

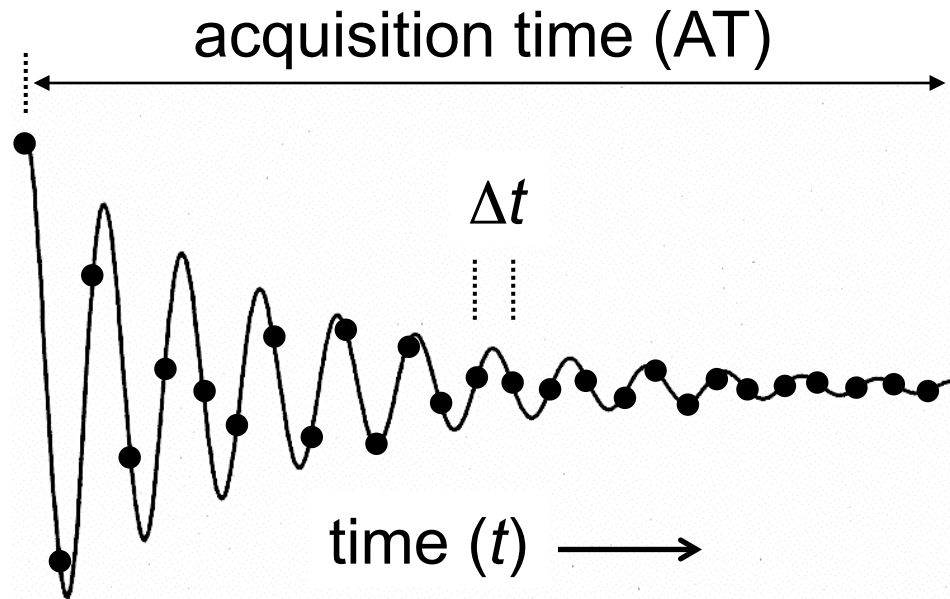
BCMB/CHEM 8190

**Data Acquisition and
Processing**

Fourier Transformation

Digitizing the Free Induction Decay

- The exponentially decaying sinusoidal NMR signal is referred to as the free induction decay (FID)
- The rotating frame FID is sampled at regular time intervals (digitized) by an analog-to-digital converter (ADC)
- Both real (i.e. y -axis) and imaginary (i.e. x -axis) amplitudes are sampled
- These data (signal amplitudes versus time) are stored and used to reproduce the FID and to extract frequencies (FT)

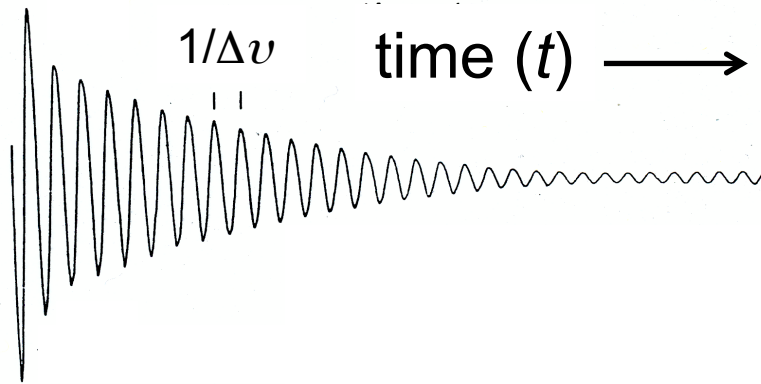


$$AT = N \Delta t$$

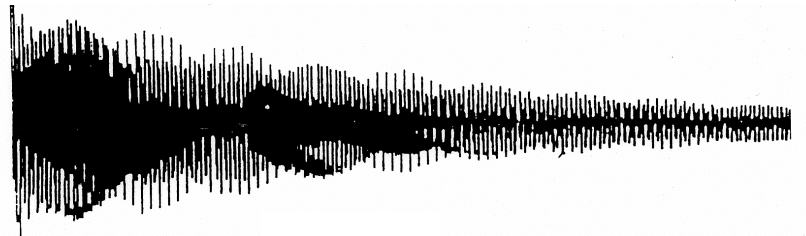
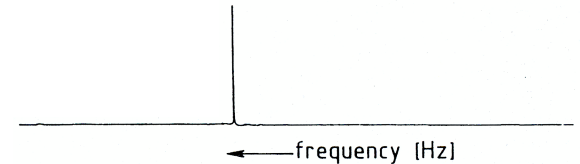
- N is the total number of (complex) points sampled
- Δt is the time interval between points sampled (also known as the *dwell time* (DW))

Frequency/Time Domains

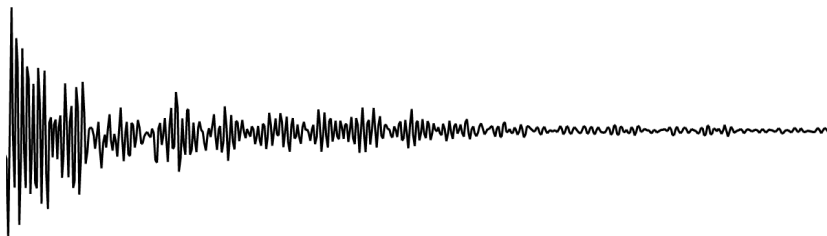
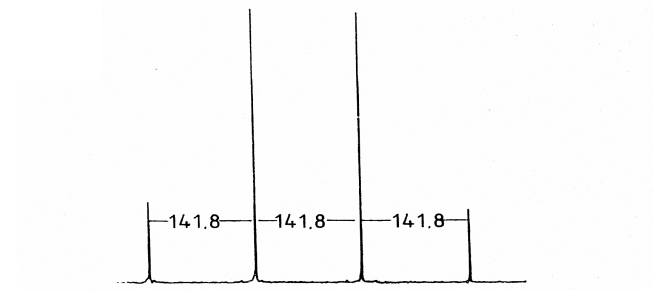
- NMR signals (FIDs) are superpositions of exponentially decaying sinusoidal signals, and frequencies can be extracted directly from only the simplest signals. So, FT is essential.



