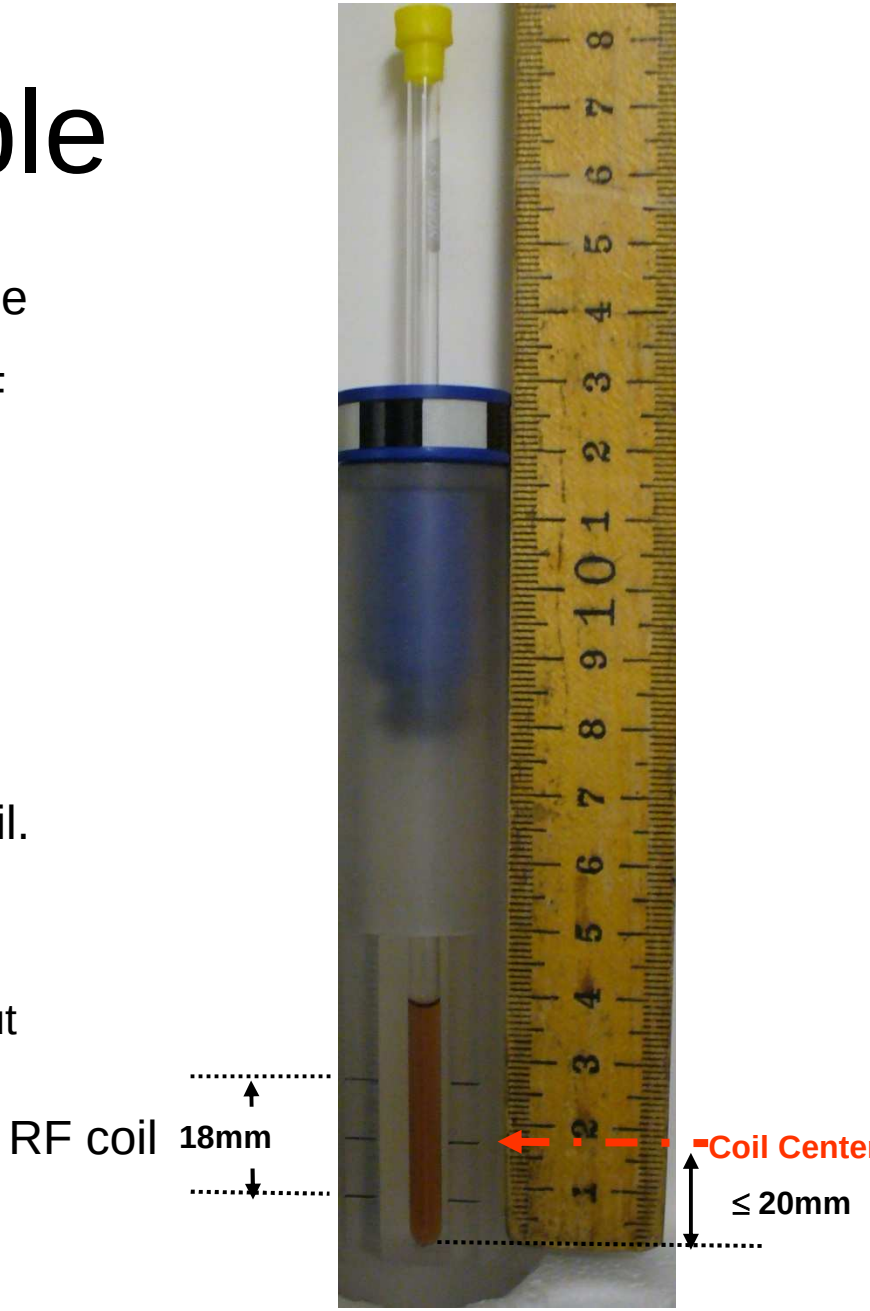
Two Insider Scoops

- Receiving efficiency
  - conceptually, it is similar to extinction coefficient in UV spectrospcopy; it characterizes how efficient a unit magnetization can produce a signal by a given NMR receiver
  - NMR signal size is proportional to receiving efficiency
  - Receiving efficiency can be pre-calibrated as a function of 90° degree pulse length
  - receiving efficiency is the same for all nuclei of the same type (indifferent to chemical shifts) in the same sample

- Solvent signal offers a universal and robust concentration internal standard
  - Normalized NMR signal size is strictly proportional to concentration for a given sample, regardless how concentrated or dilute the sample is
  - Unit magnetization generates the same amount of total NMR response, which is indifferent to chemical shift or line-shape

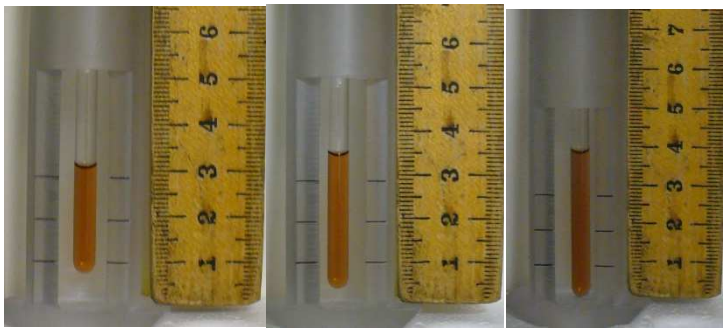
Sample

- Rule #1: for Bruker NMR spectrometers, the NMR tube insert cannot exceed max depth (19mm or 20mm) from the center of the RF coil
  - Longer insert than recommended may present problems for the probe, as well as cause frictions during spinning
  - Varian is more flexible in allowing longer insert
- Rule #2: center of NMR sample should be as close as possible to the center of RF coil.
  - Normal sample needs to about 500 ul or slight more
  - Too much solvent is a waste!
  - Too little solvent may make shim difficult, but it does work!
- 10% deuterated solvent is sufficient for locking



