What determines the sensitivity?
– the numbers of scans and sampling –

IPR-seminar

Practical Aspects of Non-uniform Sampling
in Multi-dimensional NMR Spectroscopy
and Application for Biological Systems

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Nagoya Univ.

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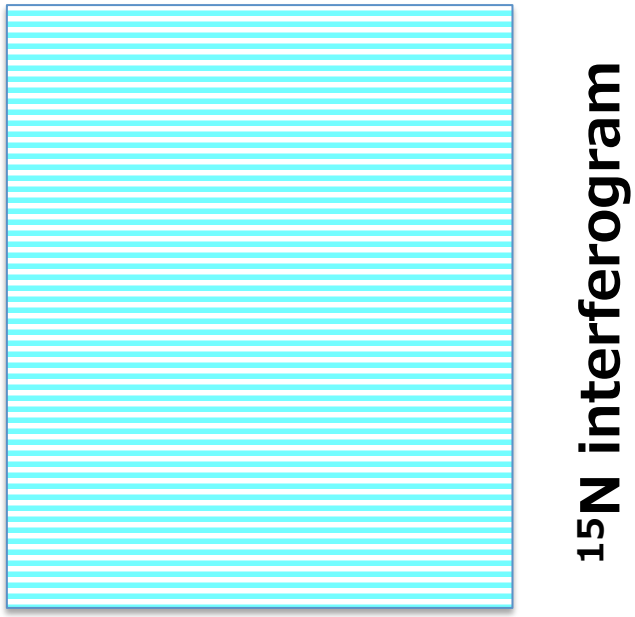
I just want to increase
the number of scans (NS) and
the number of total sampling data (TD_1)
to get 3D spectra of higher sensitivity and resolution.

But, to do this, the experimental time would extend to 7 days!

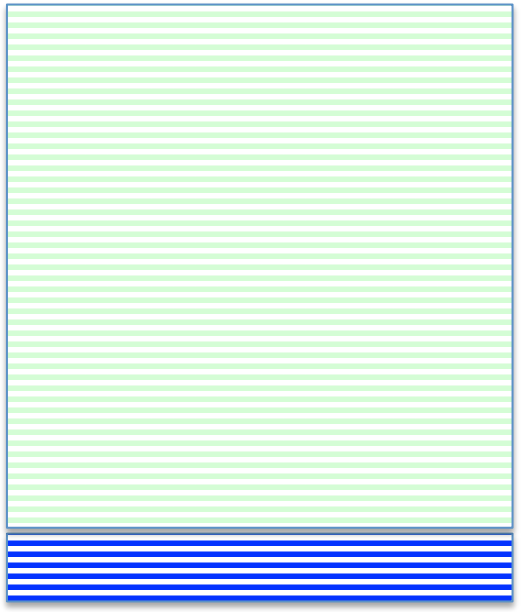
I tried decreasing TD of the ^{15}N indirect dimension, and at the same time, increasing NS, so that the experimental time was conserved.



