A Practical Introduction to DOSY

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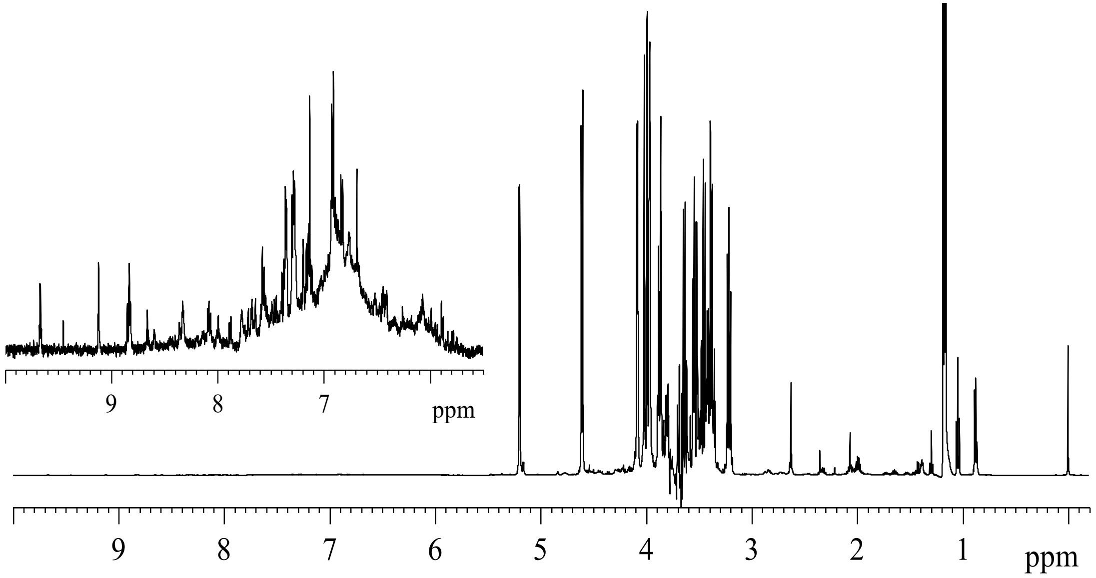
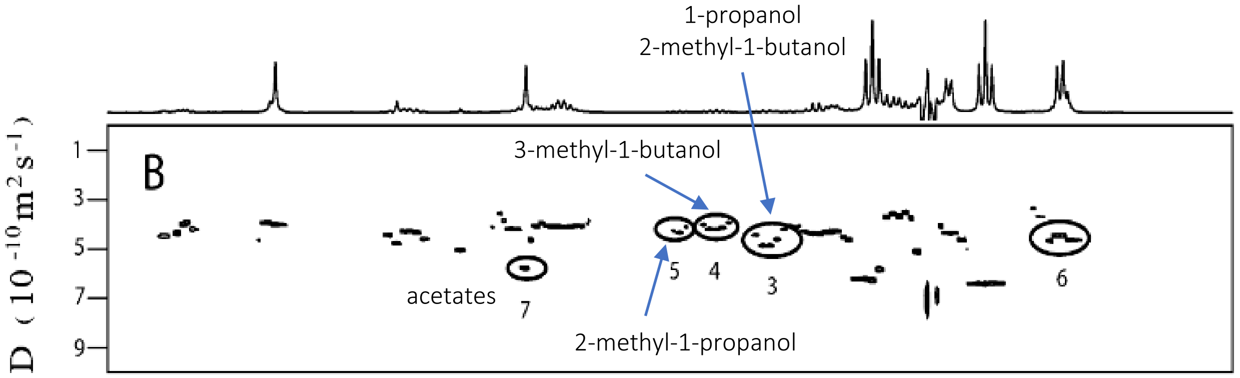
Overview

- What are DOSY and its applications?
- Effect of pulsed field gradients and how to use them to measure diffusion
- What are the issues with the basic PFGSE diffusion experiment?
- Practical considerations and experiment setup
- Convection
- Data processing with JASON
- Summary

What is DOSY?

- Diffusion-Ordered SpectroscopY
- Separate signals in the NMR spectrum based on their diffusion coefficient ("D value")
- Present results in a 2D plot with chemical shift along x-axis and D value along y-axis
- DOSY is **NOT** the same as diffusion NMR - no 2D plot, not DOSY





J. Agric. Food Chem. **2004**, 52, 3736

500 MHz proton and DOSY spectra of port wine

Applications of DOSY

Metabolomics
Mixture analysis, compound/biomarker identification

Polymer science
Molecular weight determination, aggregation, molecular interactions

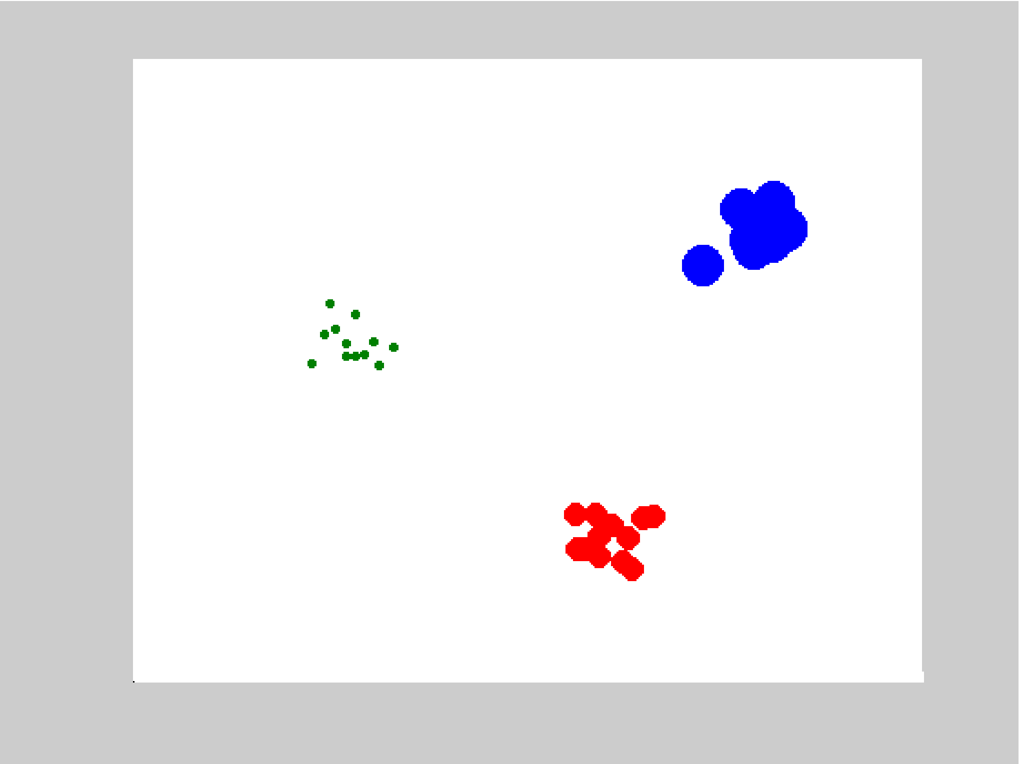
Drug development & analysis
Mixture analysis, compound ID, counterfeits, adulteration

Process chemistry
Identification of reactive intermediates, structure determination

Food science
Food profiling, adulteration, compound ID, structure determination

Materials research & characterization
Batteries, catalysts, paints, emulsions, reagents, etc.

Self-Diffusion



- Molecules experience both rotational and translational Brownian motion
- Stokes-Einstein equation (spherical molecules) can be used to determine the mobility of the molecules

$$D = {k_B T}/{6\pi\eta r_H}$$

k_B : Boltzmann constant

T: temperature

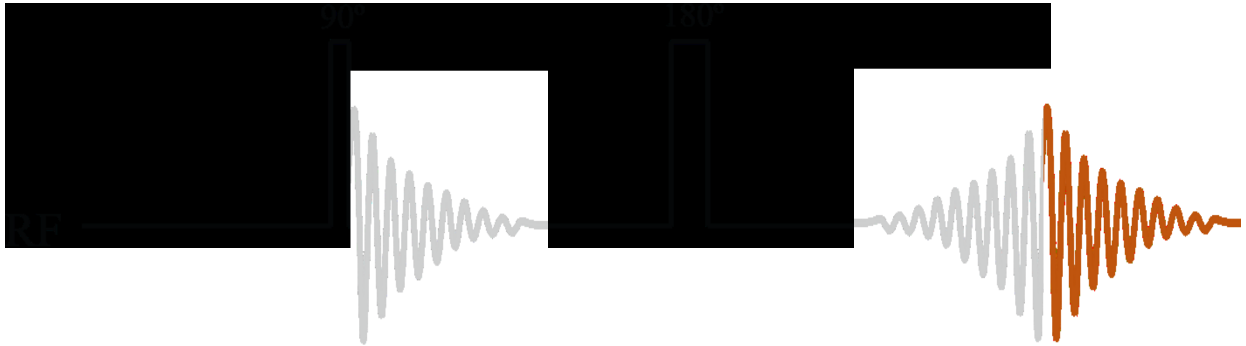
η: viscosity

r_H: hydrodynamic radius

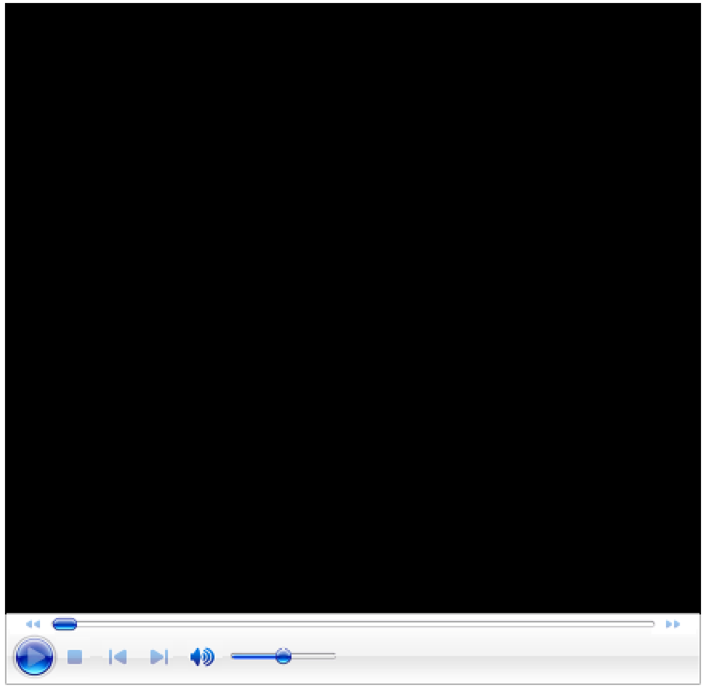
- The equation is valid for solute molecules at infinite dilution diffusing through a continuum solvent (i.e. where the solvent molecules are much smaller than the solute).

Spin echo

Signal is refocused…



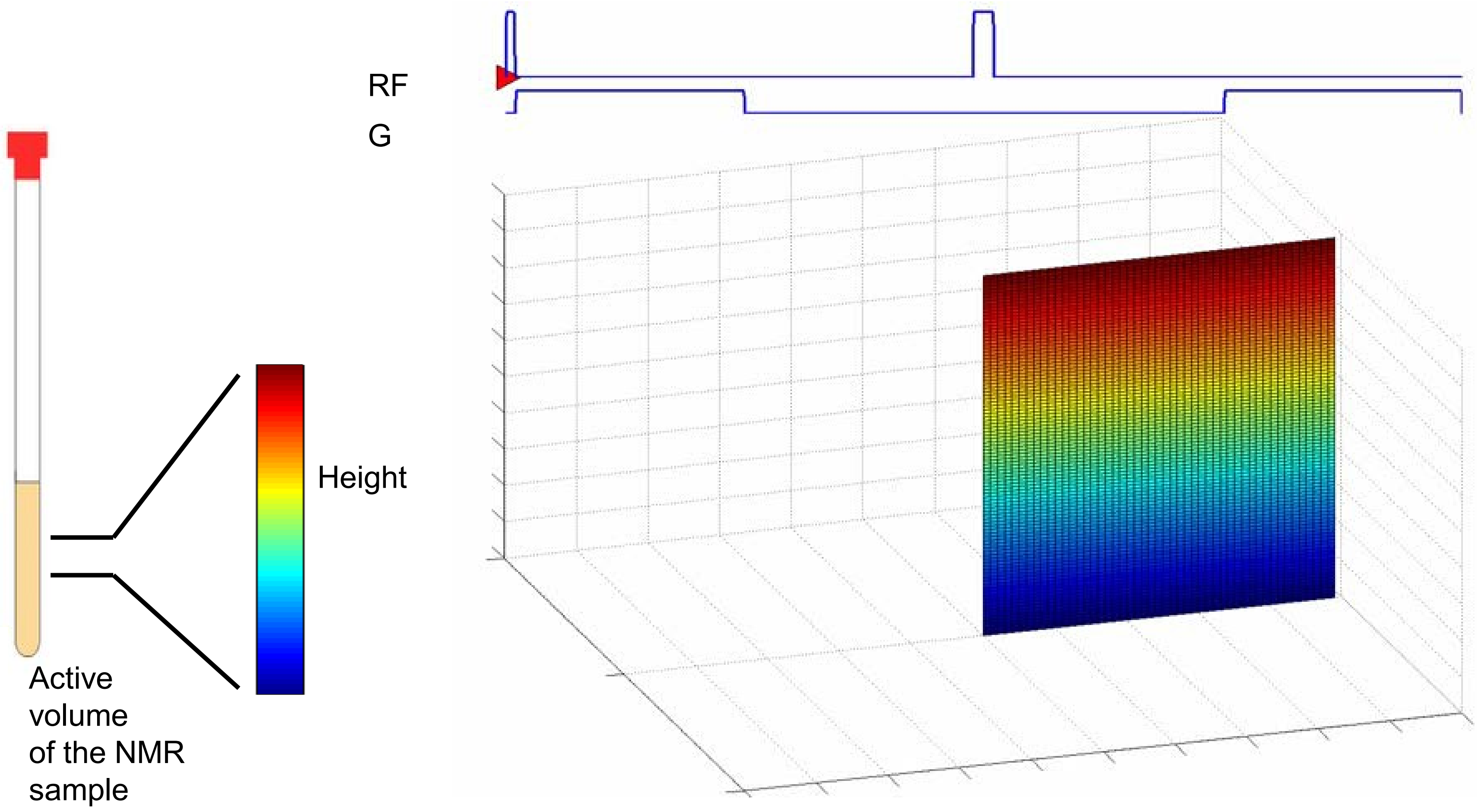
as droplets in corn syrup



https://twitter.com/wonderofscience/status/1320692387925032961

Pulsed field gradient spin echo

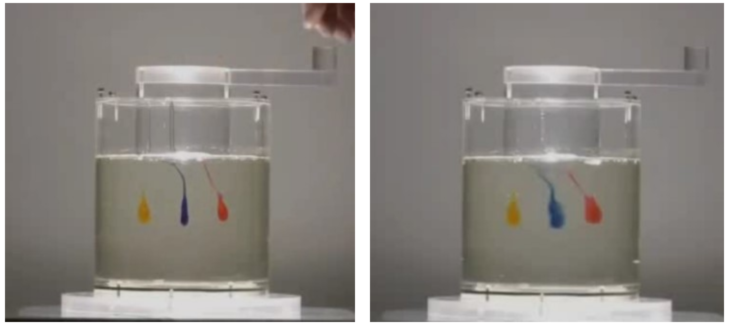
- Magnetization evolution without diffusion