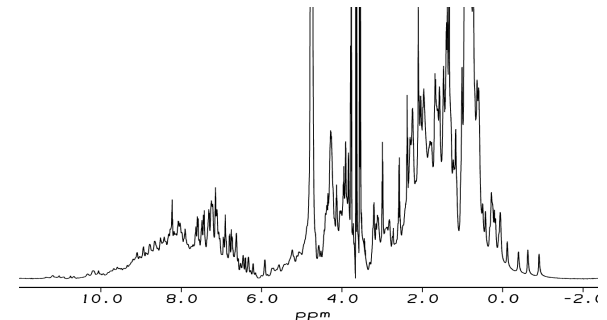
FT →



FT →

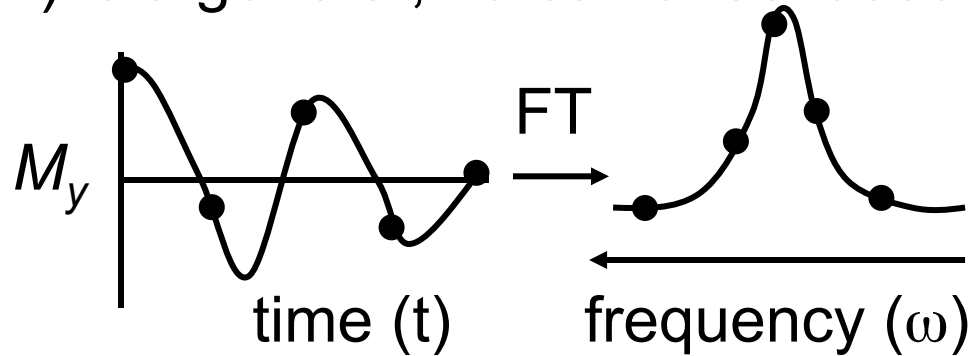


FT →



Fourier Transformation

- The Fourier transformation (FT) is a general, versatile tool used in many diverse applications
- Used in NMR to interconvert time and frequency domains



$$S(\omega) = \int_{-\infty}^{\infty} S(t) \exp(-i\omega t) dt \quad S(\nu) = \int_{-\infty}^{\infty} S(t) \exp(-i2\pi\nu t) dt \quad (\omega = 2\pi\nu)$$

$$S(\omega) = \int_{-\infty}^{\infty} S(t) (\cos(\omega t) - i \sin(\omega t)) dt$$

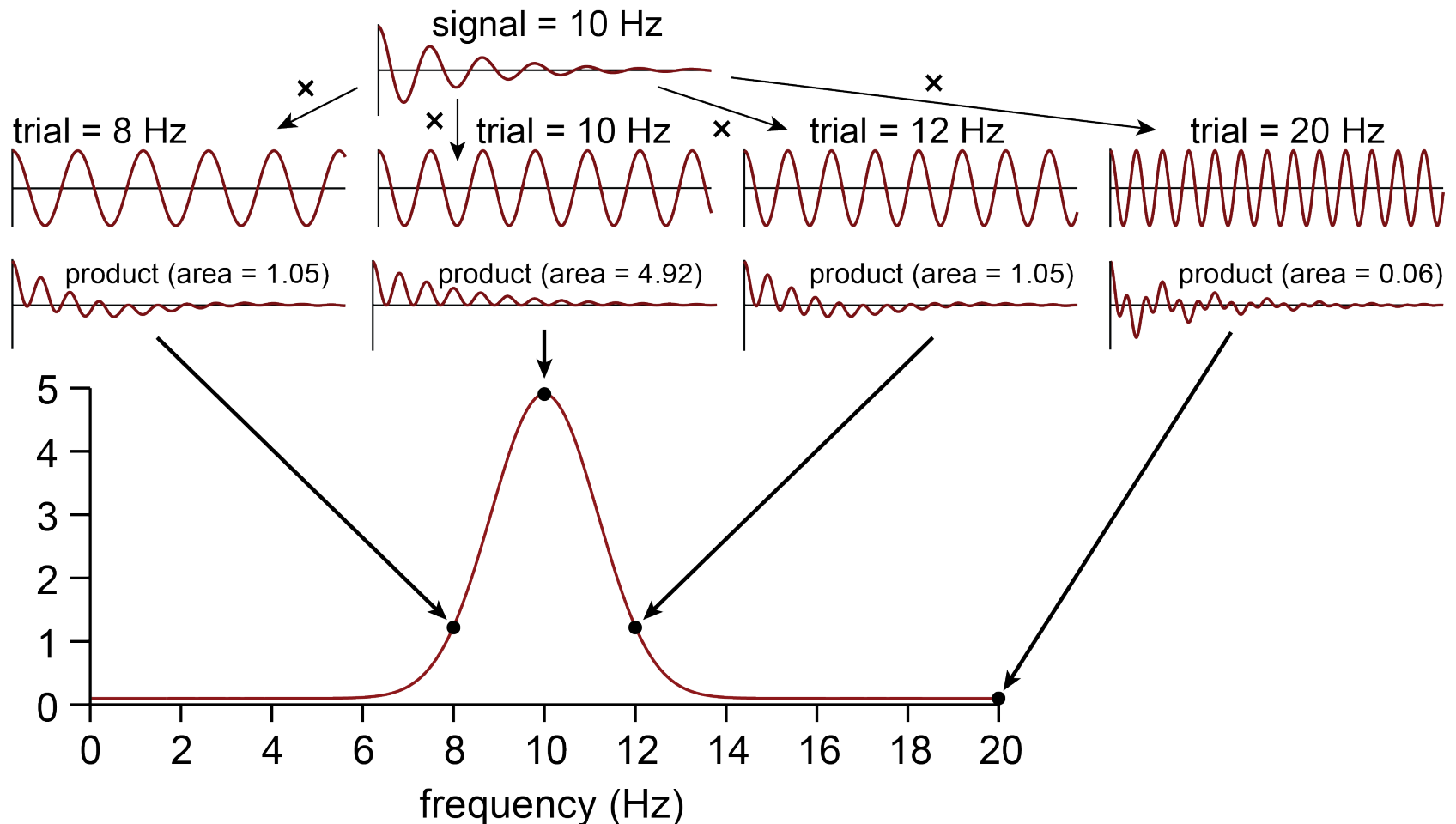
$$S(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} S(\omega) \exp(i\omega t) d\omega \quad S(t) = \int_{-\infty}^{\infty} S(\nu) \exp(i2\pi\nu t) d\nu$$