^{15}N interferogram

^{1}H FID

NS = 1
TD = 2,560



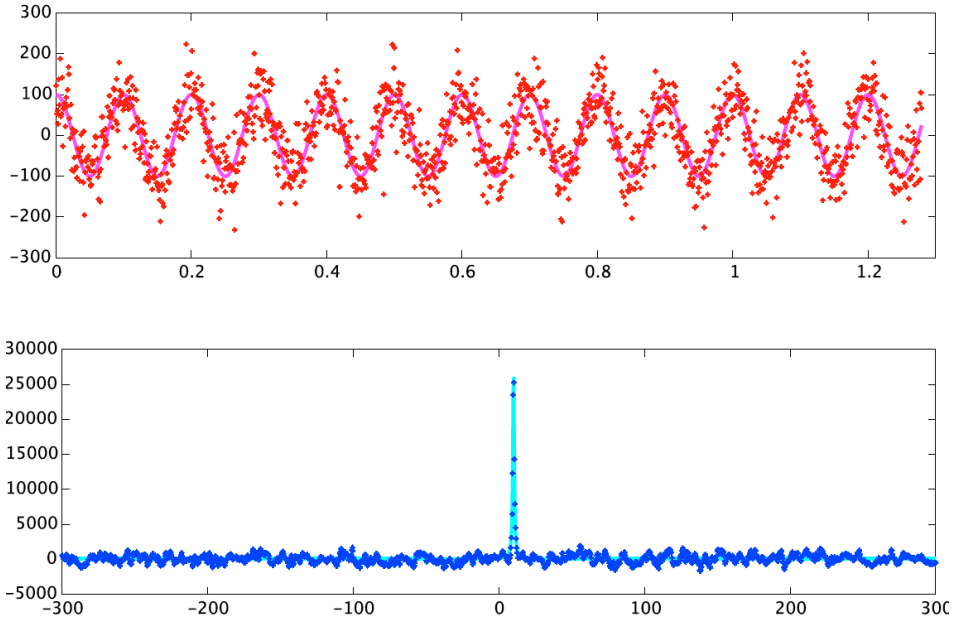
^{15}N interferogram

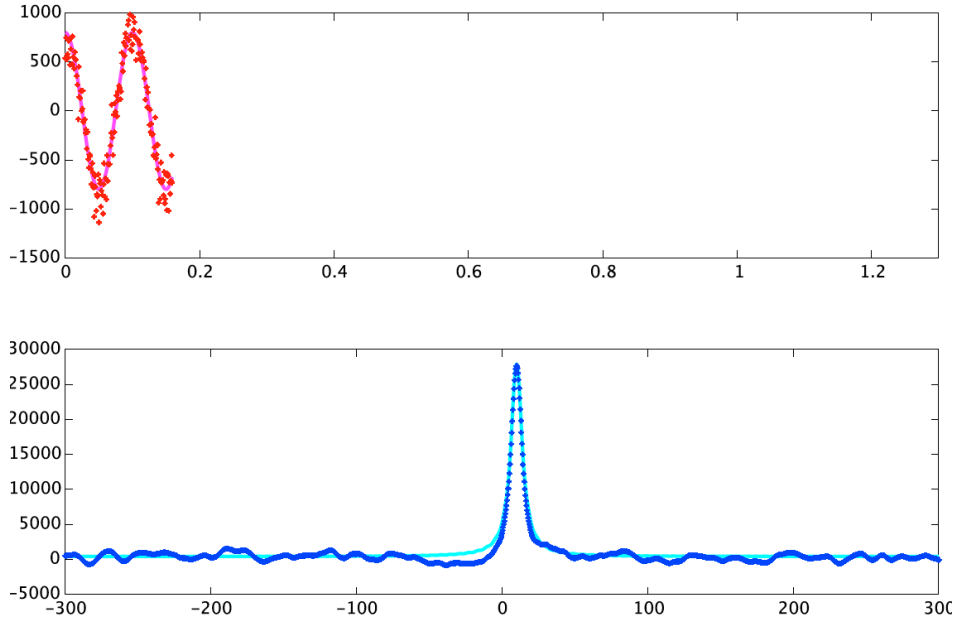
^{1}H FID

NS = 8
TD = 320

Let's assume that the interferogram be sampled in the constant-time manner (no decay).

The noise of the interferogram (NS=8) decreased to 1/√8 =35%.





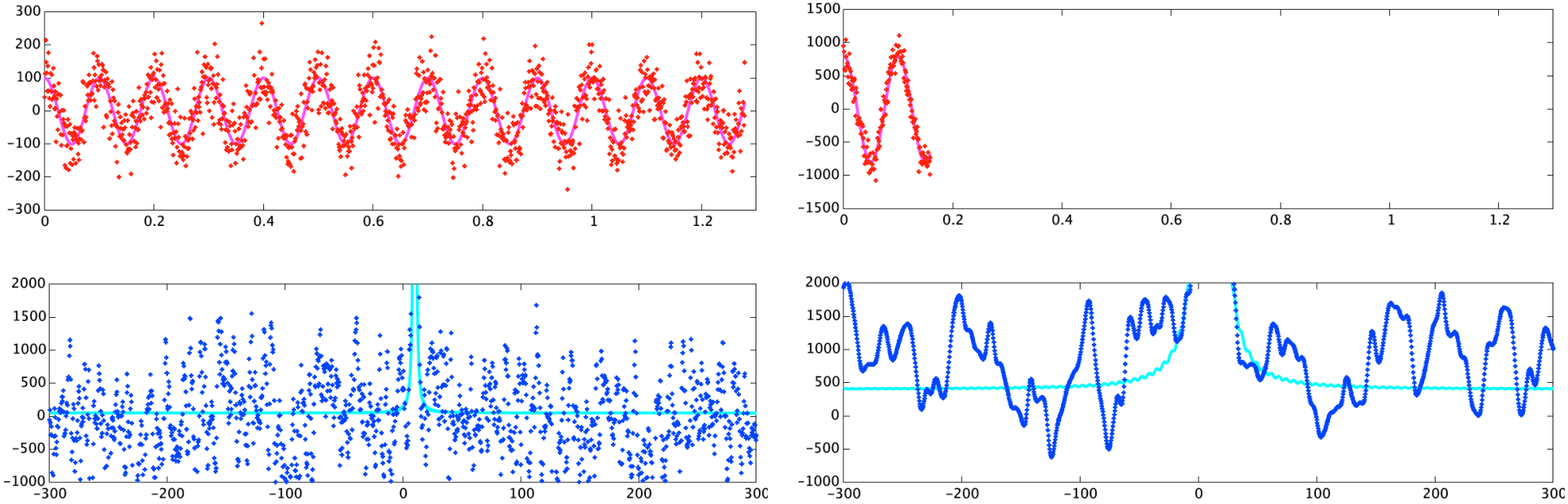
NS = 1
TD = 2,560

sharper!

NS = 8
TD = 320

broadened …

Let's magnify the spectra to compare them more carefully.



NS = 1
TD = 2,560

spectral noise : 578

NS = 8
TD = 320

spectral noise : 584

The peak and noise both broadened, but S/N was the same!

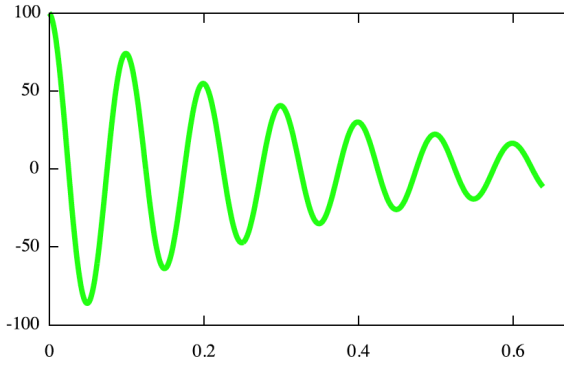
What is sensitivity ?

Signal intensity (S) = the area of FID (interferogram) after modification with a window function.

