

Sunnybrook Neuroimaging Summer School: MRS Module

Jason Rock, Peter Truong, Jamie Near

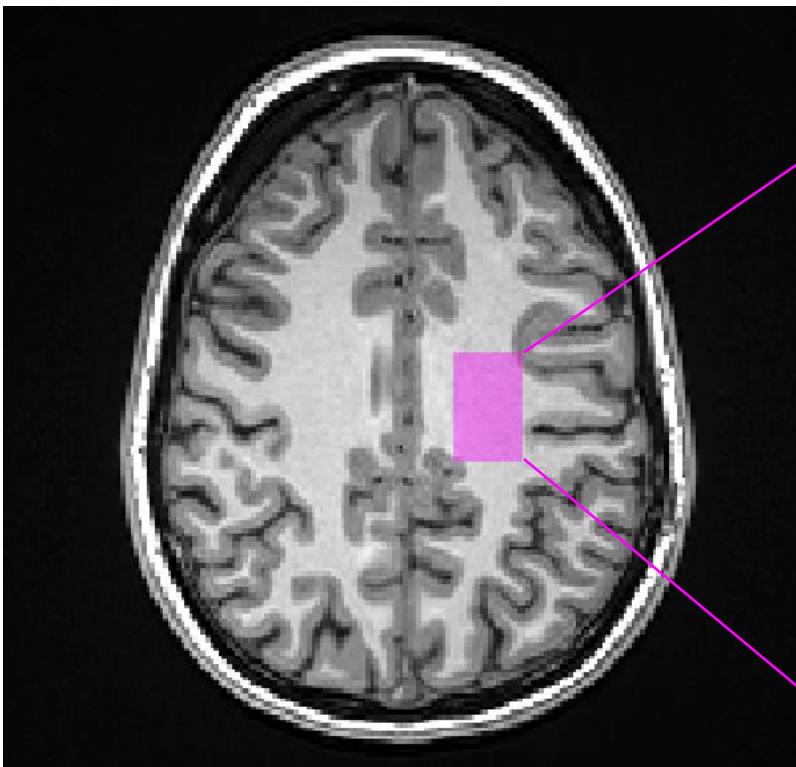
Outline: Morning Lecture

- Jason
 - Introduction to MRS
 - Localization pulse sequences (PRESS, STEAM, SPECIAL)
 - Spectral editing (MEGA-PRESS)
 - MRS data processing (Coil combination, averaging, drift correction, removal of bad averages, eddy current correction, water removal, lipid removal, etc.)
- Peter
 - MRS data analysis (peak fitting, linear combination modelling)
 - MRS quantification (Internal metabolite reference; water reference; tissue correction, quantification in absolute concentration units)
 - Software packages (FID-A, LCModel, FSL-MRS, Osprey)

Introduction to MRS

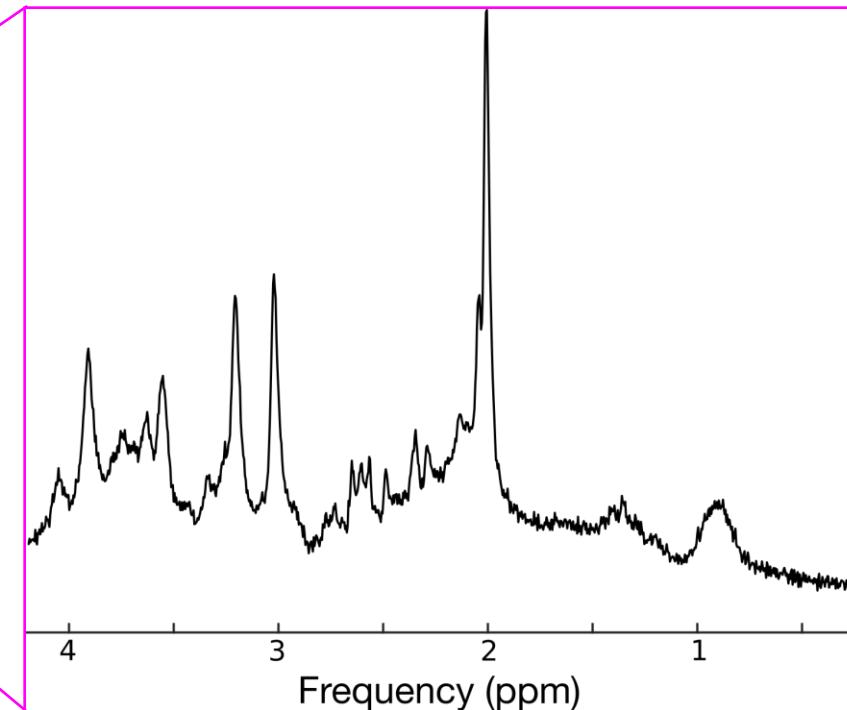
Magnetic Resonance Spectroscopy

Imaging



Imaging signal comes from water protons

Spectroscopy

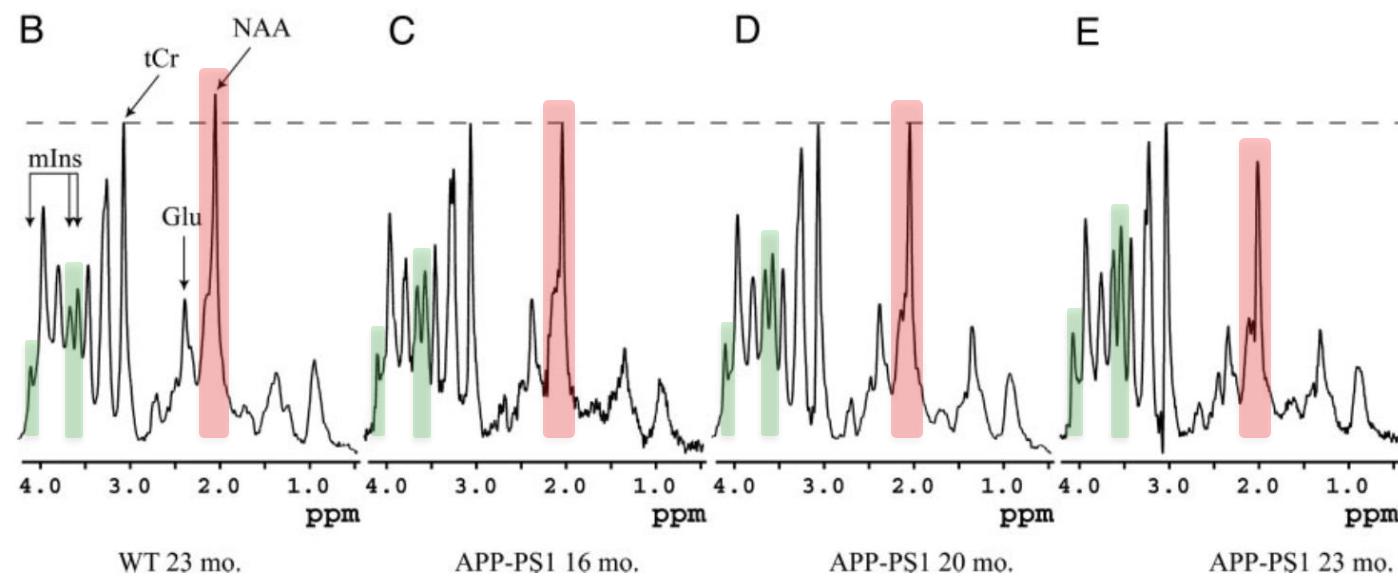


Spectroscopy signal comes from protons in other chemical environments

MRS is a powerful tool for non-invasive measurement of chemical (metabolite) concentrations in-vivo.

Motivation

- MRS enables the study of neurochemistry related to:
 - Normal brain development and function
 - Neurodegeneration
 - Cancer
 - Psychiatric disorders
 - ...



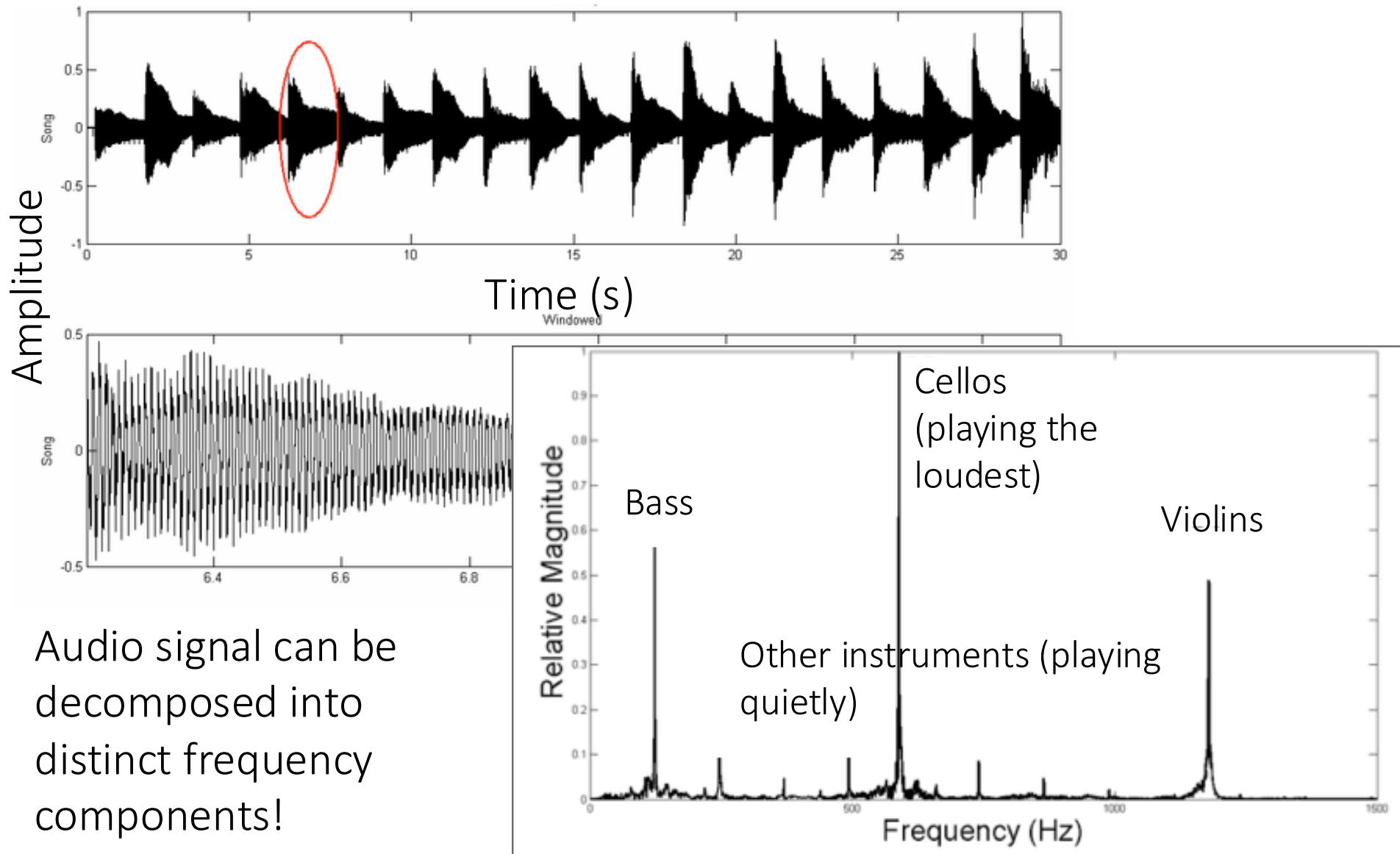
Analogy: Audio Spectroscopy

Imagine listening to an orchestra: you differentiate between instruments based on their frequency!



Slide courtesy Karla Miller

Analogy: Audio Spectroscopy

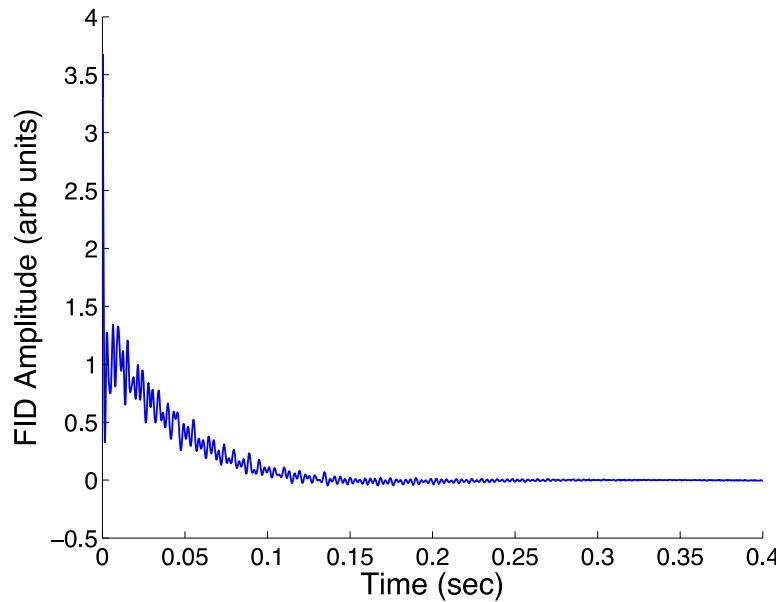


Slide courtesy Karla Miller

Magnetic Resonance Spectroscopy

(in three steps)

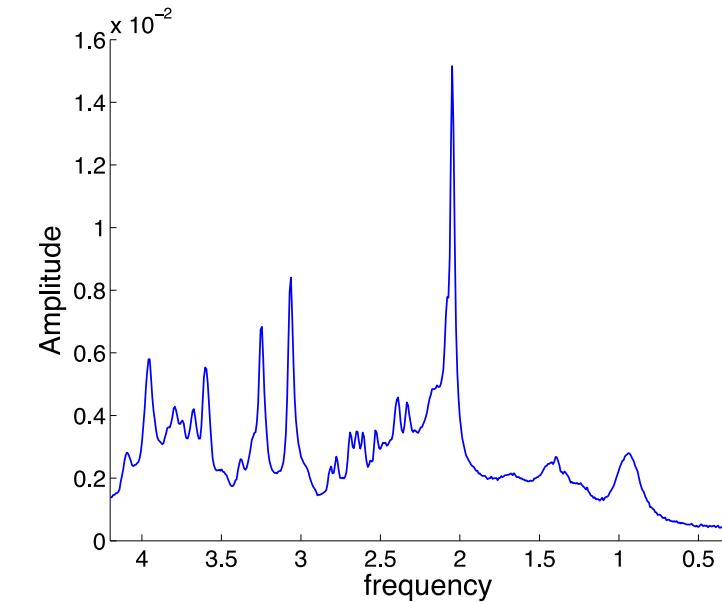
1. Listen (acquire Signal)



2. Fourier Transform



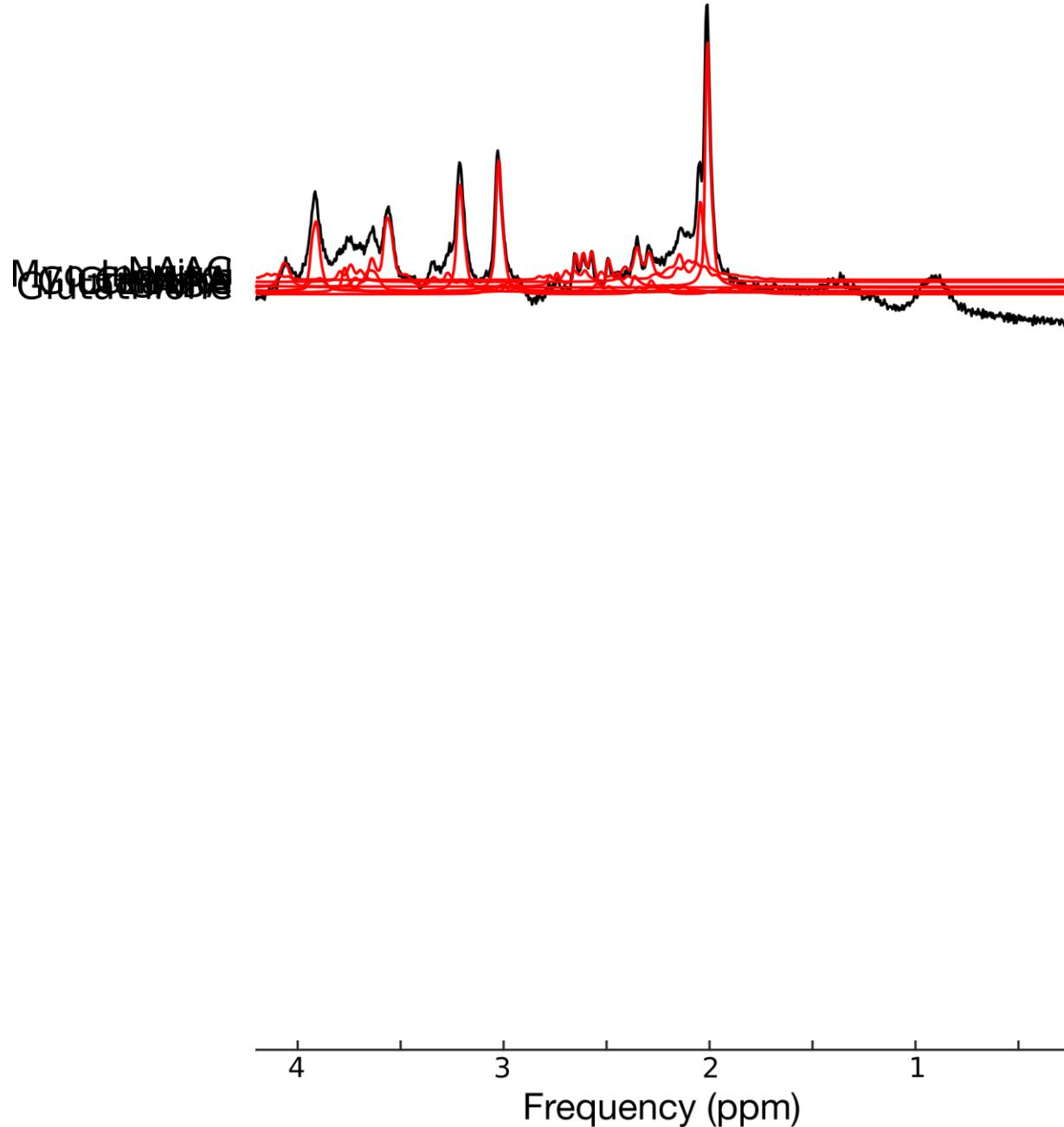
3. Voila! Spectrum



An MR spectrum is composed of signals from many different metabolites.