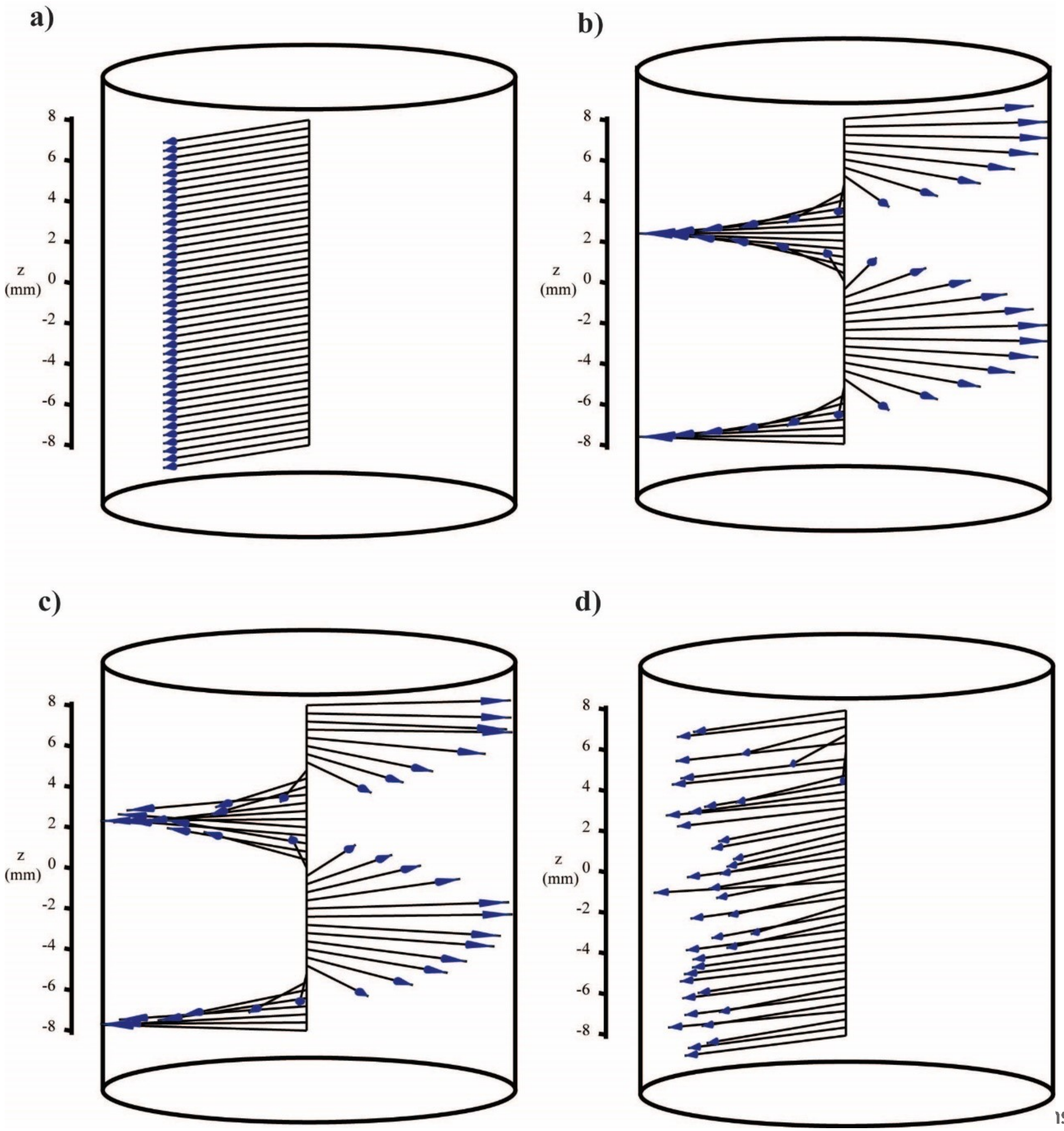


Pulsed field gradient spin echo

- Magnetization evolution with diffusion
- Diffusion results in imperfect refocusing of magnetization.
- More diffusion leads to worse refocusing, and thus more signal attenuation

No diffusion vs diffusion





Pulsed field gradient spin echo

$$S = S_0\, e^{-D\gamma^2\delta^2G^2\Delta'}$$

Stejskal-Tanner formula

S : signal amplitude

S_0: signal amplitude without diffusion

D : diffusion coefficient

γ : gyromagnetic ratio

δ : gradient pulse width

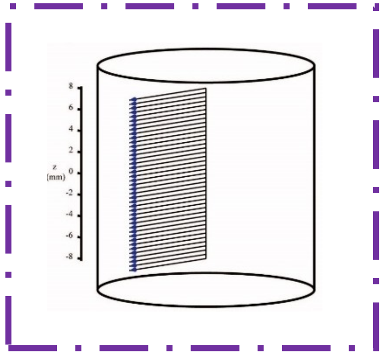
G : gradient amplitude

Δ' : corrected diffusion time

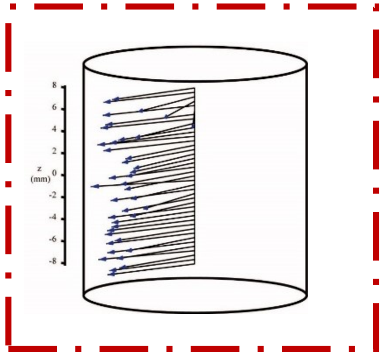
spin phases scrambled
[$\Phi = \Phi\,(z)$]

phases unscrambled

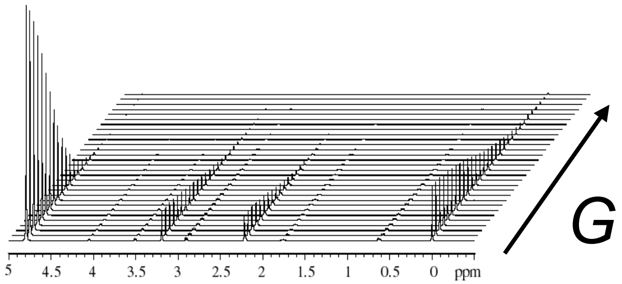
[$\Phi = \Phi\,(z) - \Phi\,(z) = 0$]
if spins haven't moved



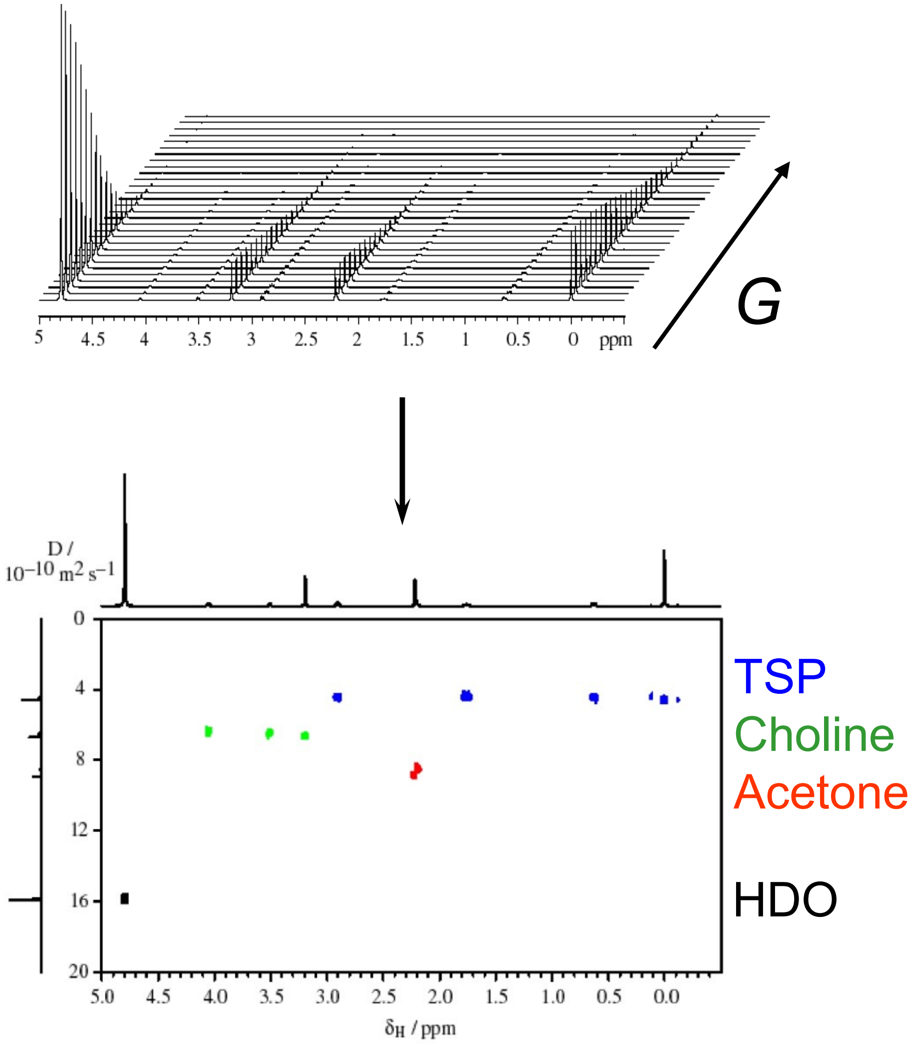
[$\Phi = \Phi\,(z) - \Phi\,(z) \neq 0$]
when spins have moved



- Application of PFG will result in a diffusivity dependent attenuation



Obtaining a DOSY spectrum



- Measure spectra as a function of *G*
- Fit peak heights to get diffusion coefficients *D*

$$S = S_0\, e^{-D\gamma^2\delta^2G^2\Delta'}$$

- Extend 1D peaks into a second dimension, with Gaussian shapes centred on the *D*'s
- Widths in Y dimension determined by the standard errors σ_D

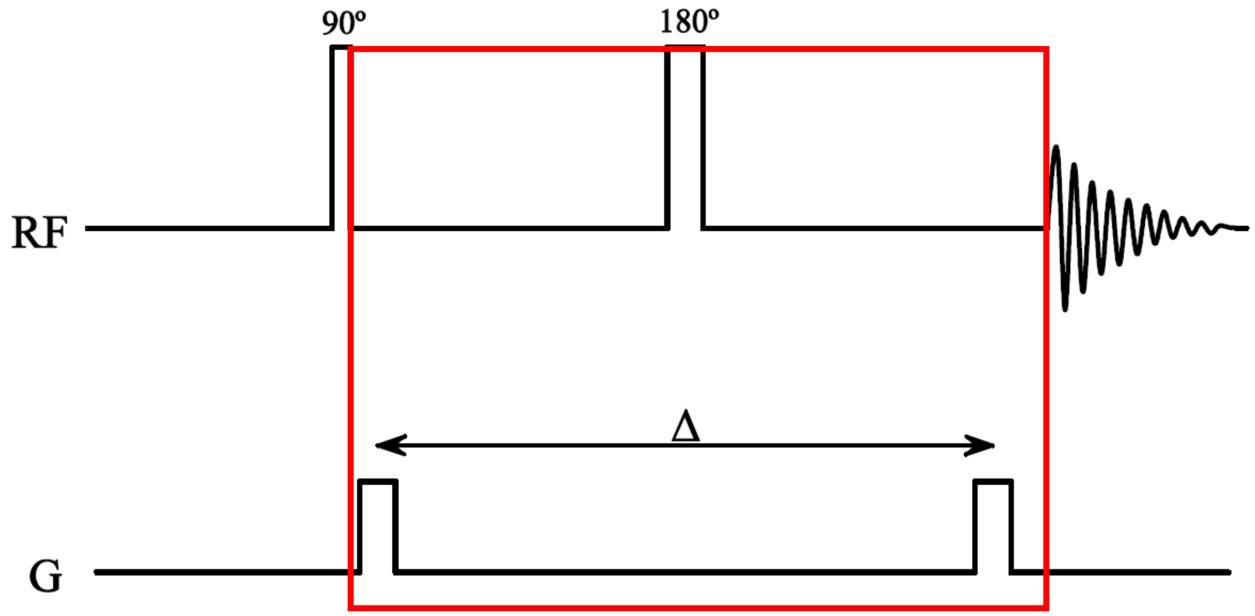
Virtual chromatography

Despite the name, DOSY is not like COSY/NOESY: spectra are statistical constructs from, not transforms of, experimental data