- Discrete Fourier transform (digital) yields amplitudes $S(\nu)$ at all frequencies $\nu = k/(N\Delta t)$ (i.e., for each point in the time domain, an amplitude is calculated in the frequency domain)

$$S(\nu) = S(k/(N\Delta t)) = \sum_{j=0}^{N-1} S(j\Delta t) \exp(-i2\pi jk/N), \quad -N/2 < k < N/2$$

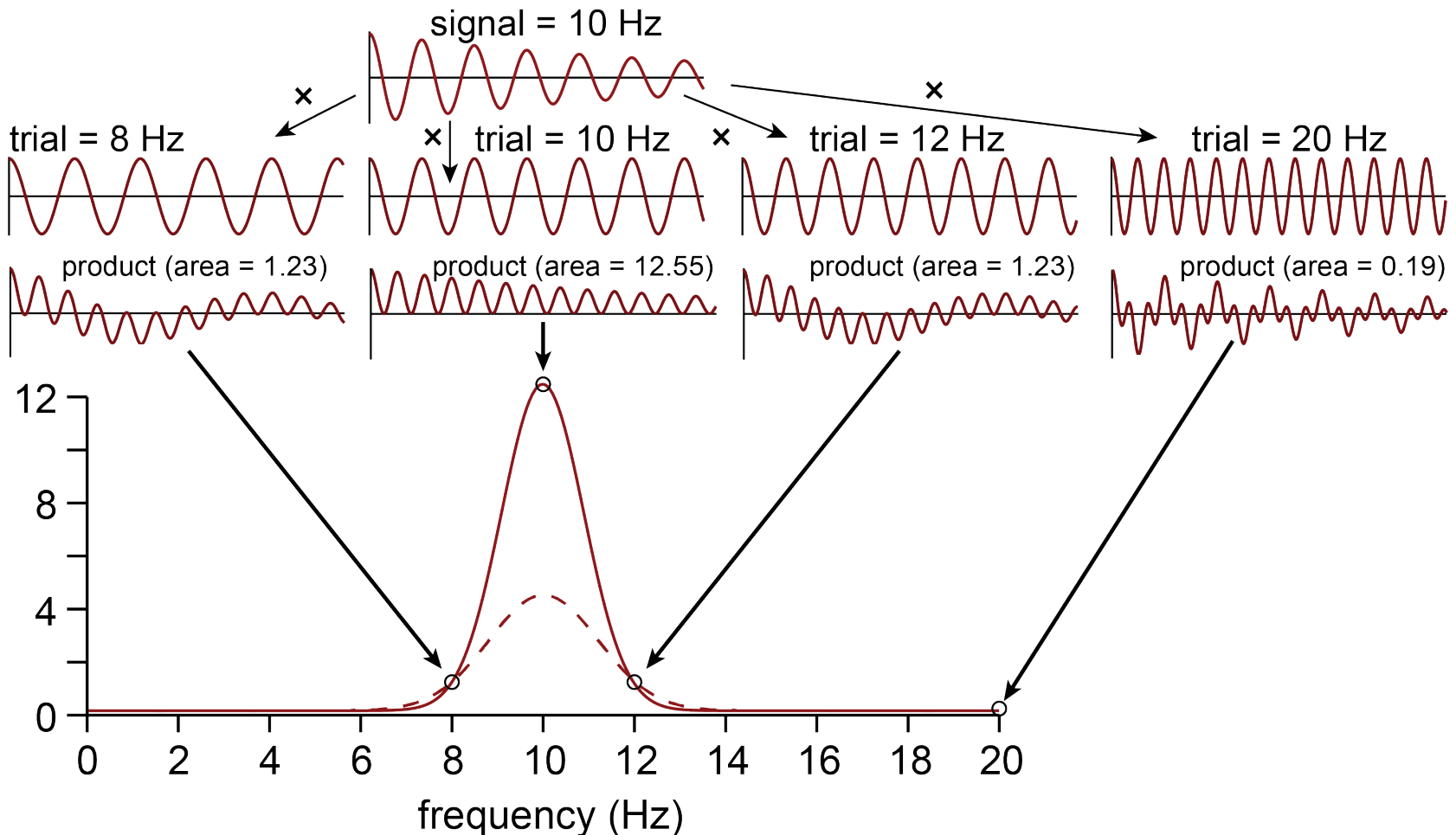
Visualization of FT

- The FID is multiplied by signals whose N frequencies correspond to the frequency range in our spectrum
- The integrals (areas under product signals) are largest for frequencies near those in our spectrum



Visualization of FT

- As expected, a more slowly decaying time domain signal gives sharper (narrower) lines in the frequency domain spectrum



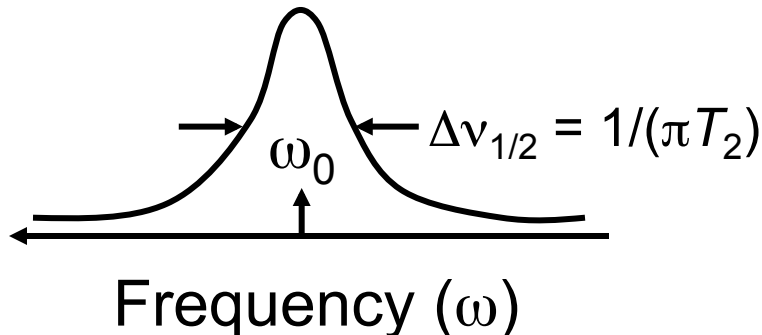
Lorentzian Lines

- The lineshape of the absorptive component of the Fourier transform of an exponentially decaying sinusoidal function is known as *Lorentzian*

