Setting Tube Depth
Varian

0.3 mL
Positioned too low!
Solution not in detected region

0.4 mL
Centered!
Solution covers detected region

0.7 mL
Adjusted to maximum depth!
Recommended sample volume
Setting Tube Depth

Bruker

0.3 mL

Positioned too low!
Solution not in detected region

0.4 mL

Centered!
Solution covers detected region

0.7 mL

Adjusted to maximum depth!
Recommended sample volume
Locking

- Compensates for the transient variations in magnetic field strength.
- The locking circuit keeps the field $B_0$ at a constant value, so that $\Delta \nu$ is constant, and the peaks are narrow.
  - Remember this? Field and frequency are directly proportional!

\[ \nu = \frac{\gamma}{B_0} \]

- Typically lock on $^2\text{H}$ signal. With that frequency regulated, all others get regulated as well.
Locking
Accumulation over time

$B_0$ constant

$\Delta \nu = \text{small}$

$B_0$ not constant

$\Delta \nu = \text{large}$

Can run unlocked, but run risk of getting broad peaks.

**Locks and then re-locks** – lock exactly on resonance or this could happen.
**Locking**

**How does it work?**

**TRANSMITTER** set for fixed deuterium frequency

**COMPARE** \( \nu(\text{trans}) = \nu(\text{received}) \)

**PULSE - LOCK POWER**

**ADJUST** \( Z_0 \ (B_0) \)

\[ \gamma \sim \nu / B \]

**LOCK GAIN**

amplifies returning signal

**PROBE - COIL**

**Goal of locking** – match transmitted frequency (\( \nu \text{ trans} \)) to the received frequency (\( \nu \text{ received} \)). Once matched, said to be *on resonance*. Feedback loop is engaged (lock turned ON), and the instrument will “follow” any drift in the magnetic field.
Locking
Saturation

- Too much power!
  - Get no signal or an oscillating signal because there is an equal populations of spins in all energy states (remember Boltzmann??)
SHIMMING is to make the field more HOMOGENEOUS.

$$\Delta B \text{ small } \Rightarrow \Delta \nu \text{ small}$$

$$\frac{\nu}{B_0} \sim Y$$

An aldehyde $^1$H at the top of the tube will resonate at the same frequency as an aldehyde $^1$H at the bottom of the tube.
Bad Shimming
Wide, asymmetrical lines

$\Delta B_0$ large
$\Delta \nu = $ large

Bad! 😞
NOT well shimmed

$\Delta B_0$ small
$\Delta \nu = $ small

Good! 😊
Well shimmed
Bad Shimming
Wide, asymmetrical lines

- How do we know when the shimming is good?
  - Amplitude of the lock
    - Have you adjusted the shims so that you have maximized your lock level?
  - Decay of the FID
    - Short, stubby FID = bad shimming
  - Examine actual peaks in finished spectrum
    - Are they all symmetrical? No tails or bumps?

Shoulder  Tail to the left  Splitting
Observe
What nucleus we are detecting

TRANSMITTER set for frequency of observed nucleus (in MHz).
• PROTON = 400 MHz
• CARBON = 100 MHz

Computer

Stored every BS (block size in Varian)

Typically, 2 observe channels – High frequency (\(^{1}\text{H},^{19}\text{F}\)) and Low frequency (\(^{13}\text{C},^{15}\text{N},^{31}\text{P}\)).
Observe
Which probe should I use?

Standard Broadband
X channel (usually Carbon) coil inside

Standard Inverse
Proton coil inside

Sample tube
Shim coils
Proton coil
(in pink)
X channel
coil
(in yellow)

Sample tube
Shim coils
Various Things to be Aware of
Common Problems and Things to Think About

- Phasing
- Baseline Correction
- Foldover
- Clipping and Truncation
- Signal to Noise
- Relaxation
  - T1
  - T2
- Apodization

NMR is cool!
Phasing
Zero Order vs. First Order

BAD First Order Phasing
frequency dependent

BAD Zero Order Phasing
Not frequency dependent

Nicely phased!!!
Baseline is even on both sides of the peak.
Baseline Correction
Why we want a flat baseline

• Easier to differentiate between noise and signal.
• Integration
  – Flat baseline = accurate integrations
• Presaturation – irradiates a selected frequency.
  – Solvent suppression – enables us to suppress an especially strong signal, allowing us to ‘see’ weaker signals better.
• 2D NMR! Now we have a flat ‘surface’ to work with.

Rolls and waves in baseline can be caused by receiver overload/especially concentrated sample.

Reducing the pulse width and increasing the acquisition time can fix this.
Foldover
aka Aliasing

- Spectral window too small.
  - Any peak outside the detected range gets ‘folded’ back into the spectrum.
    - Bruker folds peaks in on same side.
    - Varian folds peaks in on the opposite side.
  - Impossible to phase!

Bruker example
Clipping and Truncation
Cutting it short

- Clipping – when the receiver gain is too high, and the top of the FID is clipped off. Produces broad peaks.

- Truncation – when the acquisition time is too short, and the end of the FID is cut off. FID not allowed to decay to zero.
  - Produces Fourier ripples on either side of your peak.
Signal to Noise
S/N ratio and Number of Scans

• S/N ratio increases with the number of scans.
  – In order to double the current S/N, you need to quadruple the number of scans.