PFG Spin echo

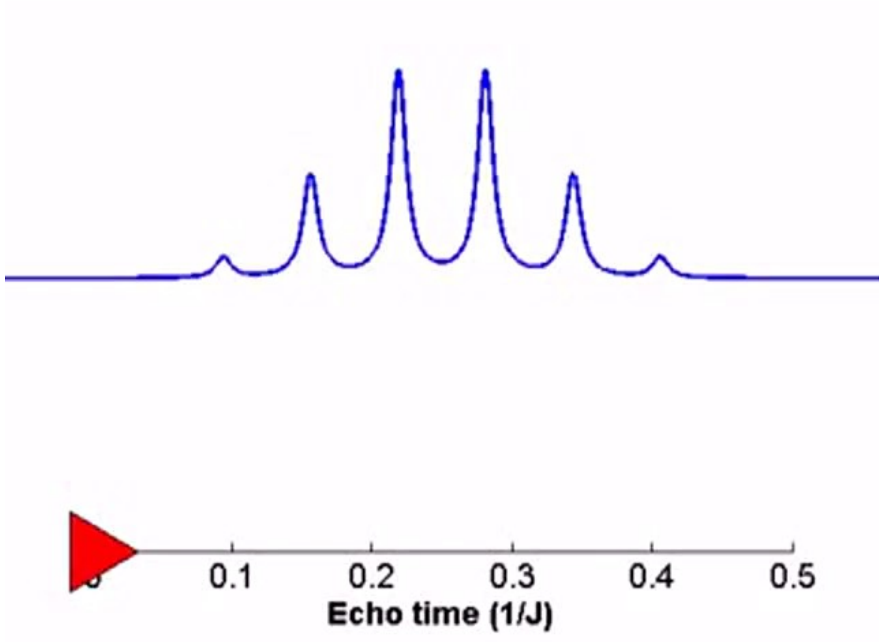
Field gradient pulses result in signal attenuation

$$S = S_0\, e^{-D\gamma^2\delta^2 G^2 \Delta'}$$



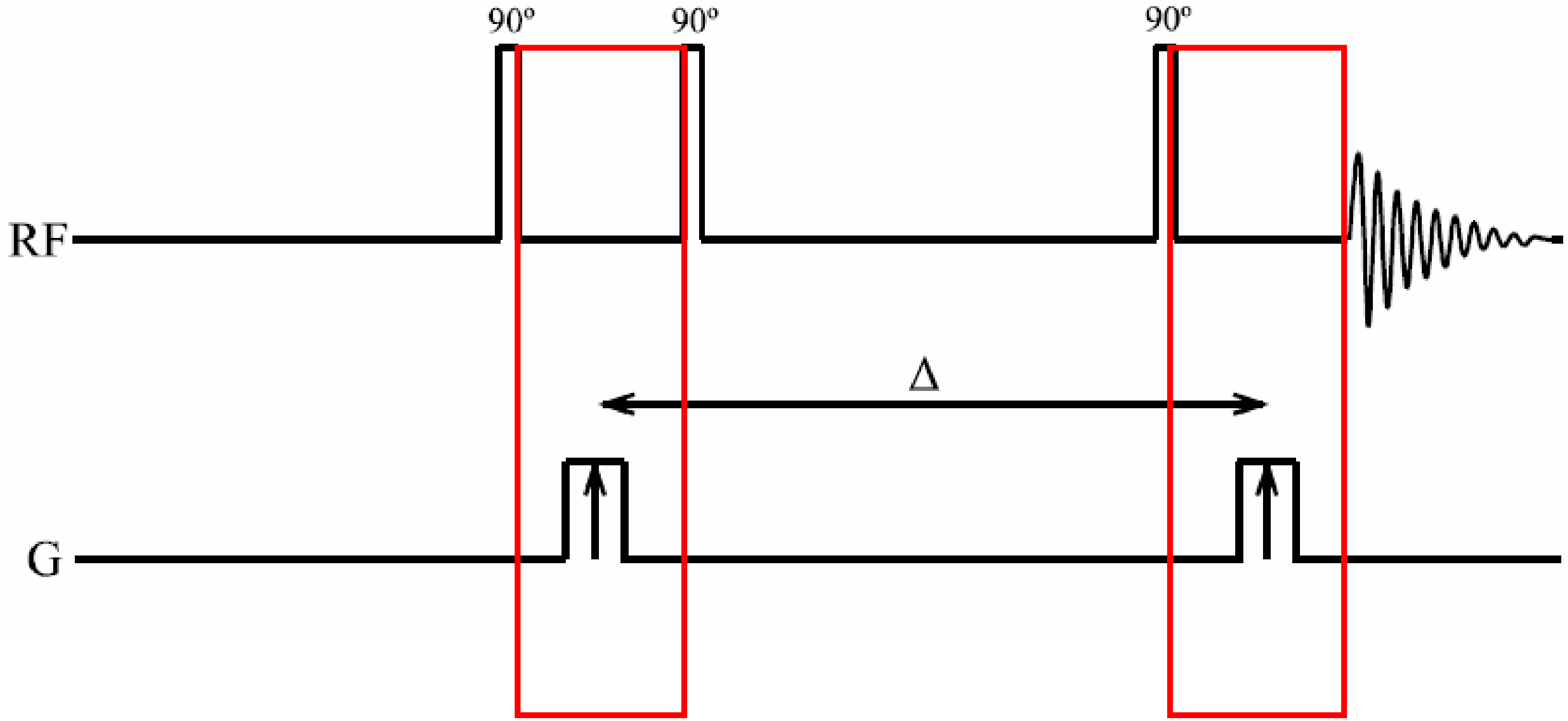
spin phases scrambled
[*Φ* = *Φ (z)*]

phases unscrambled
[*Φ* = *Φ (z)* – *Φ (z)* = 0]
if spins haven’t moved



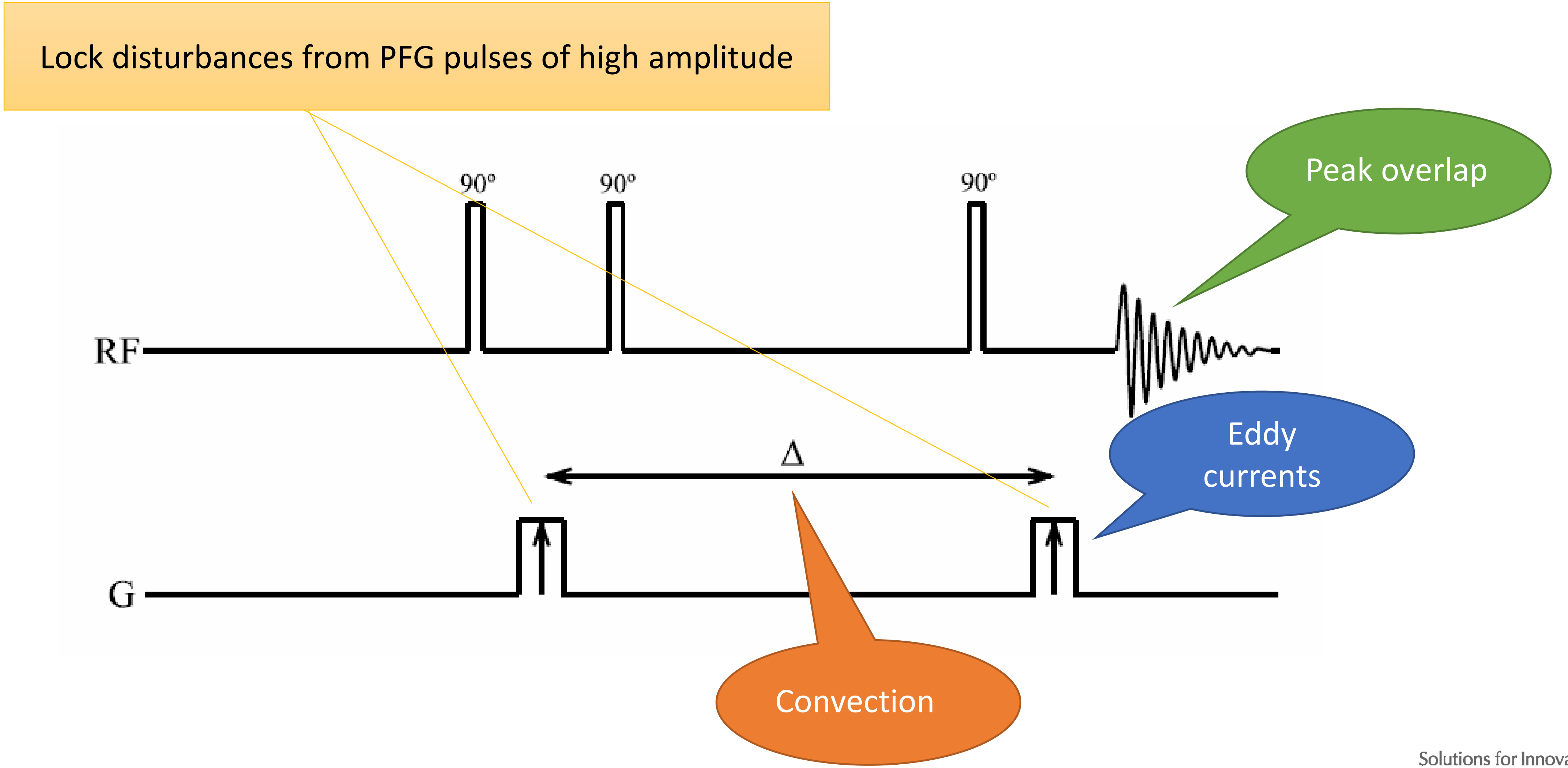
Solution: PFG Stimulated echo

- Magnetization is stored along the z-axis for most of Δ
- Reduced *J*-modulation
- Lose 50% of magnetization



Challenges with DOSY

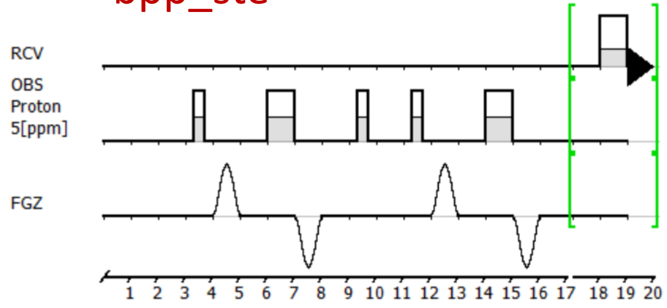
➔ Goal: record high-resolution spectra shortly after executing high-amplitude gradient pulses



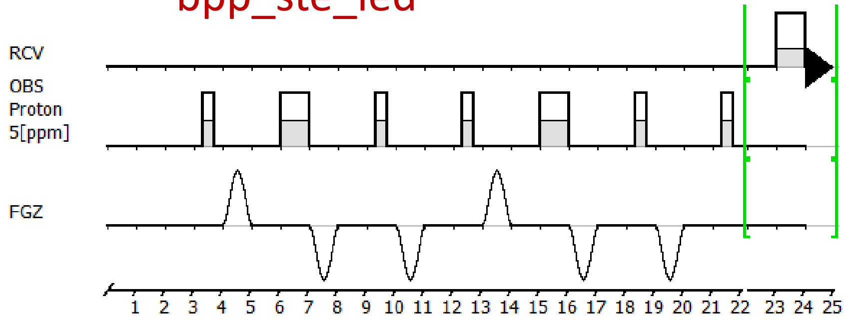
Modern DOSY pulse sequences

2D DOSY

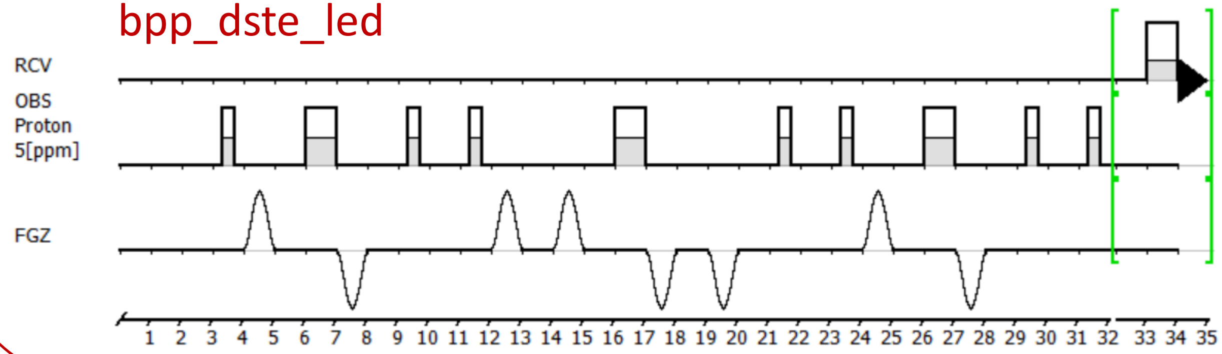
bpp_ste



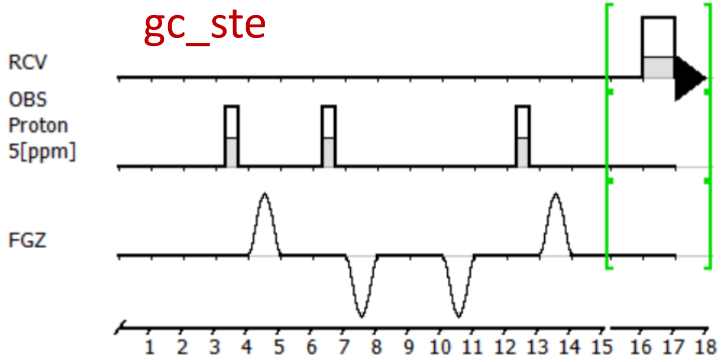
bpp_ste_led



bpp_dste_led

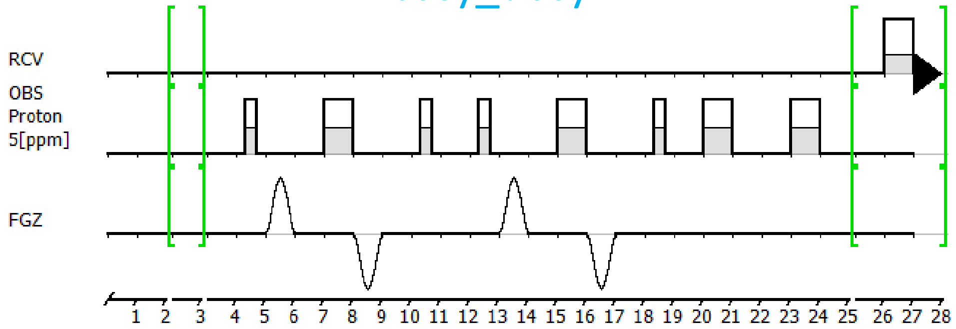


gc_ste

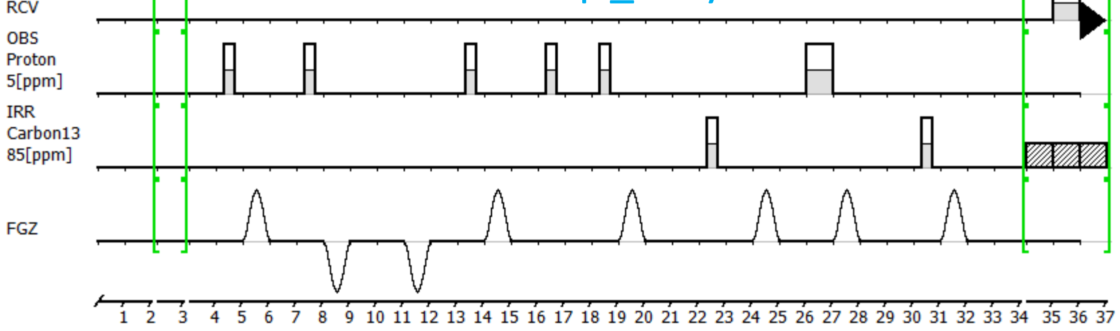


3D DOSY

cosy_dosy

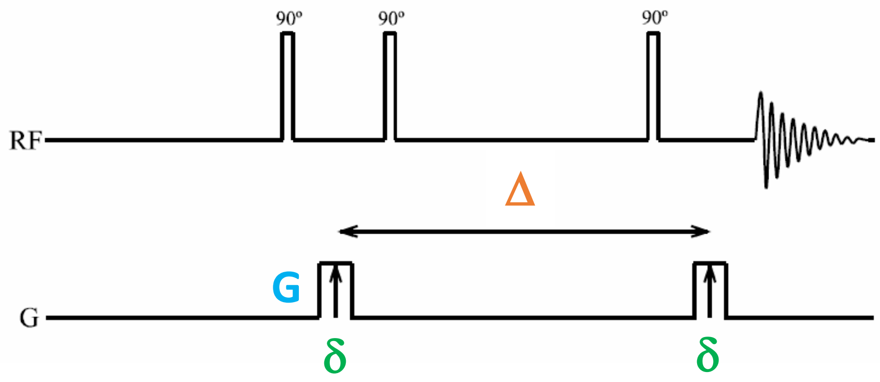


hmqc_dosy



Practical consequences of the Stejskal-Tanner Eqn

Stejskal-Tanner formula for signal intensity:



$$S = S_0\, e^{-D\gamma^2\delta^2G^2\Delta'}$$

- **$(\gamma\delta G_{zi})^2$ - gradient area**

  nuclei with high γ values are more sensitive for diffusion (^1H, ^{19}F, ^{31}P)
  (i.e. ^1H is 16 times more sensitive than ^{13}C)