$$S(\omega) = M_0 T_2 / (1 + T_2^2 (\omega_0 - \omega)^2)$$

- Here ω_0 is the center of the signal (Larmor frequency)
- When $\omega = \omega_0$, the denominator $\rightarrow 1$, and $S(\omega)$ is maximum
- As the difference between ω and ω_0 increases, the denominator increases, $S(\omega)$ decreases, giving the characteristic lineshape
- At $1/2$ the maximum height (when $S(\omega) = S(\omega_0)/2$)

$$\Delta\nu_{1/2} = 1 / (\pi T_2) \text{ or } = 1 / (\pi T_2^*)$$



when $S(\omega) = S(\omega_0)$

$$M_0 T_2 / (1 + T_2^2 (\omega_0 - \omega)^2) = M_0 T_2 / 2$$

$$1 + T_2^2 (\omega_0 - \omega)^2 = 2$$

$$T_2^2 (\omega_0 - \omega)^2 = 1$$

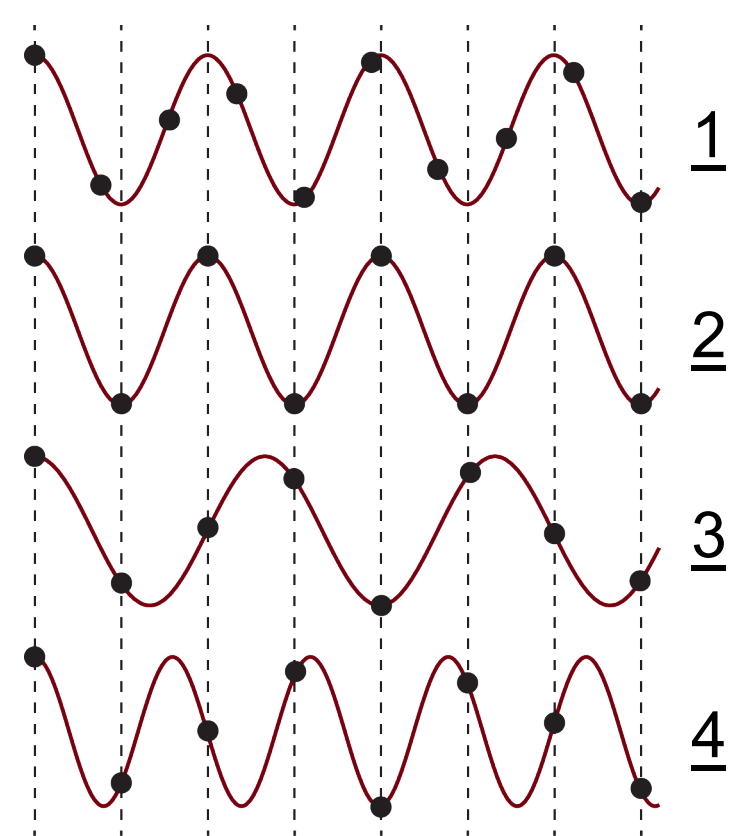
$$\omega_0 - \omega = 1 / T_2$$

$$2(\omega_0 - \omega) = \Delta\omega_{1/2} = 2 / T_2$$

$$\text{because } \omega = 2\pi\nu, \Delta\nu_{1/2} = 1 / (\pi T_2)$$

Digitizing Data and Setting Sweep Widths (SW)

- The sampling theorem asserts that a sinusoidal signal of frequency ν must be sampled minimally at 2ν (2 points/cycle, *Nyquist frequency*) for the signal to be reproduced correctly
- Sampling frequency, dwell time (DW) and sw are interdependent (sampling frequency = $2 \cdot \text{SW} = 1/\text{DW}$)
- Sampling below the Nyquist frequency results in aliasing



1 Frequency = ν_A , sampled at $> 2\nu_A$

2 Frequency = ν_A , sampled at exactly $2\nu_A$

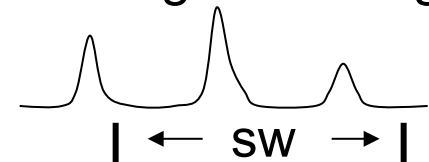
3 Frequency = $\nu_B = \nu_A - \Delta$, sampled at $2\nu_A$

4 Frequency = $\nu_C = \nu_A + \Delta$, sampled at $2\nu_A$

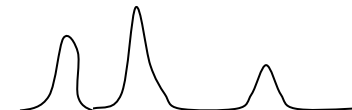
(note: for '3' and '4' amplitudes of sampled points are identical!)

For '4', the signal is sampled below the Nyquist frequency, resulting in aliasing

actual spectrum

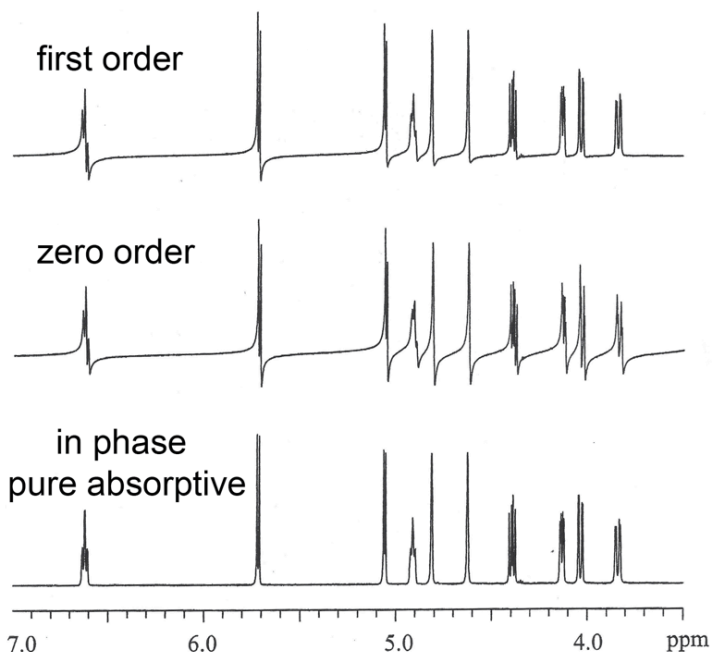
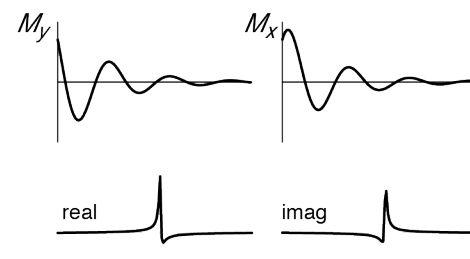
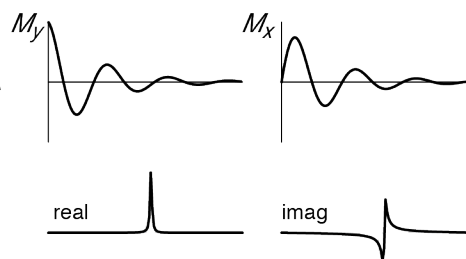
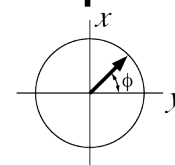
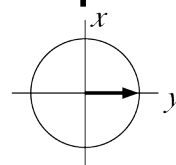