Each metabolite is identified by a unique and highly-reproducible frequency distribution.

Differences in frequency caused by differences in electronic shielding



What can we detect with MRS?

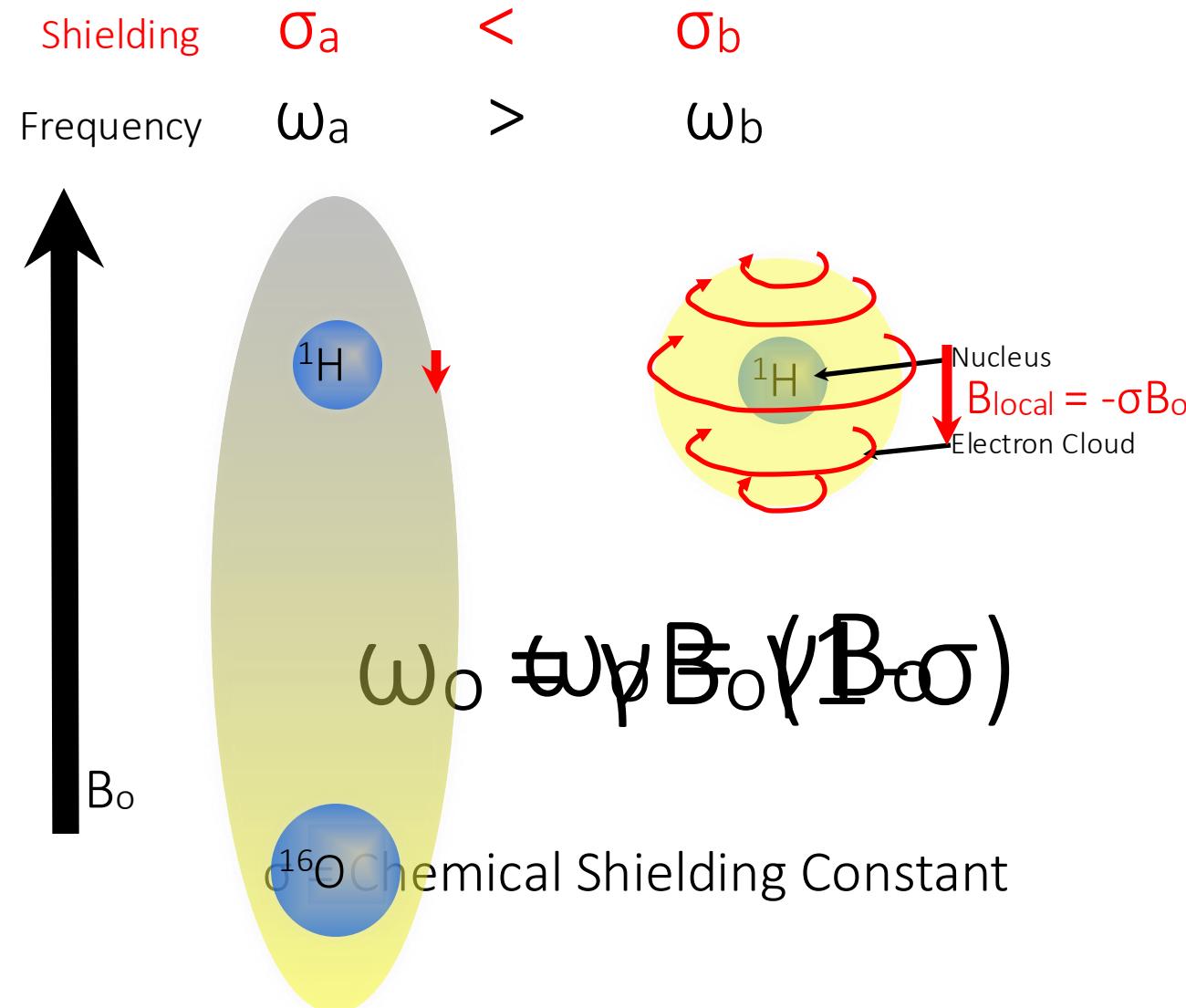
Name	Approximate Concentration (mmol/L)
Water	35,000
NAA	12
Glutamate	10
Creatine (Cr + PCr)	8
Myo-Inositol	6
Glutamine	3
Cholines (GPC + PCh)	2
Glutathione	2
Taurine	2 (human), 6(rodent)
NAAG	1.5
Aspartate	1.5
Glucose	1.5

Name	Approximate Concentration (mmol/L)
GABA	1.3
Serine	1
Ethanolamine	1
Alanine	1
Ascorbic acid	1
Glycine	0.8
Scyllo-Inositol	0.5
Acetate	0.5
Homocarnosine	0.4
Lactate	0.2
NAD ⁺	0.2
B-hydroxybutyrate	0.05

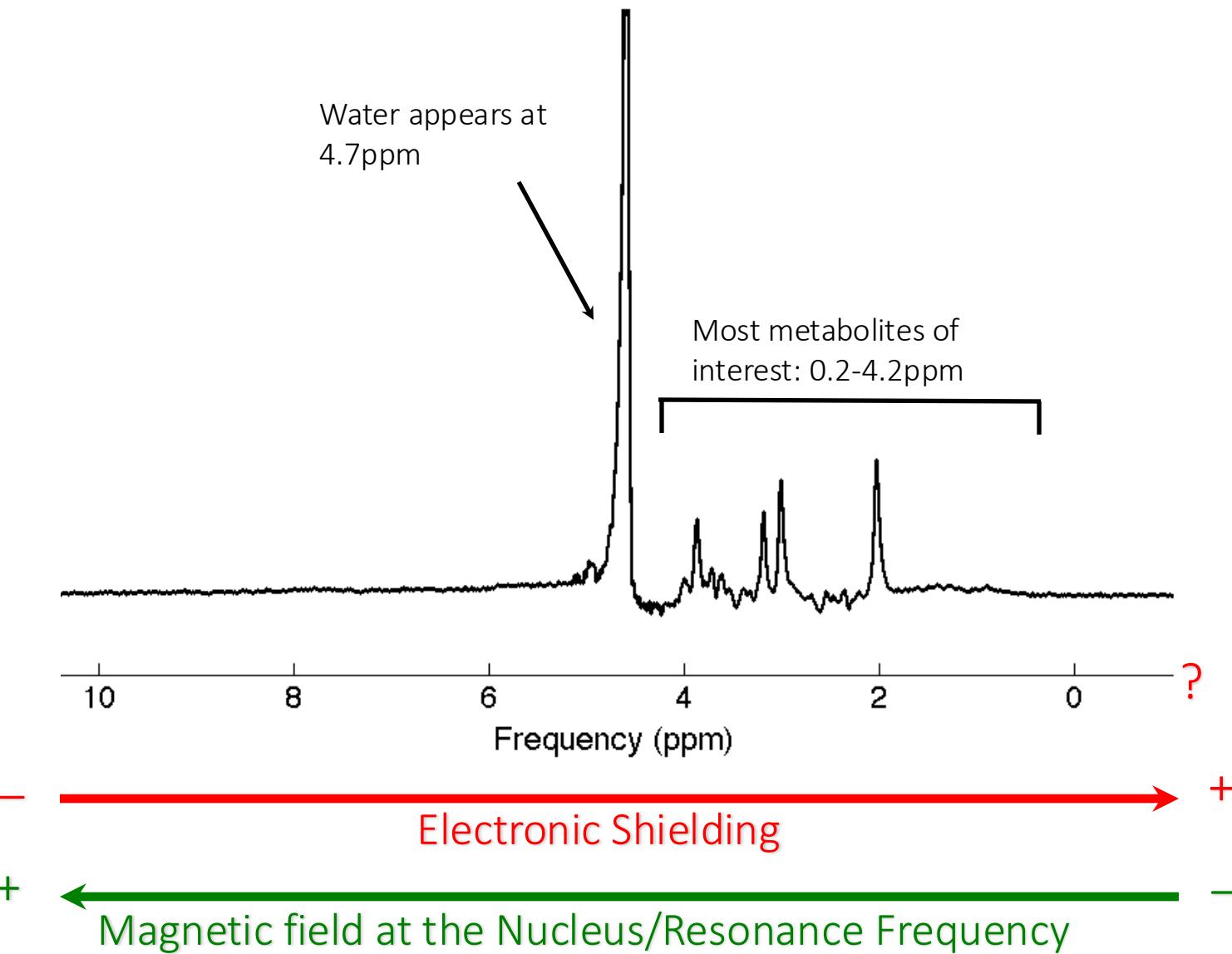
What can we NOT detect with MRS?

- Compounds with concentration < 0.1 mmol/L
 - Dopamine
 - Serotonin
 - Acetylcholine
- Large molecules (>3500 kDa)
 - Proteins
 - Enzymes
- Molecules that are immobile or tightly bound

Basics of MRS: Shielding and Chemical Shift



Spectral Appearance



The ppm Frequency Scale

You can think of “ppm” in the same way that you think of “%”, only smaller:

%	ppm
$1\% = 1/100$	$1\text{ppm} = 1/1000000$

Therefore:

1 ppm = one millionth of the Larmor frequency

Predicting Spectra

Given what we now know about shielding and magnetic resonance, we can (roughly) predict the MR spectrum of a compound based on its chemical structure!!!

Let's try and predict the following spectra:

- Water
- Lactate
- GABA

Water:

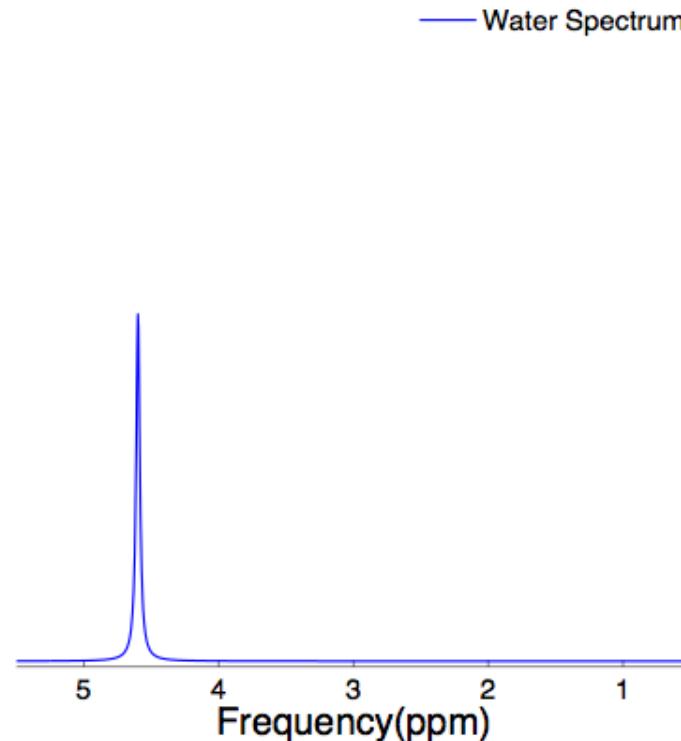
Structure:



One pair of “equivalent” protons:

- Same Resonance Frequency
- Single Peak

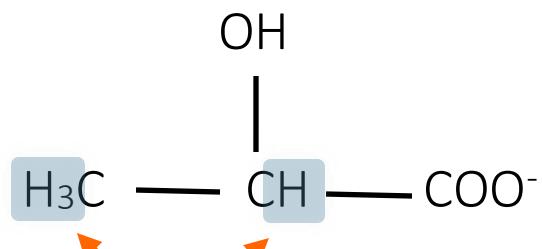
Shielding is low, due to
electronegative Oxygen atom.



By convention, the water peak appears
at a frequency of 4.7ppm

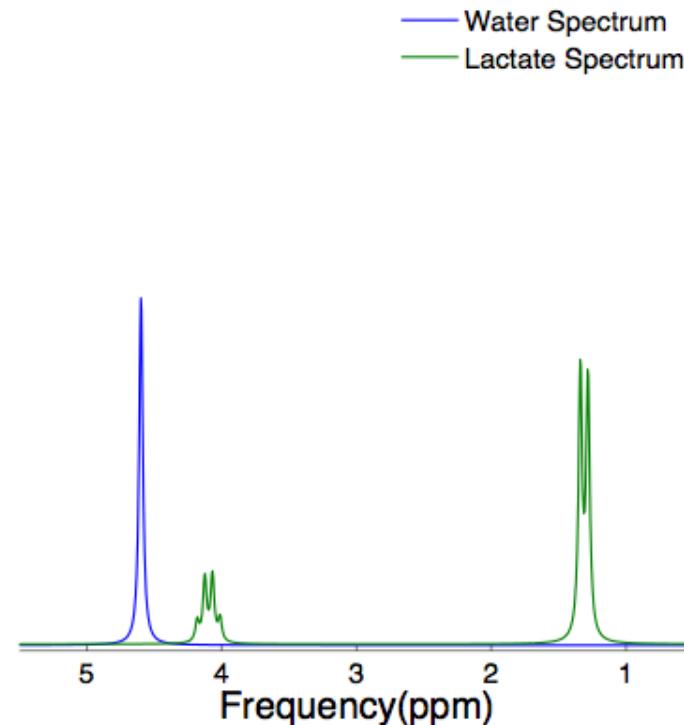
Lactate:

Structure:



- One methyl group (3 equivalent protons)
Not quite done yet!!
- Because the Methyl and one methine group (CH) Methine groups share a bond, they are said to be “**Coupled**”

Coupling results in High Peak
Splitting
Methine Group: Low



Note: Peak ~~bright~~ corresponds to number of Protons in Group

GABA



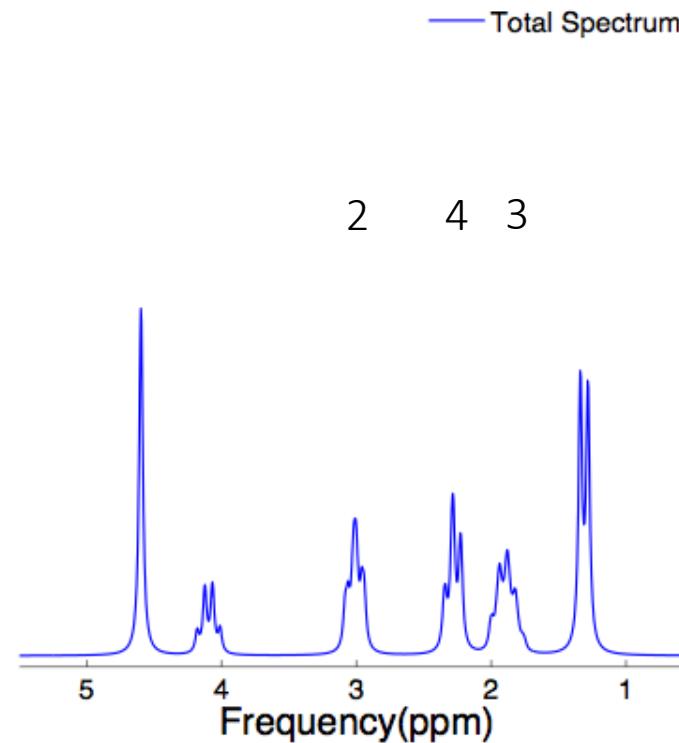
Three Methylene groups
(2 protons per group)

Shielding:

- Less shielding than CH_3 groups
- More shielding than CH groups

Coupling:

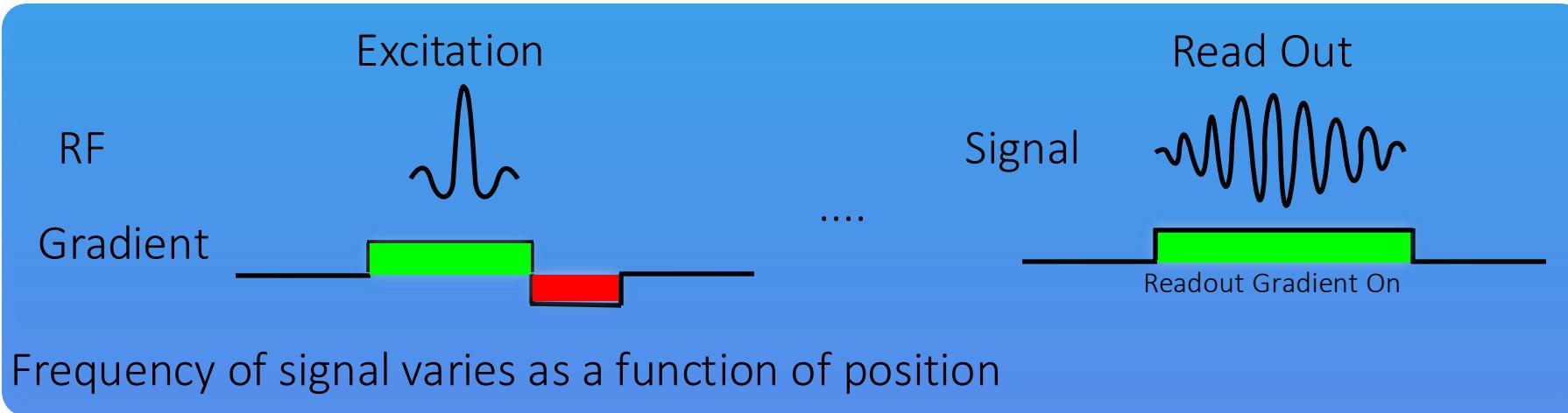
- 2 is coupled to 3
- 3 is coupled to 4



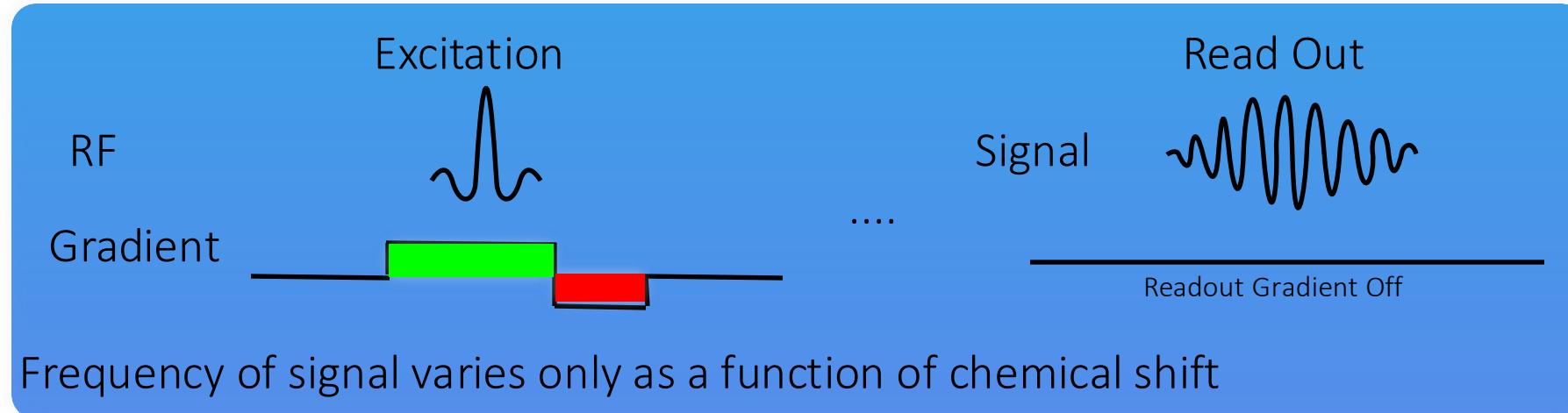
Total spectrum is given by the sum of the individual metabolite spectra

MRS Acquisition:

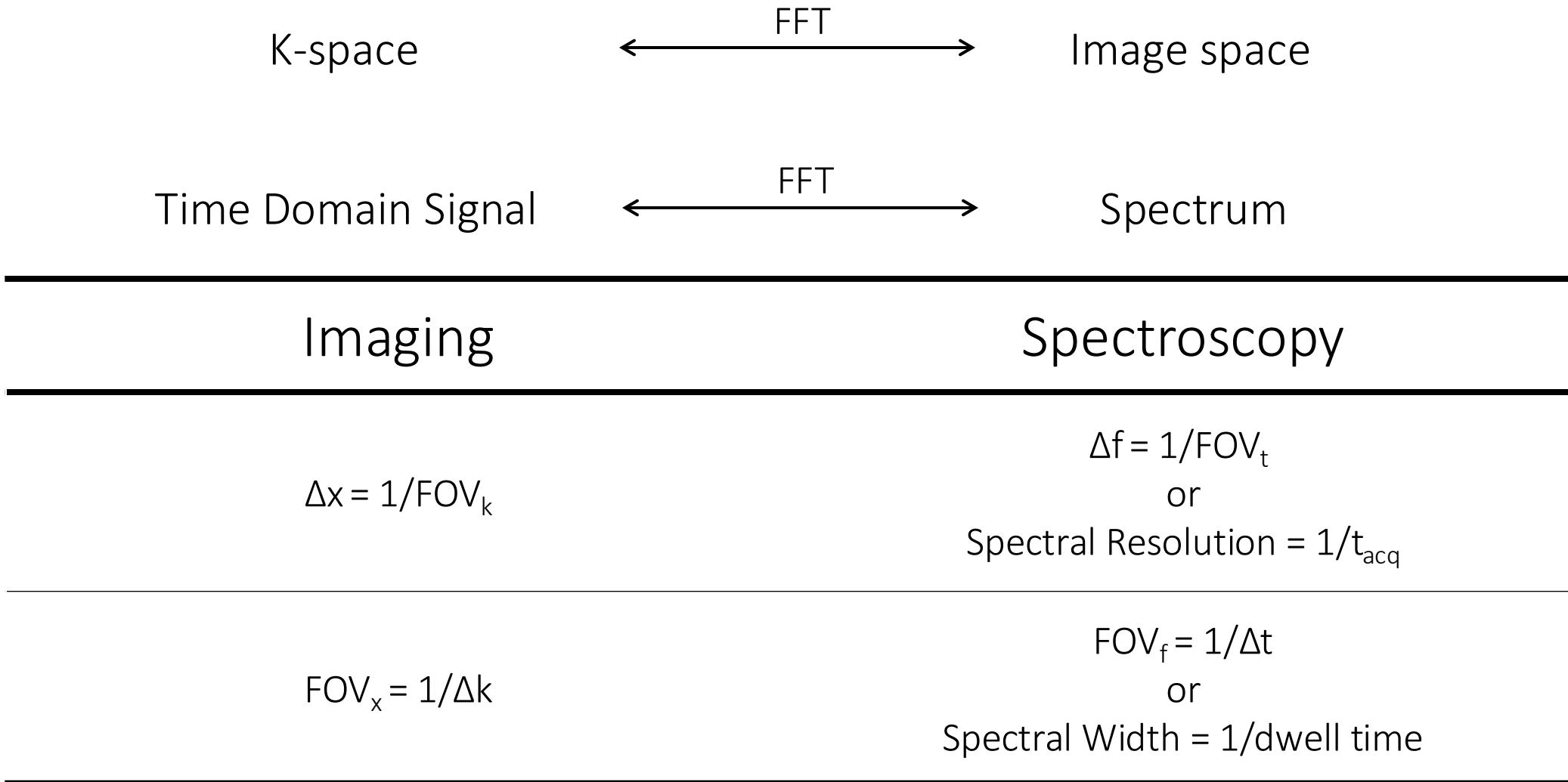
Imaging:



Spectroscopy:



Fourier Relationships in MRS



Fourier Relationships in MRS

Imagine a simple Experiment

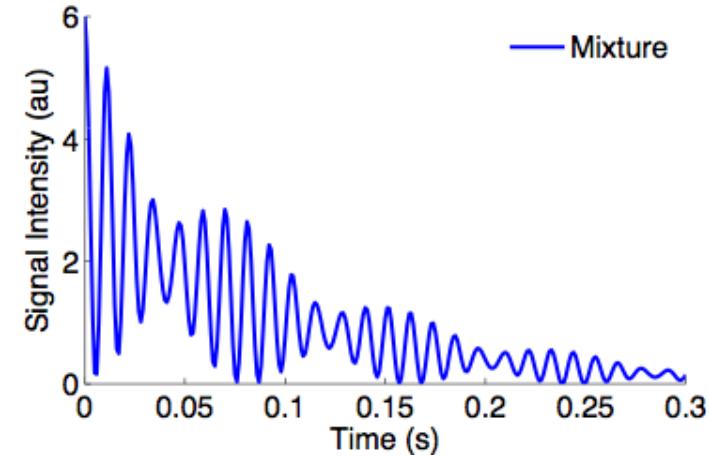
Mix together:

- Three parts water (4.7 ppm)
- Two parts “Metabolite A” (4.0 ppm)
- One part “Metabolite B” (3.9 ppm)

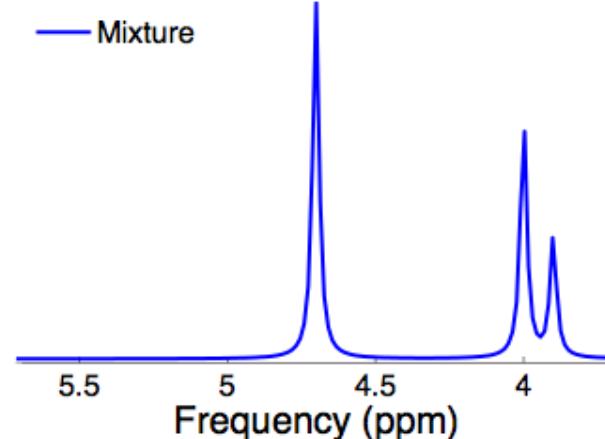
What does the signal look like?

How does the way we sample the signal impact on the appearance of the spectrum?

Time Domain: Free Induction Decay (FID)

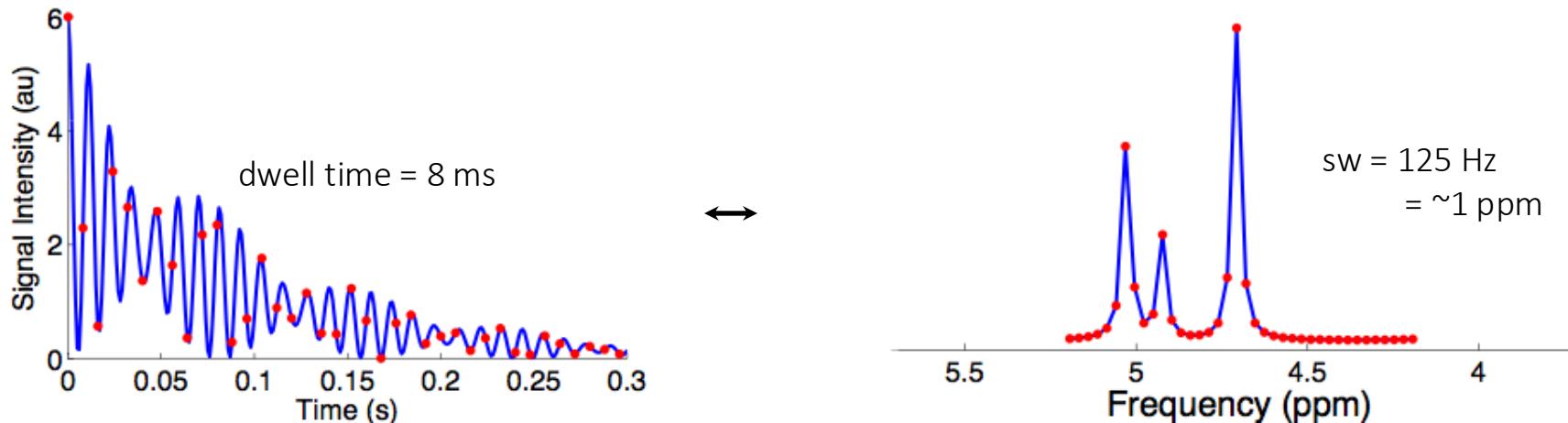
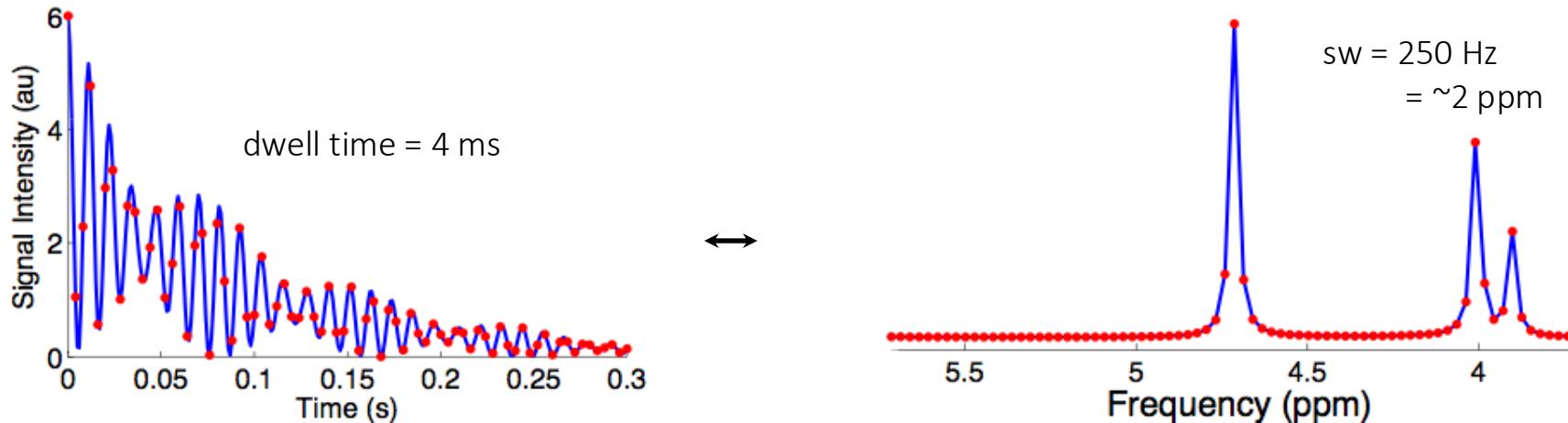


Frequency Domain: Spectrum
FT



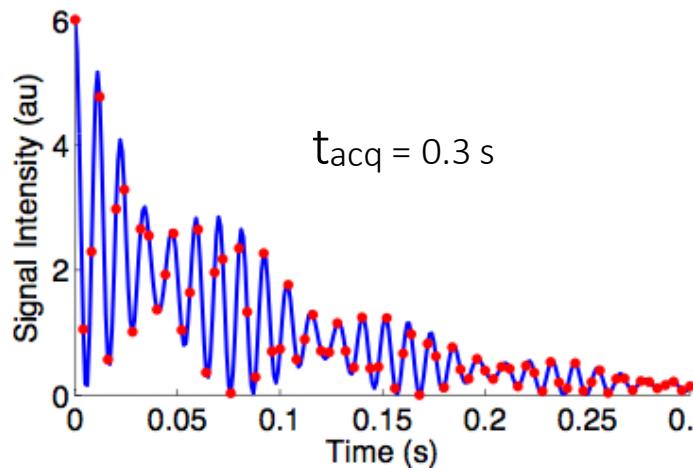
Fourier Relationships in MRS

- Effect of “dwell time” on spectral width:
 - spectral width = $1/\text{dwell time}$

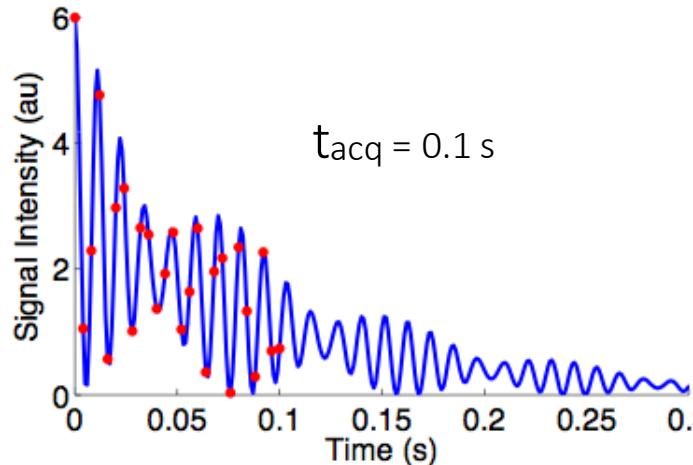
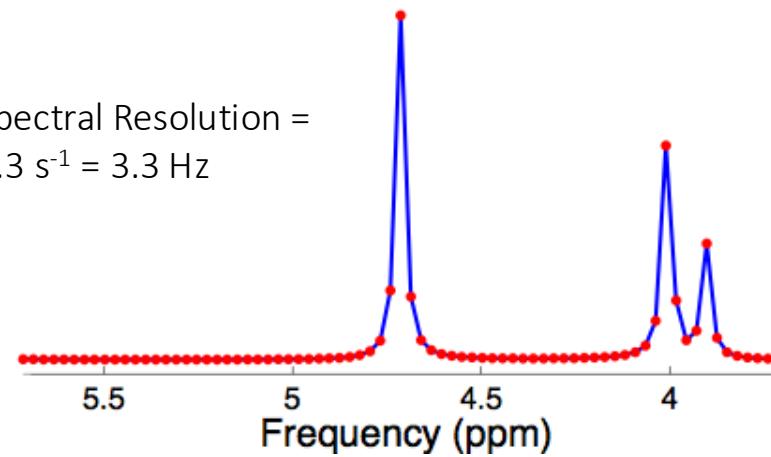