- **δ should be kept short**

  during δ the magnetization is transverse, homonuclear *J*-couplings evolve

- **G the more, the better**

  provided the gradient hardware allows it

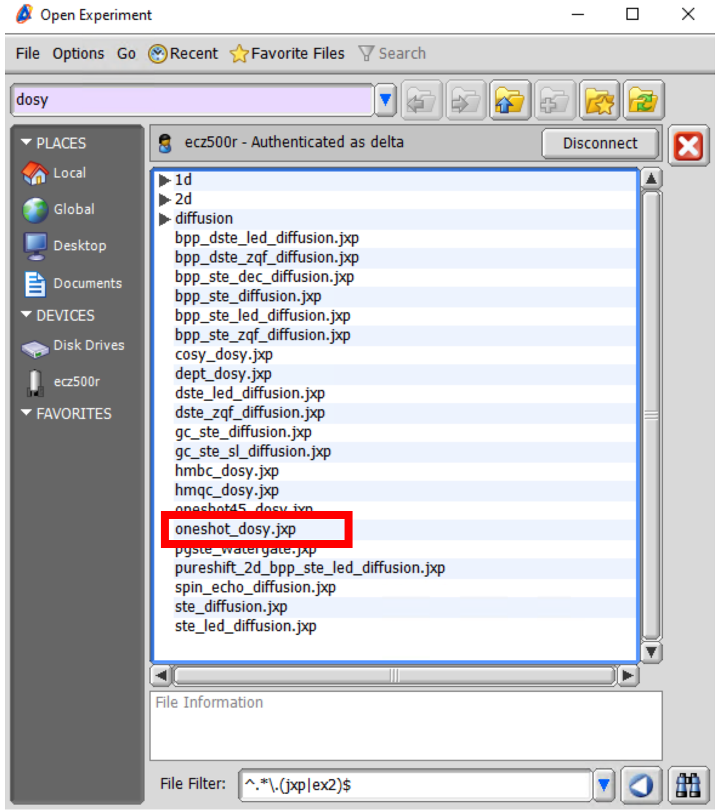
- **Δ should be kept short:**

  to minimize convection effects

Setting up a DOSY experiment

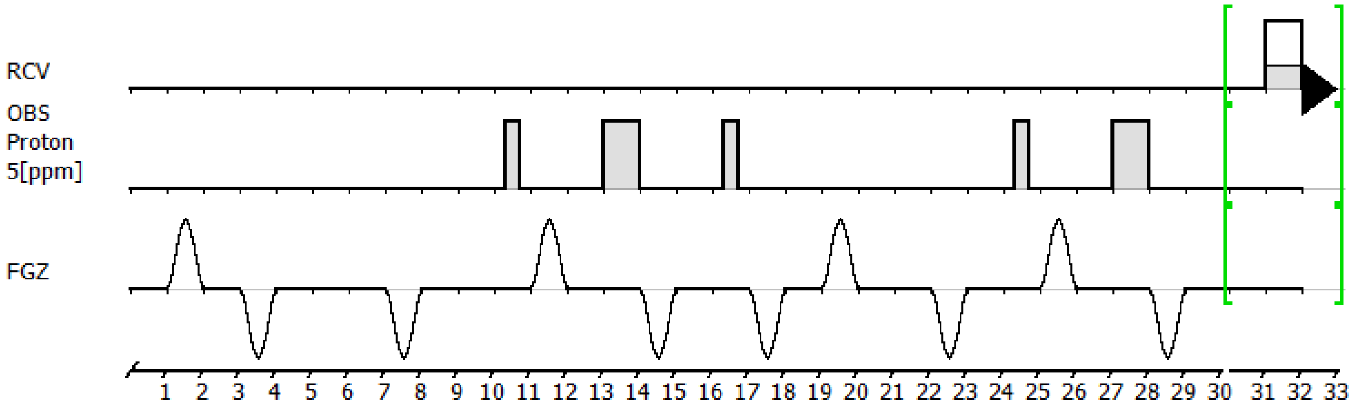
Preparation

- Insert sample into magnet
- Leave VT off if possible and <u>let sample equilibrate for several minutes</u>
- Lock, tune probe, shim
- Ensure lock phase is correctly set
- Create a job and collect a standard proton spectrum and check data quality (e.g. shimming)



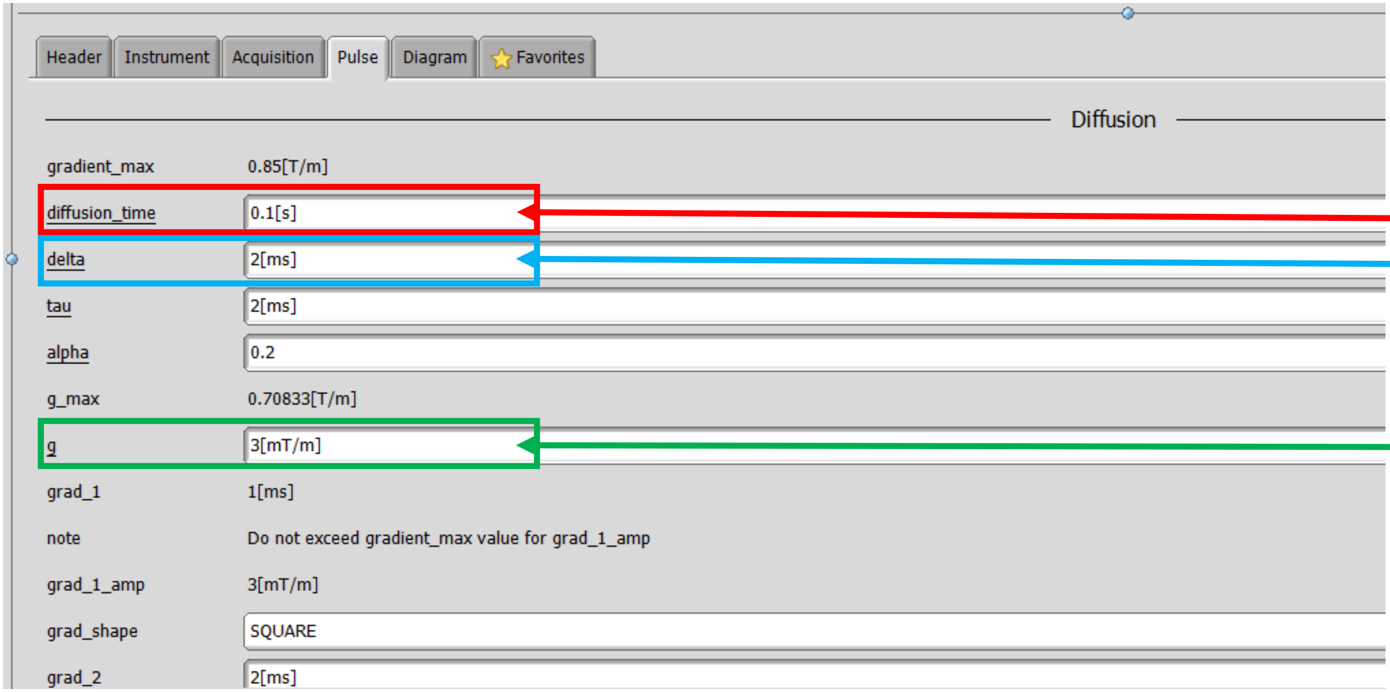
- Select the DOSY experiment from the DOSY folder

  Suggested first experiment to try: oneshot_dosy.jxp



Setting up a DOSY experiment

$$S = S_0\, e^{-D\gamma^2 \delta^2 G^2 \Delta'}$$

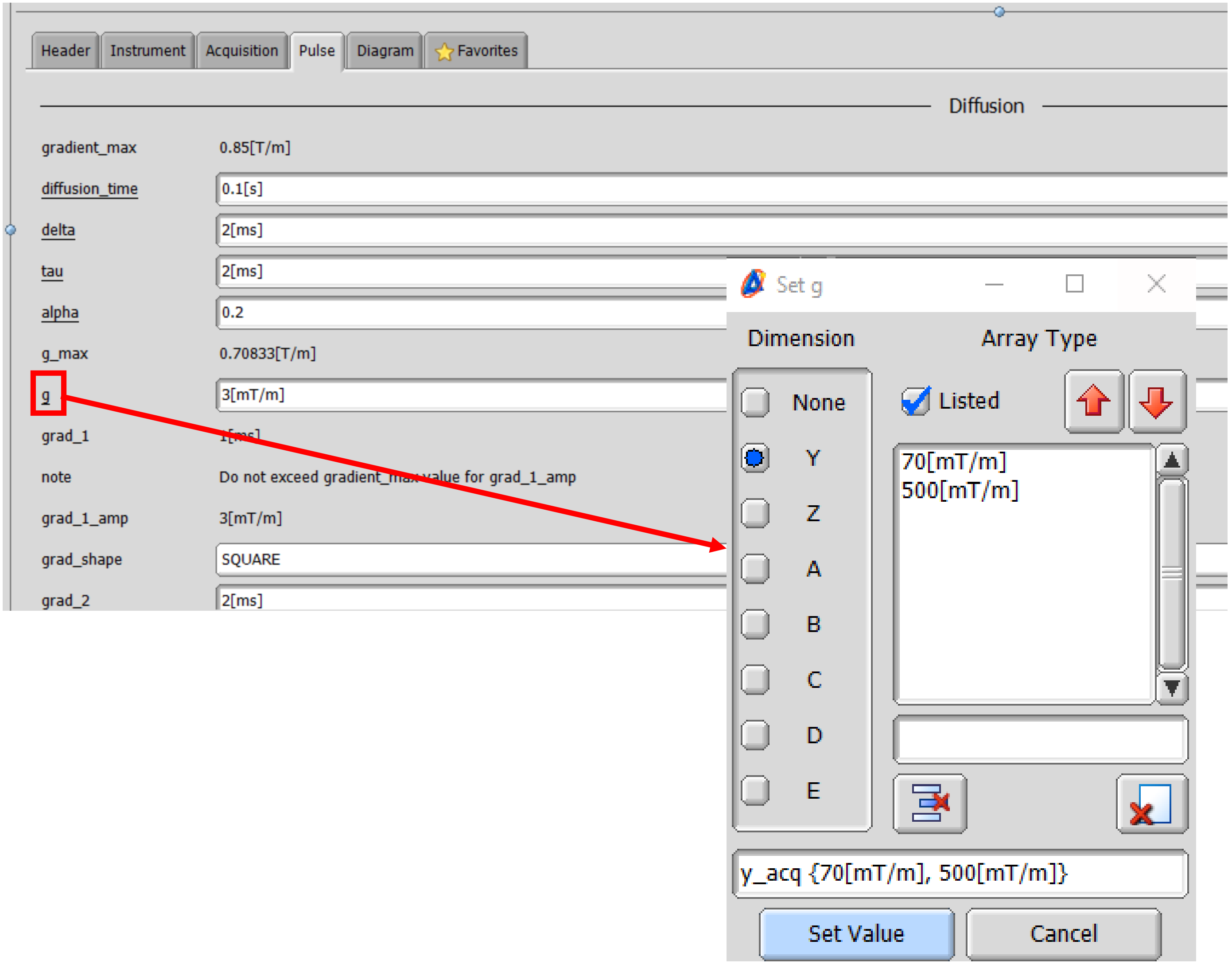


Keep diffusion time as short as possible

Keep delta short – 2[ms] is good for many samples

We need to set up an array of G values (typically 10-30) between a “low” and a “high” value