observed spectrum



Zero Order Phase Correction

- If the reference signal and actual signal are perfectly in phase, the signals along the y- and x-axes are pure absorptive and dispersive, respectively, and all absorptive peaks are in phase
- Generally, this is not the case, so signals are combinations of absorptive and dispersive components, and appear out of phase (zero order phase correction necessary)



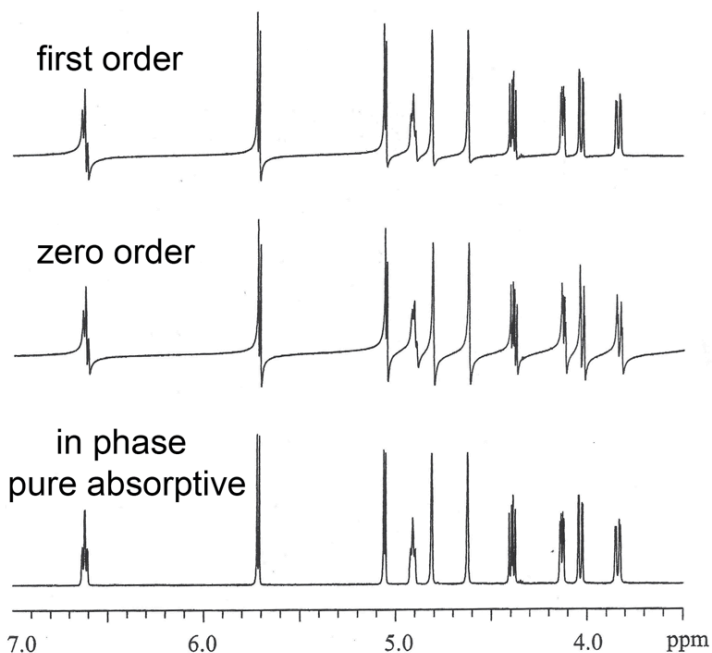
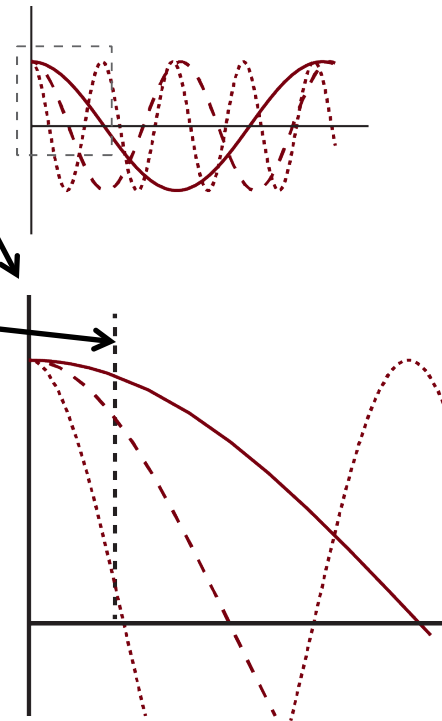
- Typically, M_y and M_x are combinations of real (absorptive) and imaginary (dispersive) components:

$$M_y = M_r(\cos \phi) + M_i(\sin \phi) \quad M_x = M_i(\cos \phi) - M_r(\sin \phi)$$

- Cos and sin weighted average of absorptive and dispersive parts
- Can adjust weighting interactively to give pure absorption spectrum (zero order phasing)

First Order Phase Correction

- If data collection began immediately after the pulse, all signals would have the same phase
- However, there is a delay following the pulse before data collection begins, which causes signals to be out of phase (linear with frequency, highest frequencies need largest corrections)



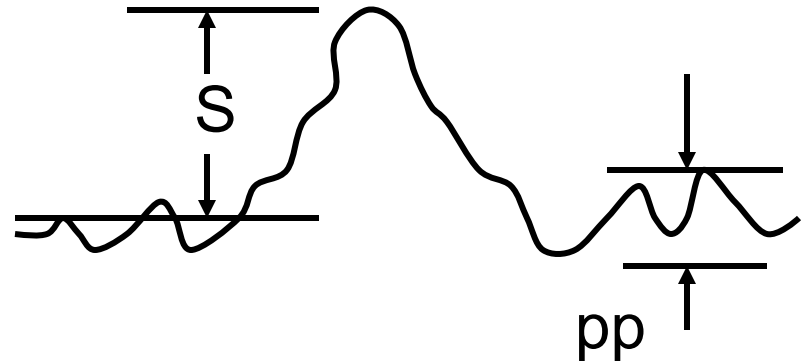
- As with zero order correction, adjustments are made to the phase interactively to account for the frequency dependent (first order) correction

$$\phi_0 + \nu \phi_1$$

Signal Averaging

NMR is not a high sensitivity technique:
signal averaging improves the signal to noise (S/N) ratio