Samples of smaller volumes

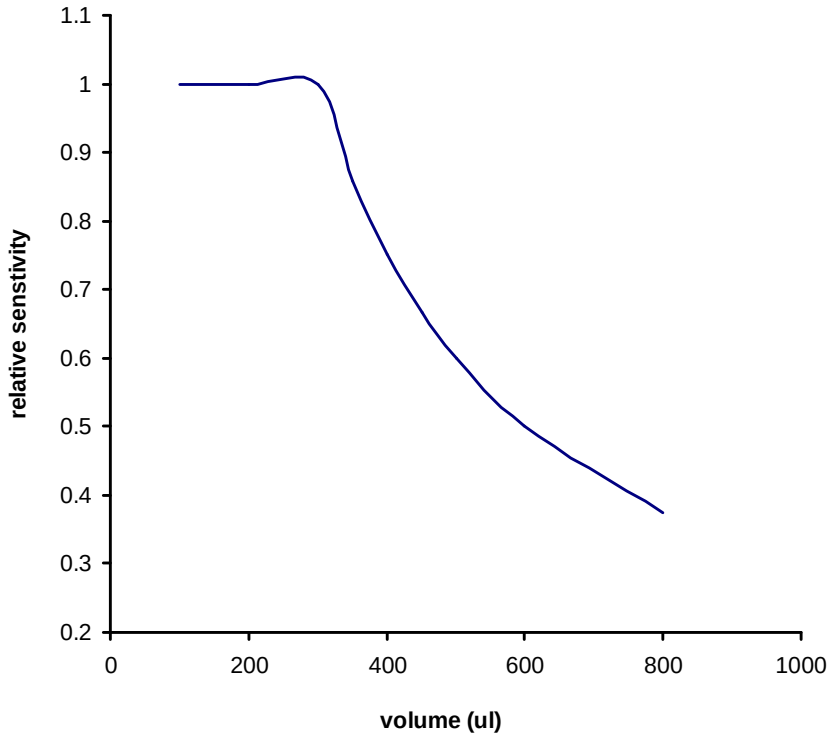
- Follow rule # 1 and then rule #2
- Shimming might be challenging due to air/glass and air/solution interfaces
- Consider Shigemi tubes
- Be careful with spinning
  - Non-spinning is recommended for volume ~ 300 ul or less



300ul 400ul 500ul

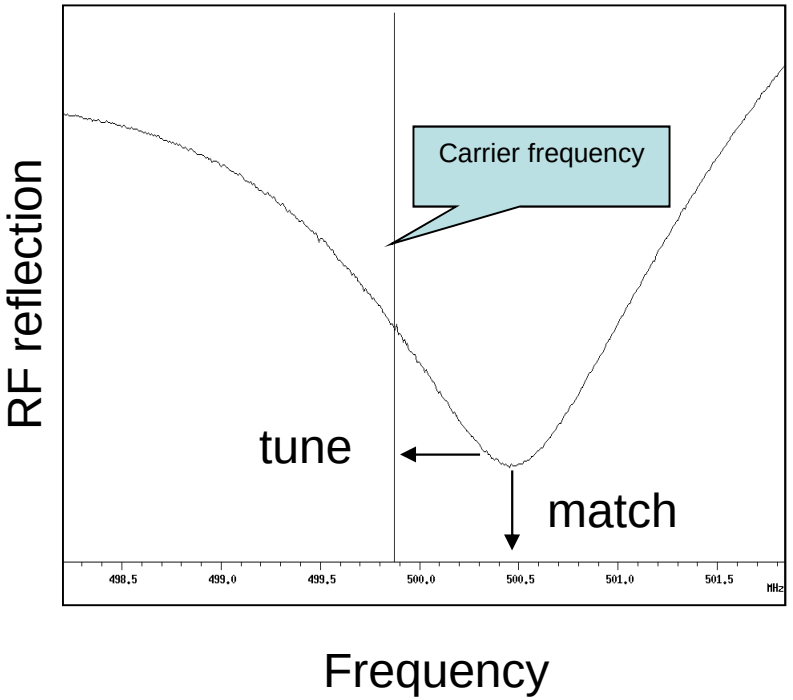
Sensitivity for smaller volumes

- Volume less than 300 ul may not offer additionally sensitivity improvement over that achieved by 300 ul, if the total amount of analyte is constant