Setting up a DOSY experiment

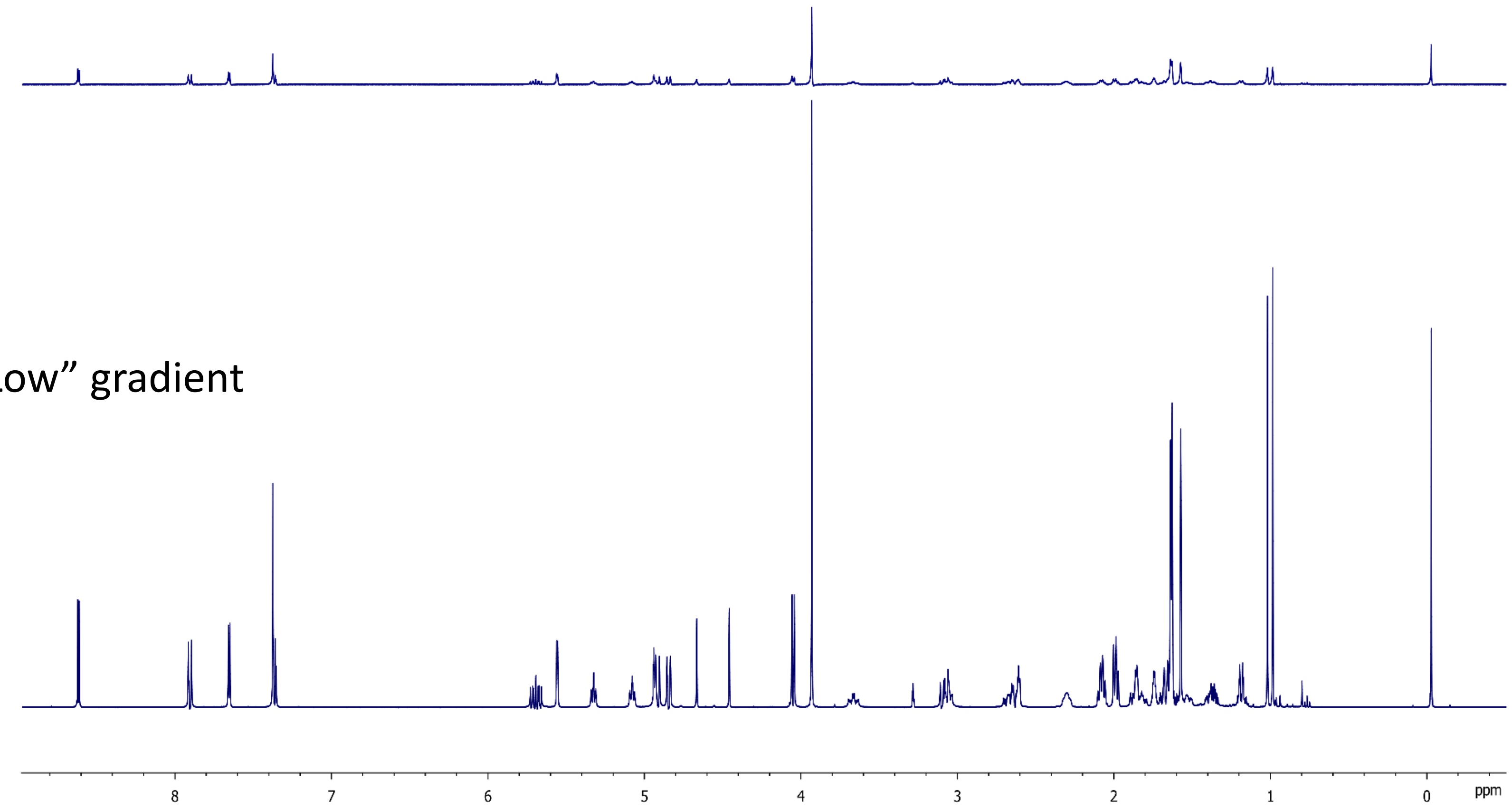


- Use most of the gradient amplitude range available
- Set two gradient values g to start with, a “low” value and a “high” value
- Do not set “low” too low → default 3[mT/m] is way too low. 100[mT/m] is better
- Aim for around 90% attenuation of the fastest diffusing component, or at least 50% attenuation of the slowest-diffusion component
- If possible, reduce diffusion_time to reduce attenuation rather than maximum g

Setting up a DOSY experiment

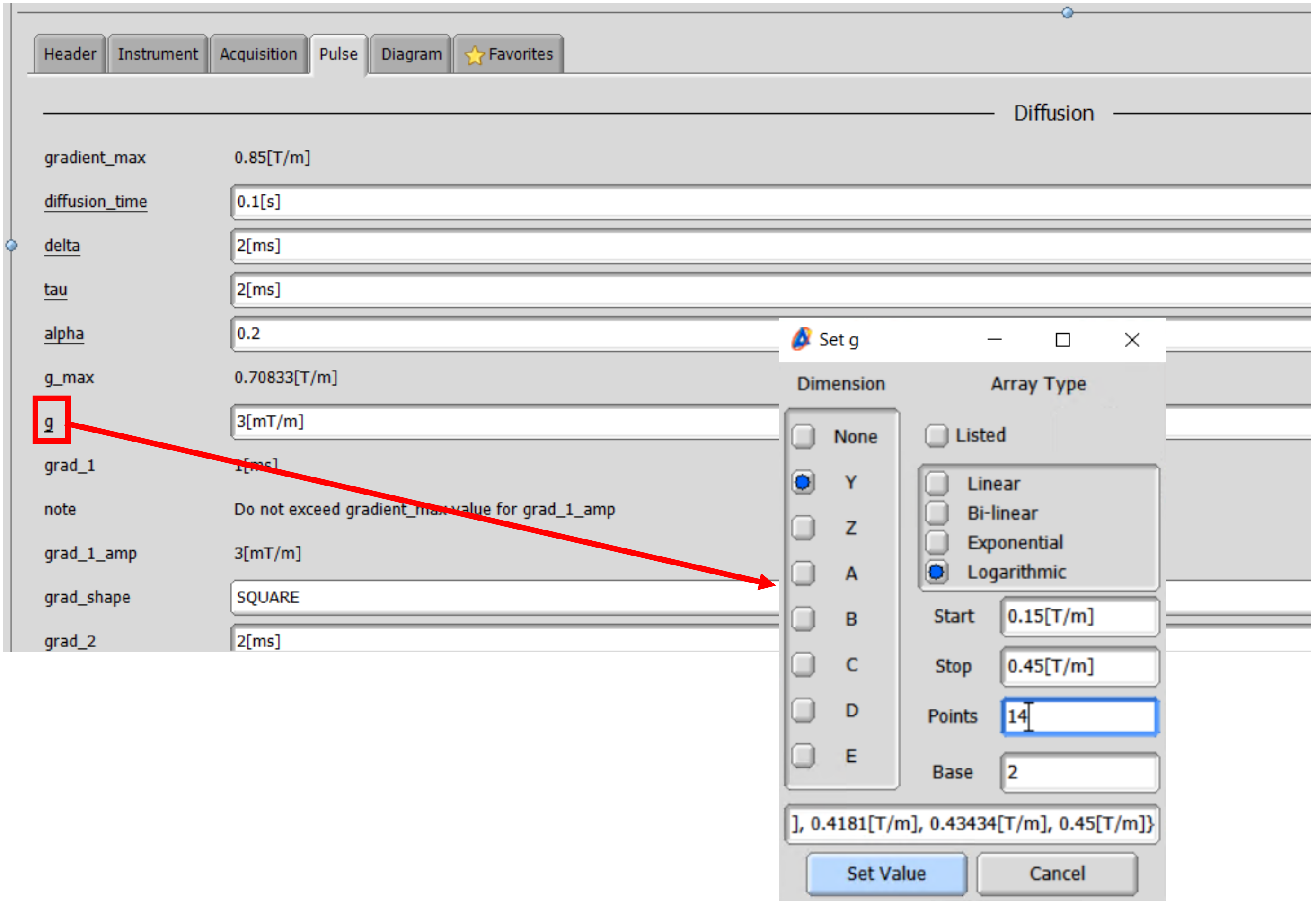
"High" gradient (~ 90% attenuation)

"Low" gradient



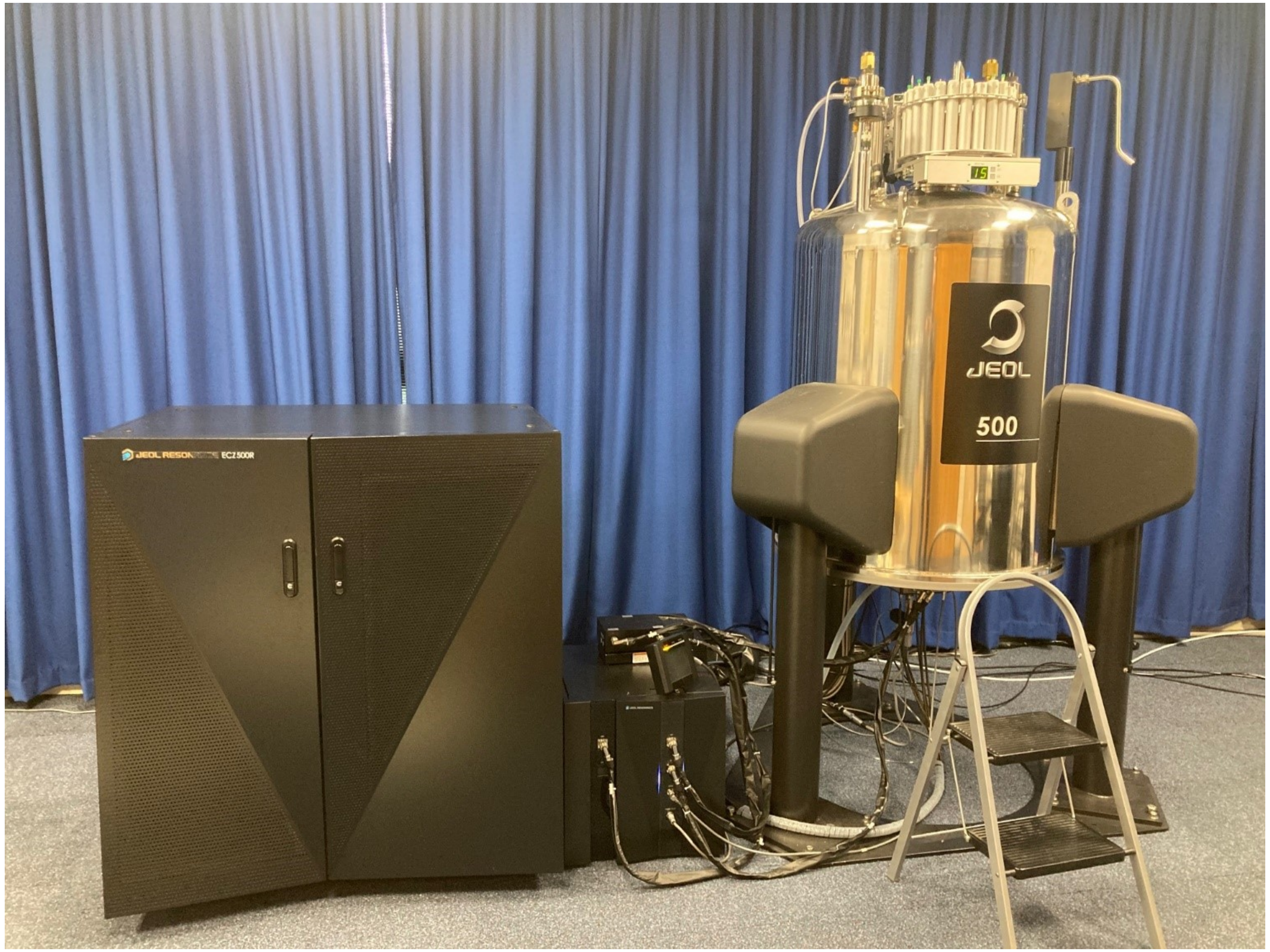
- ✓ Check peak attenuations
- ✓ Check the baselines
- ✓ Check the phasing, should be the same and good in both spectra. If not:
  - Convection?
  - Badly adjusted lock?
  - "Lock hold" function not enabled?
  - Gradient recovery delay too short?
  - Insufficient scans for minimum phase cycle? Even oneshot benefits from phase cycling

Setting up a DOSY experiment



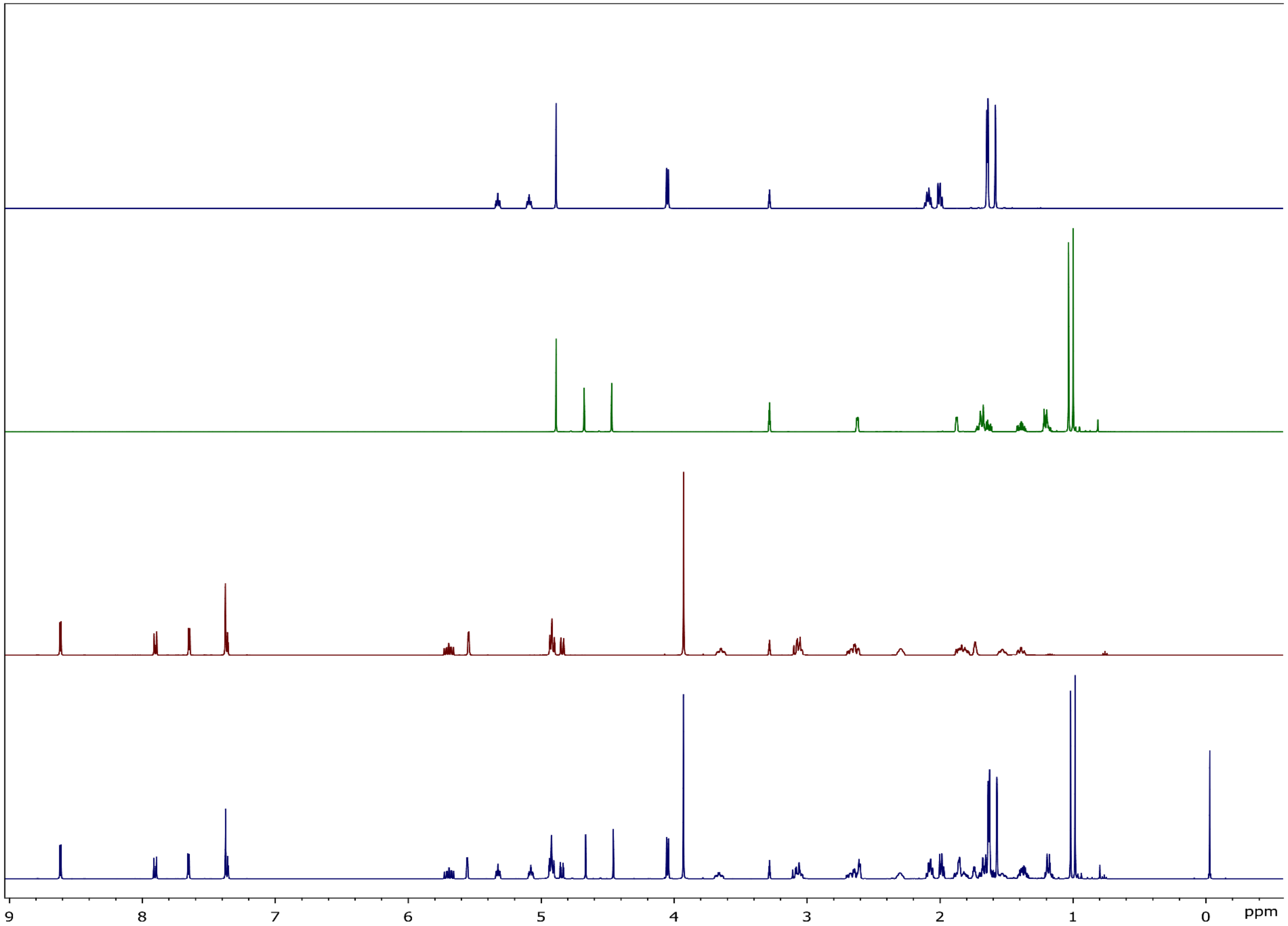
- Once the attenuation level and phasing for the “low” and “high” spectra look good, set up full DOSY array
- Uncheck “Listed”, select “Logarithmic” option and set “Base” to 2
- Use 10 – 30 gradient values. If sample has wide range of D values, more is helpful
- Make sure you use enough scans (not just for SNR but for phase cycling)