S/N defined as: $2.5 \times S/pp$



On averaging signal adds as number of scans (NS),
noise adds as the root of the number of scans

$$S/N \propto (NS)/(NS)^{1/2} = (NS)^{1/2}$$

$$*** 4 \times \text{scans} = 2 \times S/N ***$$

Best Single Scan Parameters

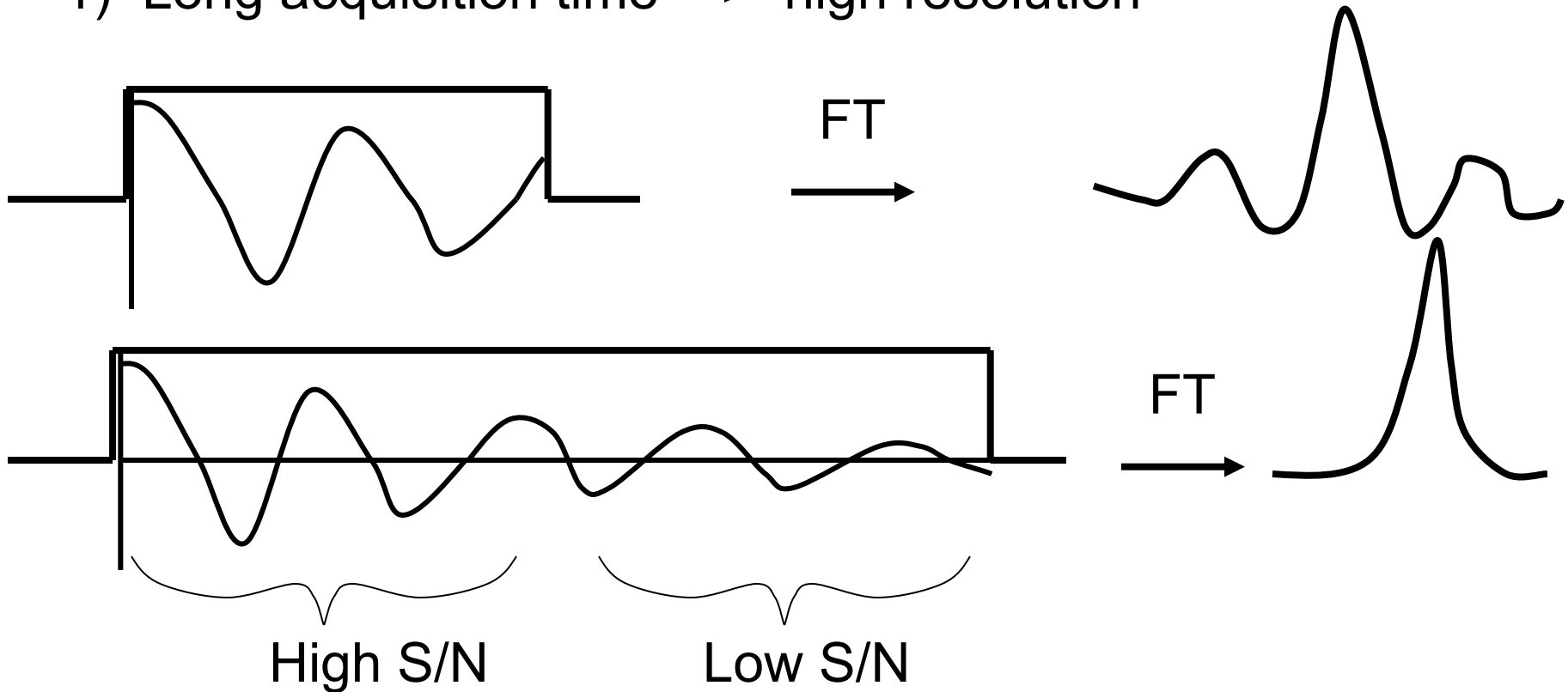
- Recycle times? Pulse angle?
- These are opposing factors
 - recovery of magnetization:
$$M_z(t) = M_0(1 - \exp(-t/T_1))$$
$$3 \times T_1 \text{ for } \sim 90\% \text{ recovery, } 5 \times T_1 \text{ for } \sim 99\%$$
 - more scans / unit time:
$$S/N \propto N^{1/2}$$
- Compromise: use $\beta < 90^\circ$
 - Ernst angle: $\cos\beta_{\text{opt}} = \exp(-t/T_1)$

Ernst Angle Example

- Small molecule: T_2 limited by shimming
 $T_2^* = 0.3\text{s}$ (1 Hz); $T_1 = 2.0\text{s}$
- Collect 0.6s to optimize resolution
- $\cos\beta_{\text{opt}} = \exp(-0.6/2) \Rightarrow \beta_{\text{opt}} = 42^\circ$
- Cost: may not have unit intensity for all resonances since T_1 s differ
- Comparison to waiting $3 \times T_1$:
10 scans / min @ unit intensity = $\sqrt{10} = 3.2$
100 scans / min @ 0.67 intensity = 6.7

How long should acquisition time be?