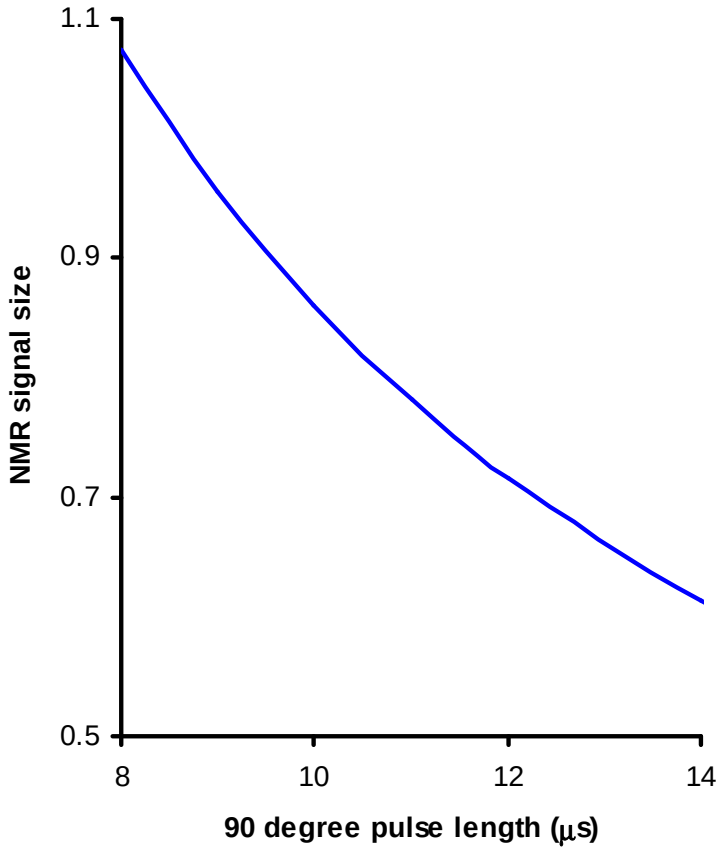
Tune and match the probe

- Only higher fields (500, 600 and 800 HMz) in our facility need tuning
- Most of the time only proton channel requires tuning
- Drx500-2 with BBO needs special attention
  - Proton always needs tuning
  - BB (used for ^{13}C or ^{31}P etc) channel needs tuning, by first dialing the numbers to the pre-set values



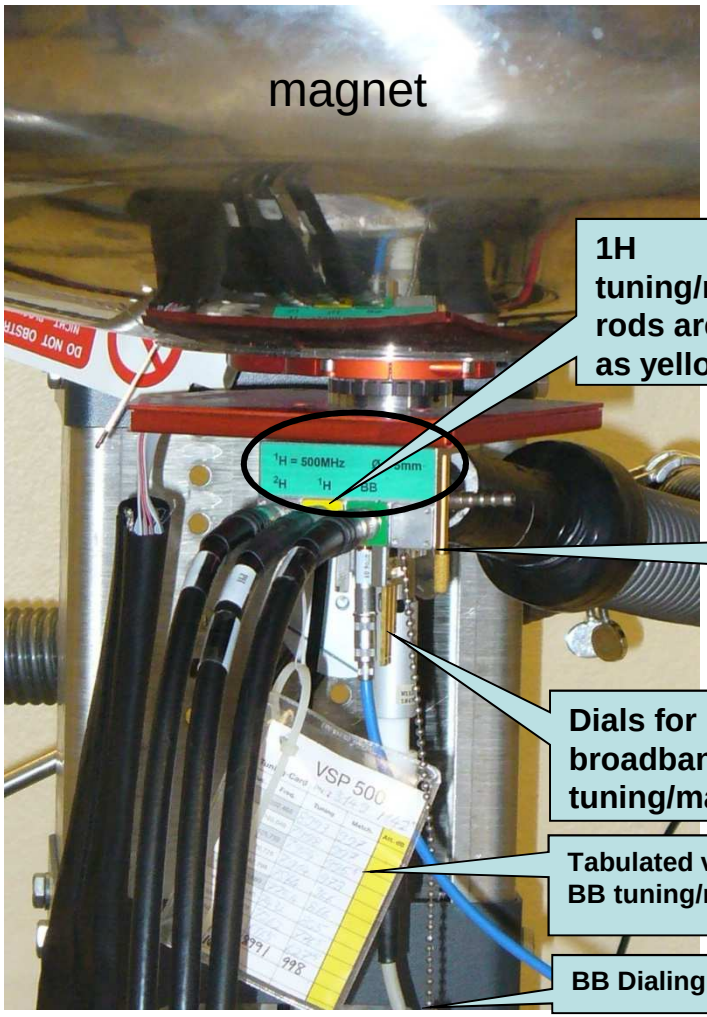
Significance of tuning/matching

- Shorter 90 degree pulse
  - More efficient use of RF power
    - Protects transmitter
  - More uniform excitation in high power
- Better sensitivity
  - Reciprocity: if excitation is inefficient, then detection is equally inefficient
- Potentially quantitative:
  - The product of NMR signal size is inversely proportional to the 90 degree pulse length