Fourier Relationships in MRS

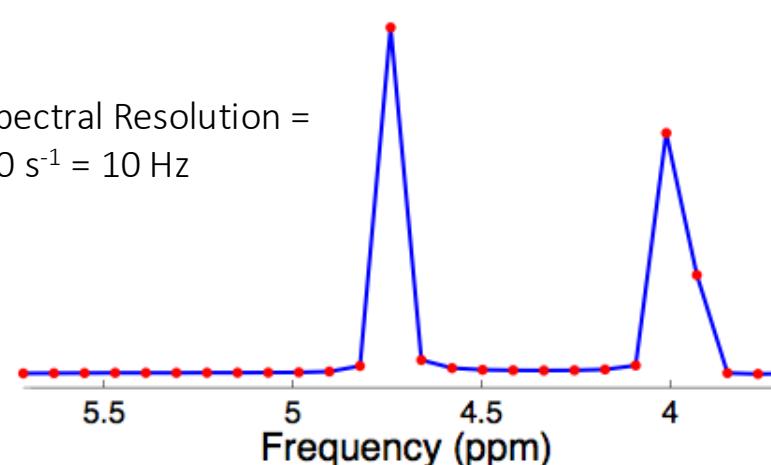
- Effects of acquisition duration (t_{acq}) on spectral resolution:
 - $\text{Spectral resolution} = 1/t_{\text{acq}}$



Spectral Resolution =
 $3.3 \text{ s}^{-1} = 3.3 \text{ Hz}$

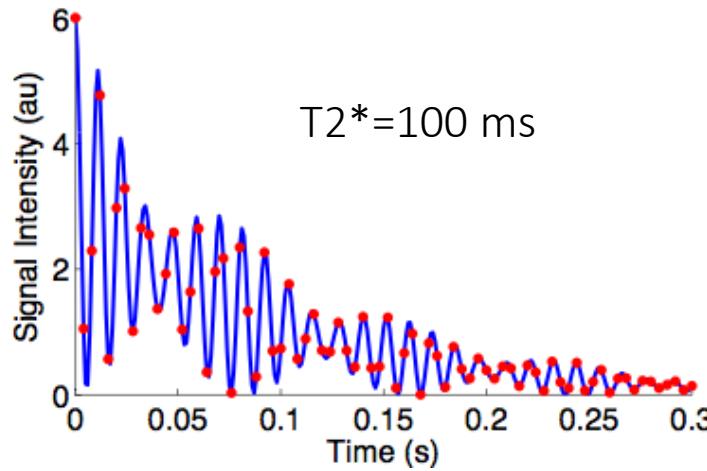


Spectral Resolution =
 $10 \text{ s}^{-1} = 10 \text{ Hz}$

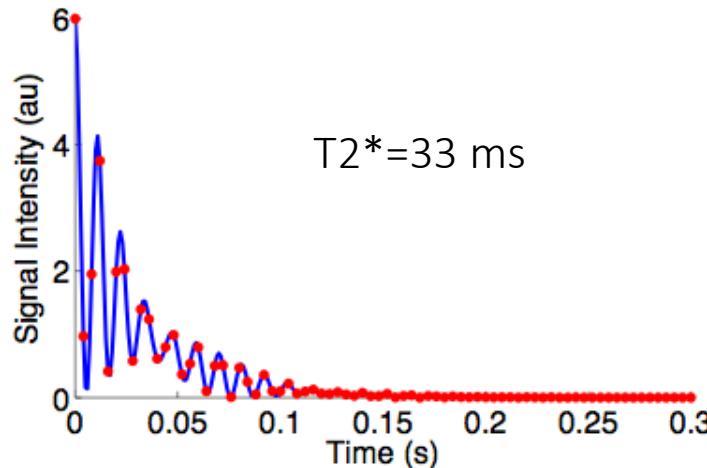
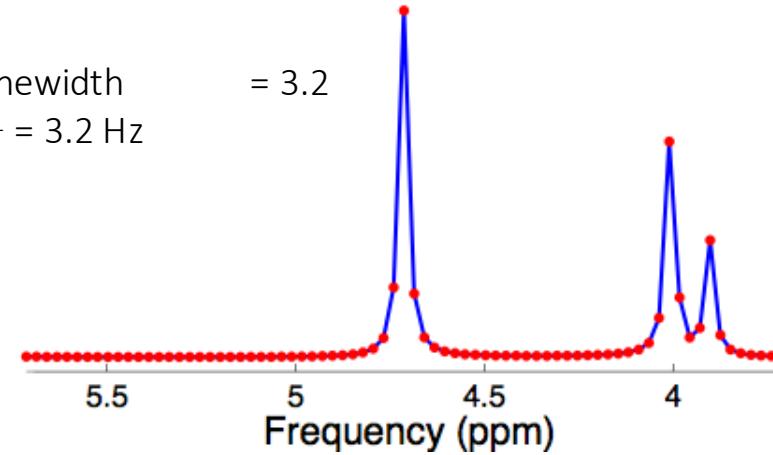


Spectral Linewidth

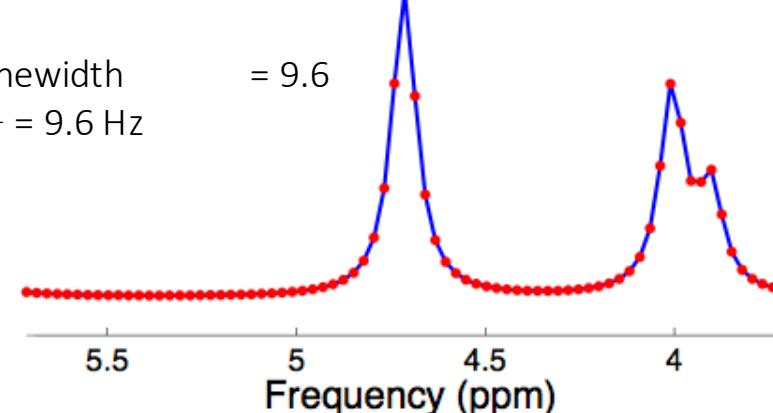
- Effect of changing $T2^*$ on linewidth:
 - $\text{Linewidth} = 1/(\pi \cdot T2^*)$



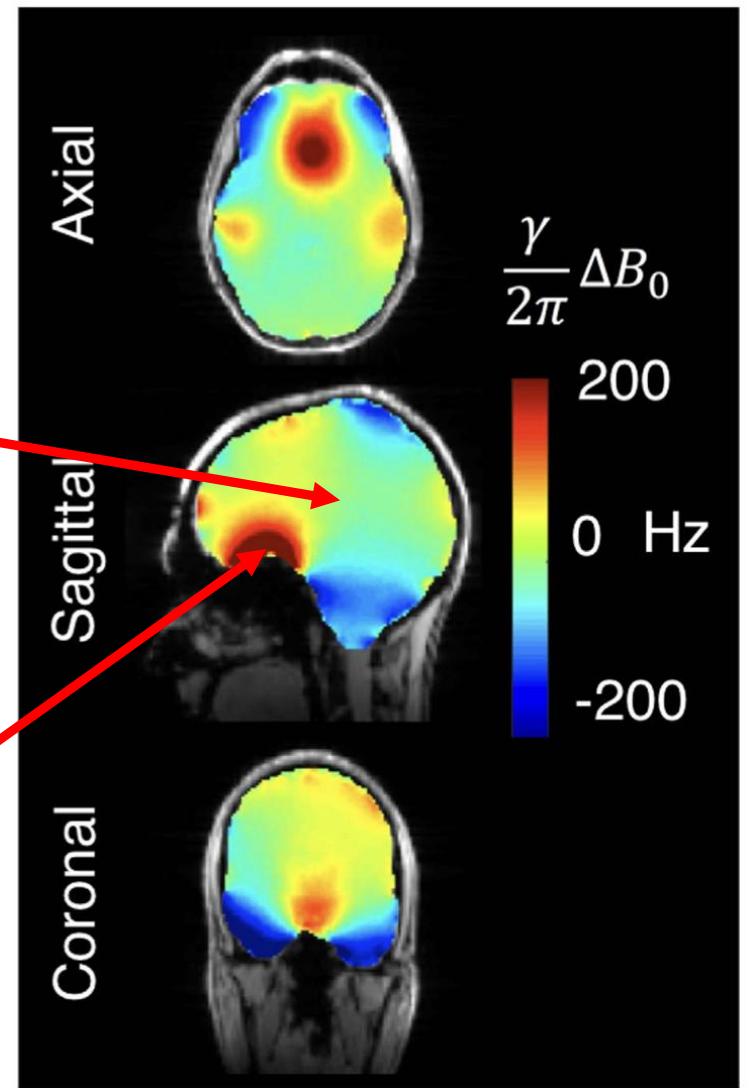
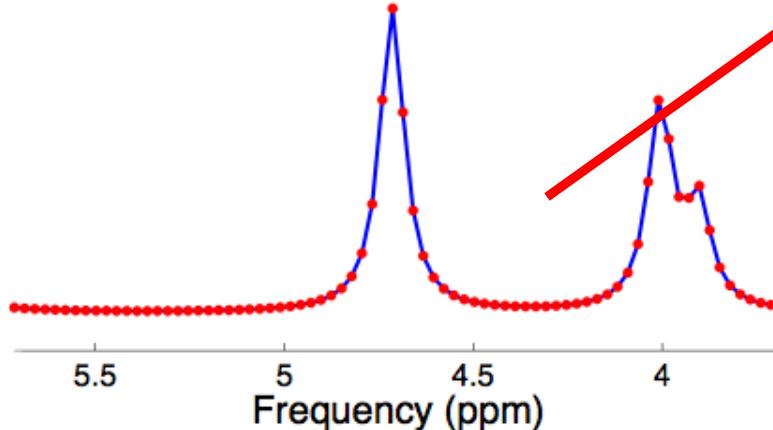
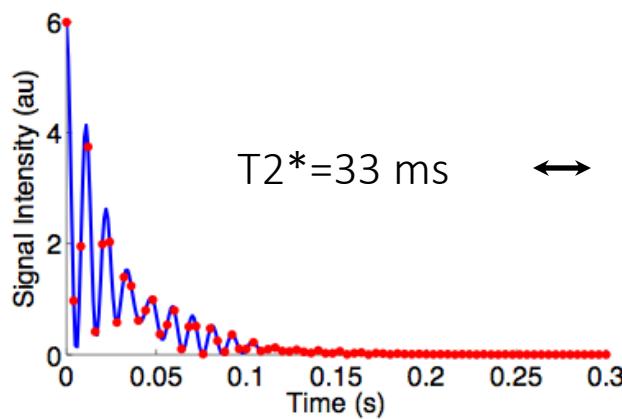
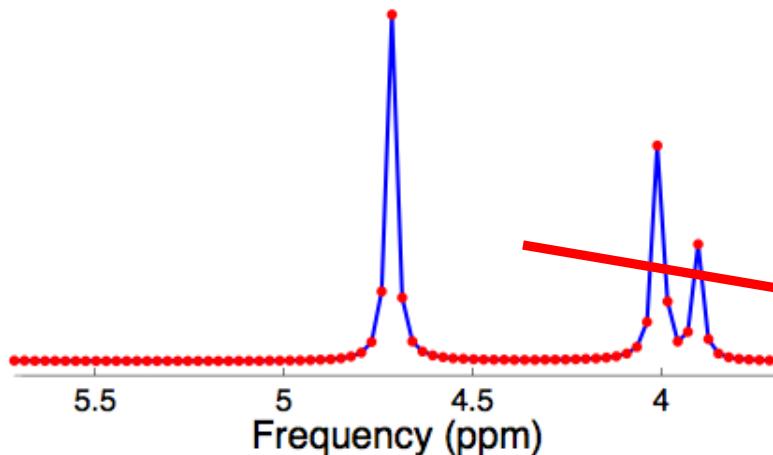
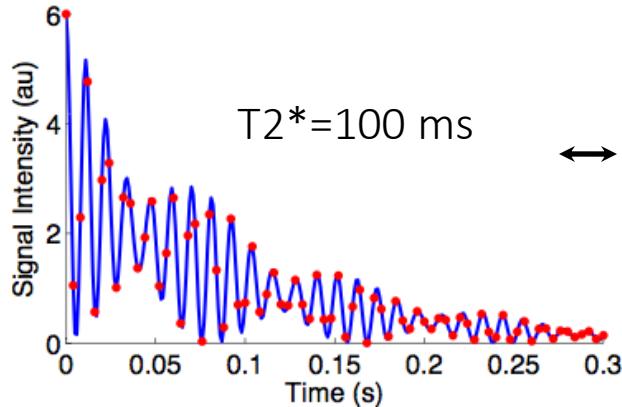
Linewidth
 $s^{-1} = 3.2 \text{ Hz}$



Linewidth
 $s^{-1} = 9.6 \text{ Hz}$



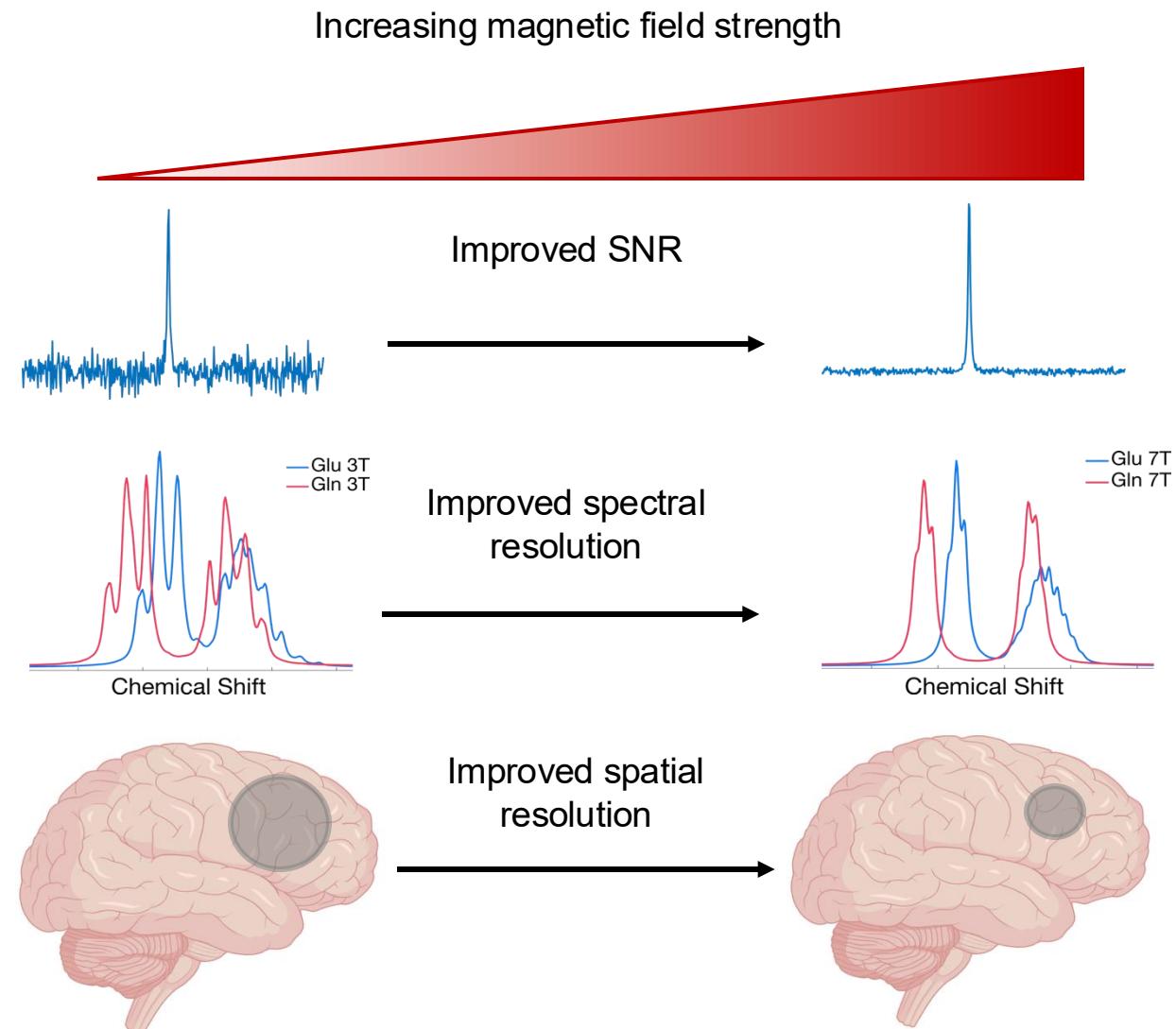
Spectral Linewidth



Jason P. Stockmann, Lawrence L. Wald, In vivo B_0 field shimming methods for MRI at 7T, NeuroImage