Sensitivity (S/N) $\propto \sqrt{N_s} \bullet \sqrt{T_d} \propto \sqrt{expt}$

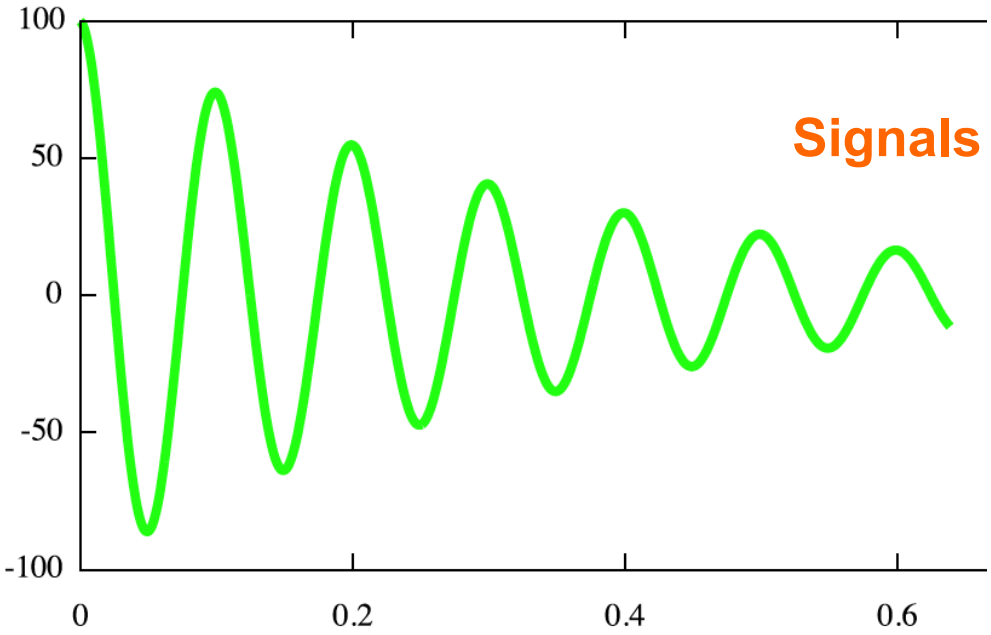
As far as the *expt* is kept at constant, S/N does not change.

N_S = 8
T_D = 256



N_S = 16
T_D = 128

Practically,
the spectrum (NS=8, sampling=16) : higher S/N
the spectrum (NS=2, sampling=64) : lower S/N

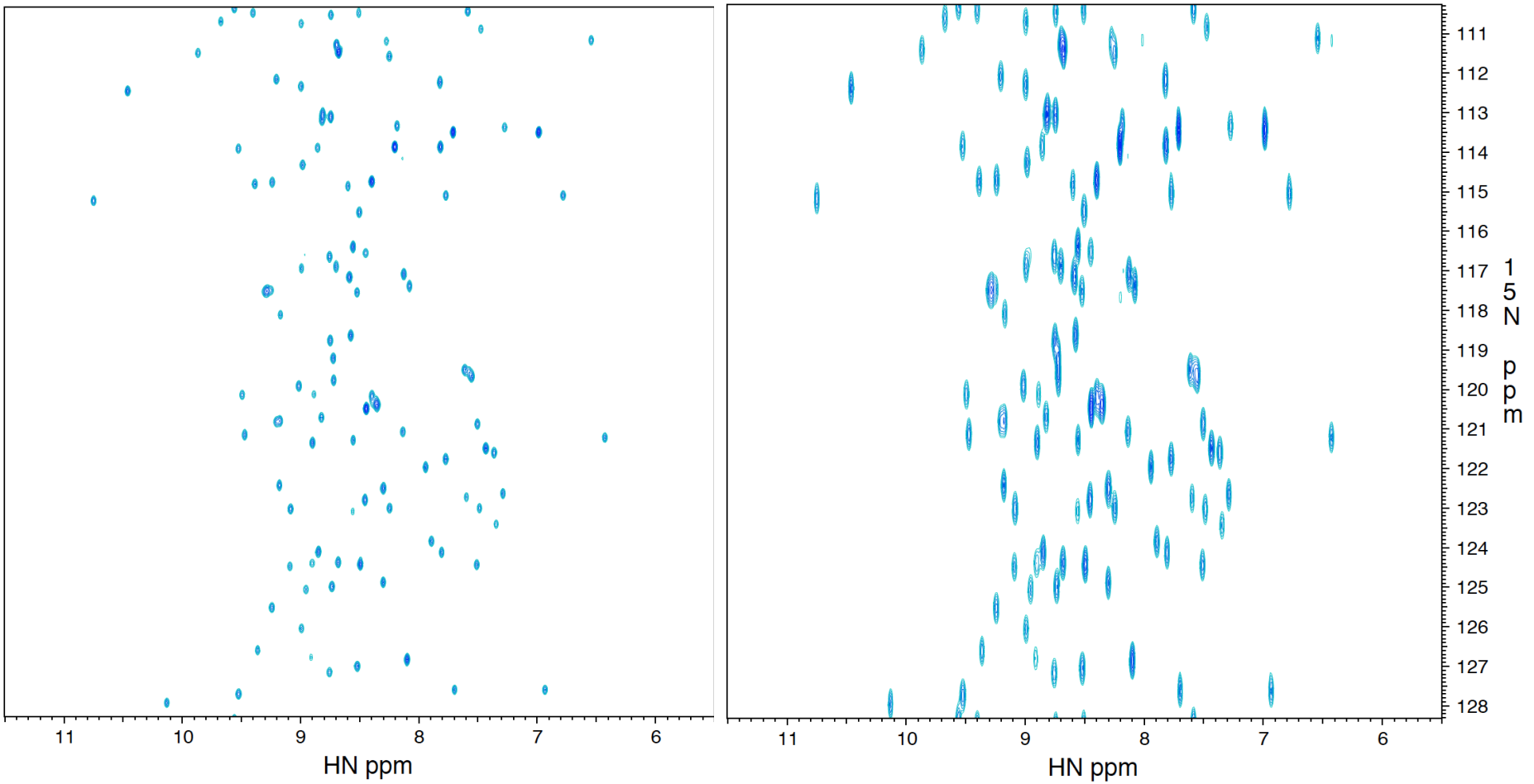


Signals alone decay!

S/N : high **S/N : low**

The noise level is the same throughout the interferogram irrescpective of relaxation.

Each measurement took 30 min.