Recognizing Bruker probe types

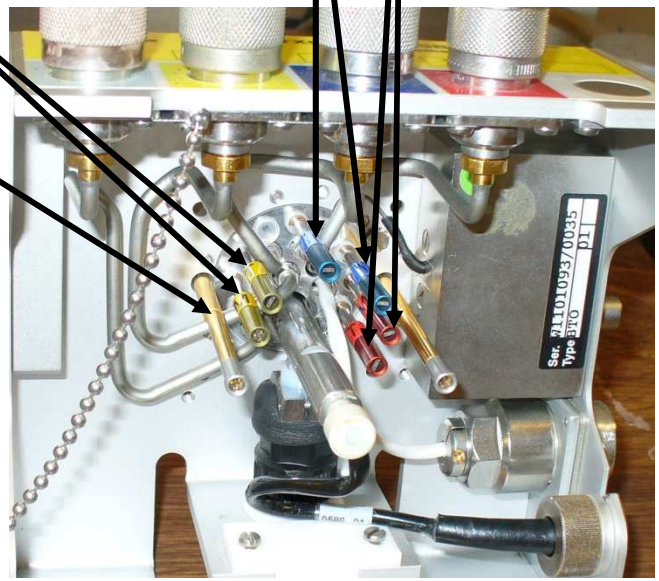
side view



BBO probe on drx500-2

side view

bottom view



TXI probe

Lock

- Lock depends on shim: bad shim makes bad lock
  - Initialize shim by reading a set of good shims (i.e. rsh shims.txi)
  - Inheriting a shim set from previous users may present difficulties
  - Unusual samples (esp. small volumes) may need significant z1/z2 adjustments
- Use “lock_solvent” or “lock” command
  - The default (bruker) chemical shift may appear as dramatically changed if the spectrometer assumes another solvent
- Avoid excessive lock power
  - Lock signal may go up and down if lock power is too high due to saturation of deuterium signal
  - Apply sufficient lock power and gain so that lock does not drift to another resonance (this may happen by autolock if multiple deuterium signals exist)

Shim

- The goal of shim is to make the total magnetic field within the active volume homogeneous (preferably <1Hz).

  Total magnetic field =

  static field (superconductor) + cryoshim (factory set) + RT shim (user adjust)

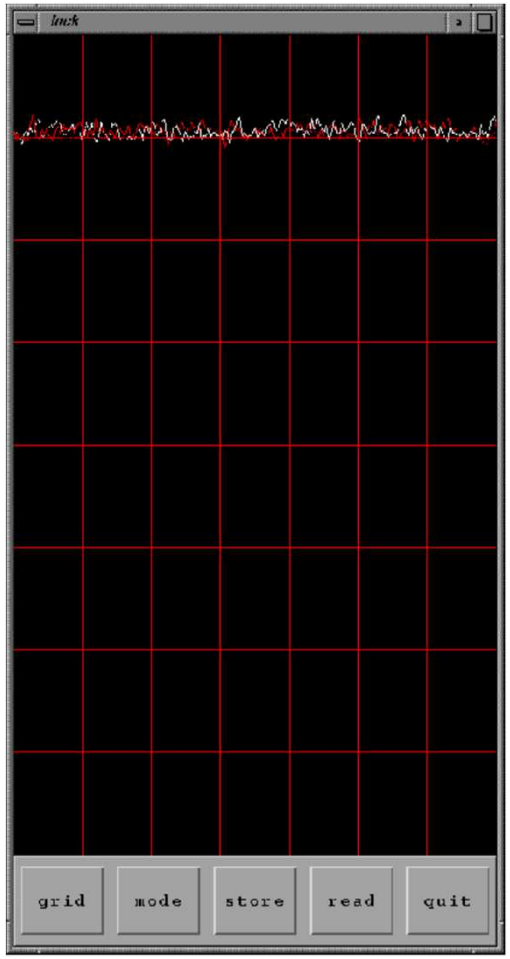
- Shim can be done either manually or by gradient, which can be very efficient and consistent if done properly

- Sample spinning may improve shim
  - However, spinning-side band appear

- Recommendation:
  - Start from a known good shim set (by rsh on bruker or rts on varian).
  - Do not inherit shims from other users unless you know they’re good
  - Non-spinning and higher order (spinning) shims should not change dramatically from sample to sample for most applications