MRS and field strength

- Higher magnetic field strength has the following advantages:
 - Improved SNR
 - Improved spectral resolution; better separation of neighbouring peaks (i.e. Glutamate / Glutamine)
 - Improved spatial resolution and/or temporal resolution
 - Enables detection of more metabolites with better precision



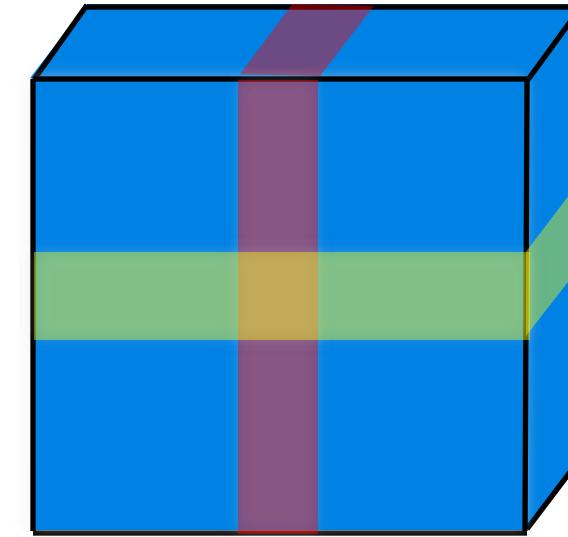
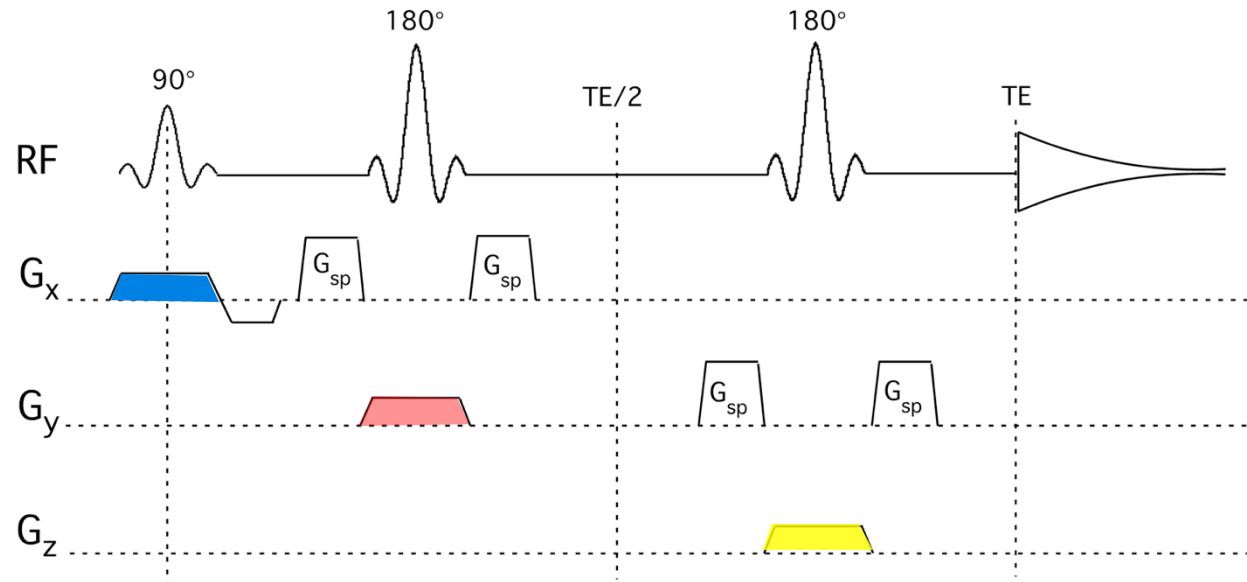
Localization pulse sequences

Localization

- So far, we have no spatial information: Spectral information will come from everywhere!
- It is more useful to get spectral information from a specific region of interest
- Localized spectroscopy enables the observation of spectral information from a specific region/voxel
- Two main types of localization are typically used:
 - Single Voxel Spectroscopy (PRESS, STEAM, SPECIAL, etc.)
 - Magnetic resonance spectroscopic imaging (MRSI/CSI)

Single-voxel spectroscopy (PRESS)

Point RESolved Spectroscopy (PRESS)



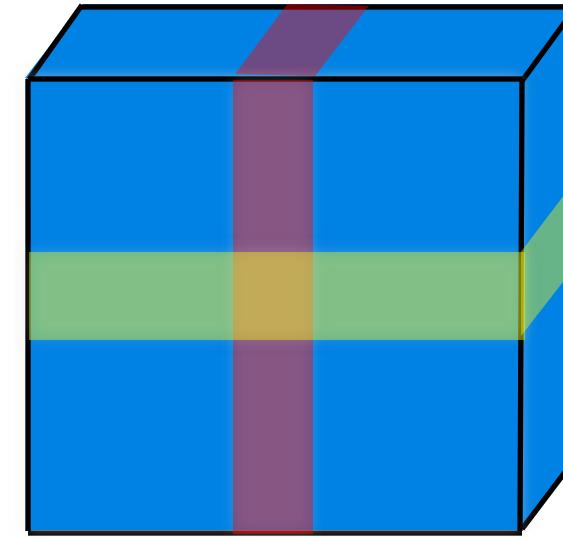
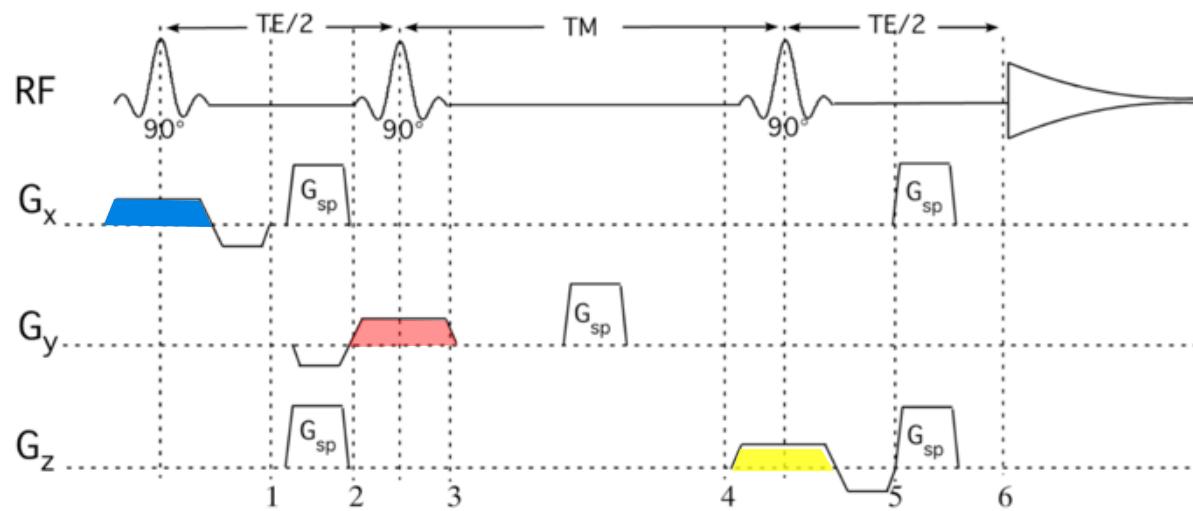
Double spin-echo sequence consisting of three slice selective pulses (90° , 180° , 180°) in three orthogonal planes

Signal comes from the intersection of the three planes (the voxel)

T2 decay occurs during TE

Single-voxel spectroscopy (STEAM)

STimulatEd Acquisition Mode (STEAM)



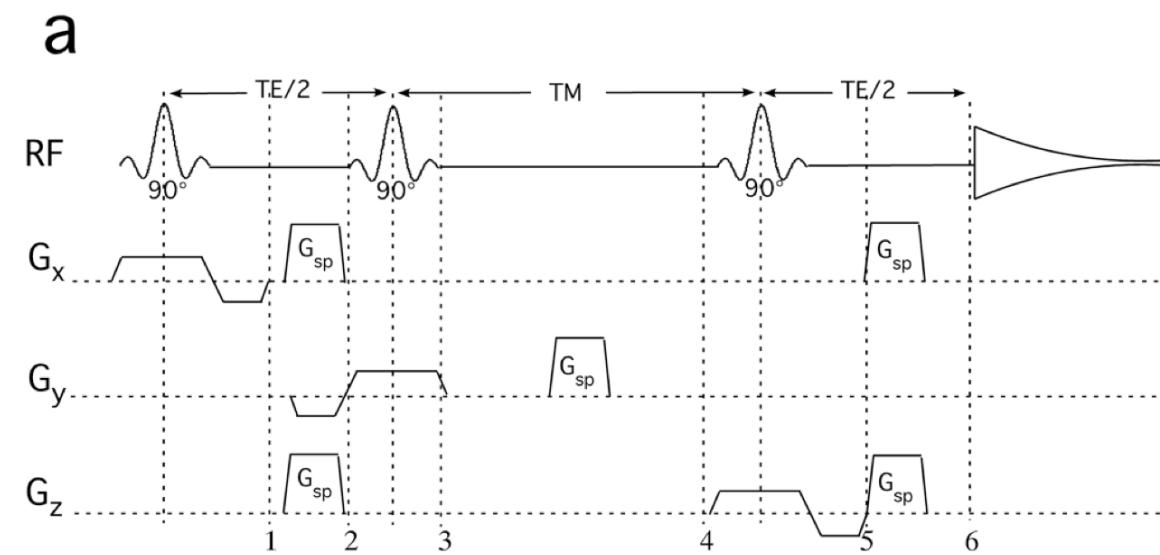
Three orthogonally slice selective 90° pulses

Again, signal comes from the intersection of the three planes (the voxel)

T2 decay occurs during TE, but not during the mixing time (TM)

STEAM: Stimulated Echoes

- Three 90° pulses combine to create a “stimulated echo”.
- Compared with PRESS:
 - Stimulated echo amplitude is only half the size of a true PRESS spin-echo (50% less SNR than PRESS)
 - T2-decay does not occur during TM (STEAM enables shorter TEs)
 - Significantly lower SAR compared to PRESS

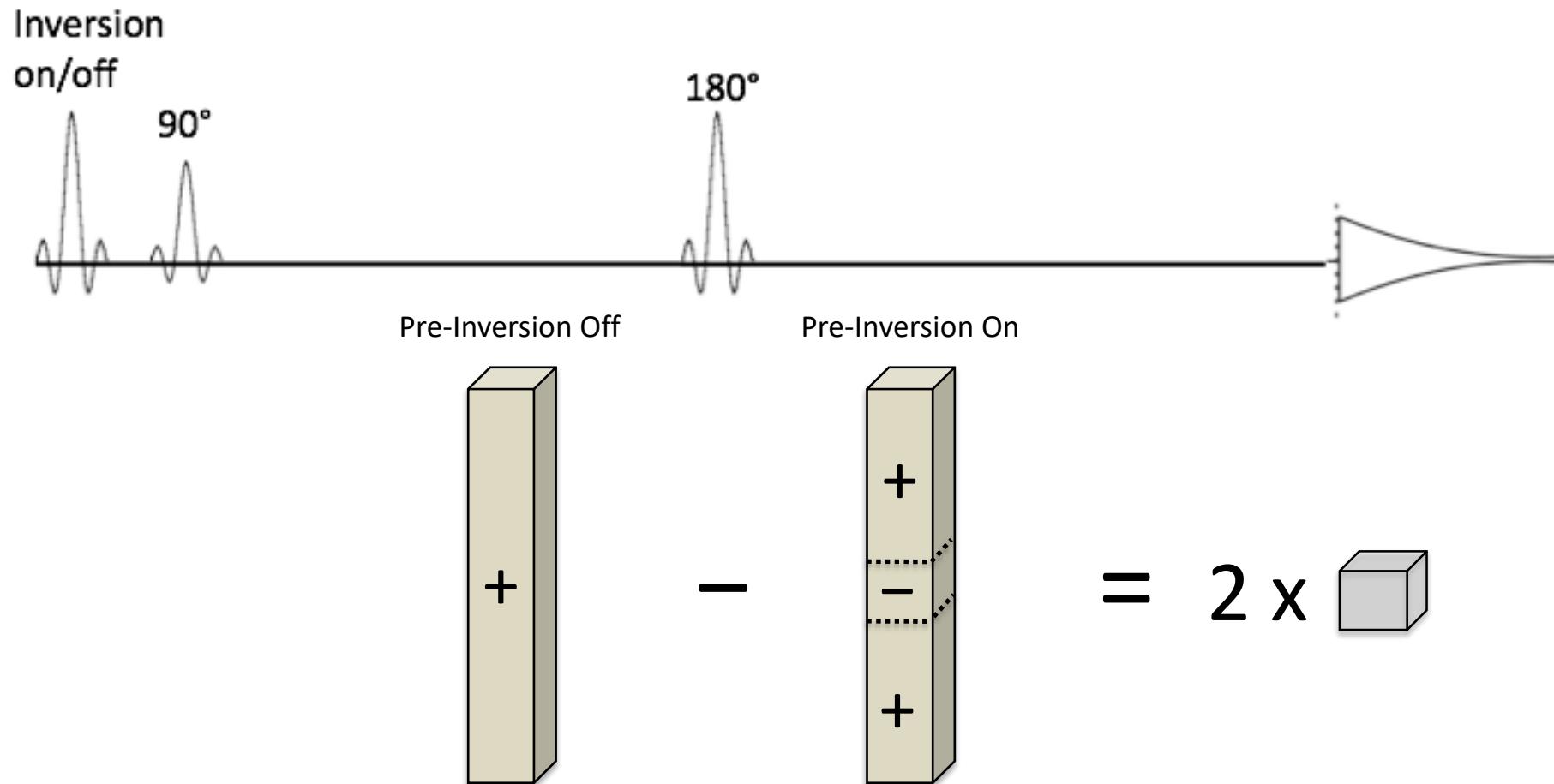


PRESS vs. STEAM

- PRESS
 - PROS: High SNR
 - CONS: High SAR, short TEs not possible
- STEAM
 - PROS: Low SAR, Short echo-times possible
 - CONS: Low SNR
- Is there a pulse sequence that provides both high SNR and short echo-times.... SPECIAL sequence.