NS = 4
TD = 256

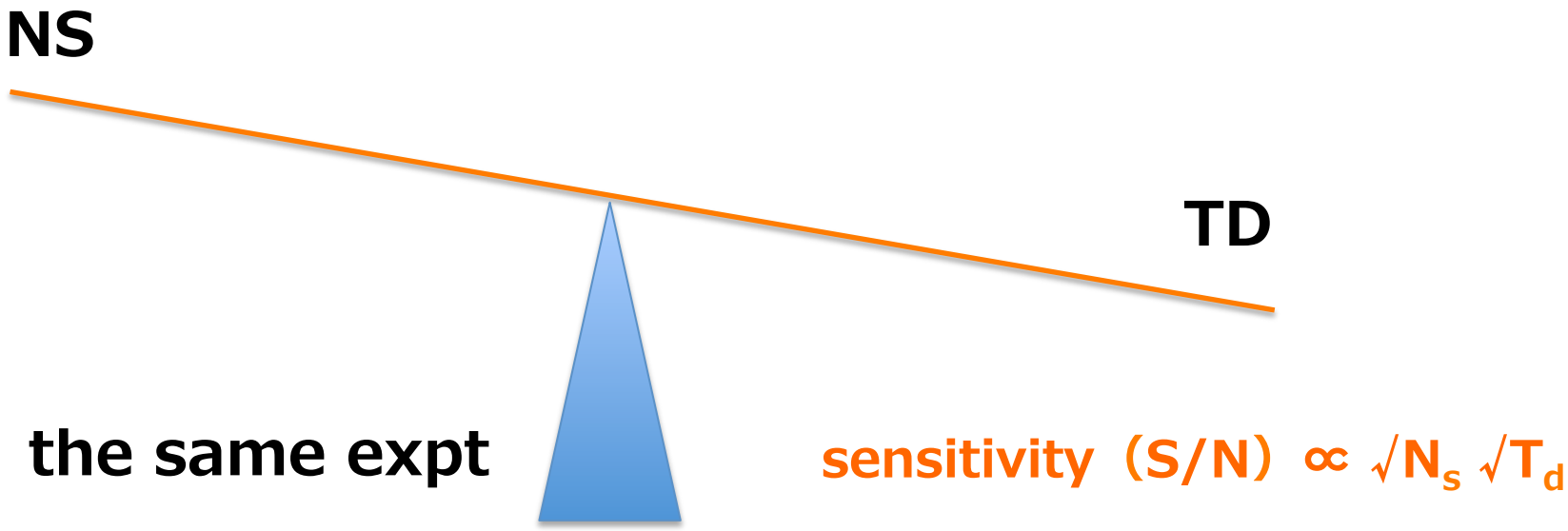
S/N → 304

Slightly higher S/N owing to ^{15}N T_2 relaxation. →

NS = 16
TD = 64

S/N → 335

Any good way to enhance the resolution without extending expt?



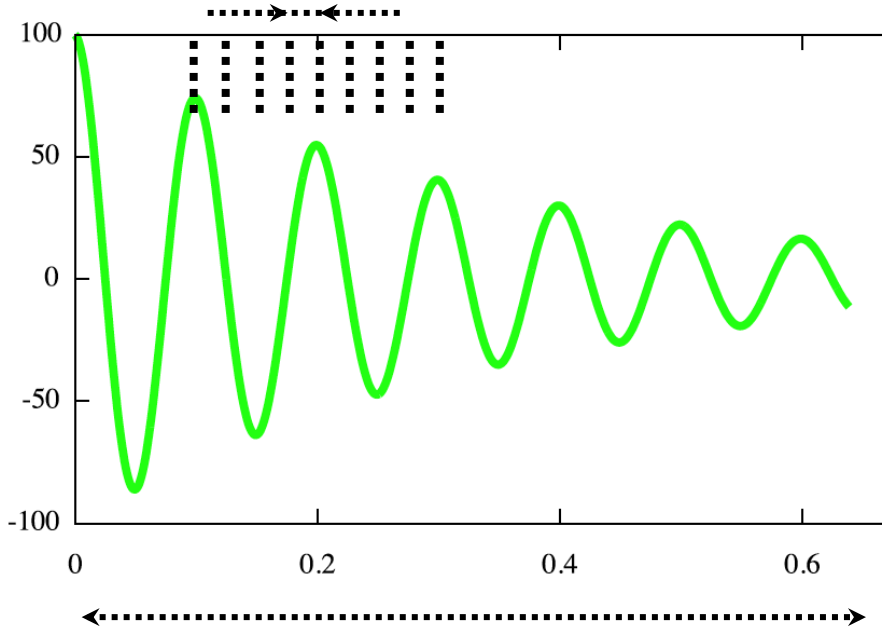
relaxation

- NS: min. and TD: max. → not enough phase cycles
- sampling t_1 non-uniformly = NUS
- just increasing Δt_1 = *i.e.*, narrowing SW