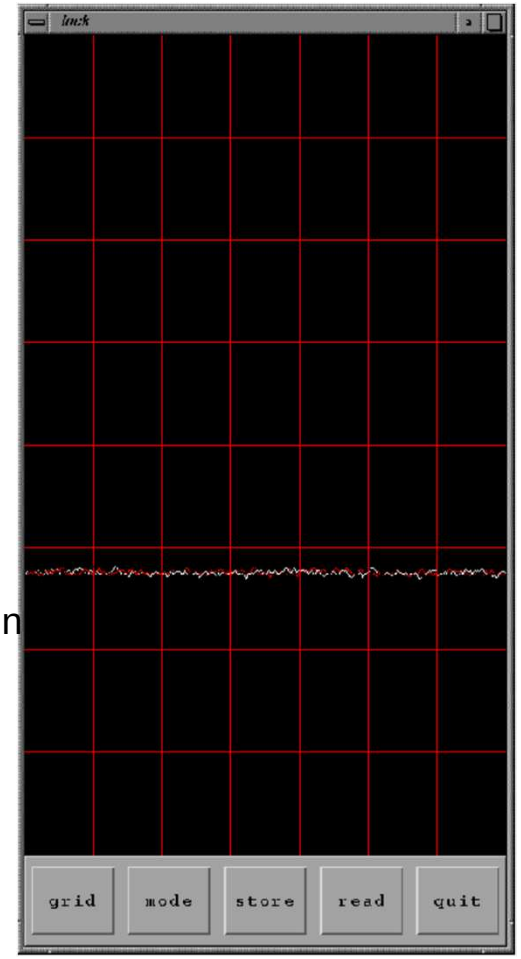
Lock: lock gain

recommended



higher lock gain

not recommended

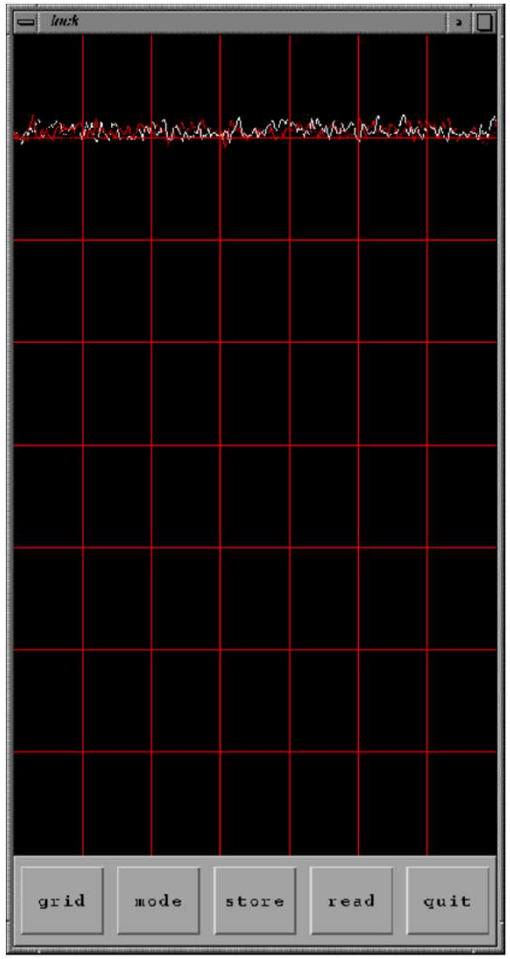


Lower lock level
due to lower lock gain

may easily lose lock;
change in lock level (during shimming) is less visible

Lock: avoid high lock power

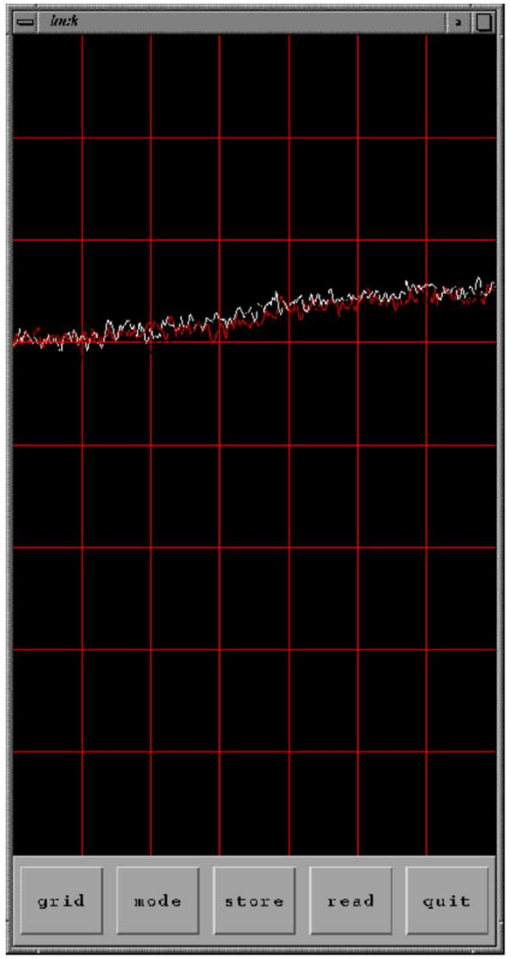
Good lock



Lock power okay

Lock power too high

Bad lock



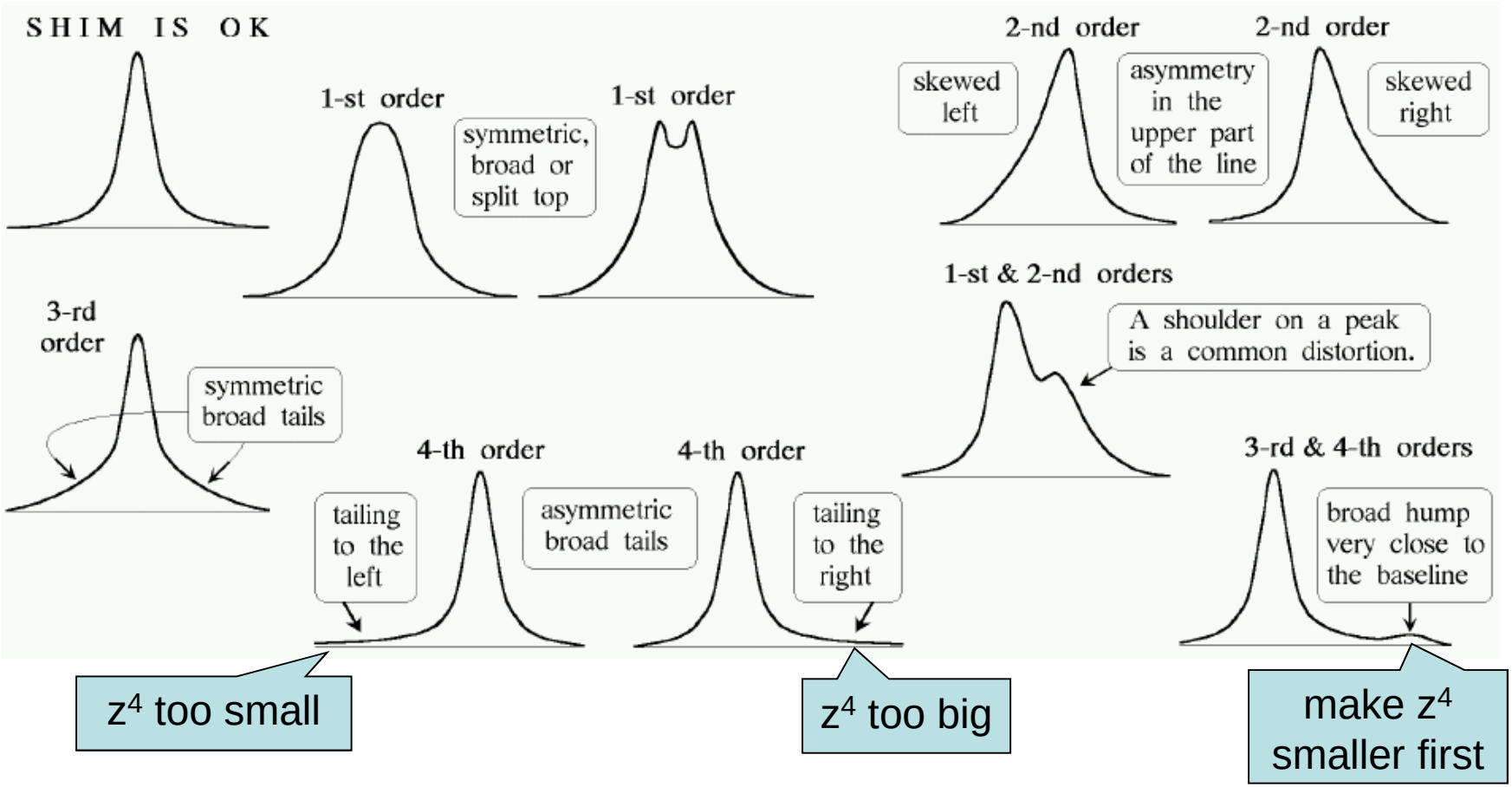
unstable lock and lower level

Evaluate shims

- Look for a sharp peak
  - No clear distortion
  - Full width at half height should be about 1 Hz or less for small molecules
  - Small (1% or smaller) or free of spinning side-bands
- Check if peak distortions are individual or universal
- Make sure that phasing is not causing peak distortions
- Maximize the lock level
  - Higher lock level => better shim
- Lock level does not drop significantly when spinning is turned off

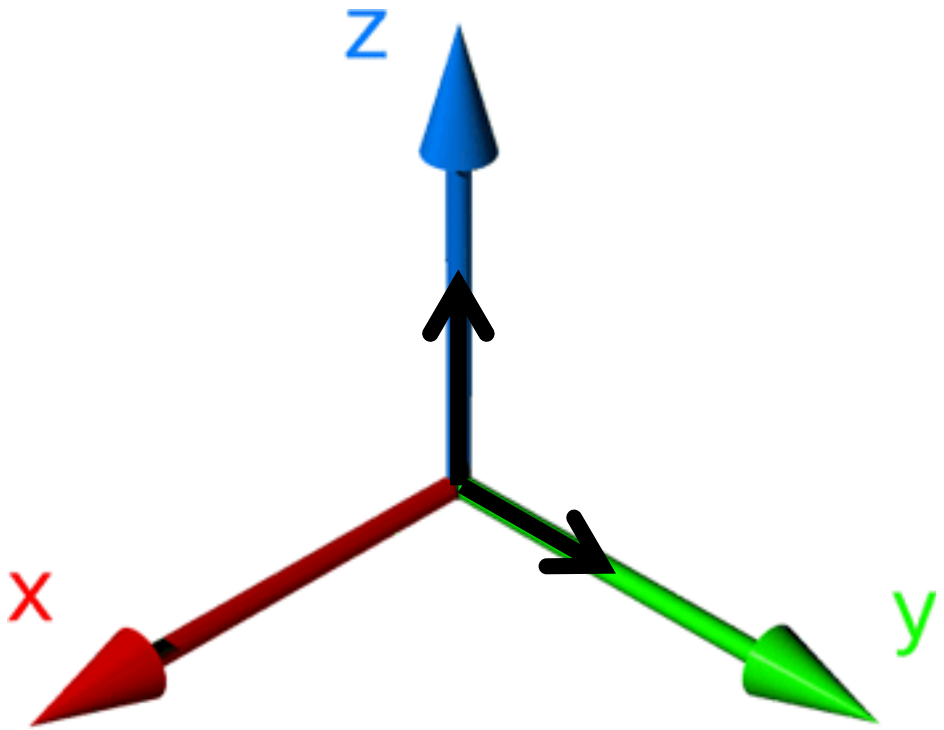
Shim by line-shape

Plot made by G. Pearson, U. Iowa, 1991



Understanding NMR

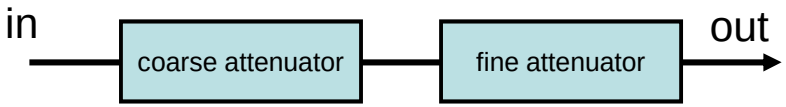
- Modern NMR spectrum is an emission spectrum
- Equilibrium state
  - Magnetization is along +z axis
  - It is desired to have the largest +z magnetization prior to excitation
- Excitation by a RF pulse
  - A projection of magnetization is made on xy plane
  - It is desired to have the largest xy plane project for observation
- Observation
  - Precession of the projected xy-plane magnetization



RF pulses

- RF pulse manipulates spins
  - Important in excitation and decoupling
  - Defined by length, power and shape

- RF power is expressed in decibels
  - Bruker
    - Power range: typically 0db (high power) to 120db (low power)
  - Varian:
    - Coarse power: typically 60db (high) to 0db (low); 1 db increment; absolute
    - Fine power: 4095 (high) to 0 (low); default is 4095; relative
      - e.g. 54.5db can be roughly achieved through setting coarse power to 55 and fine power to 3854