SPECIAL sequence

- SPin ECho full Intensity Acquired Localized spectroscopy (SPECIAL, Mlynarik et al, 2006).
- Consists of a single spin echo, which excites a column of tissue.
- A slice selective inversion pulse is applied on odd acquisitions only.
- Localized spectrum is obtained by subtracting even and odd scans



SPECIAL sequence

- PROS:
 - Single spin echo enables shorter echo times compared to PRESS (comparable to STEAM)
 - Spin echo results in full intensity acquisition (two-fold increase over STEAM)
- CONS:
 - Subtraction scheme results in more motion sensitivity than either PRESS or STEAM

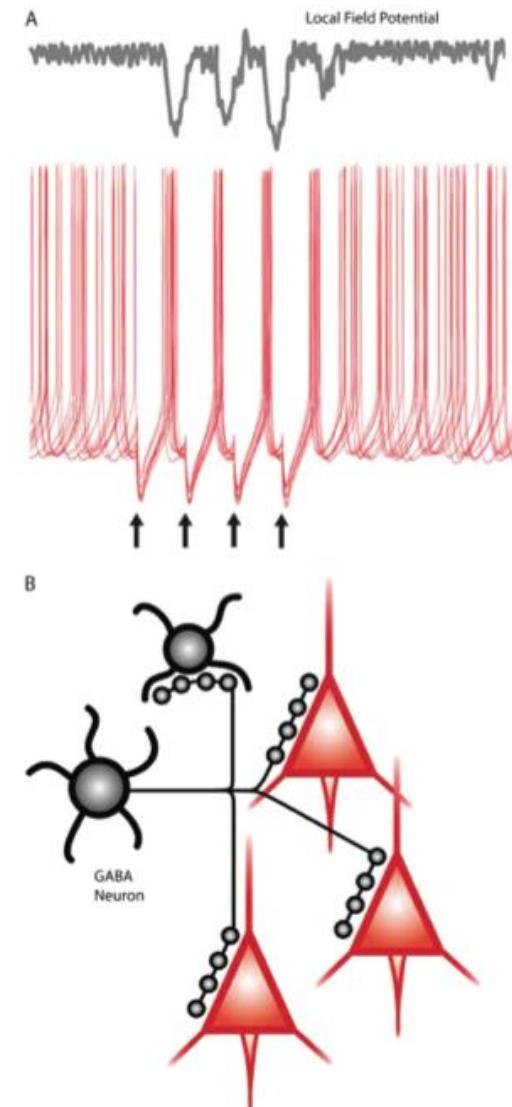
Spectral Editing (MEGA-PRESS)

Targeting Specific Compounds

- Certain metabolites may not be detectable using standard MRS methods.
- In these cases, it is necessary to implement tailored MRS acquisitions for detection.
- Examples include:
 - GABA
 - 2-Hydroxyglutarate
 - Glutathione
 - Lactate

GABA Background

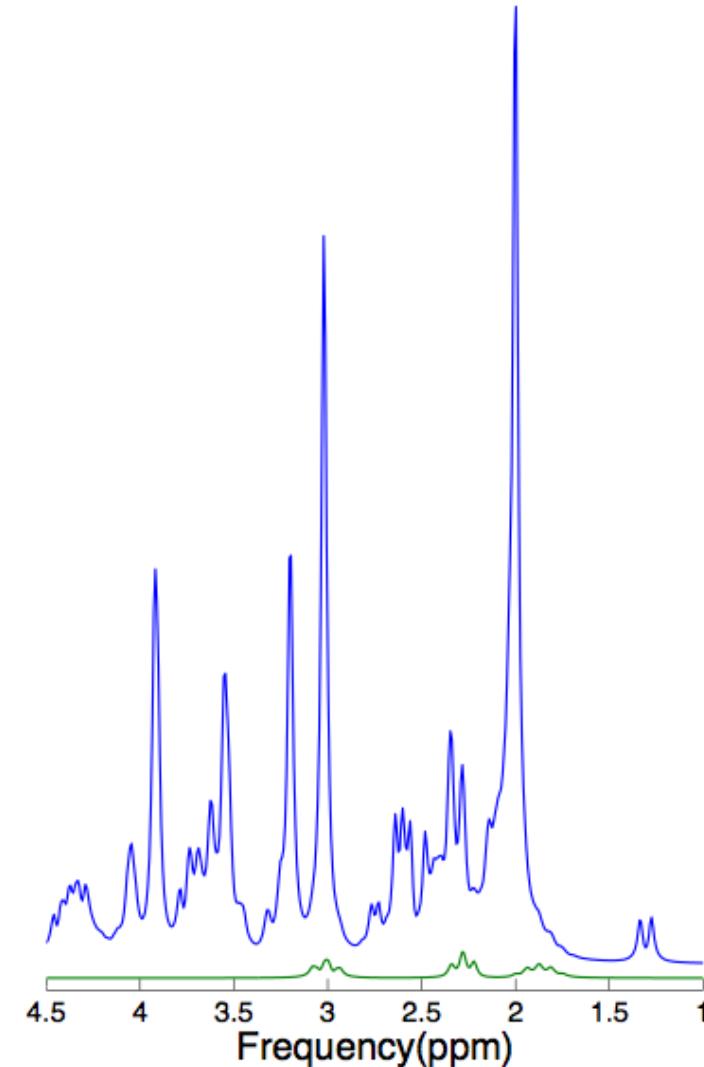
- GABA is the main inhibitory neurotransmitter in the adult brain
- Involved in regulating neuronal excitability and neuronal activity
- A single GABAergic interneuron can influence many excitatory pyramidal cells. As a result, GABAergic cells are responsible for coordinating the synchronous firing of large populations of excitatory neurons.
- Abnormal levels of GABA in the brain have been reported in various conditions, including epilepsy, schizophrenia and depression



Measuring GABA

Total Spectrum
GABA

- Recall: GABA is difficult to measure because:
 - Low concentration (1-2 mM in brain)
 - Overlapping with much larger resonances (Creatine, Glutamate and NAA)

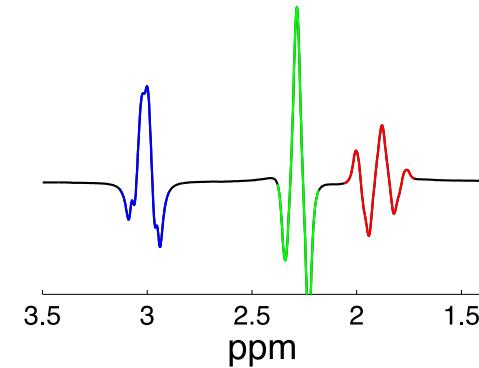


J-Difference Editing of GABA

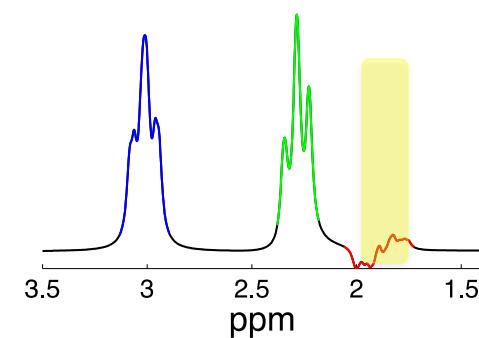
- Step 1: Acquire one spectrum by choosing the echo time such that the peaks are inverted.
- Step 2: Acquire a second spectrum using a pair of “editing pulses” applied at the frequency of the C3 protons (1.9 ppm). This has the effect of refocusing the scalar signal of the coupling partners.
- Edited spectrum is obtained by subtraction (2-1). Residual signal after subtraction is called a “difference signal”



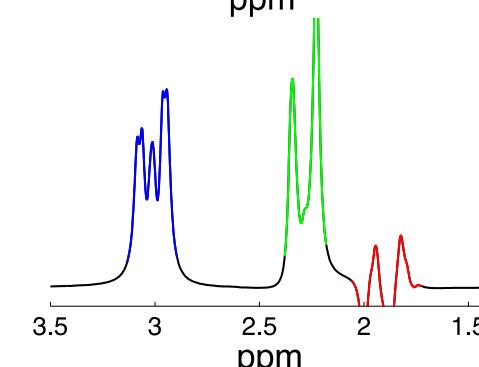
EDIT OFF



EDIT ON

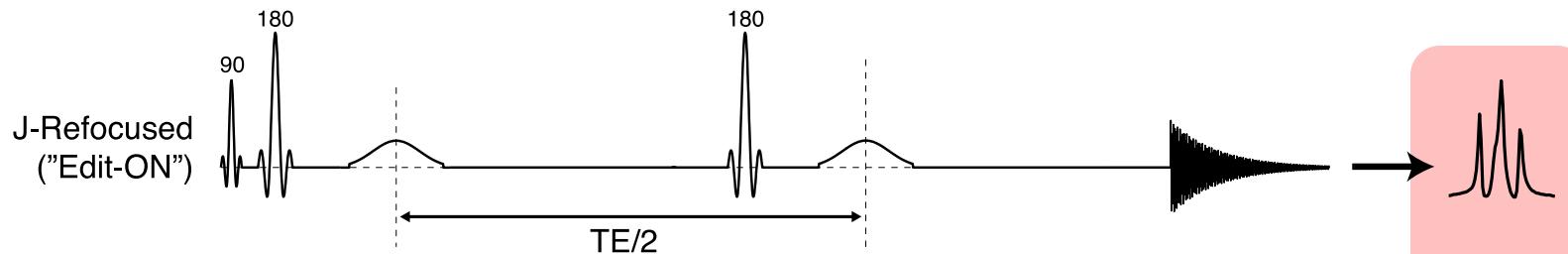


Difference

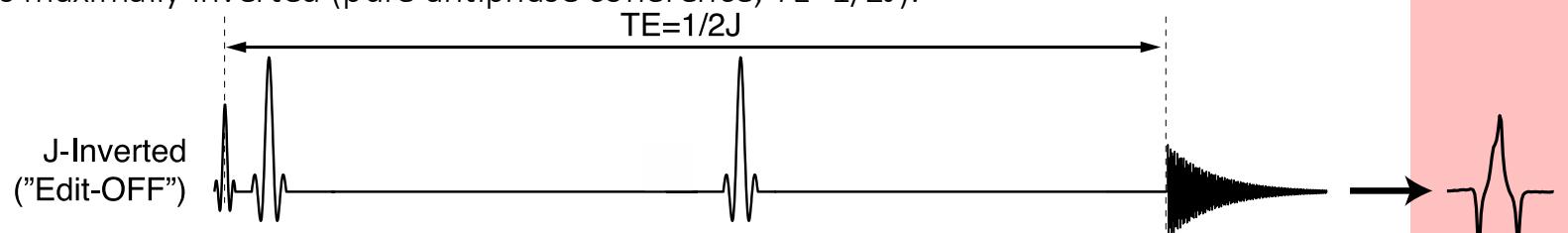


MEGA-PRESS

- Consists of an “edit-ON” and an “edit-OFF” scan.
- In the “edit-ON” scan, the spacing between editing pulses is set to $TE/2$, resulting in full J-refocusing (pure in-phase coherence).



- In the “edit-off” scan, no editing pulses are applied. TE is chosen such that the GABA resonance is maximally inverted (pure antiphase coherence, $TE=1/2J$).

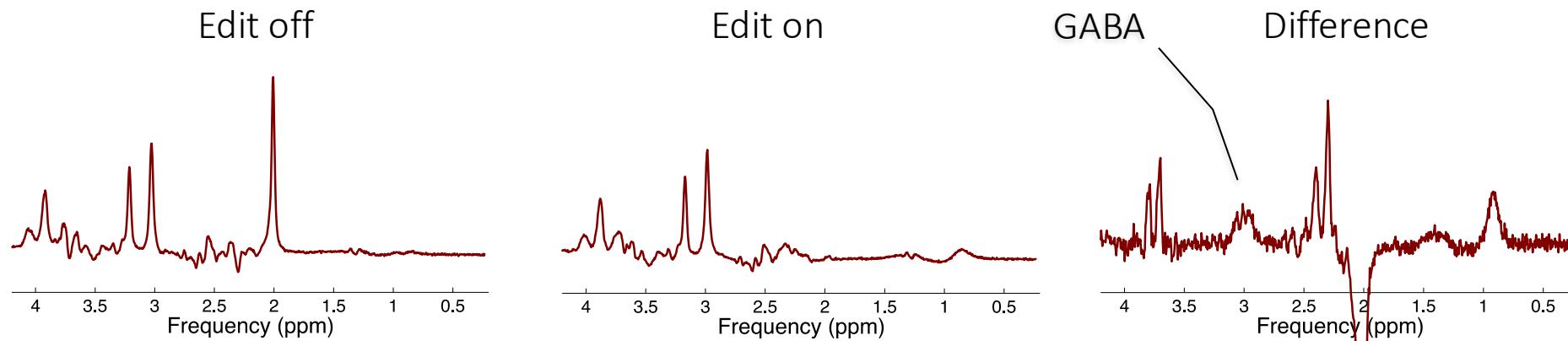


- Subtraction of an anti-phase from an in-phase coherence gives rise to the maximum difference signal.

$-$
 $=$
 $=$

Example MEGA-PRESS Data

- 3 x 3 x 2 cm voxel in human dorsolateral prefrontal cortex
- Acquisition time: 9 minutes

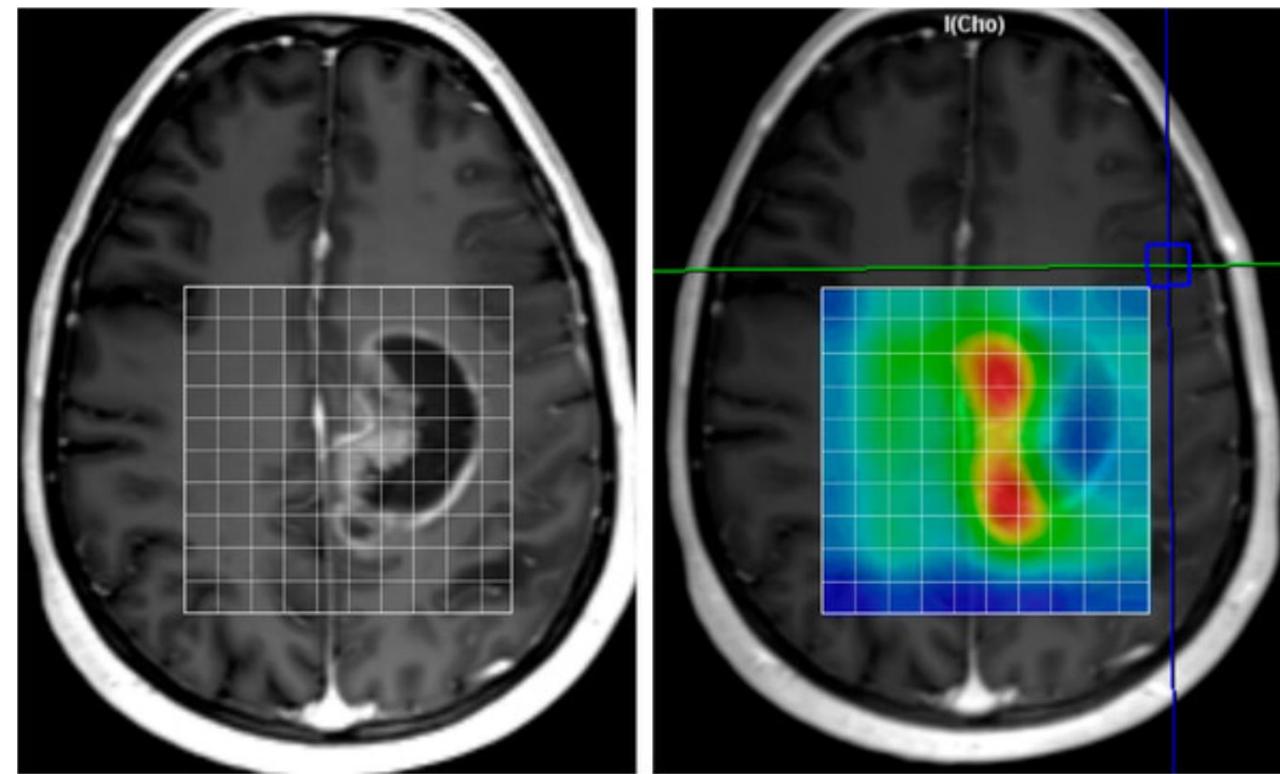


- A similar technique can be used to detect GSH, Lactate

Spectroscopic Imaging

With more advanced pulse sequences, we can spatially localize multiple spectra

- Can create metabolic maps
- See how metabolite vary spatially
- Potentially informing clinicians for treatment plans



Sitter B, Sjøbakk TE, Larsson HBW, Kvistad KA. Clinical MR spectroscopy of the brain. Tidsskr Nor Laegeforen. 2019 Mar 25;139(6). Norwegian, English. doi: 10.4045/tidsskr.17.1099. PMID: 30917645.

MRS Data Processing

What is processing?

- Steps taken to prepare/improve data
 - After data collection
 - Before data analysis/fitting

Why do processing?