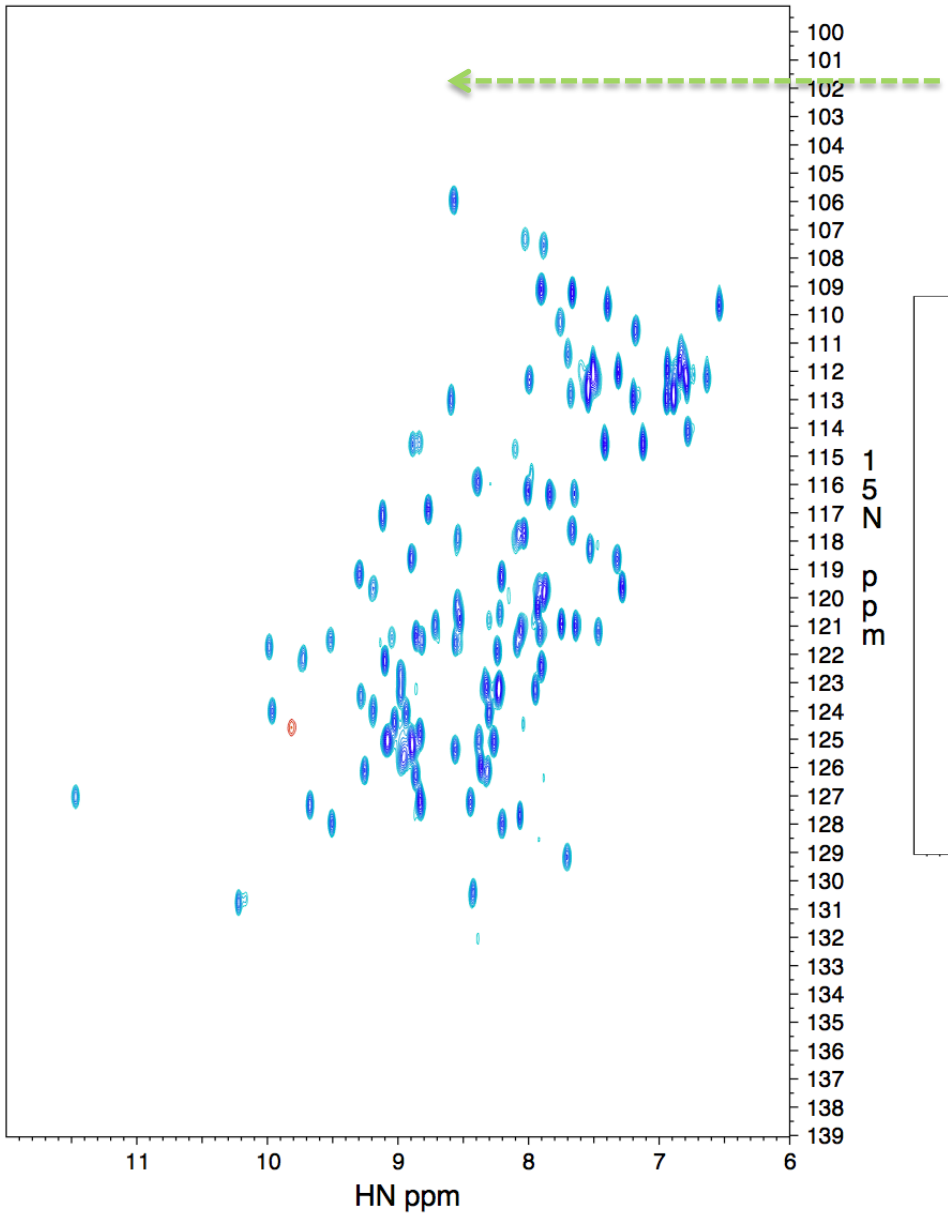
narrower spectral width → higher resolution

$$acquisition\ \ time = \frac{point\ \ number}{spectral\ \ width} = \frac{1}{resolution}$$

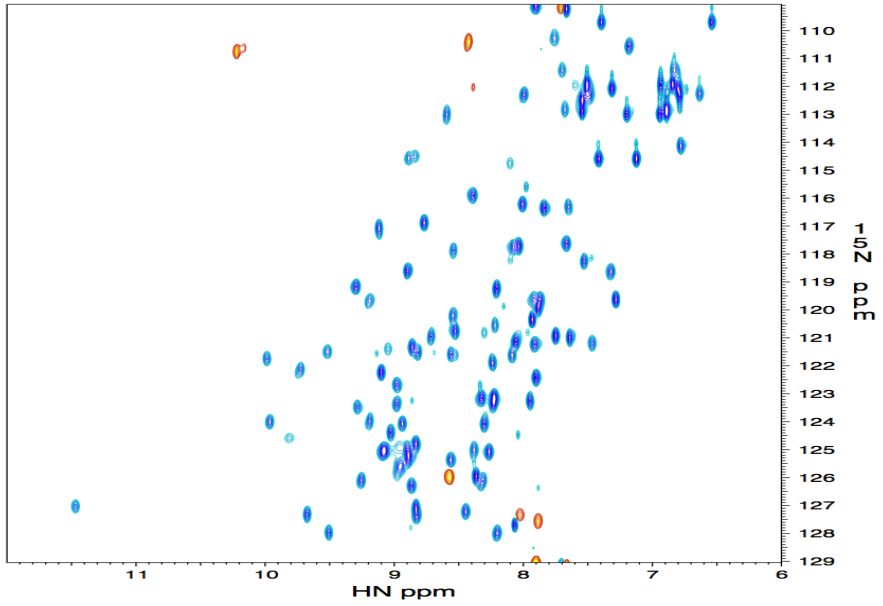
1/SW (*spectral width*)