<table>
<thead>
<tr>
<th>Scans</th>
<th>S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>10/1</td>
</tr>
<tr>
<td>32</td>
<td>20/1</td>
</tr>
<tr>
<td>128</td>
<td>40/1</td>
</tr>
</tbody>
</table>

Notice that the longer you run, the less you gain over time. For example, there is not a huge gain in S/N between 3000 and 4000 scans.
Remember what happens when a pulse is applied?
- A second magnetic field is applied ($B_1$) at radio frequencies (a pulse).
- The length of the pulse (in microseconds) determines how much of the net magnetization is tipped from the z direction into the xy plane.

We only detect what is tipped into the xy plane.
- Relaxes back to ground state (along z-axis) and awaits next pulse.
  - Two types of relaxation – T1 and T2.
Relaxation
Spin-Lattice or T1

- **T1 relaxation** – how long it takes for the net magnetization to return to the ground state (z axis).
  - Different nuclei relax at different rates
  - Dipole-dipole interaction help with relaxation. The more neighbors, the faster the relaxation.
    - Smaller, organic molecules tend to be slow relaxers and have long T1s.
  - The same nuclei in different magnetic environments also relax at different rates.
    - Carbons are relaxed by neighboring protons. The more protons, the shorter the T1. Thus, quaternary carbons have long T1s because they have no attached protons.
  - Must wait 5 T1s between pulses to ensure that $M_0$ has fully relaxed.
    - We usually pulse less than 90°, so that we do not have to wait that long.

Net magnetization ($M_0$) Tipped by a 90° pulse

T1 – relaxing back to z axis
Relaxation
Why do we care about T1?

• If you do not wait the right amount of time between pulses, you are not allowing your net magnetization vector to return to the ground state before you pulse again.
  – You will basically ‘beat down’ your signal by pushing it more and more past the xy plane. The less signal in the xy plane, the less you will detect.
    • If you suspect that you are losing some of your signal, increase the delay, take a few scans, and check your spectrum.
  – Aromatics especially sensitive to being ‘beaten down’.
  – In order to avoid having to wait 5 T1s (this can be long for certain carbons), we rarely use a full 90° pulse.
    • 45° and 33° pulses tip less M₀ into the xy plane, so we don’t have as long for it to relax back to equilibrium.
    • Sacrifice some signal so that we can take more scans in less time.
• This is how solvent suppression (presaturation) is done! The delay between pulses (τ or τ) is set so that the selected peak is ‘beaten down.’
Relaxation
α-santonin

300 scans
2 second delay (τ or tau)

Shorter pulses – not as much $M_0$ tipped
So delay is long enough

90° pulse – delay too short
Losing aromatic peaks
Relaxation
Spin-Spin or T2

- T2 relaxation – how long it takes for the spins to lose phase coherence, allowing the net magnetization ($M_0$) decay to 0 in the xy plane.
  - Spin flipping ($+\frac{1}{2} \leftrightarrow -\frac{1}{2}$) due to fluctuating local dipolar magnetic fields (inhomogeneity of the field) causes some spins to $\uparrow$ (increase) in energy, some to $\downarrow$ (decrease), some to precess faster, others slower. Dephasing occurs.
  - If the acquisition time is shorter than T2, the end of the FID will be cut off (truncation!).
- Meanwhile, T1 relaxation is taking place.
  - T2 is always less than or equal to T1.
Apodization
FID manipulation

**Apodization** – when the FID is multiplied by some mathematical function to modify the spectrum.
  - Allows you to emphasize some quality of the spectrum at the expense of another.
  - This is done after the spectrum has been acquired.
  - Several different types of apodization.
    - **Sensitivity Enhancement** (line broadening) – enhances the first part of the FID, increasing sensitivity at the expense of resolution.
    - **Resolution Enhancement** (Gaussian, Sine Bell) – enhances the later part of the FID, increasing resolution at the expense of S/N.
Apodization
FID manipulation

Line broadening

Increased sensitivity
Decreased resolution

Decreased sensitivity
Increased resolution

Gaussian
Sinebell
Zero Filling
FID manipulation

• When an FID is Fourier transformed, the data (np, or number of points) consists of 2 parts.
  • **Real points** (np/2) from the cosine portion.
  • **Imaginary points** (np/2) from the sine portion, which are not displayed in the actual spectrum.
• Zero filling doubles number of real points used by adding an equal number of zeroes.
  • Increases the digital resolution.
  • FID needs to have decayed to 0 to be zero filled.

If np = 32,768, then 16,384 points would typically used to create the actual spectrum. If it were zero filled, 16,384 zeros would be added to the end of the FID so that 32,768 points would be used to create the spectrum.
Credits
Special thanks to.....

Kathleen Gallagher, University of New Hampshire.

Jeremy N. S. Evans - BIOMOLECULAR NMR SPECTROSCOPY, Oxford Press, 1995

Varian Associates, Inc.

Joseph P. Hornak – Rensallear Institute of Technology

http://www.cis.rit.edu/htbooks/nmr/bnmr.htm
End of Part 2