Practical session 1: quick oneshot DOSY

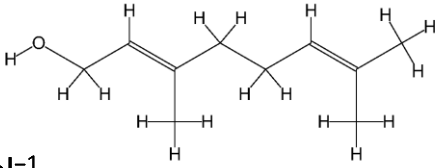


- JEOL ECZ500R Spectrometer
- ROYAL HFX probe
- Maximum gradient strength 0.9 T/m (90 G/cm)
- ASC 24 autosampler
- Autotune module

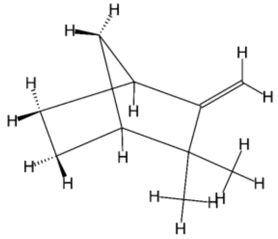
Practical session 1: quick oneshot DOSY



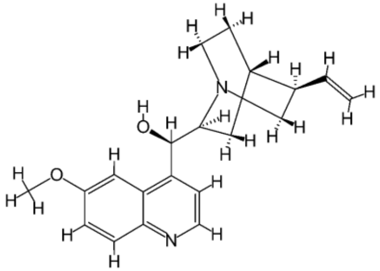
Geraniol
$C_{10}H_{18}O$
154.253 g·mol^{-1}



Camphene
$C_{10}H_{16}$
136.238 g·mol^{-1}



Quinine
$C_{20}H_{24}N_2O_2$
324.424 g mol^{-1}



"QCG" + TSP-d4 mixture in CD_3OD

Practical session 1: quick oneshot DOSY

Steps

1. Preparation: sample equilibrated, locked and shimmed

2. Collect proton spectrum

3. Set up oneshot DOSY experiment and optimize diffusion parameters

4. Collect quick (4 scans, 14 increments) oneshot DOSY dataset (~8 minute expt)

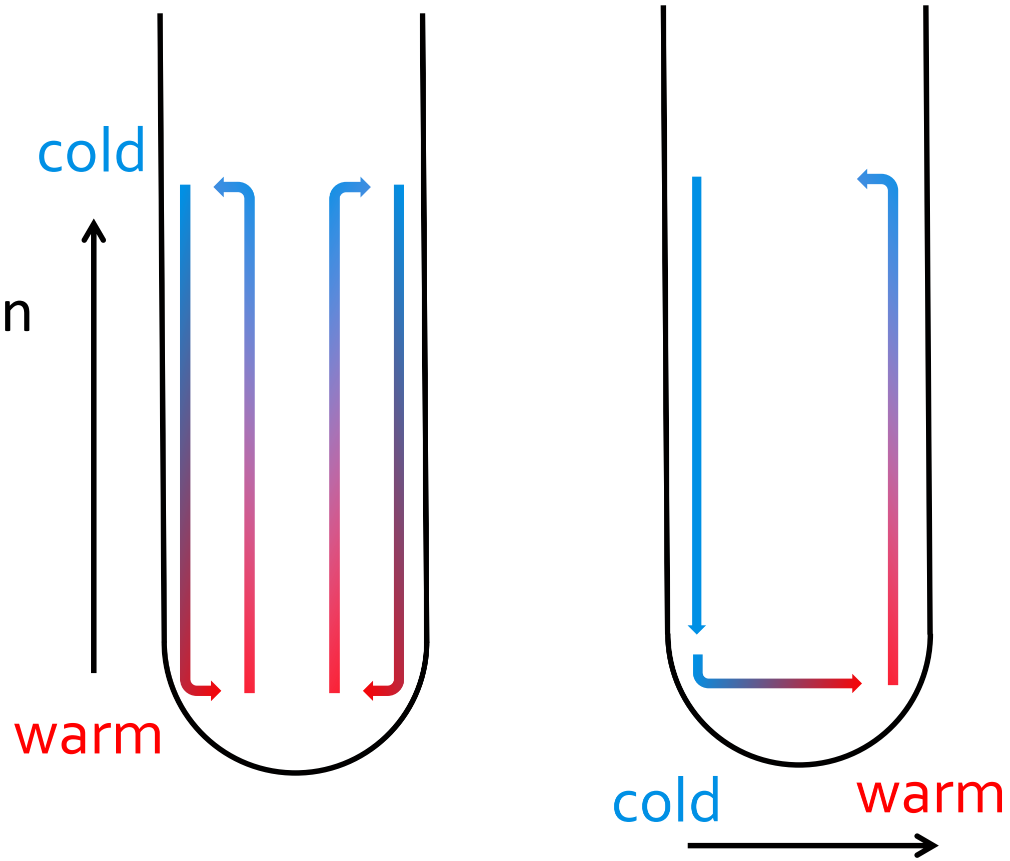
5. Process data in JASON

Interference in diffusion measurements: convection

- Once a critical Raleigh number is reached (1700 for a Benard configuration, i.e., two parallel horizontal boundaries separated by a distance *d*), natural convection appears.

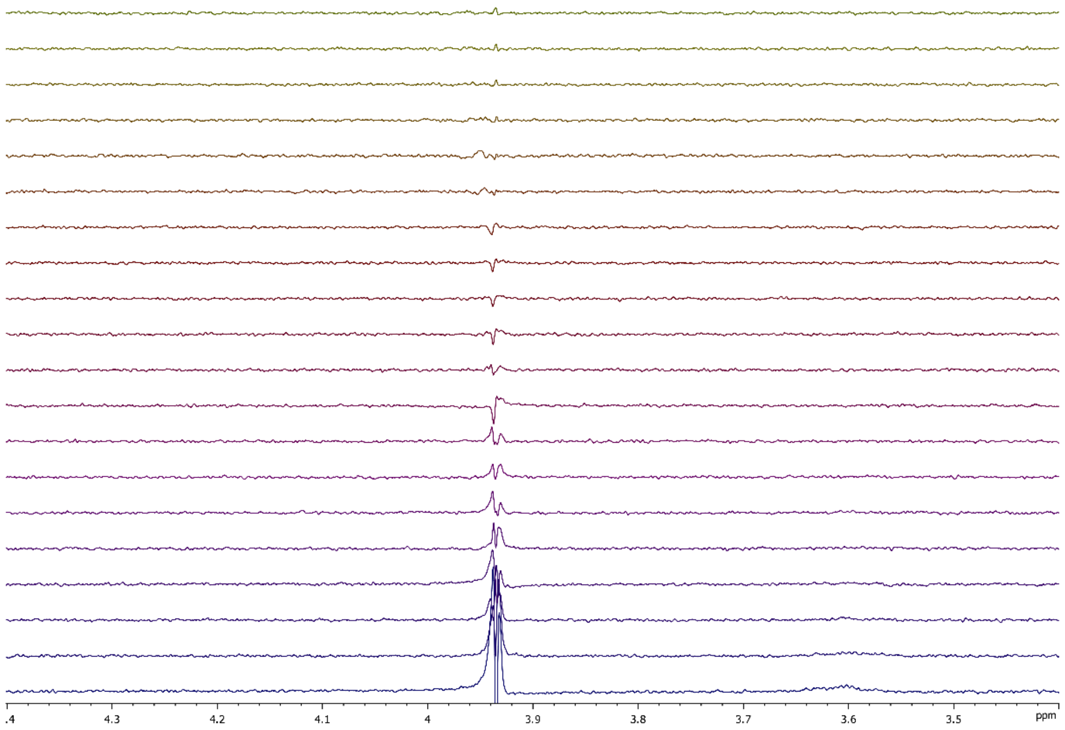
$$\mathrm{Ra} = \frac{\rho \beta \Delta T l^3 g}{\eta \alpha}$$

g: gravity
β: coefficient of thermal expansion
ΔT: temperature difference
l: length
η: viscosity
α:thermal diffusivity

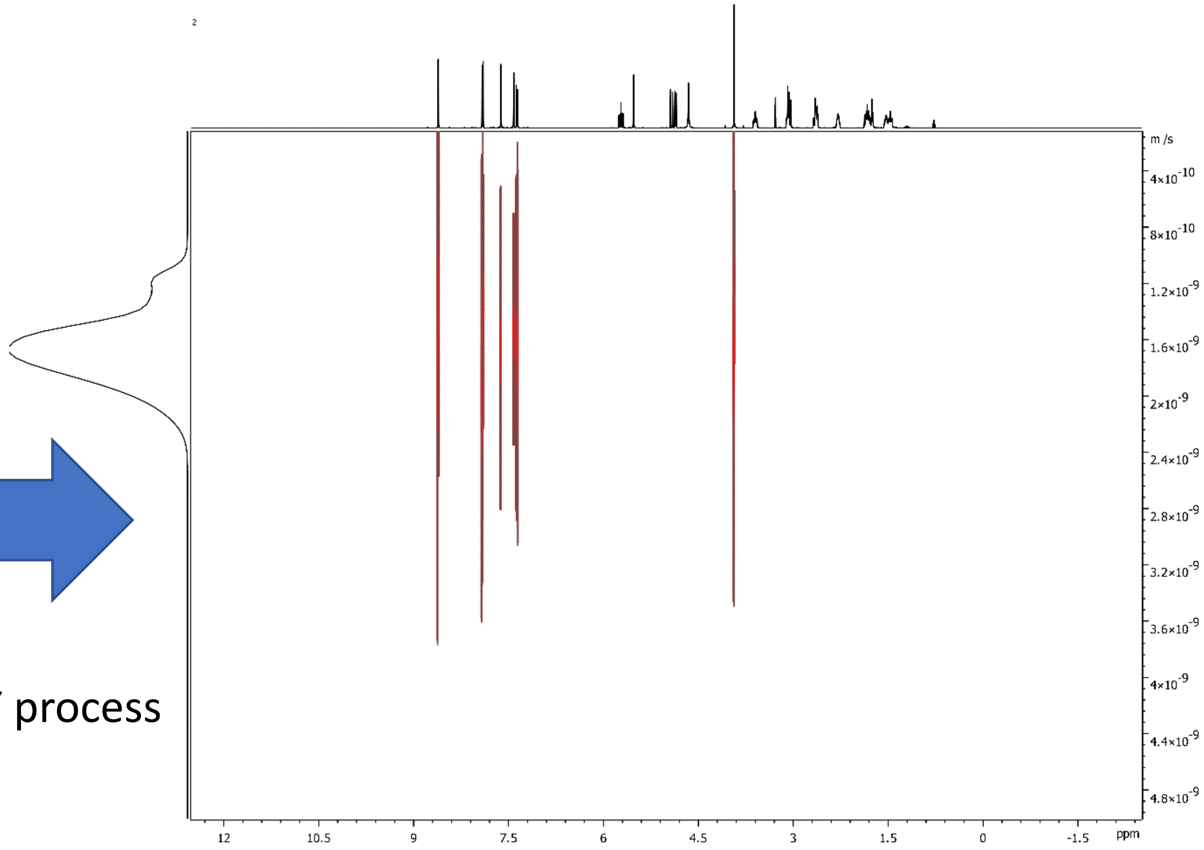


Interference in diffusion measurements: convection

Quinine in CD_3OD at 45° C – oneshot sequence



DOSY process



Reducing convection in DOSY

Use a smaller diameter tube

Probably the most effective method, but costs you (typically 50%) in sensitivity. A 3mm tube or a thick-walled 5mm tube are good choices

Use a more viscous solvent

D_2O and DMSO are good choices. Solvents like chloroform and acetone convect very easily

Turn off the VT control

Leaving the probe to equilibrate at ambient temperature minimizes temperature gradients.

Restrict the sample height

E.g. using a Shigemi tube. Significantly less effective than a small diameter but preserves more signal.

Use a sapphire tube

Expensive but the high heat conductivity helps reduce temperature gradients.