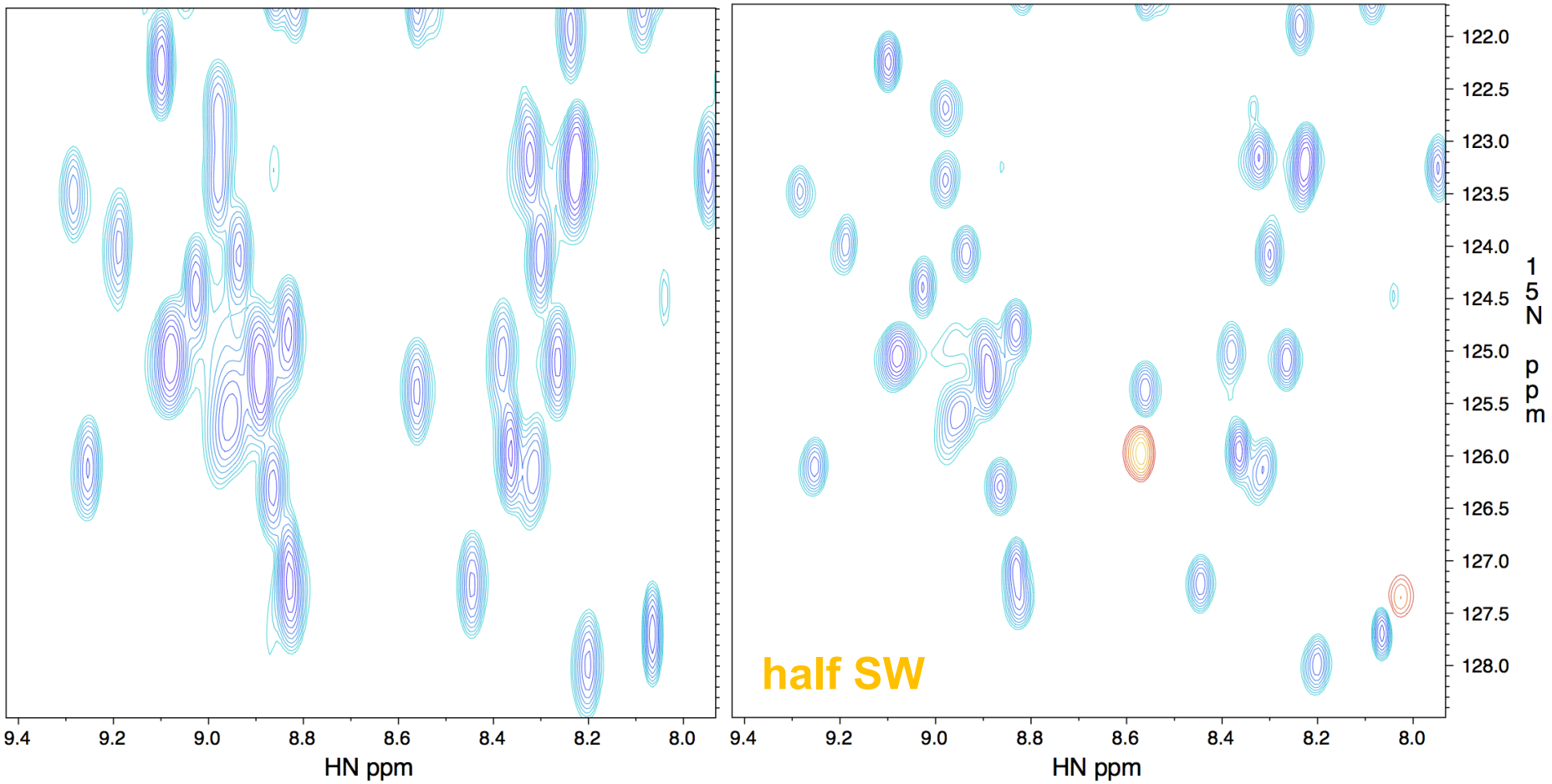
t_1 (*acquisition time*)



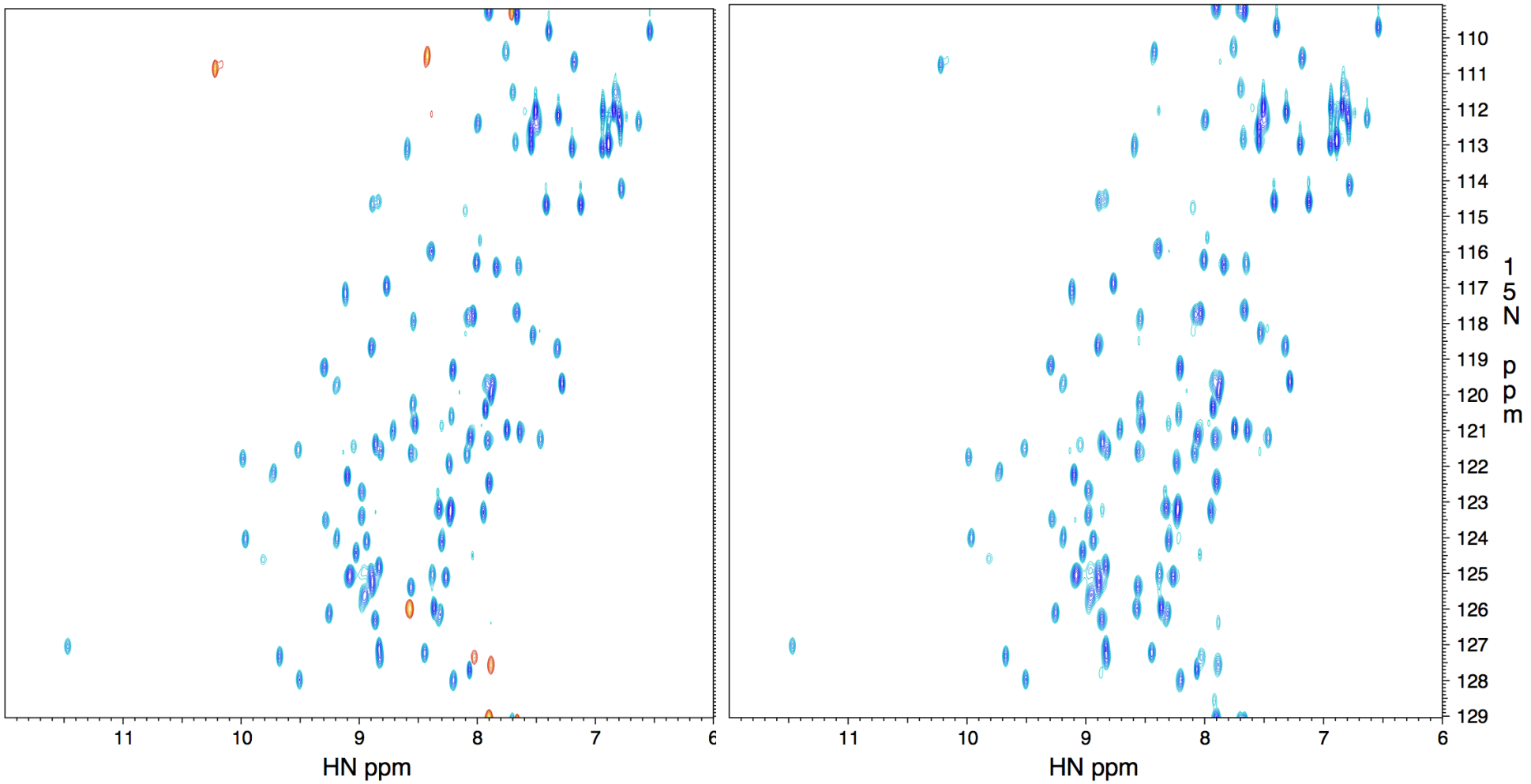
no need to observe these areas (waste of time!)