1) Long acquisition time \Rightarrow high resolution

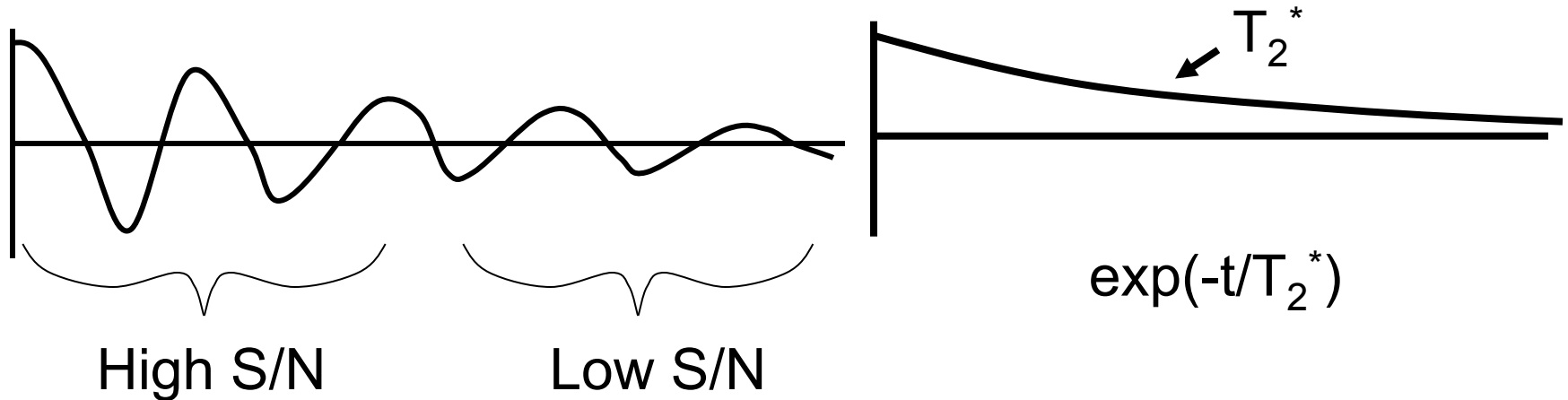


2) Long acquisition time \Rightarrow low S/N

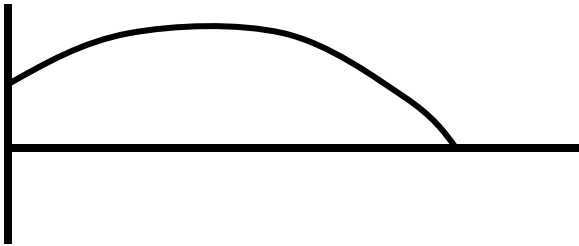
3) Compromise" $T = (1-2) \times T_2$ - optimum S/N $1.2 \times T_2$

Some other processing details

- Matched Filters – maximize S/N



- Other Filters: sine-bell



- Other Filters: kaiser

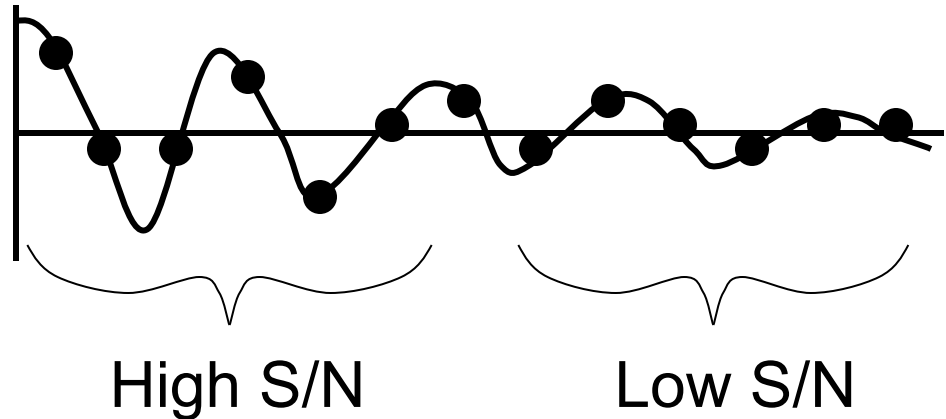


Alternate Processing Methods

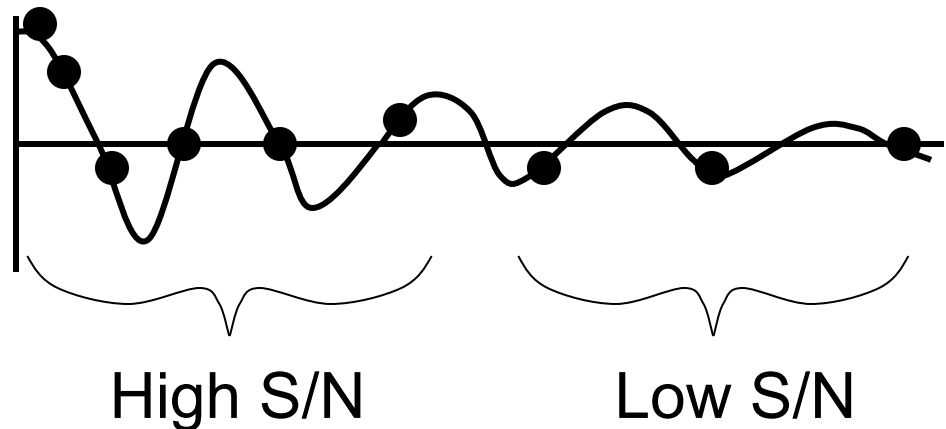
- What is wrong with FT?
 - no assumption about max # lines
 - no assumption about line shape
 - FFT uses a uniform distribution of pts
- Alternatives
 - Maximum entropy
 - Linear prediction
 - non-linear sampling
- References:
 - Rovnyak, D Hoch, JC Stern, AS Wagner, G, (2004) J. Biomol. NMR, 30, 1-10.
 - Hyberts, SG Robson, SA Wagner, (2013) J, Biomol. NMR 55, 167-178 .

Non-Linear Sampling

Normal Sampling



Non-linear Sampling



References:

Hyberts SG, Frueh DP, Arthanari H, et al. (2009) J. Biomol. NMR, 45, 283-294

Failing to collect all points results in artifacts and noise

Iterative Soft Threshold (hmsIST) method eliminates

NUS synthetic two line spectrum

FFT produces spectrum with many artifacts (point spread function, PSF)