Increase the VT air flow

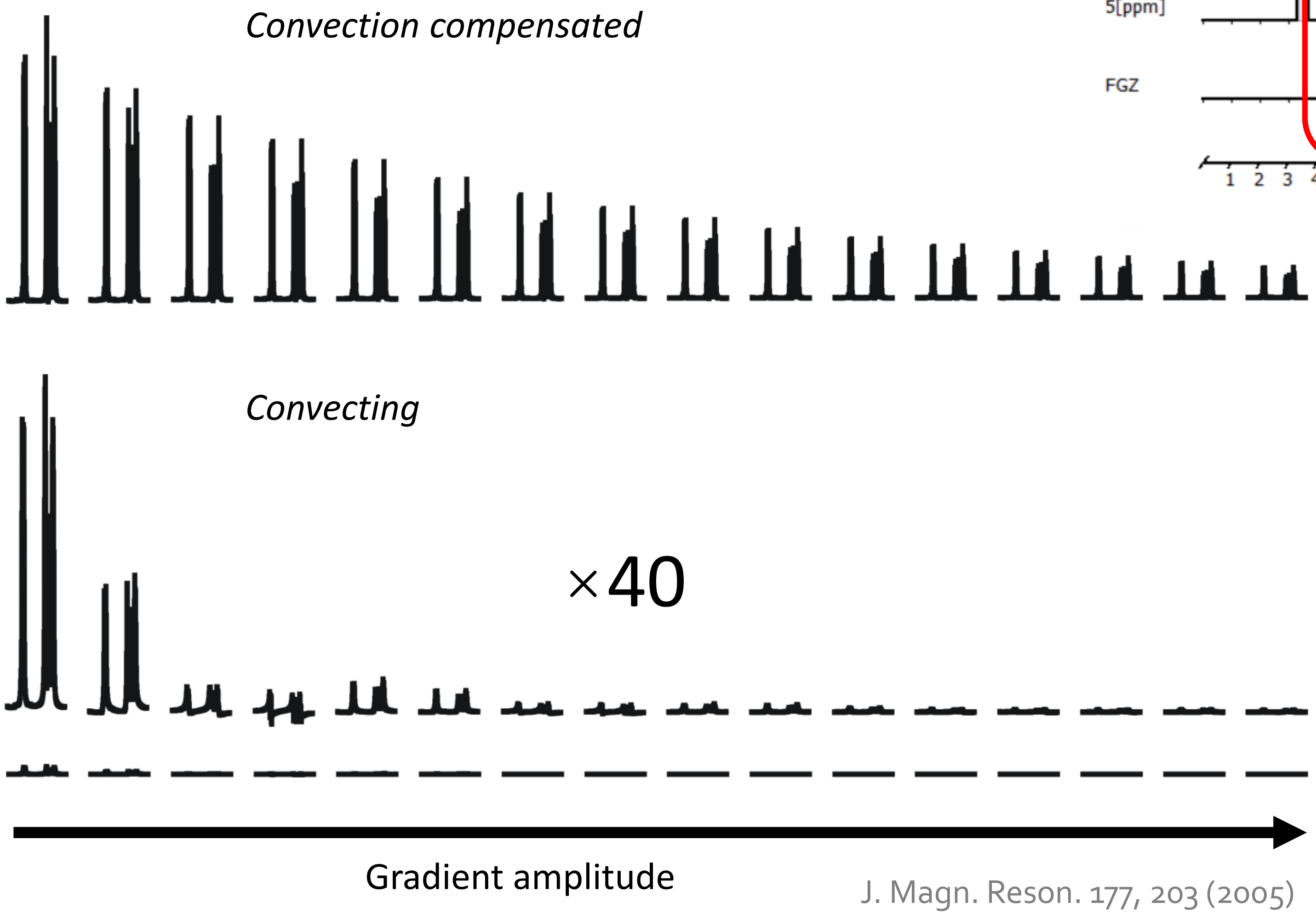
Helps reduce temperature gradients, but vibrations can disturb the measurements.

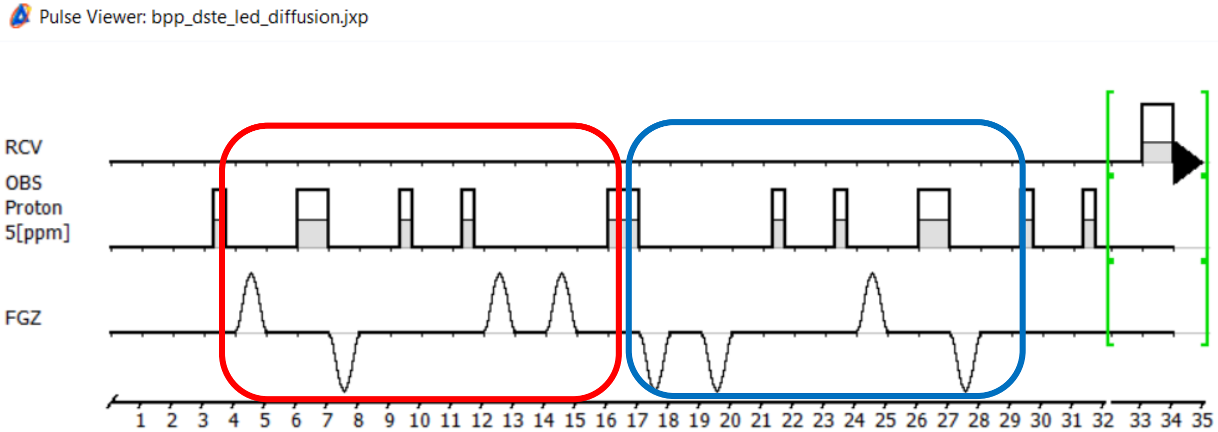
Use convection compensated sequences

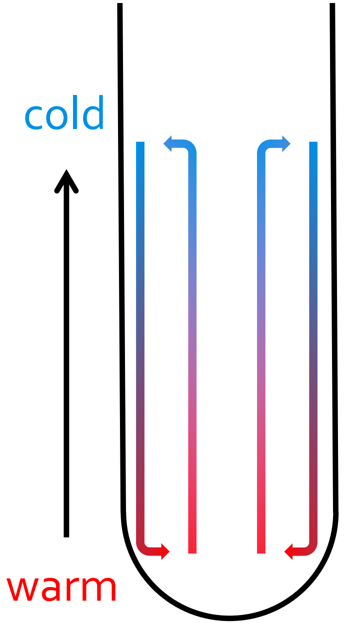
“Last resort” e.g. for high and low temperature experiments. Good but not perfect compensation. Costs 50% in sensitivity and requires a lot (64 scans) phase cycling.

Convection compensated DOSY

Quinine in $CDCl_3$ at 25 °C



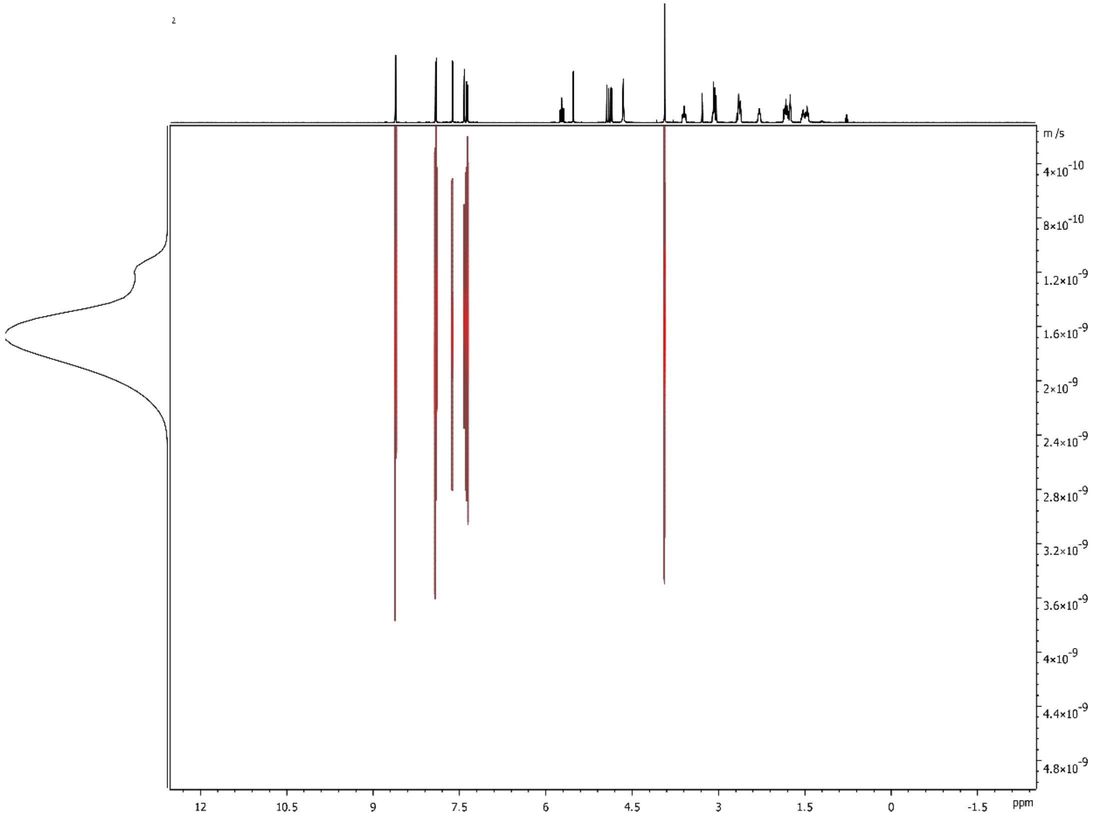




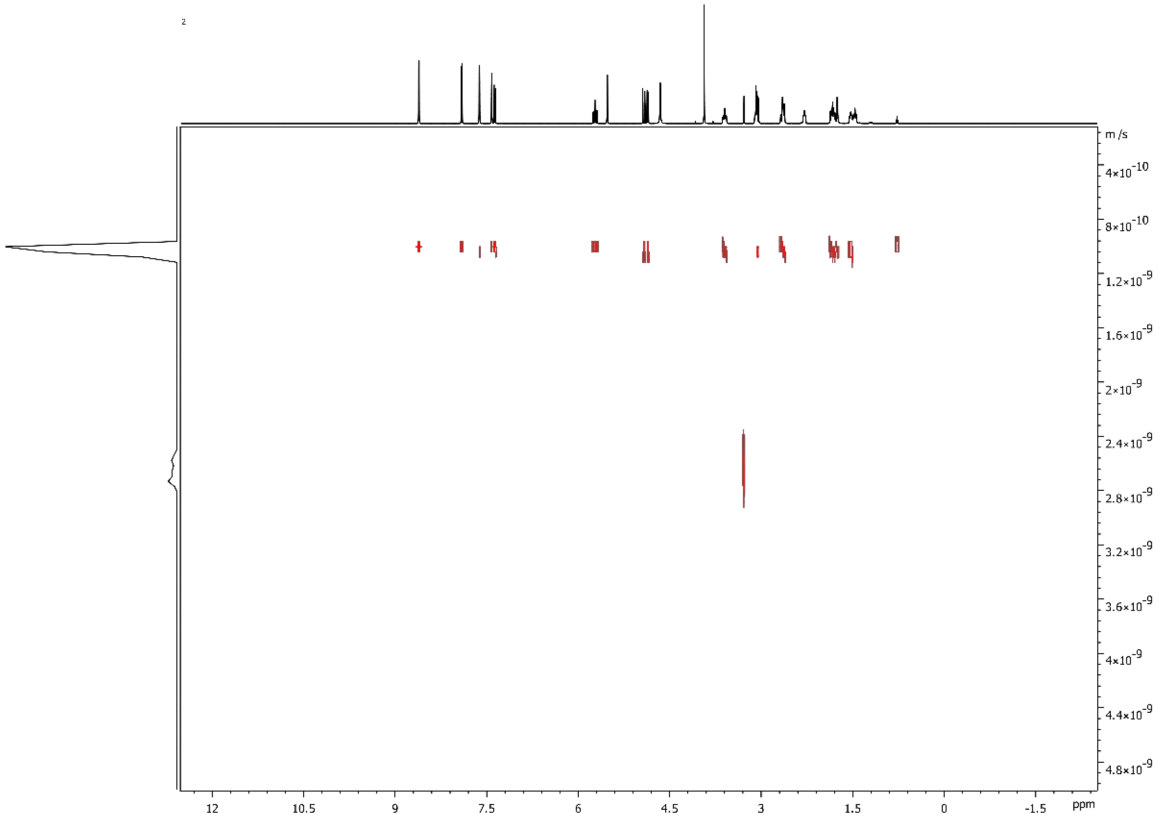
J. Magn. Reson. 177, 203 (2005)

Convection compensated DOSY

Quinine in CD_3OD at 45° C



No convection compensation
(oneshot_dosy.jxp)



Convection compensated
(bpp_dste_led_diffusion.jxp)

Processing of DOSY data

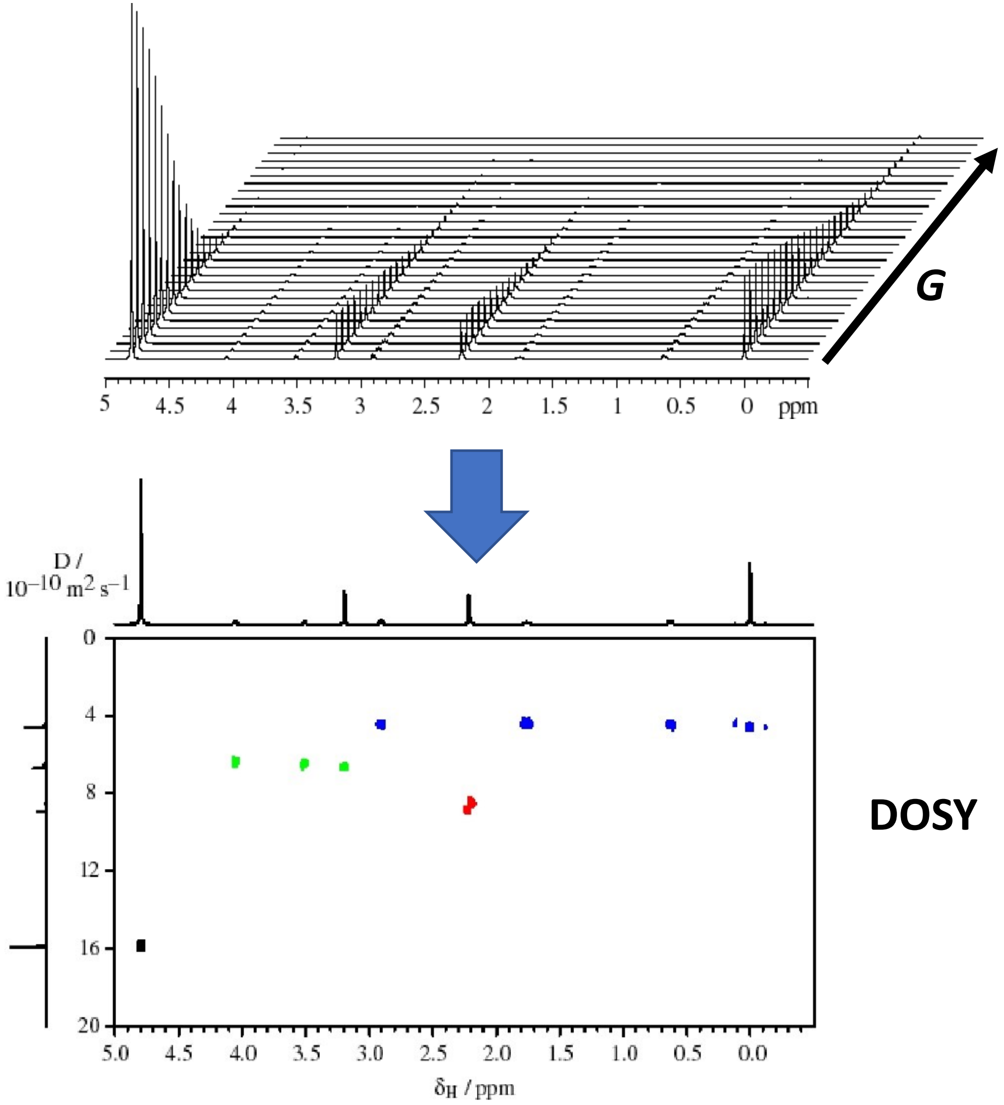
Univariate

- Mono-exponential fitting
  - Simplest and most robust
  - Each signal decay fitted separately
  - Peak overlap will give intermediate D values
  - Should be first analysis method