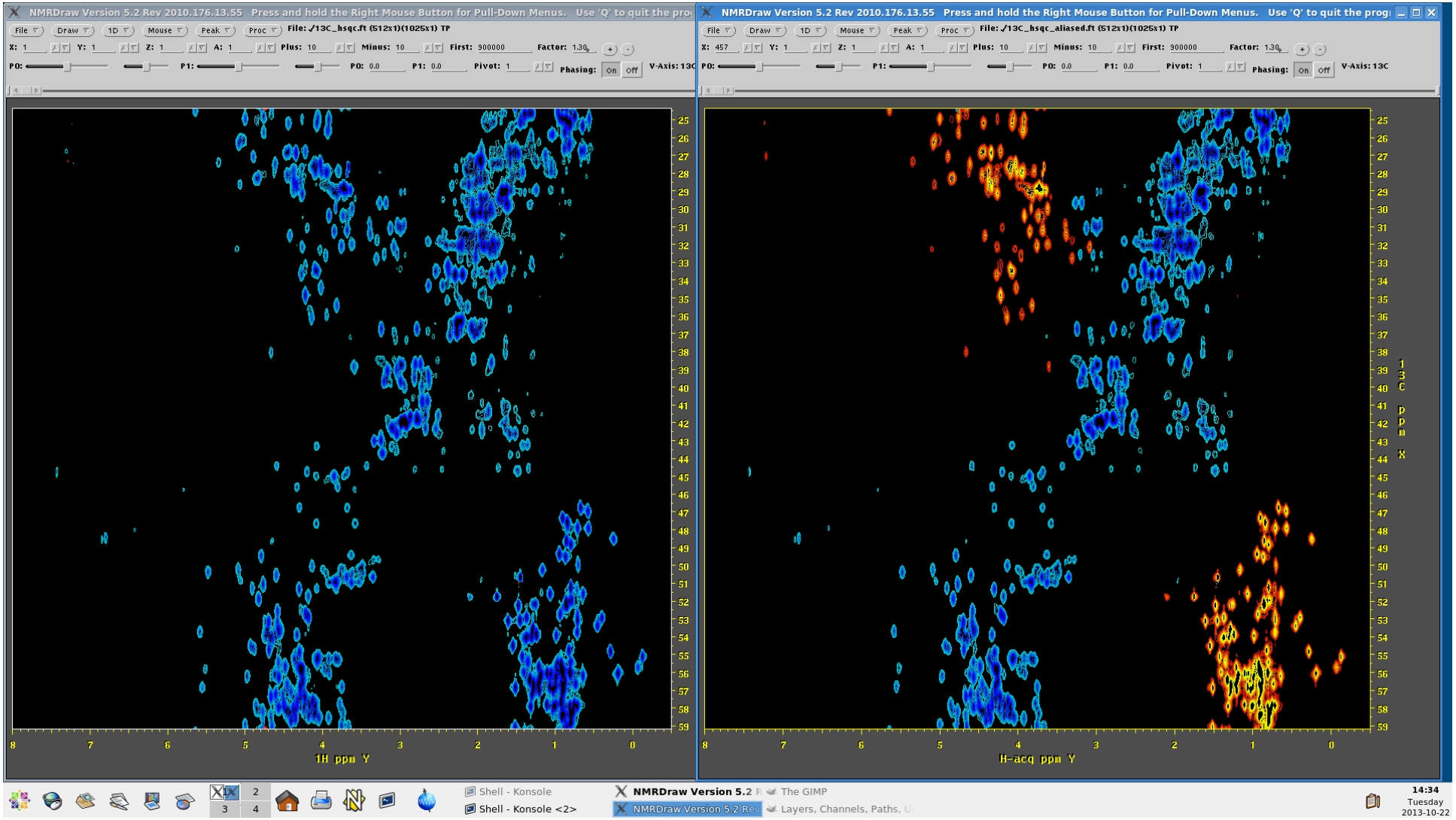
A higher resolution after the same measurement time



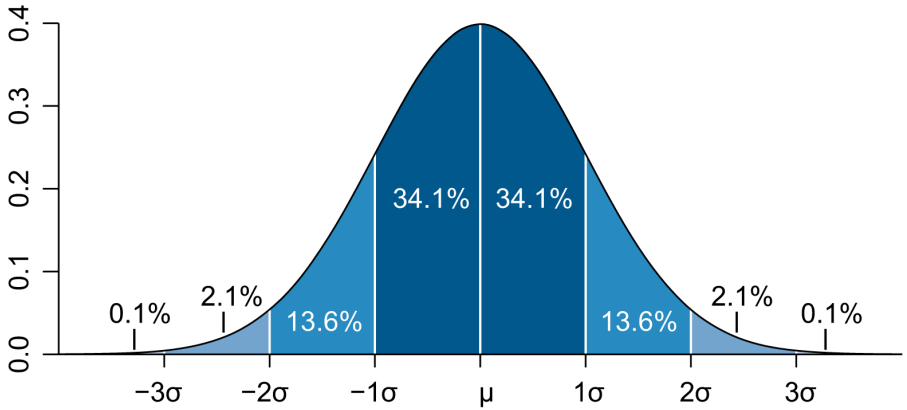
If SW of ω_1 and ω_2 each is reduced by half in 3D, expt can be shortened to ¼ (or S/N can be enhanced twice).



if $t_1(0)$ starts with $\Delta t_1/2$, aliased peaks appear negative.
Let's narrow ^{15}N SW as much as possible (protein NMR).
Never narrow ^1H_N, ^1H_C SW (FID)! ... cost of ¥ 6,000,000.-



Important spectra in protein NMR, such as 3D ^{13}C-edted NOESY, can be measured with a higher sensitivity by simply utilizing aliasing.