- Experimental imperfections are unavoidable
- Postprocessing addresses these imperfections, resulting in:
 - Improved spectral quality (SNR, linewidth)
 - Improved quantification accuracy

Common processing operations

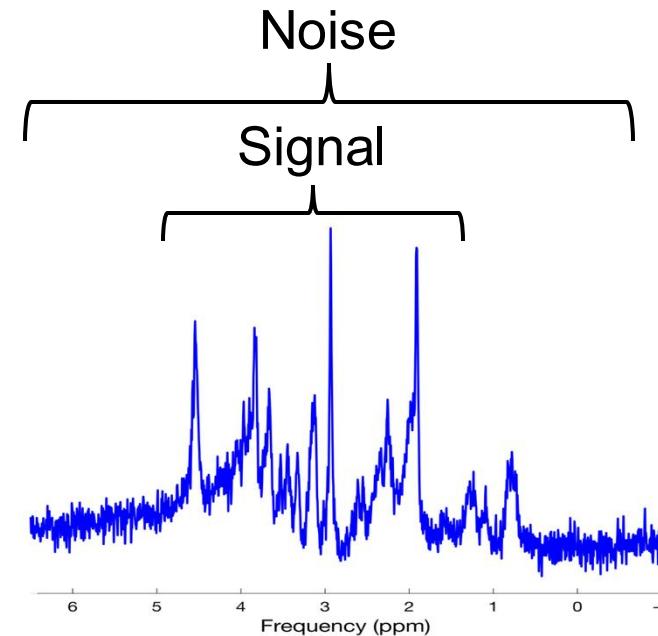
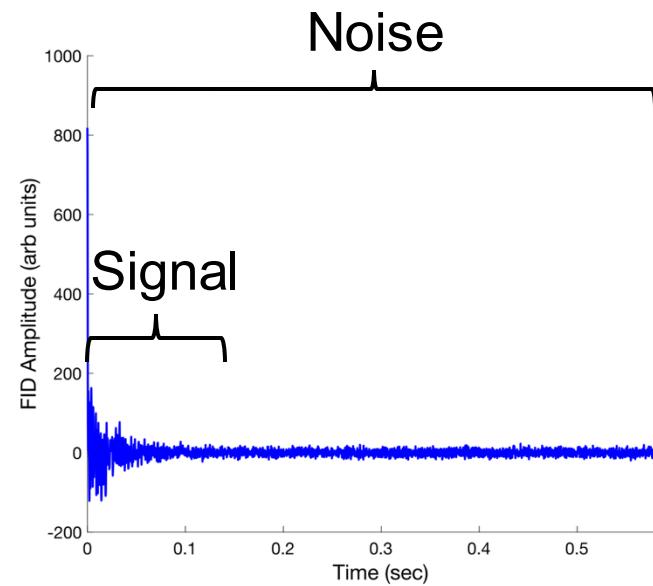
- Apodization
- Phase Correction (0^{th} and 1^{st} order)
- RF coil combination
- Drift correction
- Motion correction
- Eddy current correction
- Alignment of subtraction sub-spectra

Noise and SNR

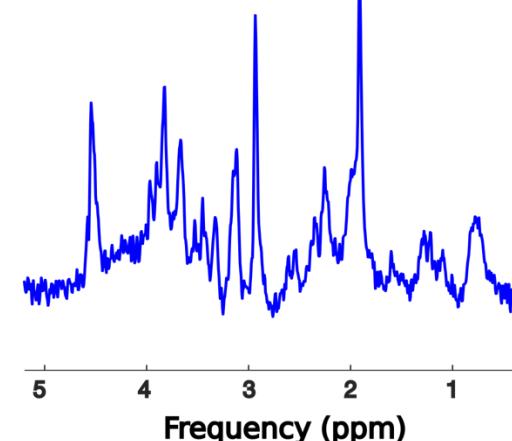
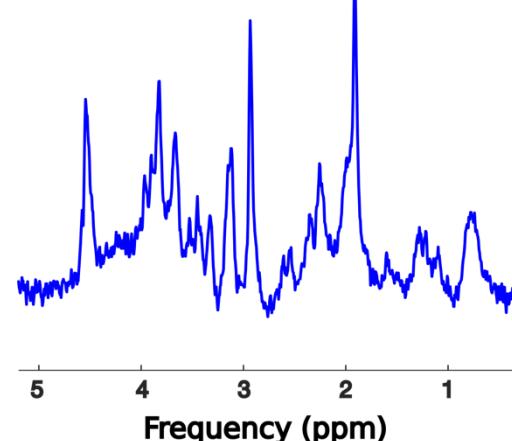
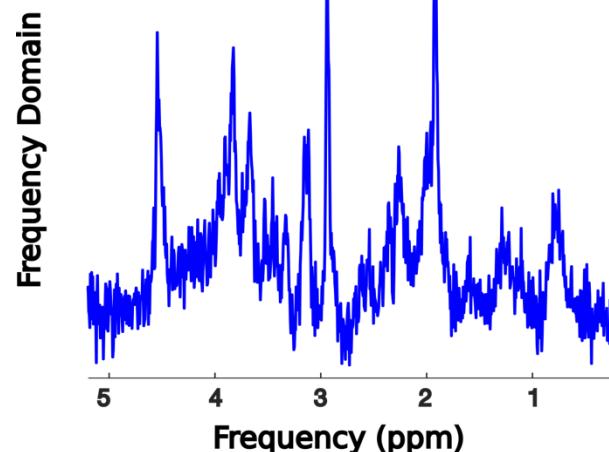
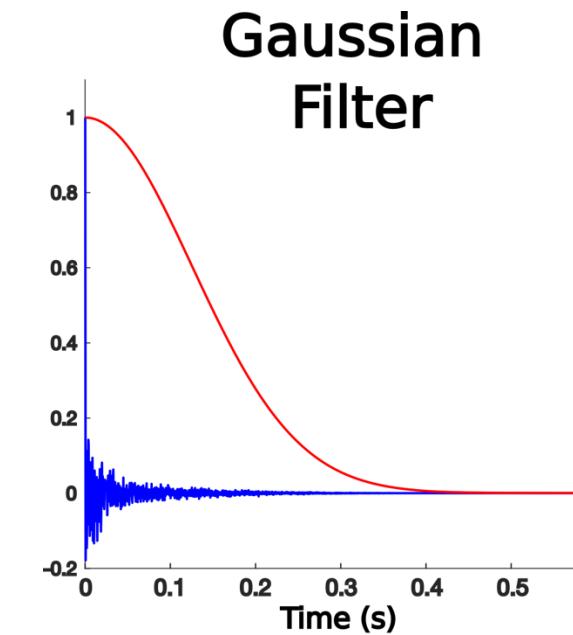
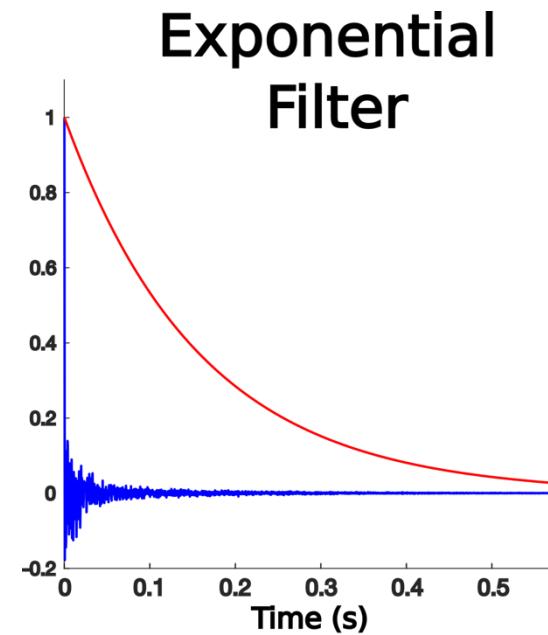
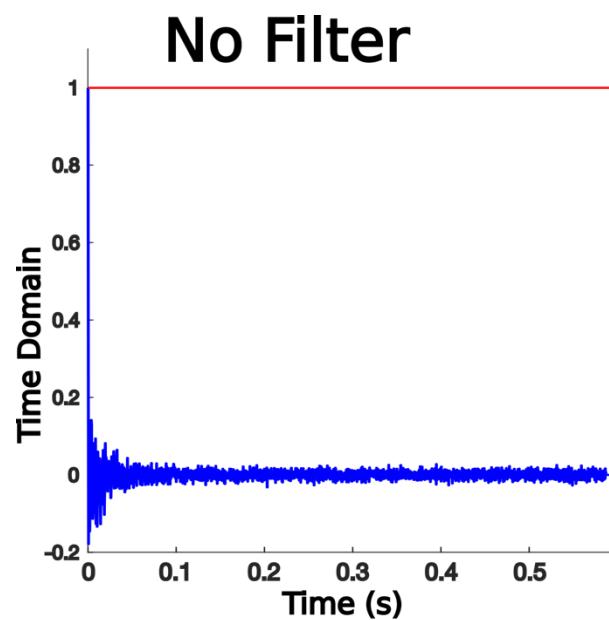
- Signal-to-noise ratio (SNR) is a key determinant of spectral quality
- $SNR = \frac{Signal}{noise}$
- Want signal to be ↑ and noise to be ↓
- Noise obscures signal. Impedes quantification.
- $SNR \propto V_{ROI} \cdot \sqrt{N_{avg}}$
- Increase SNR by:
 - Increasing voxel size ($\uparrow V_{ROI}$)
 - Increasing number of averages ($\uparrow N_{avg}$)

Noise

- Main sources of noise include:
 - RF coil / RF chain
 - Sample



Apodization



Common processing operations

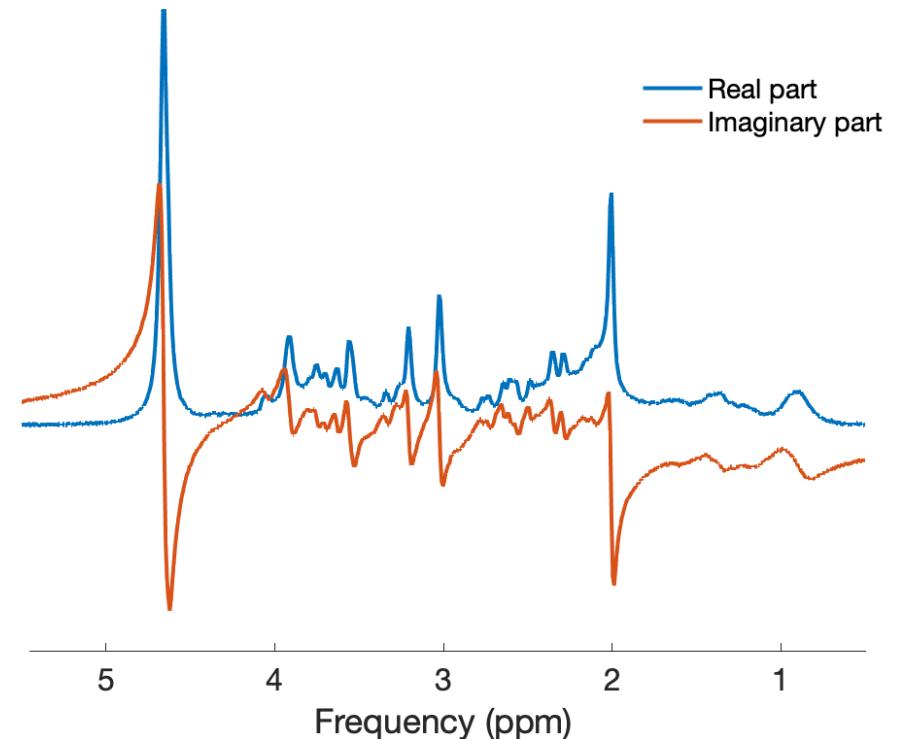
- Apodization
- Phase Correction (0^{th} and 1^{st} order)
- RF coil combination
- Drift correction
- Motion correction
- Eddy current correction
- Alignment of subtraction sub-spectra

Phase

- In MRS, the acquired signal is “complex”
 - i.e. each digital sample has a real and an imaginary part

$$S = s_{real} + i \cdot s_{imag}$$

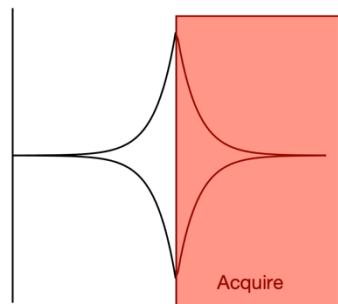
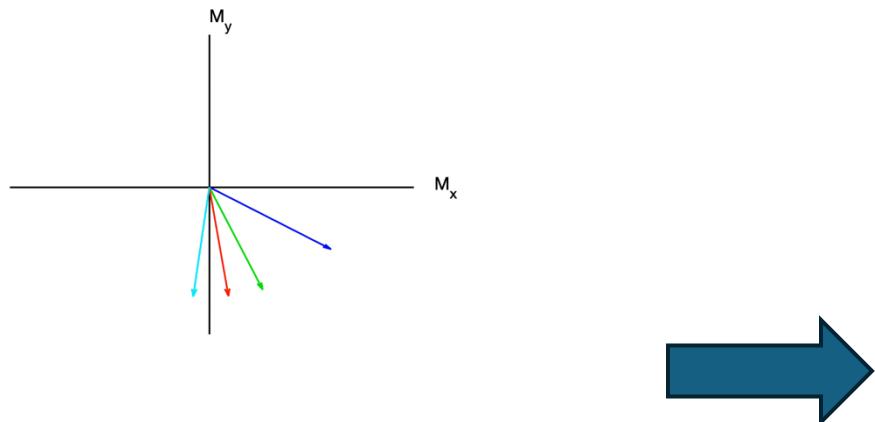
- s_{real} -> x-component of the transverse magnetization
- s_{imag} -> y-component of the transverse magnetization
- When viewing a spectrum, only the *real* part is plotted.
- When the *real* spectrum is upright, it is said to be “in phase”*



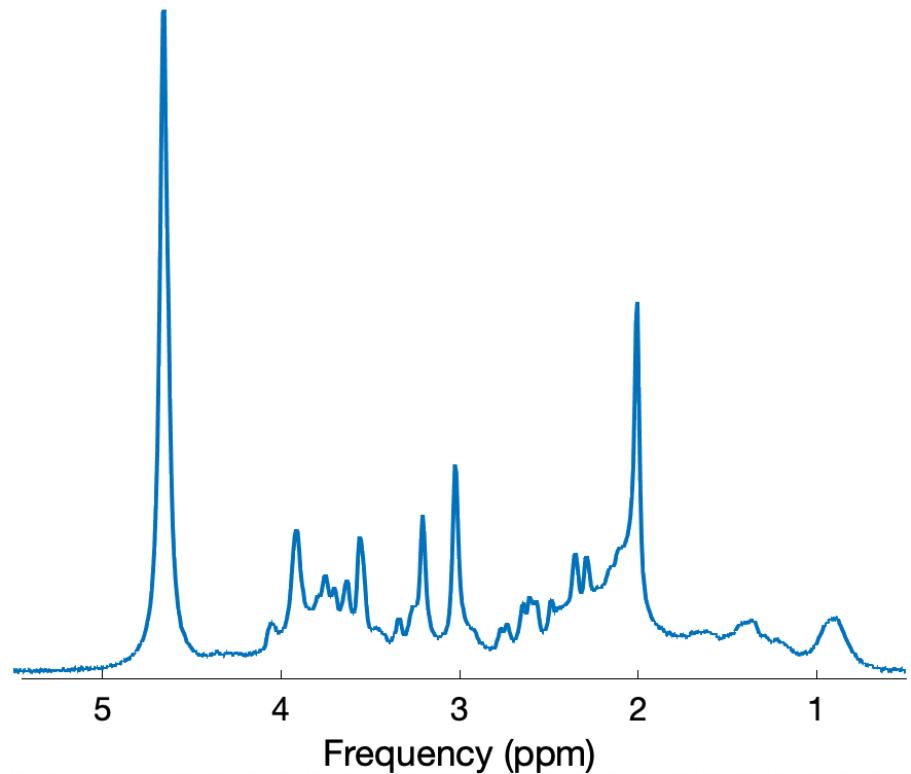
*Note: Due to subtraction, edited spectra may have inverted peaks when in-phase

zero-order Phase offsets

- Zero-order phase is related to the orientation of the spins at the beginning of the acquisition.



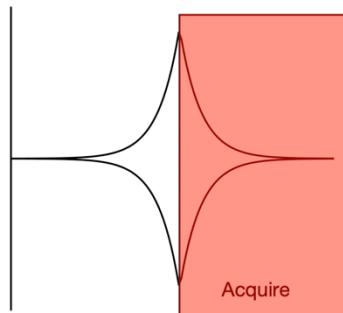
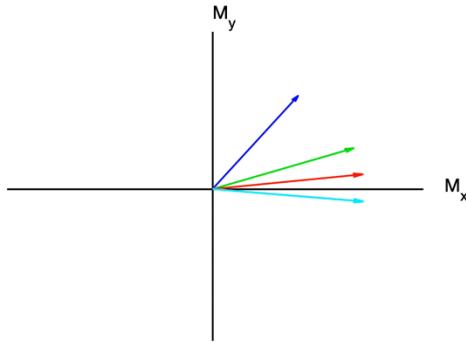
Spins on x-axis at acquisition start



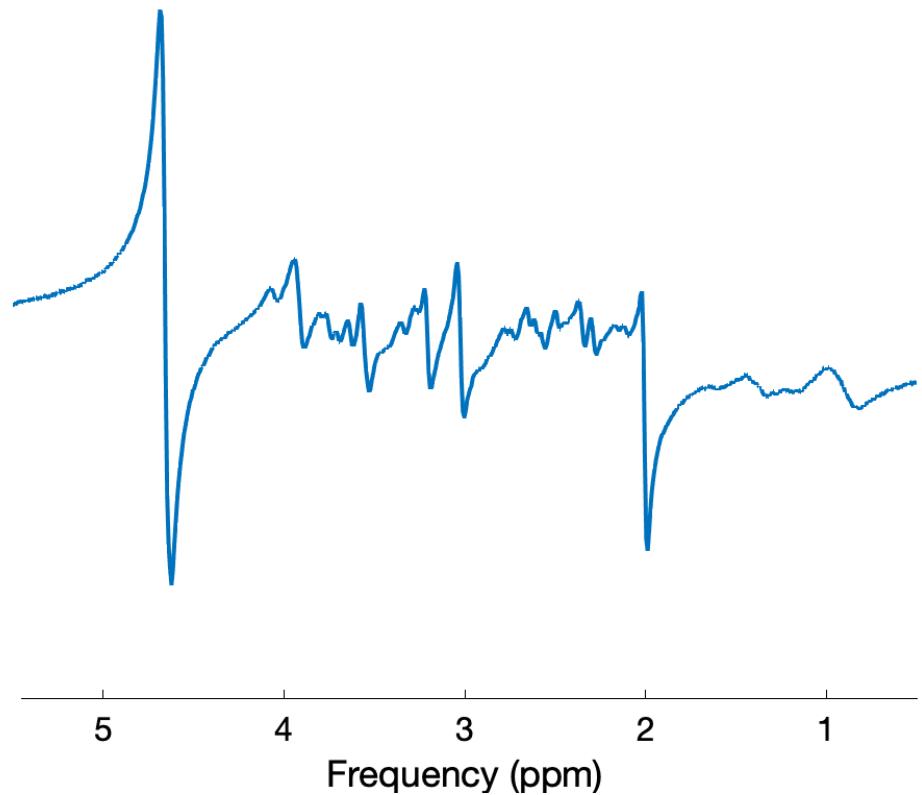
Spectrum in phase

zero-order Phase offsets

- Zero-order phase is related to the orientation of the spins at the beginning of the acquisition.