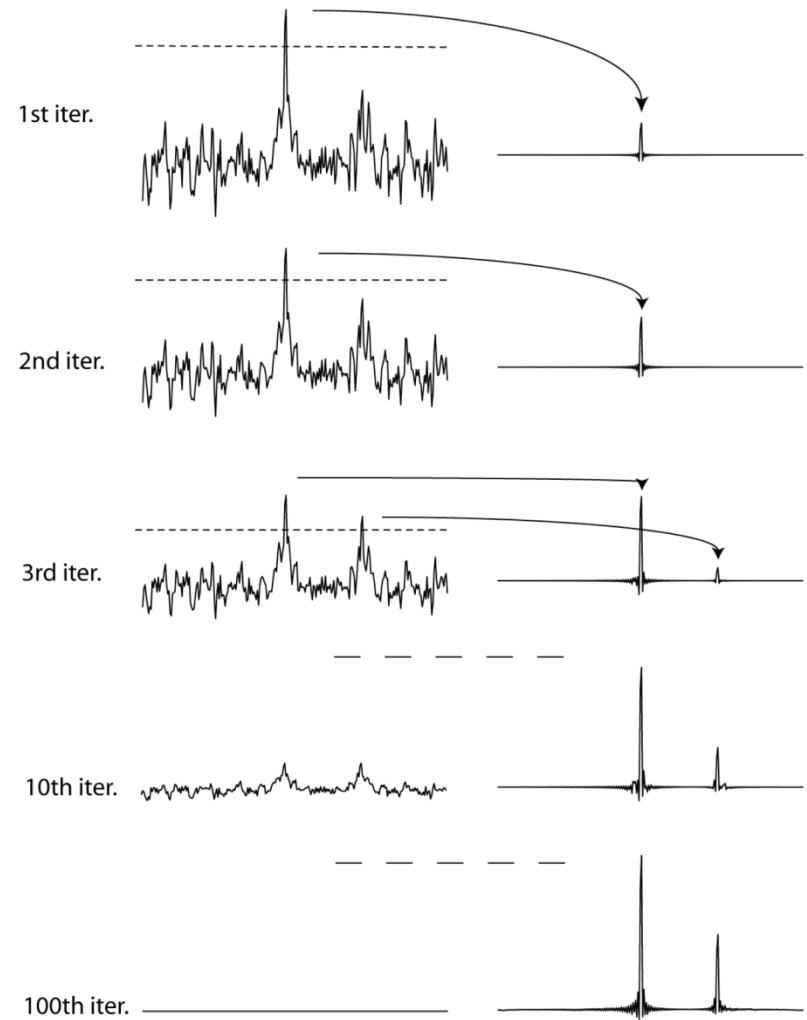
Copy top 2% of spectrum and store in different location

FFT^{-1} and zero skipped time domain data points - subtract

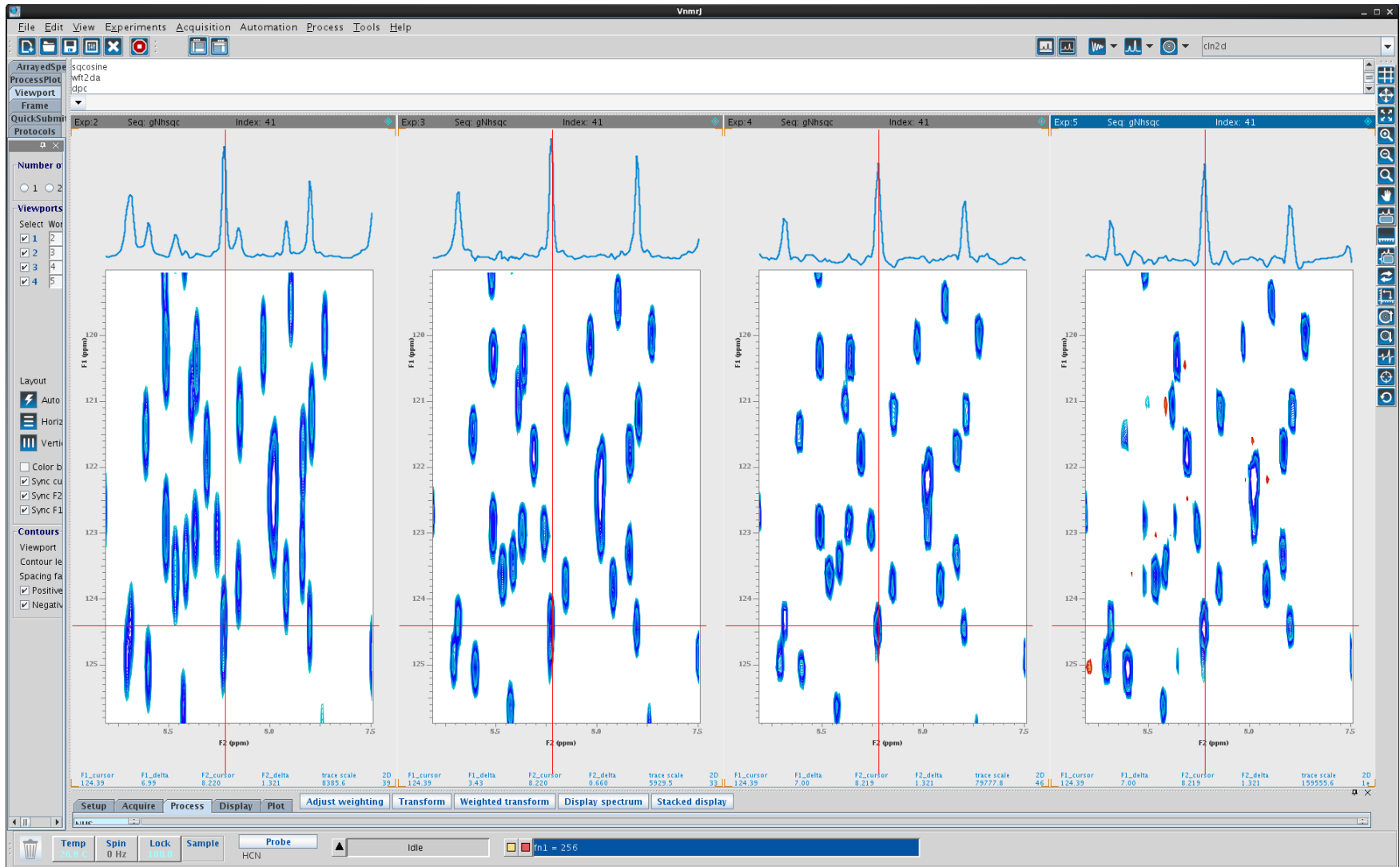
FFT and iterate

After a sufficient number of iteration all PSF artifacts are eliminated

Very fast procedure



Sparse sampling can improve resolution or S/N



std
ni=32

std
ni=32
LP=64

sparse
Max ni=64
50%

sparse
Max ni=64
25%