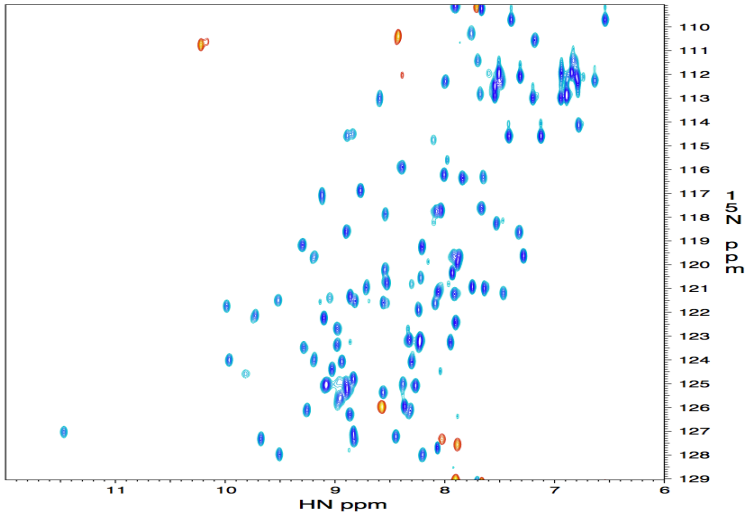


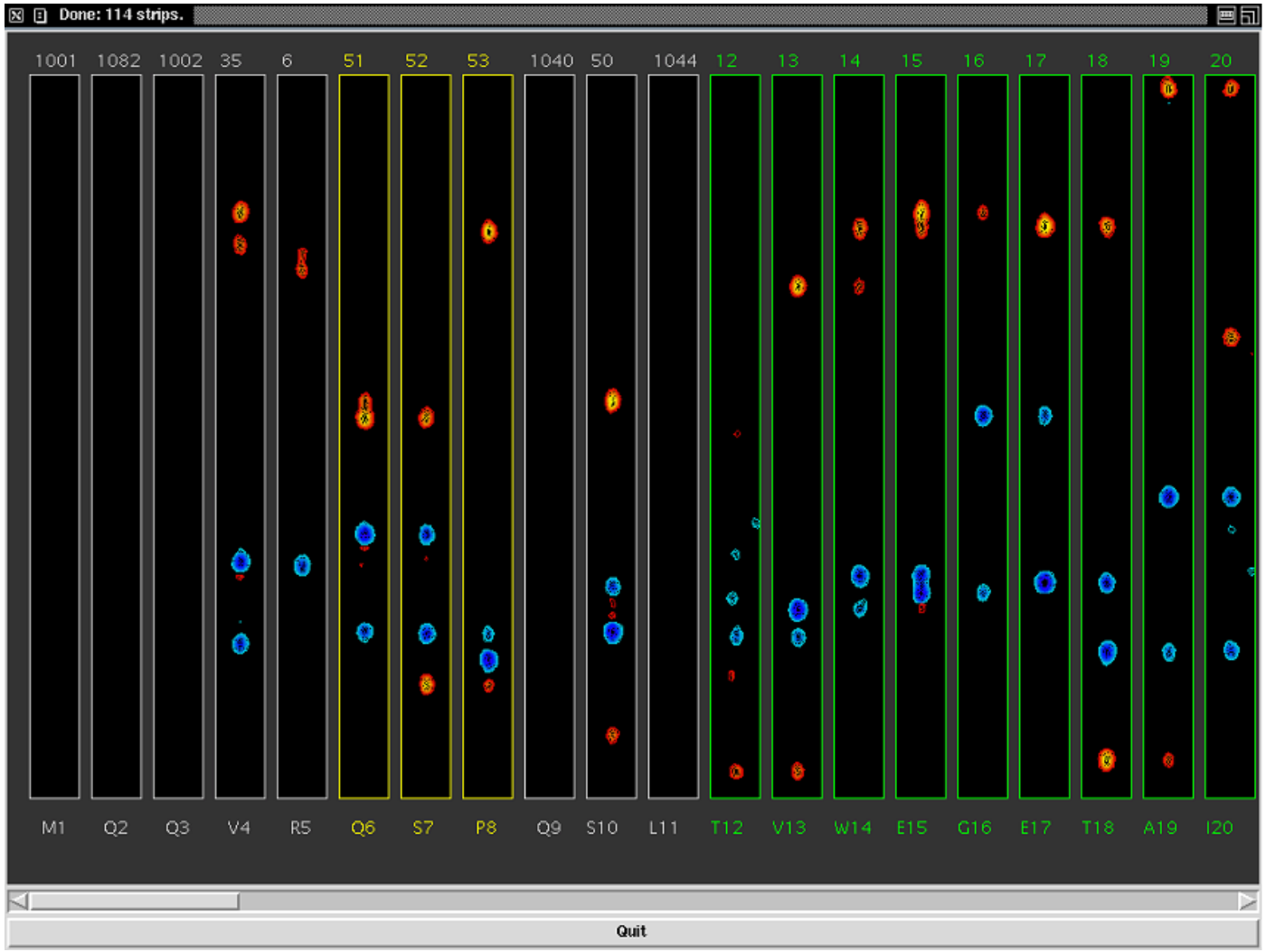
^{13}C SW

In principle, SW of ± 1.5 σ is enough, since aliased peaks do not appear beyond the center (99.7%).
Just in case, ± 2.0 σ is safer.

^{13}Co : 176.28 ppm ± 8.06 (3σ)
→ 11 ppm (2σ) is OK.

^{13}Cα : 56.335 ppm ± 15.27 (3σ)
→ 21 ppm (2σ) is OK.





You can assign the protein main-chains sequentially without picking up peaks. You just only need to arrange strips by matching patterns on your *iPad*.

http://www.nmrscience.com/nmrpipe.html (NMRWish in F. Delaglio's NMRPipe HP)