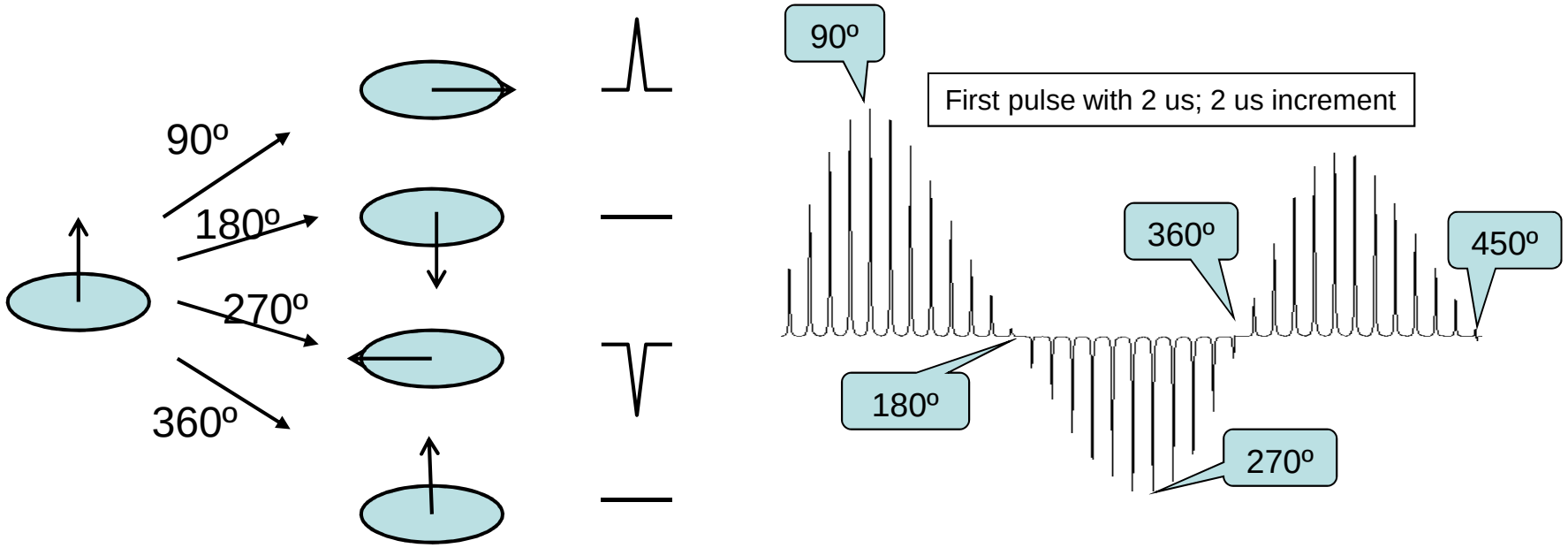


RF pulse calibration

- Hard pulse (high power pulse) can be calibrated directly or indirectly
- For best calibrations, pulses need to be on resonance (know the chemical shift or resonance frequency!)
- Soft or shaped pulsed can be first calculated and then fine-tuned to optimum
  - Shapetool (by Bruker) or Pbox (by Varian) can be used for calculation and simulation
  - Be aware of possible minute phase shift (several degrees for soft pulses), which can be critical in water flip back or watergate