- Multi-exponential fitting
  - Requires extremely high quality and high SNR data
  - Systematic sources of non-exponential decay (e.g. phase roll, gradient non-uniformity) should be removed, or accounted for in the fitting
  - Overlapped signals that have very similar D values hard to separate

Multivariate

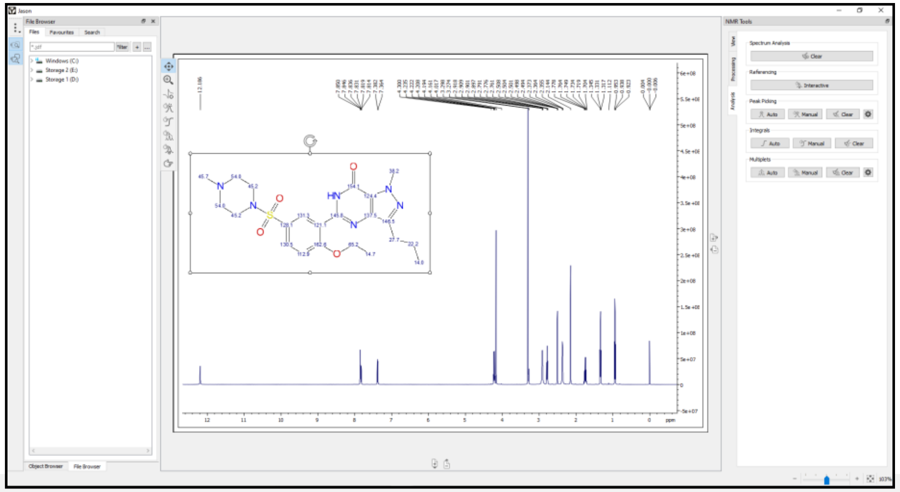
- E.g. SCORE, OUTSCORE, DECRA, MCR
  - Very sensitive to data imperfections
  - Not really advised for 2D DOSY data
  - Better with additional dimensions, e.g. relaxation, kinetics



JASON software

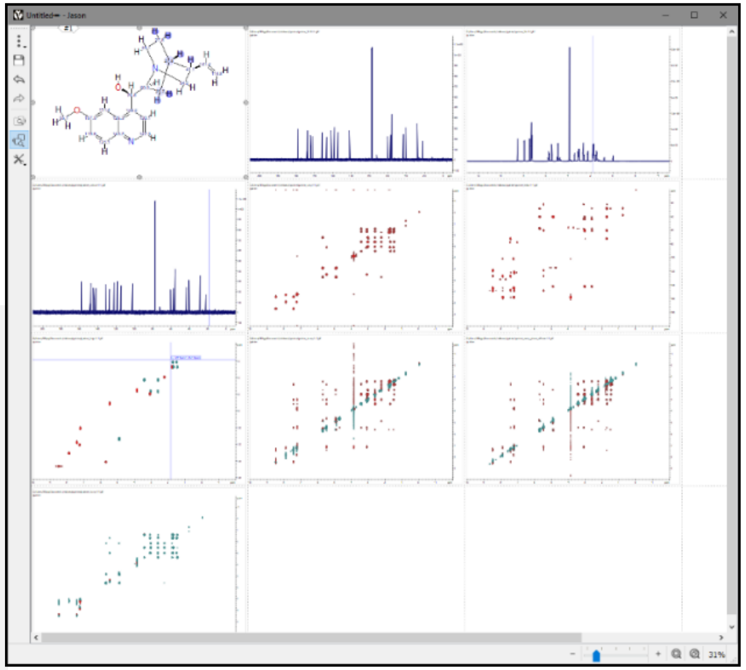
jason

JEOL Analytical SOftware Network

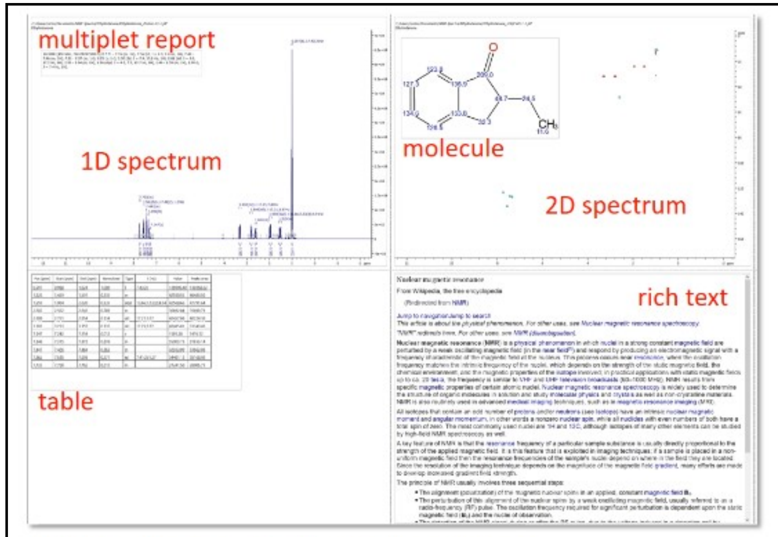


Simplified user experience:

- Easy to navigate between your data sets
- Functionality at your fingertips, no need to search through multiple layers of nested menus
- NMR analysis at a button click
- Reporting



CANVAS



JASON document contains different objects of various types

Main Features

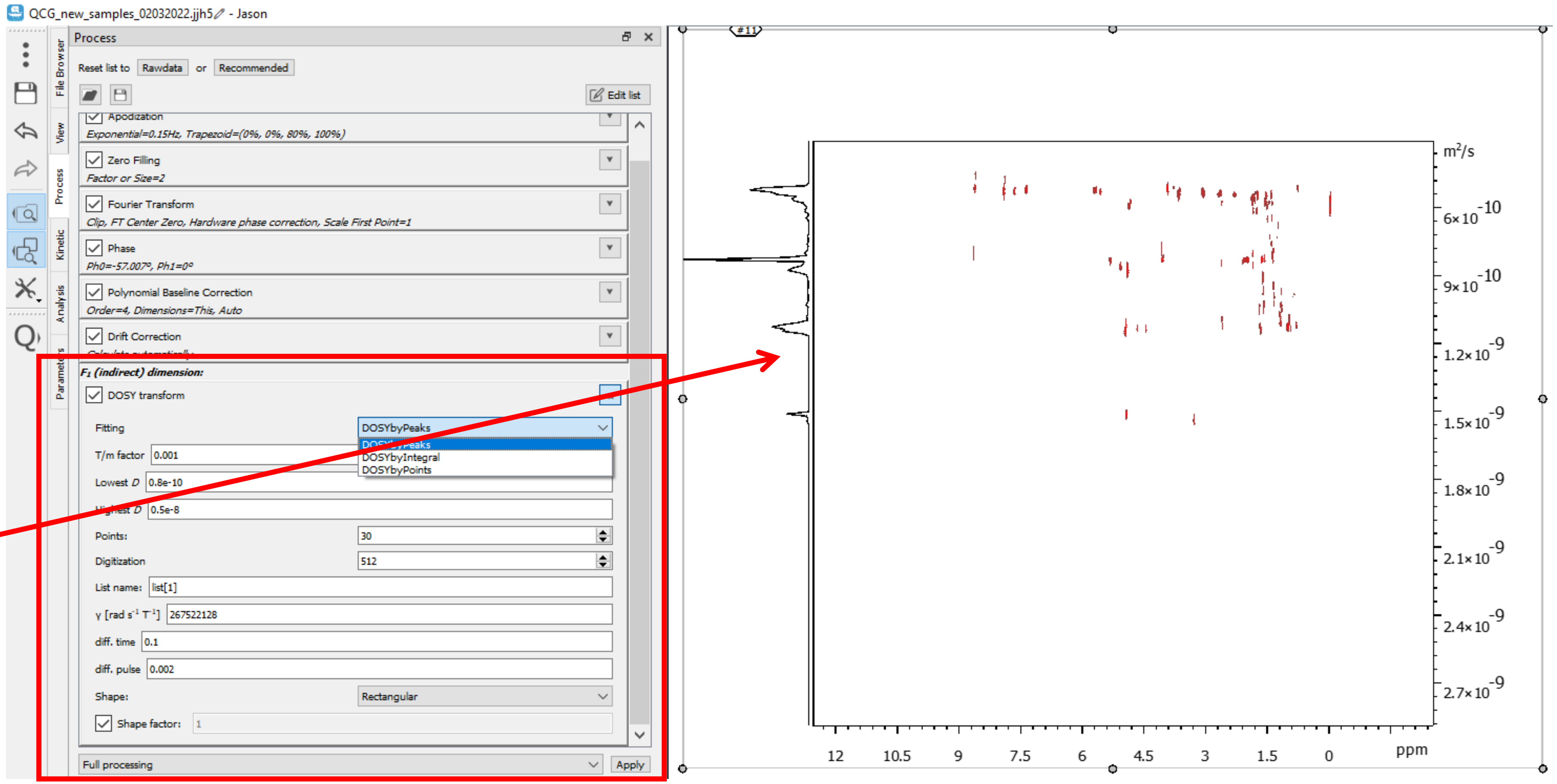
1D / 2D processing , Peak Deconvolution,
^{1}H, ^{13}C chemical shift prediction ,
Multiplet analysis,
Spin simulation, Molecular structure drawing,
Multi language support, etc

New in JASON ver. 1.3 - DOSY processing

Supported OS : Windows 10, Mac

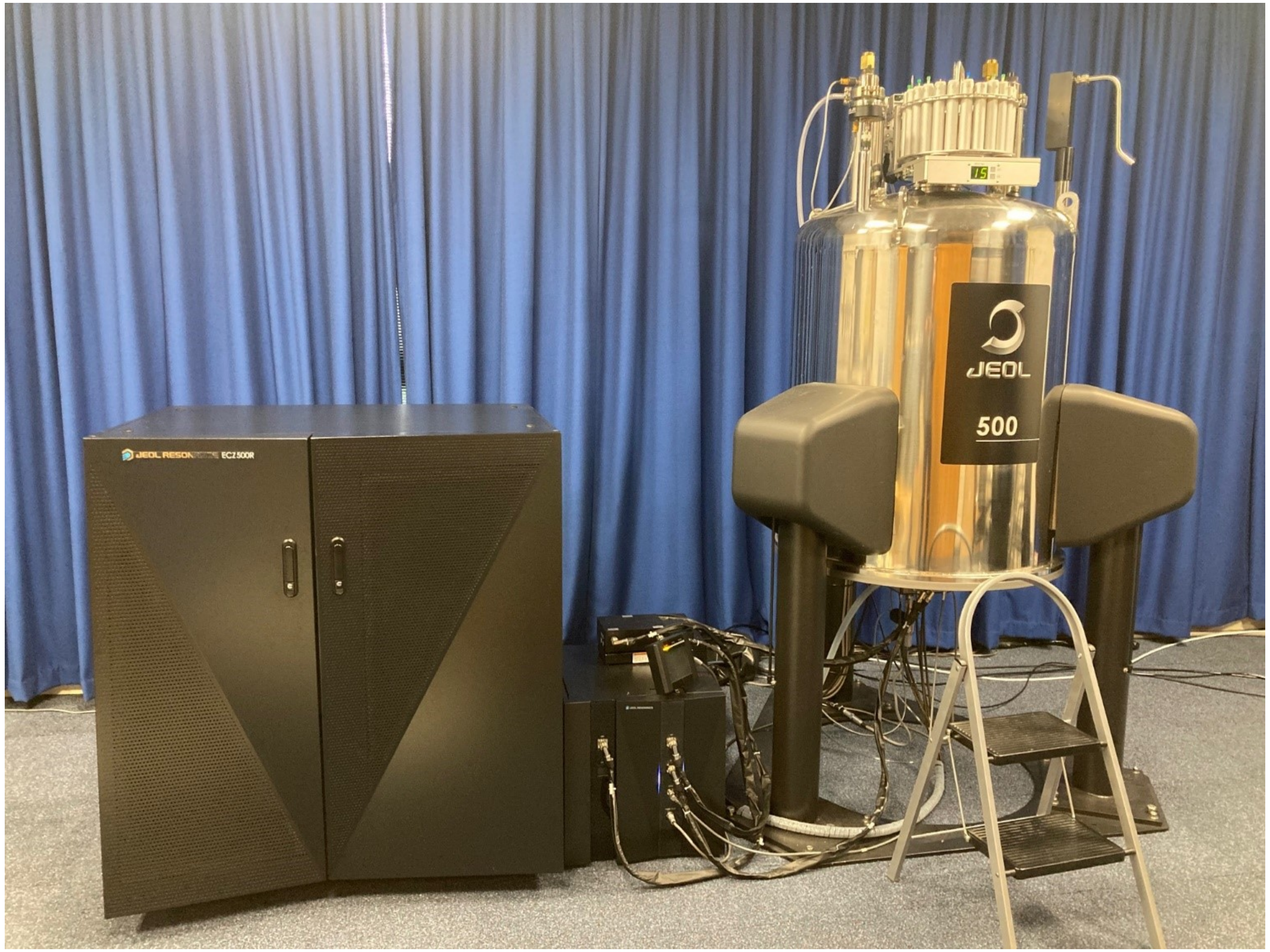
DOSY processing in JASON

- JASON automatically detects experiment type and adds "DOSY transform" step to processing list for DOSY data
- Currently supports mono-exponential fitting of peak amplitudes, integrals or points using linear least-squares
- Generates DOSY plot with diffusion projection along Y-axis



Watch DOSY processing and other JASON videos: https://www.jeoljason.com/new-version-1-3

Practical session 2: DOSY processing in JASON



Back to the spectrometer…

Summary / Take Home Message

- DOSY is a powerful NMR technique for mixture analysis, probing molecular interactions and a wide range of applications
- DOSY is not a “plug-and-play” technique and requires careful set up of experiment parameters and conditions to get good results – but it is not difficult to do once you know how ☺
- The simplest and most robust DOSY processing uses a single-component (mono-exponential fit) of peak amplitudes or peak areas
- JASON software version 1.3 features easy and intuitive DOSY processing using mono-exponential fitting

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Zeit für Q&A

Thank you

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