Proton pulse calibration

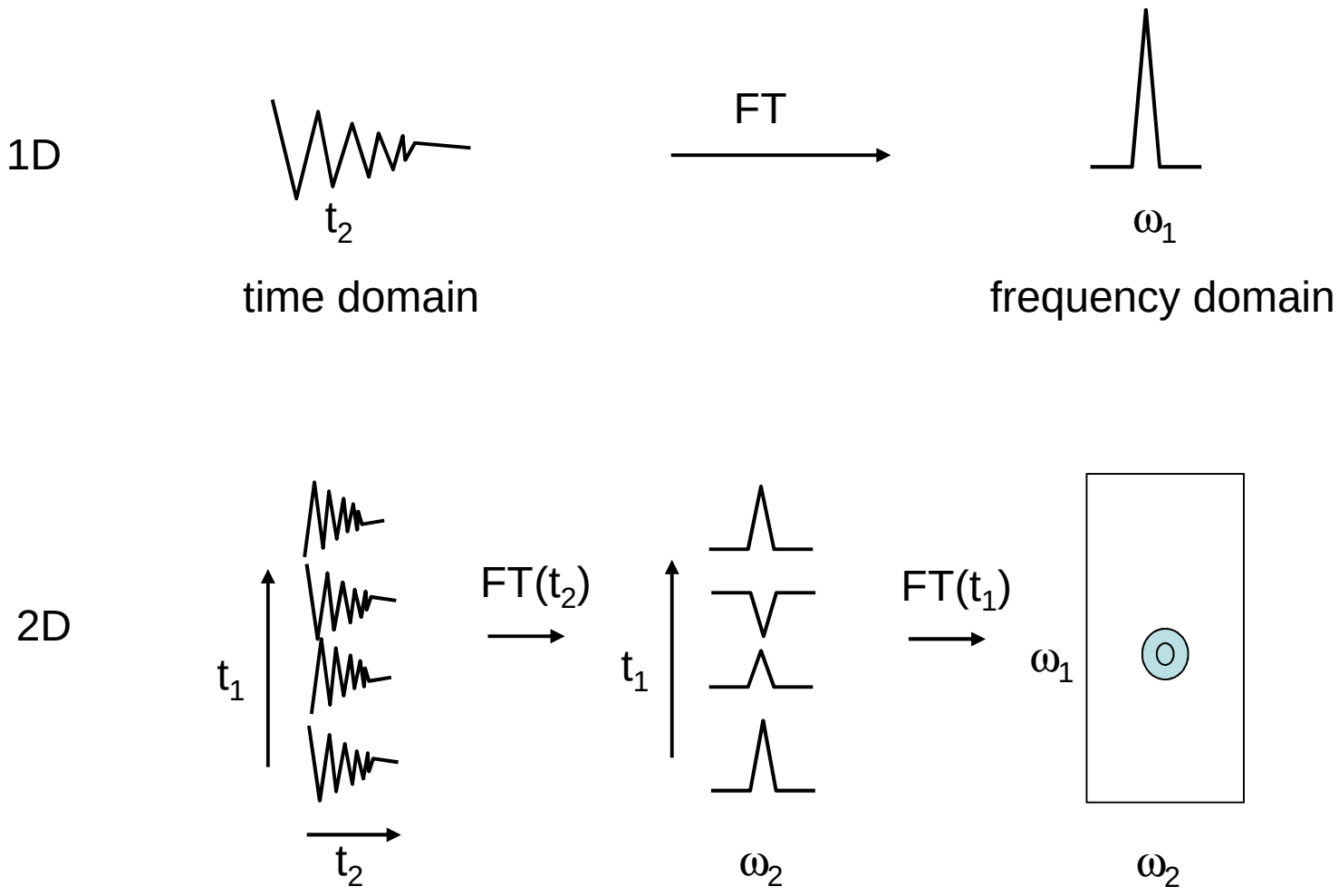
- Most hard (highest power) 90° pulses are typically from 5 us to 20 us.
- Direct observation for high power proton pulse calibration (or even for heteronuclei if sensitivity is sufficient)
  - 360° method (not quite sensitive to radiation damping or relaxation)
  - 180° method



NMR observables

- Chemical shifts
  - expressed in ppm
- Scalar couplings
  - expressed in Hz
  - 2D or nD bond correlations
- NOEs / relaxation / line-shapes
- Peak size
  - Potentially useful in quantitative analysis

From 1D to 2D


1D
FT
t_2
ω_1
time domain
frequency domain
2D
t_1
t_2
$FT(t_2)$
t_1
ω_2
$FT(t_1)$
ω_1
ω_2
time domains
frequency domains

2D NMR

- Correlate resonances through bond or space
  - COSY: coupling
    - Magnitude mode recommended.
    - 1 mg or less will do
    - Minutes to a couple of hours
  - TOCSY: coupling network
    - ~ 70 ms mixing time
    - 1 mg or less will do
    - An hour or longer
  - NOESY / ROESY: distance / NOE
    - Mixing time ranging from less than 100 ms (proteins) to 500 ms (small molecules)
    - 1 mg or more
    - Hours or longer
  - HSQC/HMQC: proton correlation to X, typically through one-bond scalar couplings (two or three bond correlation possible)
    - 1mg or less will do
    - An hour or longer
  - HMBC: proton correlation to X, through multiple bond scalar couplings
    - 1 mg or more
    - Hours or longer

2D NMR

- Resolve overlapping peaks
  - Resolution is provided largely through the indirect dimension
  - No need to have highest resolution in the direct detected dimension
    - Limit direct acquisition time to 100ms or less if heteronuclear decoupling is turned on
    - Lower decoupling power if longer acquisition time is needed
  - Change in experimental conditions may help

2D NMR essentials: acquisition

- Proton tuning and matching
- Calibration of proton (90 degree) pulse length
  - Standard pulse lengths can be used if the solution is not highly ionic (< 50 mM NaCl equivalent)
  - All proton pulses are likely getting longer if the solution is ionic and/or the probe is not tuned
- Modest receiver gain
  - rg about half of what rga gives or less
- Carrier frequency (center of spectrum in Hz) and SW (sweep width) in both dimensions (avoid aliasing unless intended to)
- Number of scans (NS)
  - The pulse program recommends NS (a integer times 1, 2, 4, 8 or 16)
  - Needs some dummy scans, especially with decoupling / tocsy
- Number of increments in the indirect dimension (td1)
  - Larger td1 improves resolution in the indirect dimension
  - Rarely exceeds 512 (except occasionally in COSY
- Detection method in the indirect dimension
  - Determined by the pulse program
  - Typically is either states (and/or TPPI) or echo-antiecho
- Acquisition time (aq) less than 100 ms with decoupling
- Modest gradients (cannot be more than the full power of 100% and typically less than 2 ms in duration)
- Go through the pulse program if you really care

2D processing

- Window functions
  - Allow FID approach zero at the end of the acquisition time
  - Sine bell functions with some shifts are recommended most of the time
- Zero filling
  - Typically double data points in each dimension
- Phasing
  - Indirect dimension zeroth and 1st order corrections are recommended in the pulprogram. If not, use 0 for both
  - Direct dimension first order phase is rarely more than 50 degrees. Zeroth order can be anywhere from 0 to 360 degrees
  - Phase in the 2D mode to best appearance
- Referencing
  - Can be done by picking a known resonance in the spectrum
  - Or referenced by (external) protons