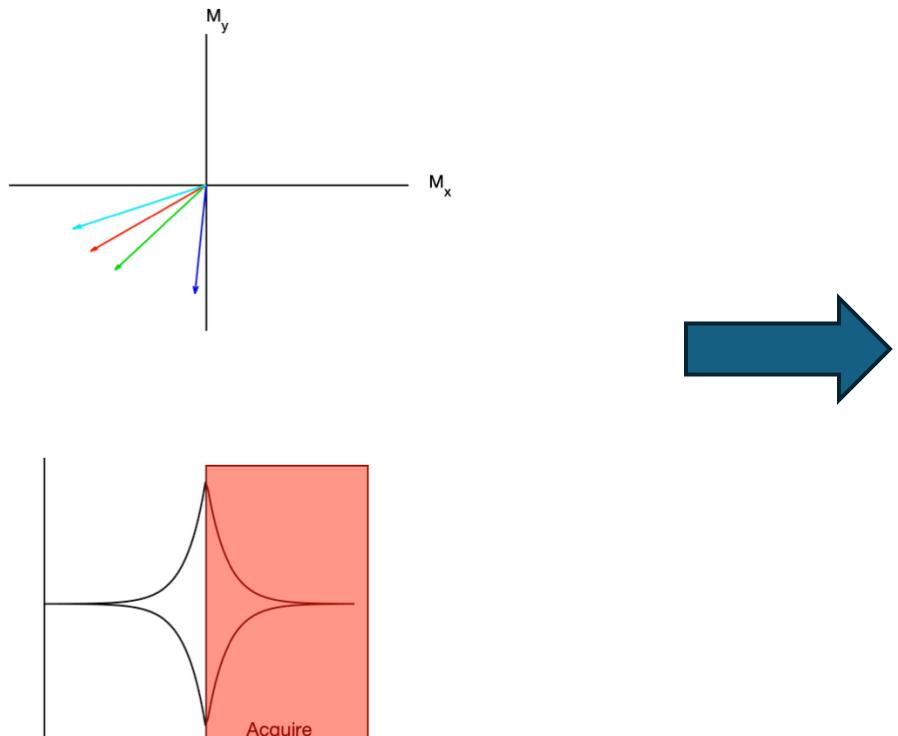
Spins on y-axis at acquisition start



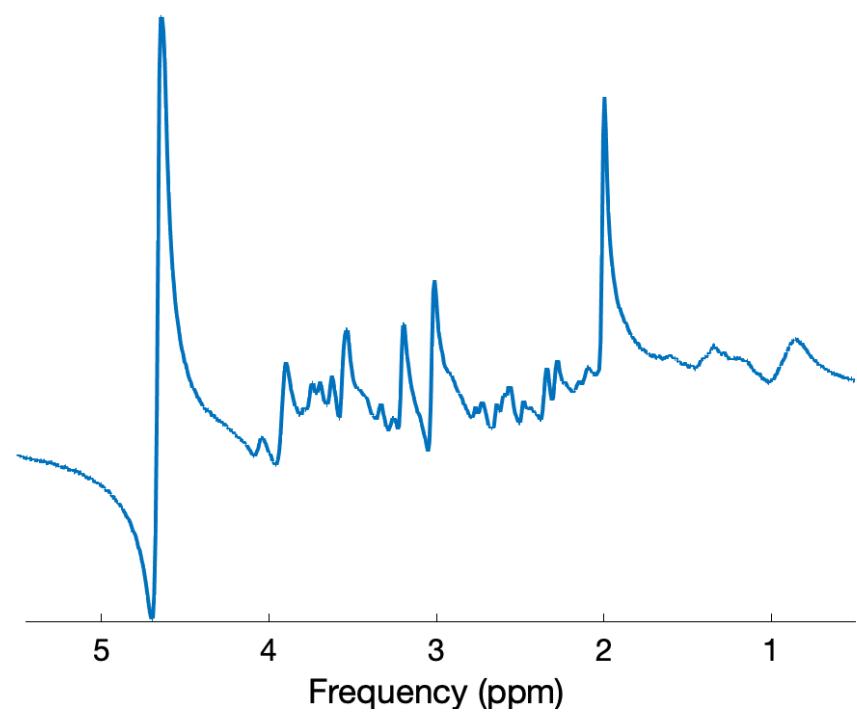
Spectrum 90° out of phase

zero-order Phase offsets

- Zero-order phase is related to the orientation of the spins at the beginning of the acquisition.



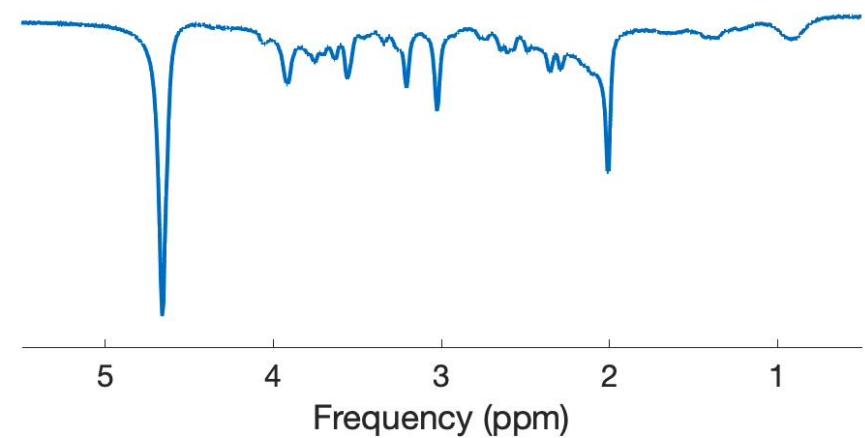
Spins at -60° at acquisition start



Spectrum -60° out of phase

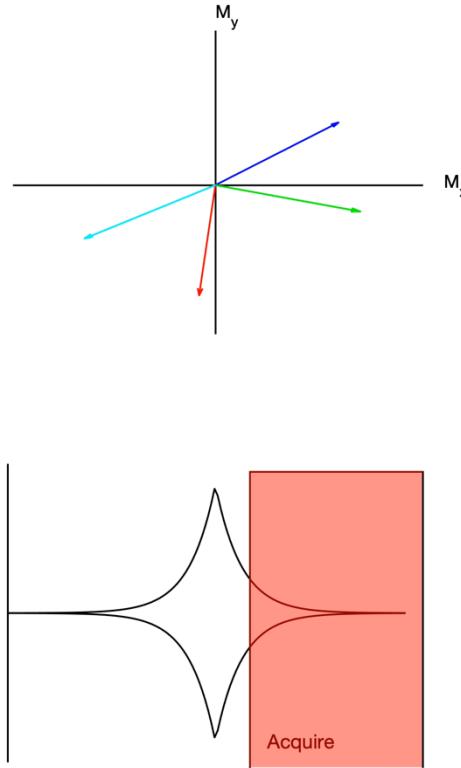
Zero-order phase Correction

- To correct a zero-order phase offset, apply a constant phase shift to every point in the spectrum until peaks are upright
- Can be done automatically, or manually
- Manual correction takes practice

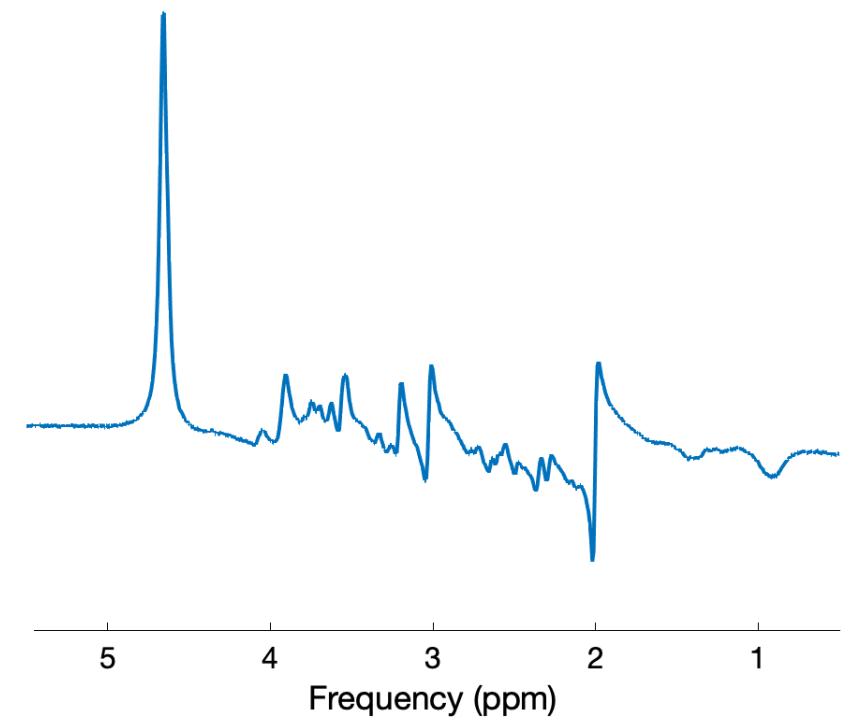


First-order Phase offsets

- First order phase offsets are related to a timing mis-match between the acquisition and the echo top.



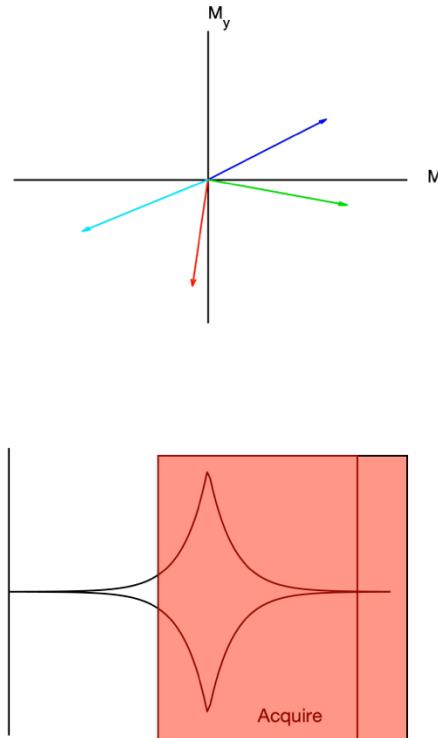
Acquisition starts late



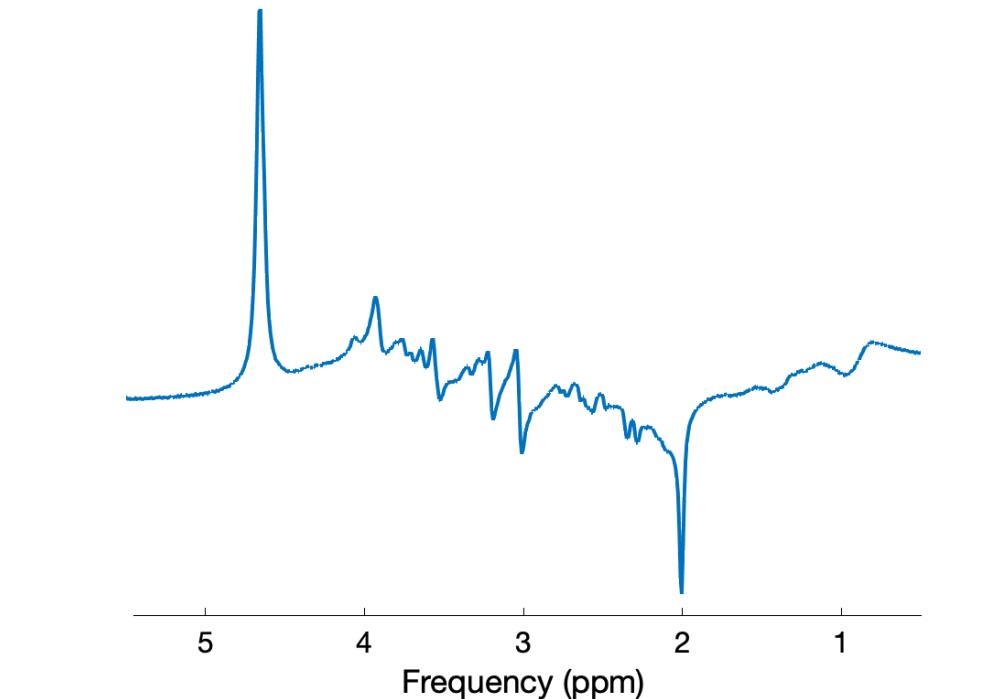
1st order phase offset

First-order Phase offsets

- First order phase offsets are related to a timing mis-match between the acquisition and the echo top.



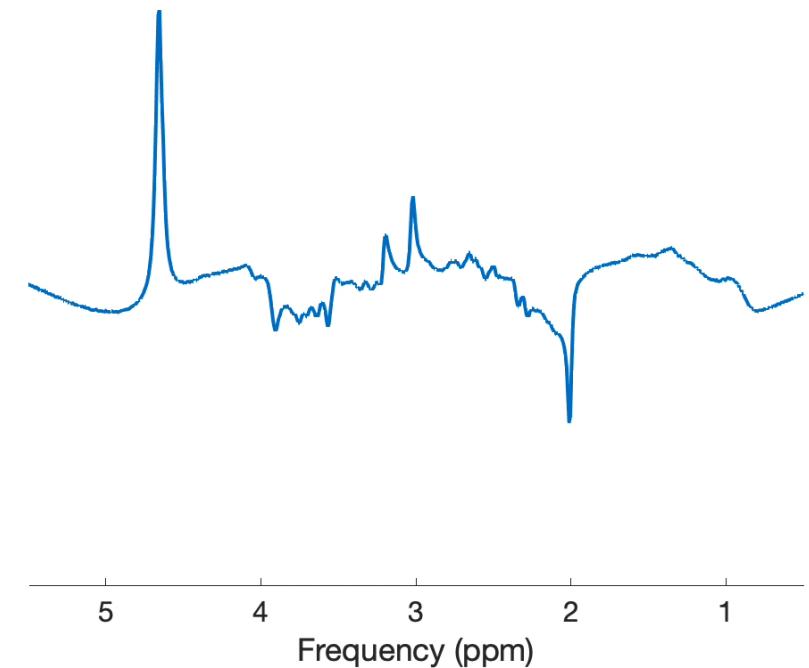
Acquisition starts early



1st order phase offset

first-order phase Correction

- To correct a first-order phase offset, apply a frequency-dependent phase shift to every point in the spectrum until peaks are upright
- If ADC was acquired early, remove ADC points from before FID
- Amount of 1st order phase shift is typically fixed for a given pulse sequence



Common processing operations

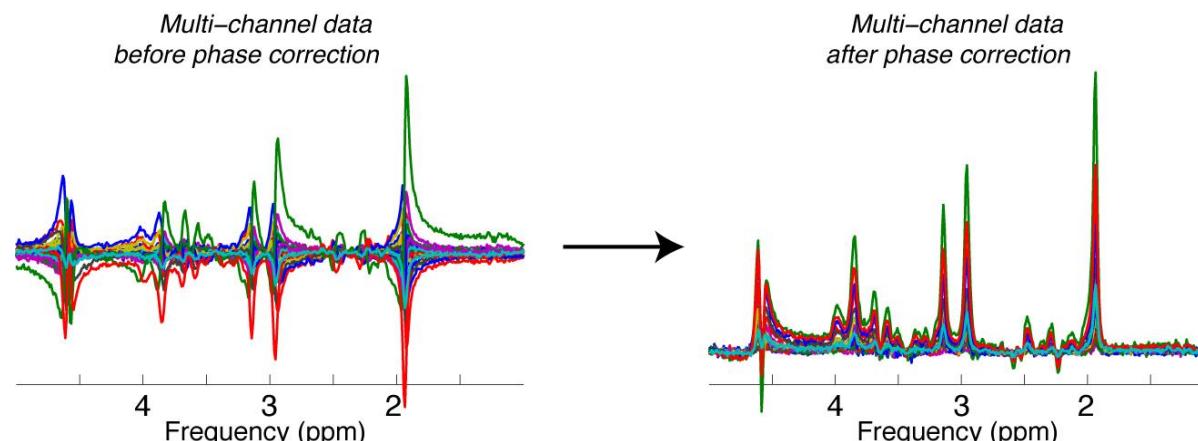
- Apodization
- Phase Correction (0^{th} and 1^{st} order)
- RF coil combination
- Drift correction
- Motion correction
- Eddy current correction
- Alignment of subtraction sub-spectra

Combining multiple RF channels

- Highly parallel RF arrays have become the standard in in vivo MRS.
- Depending on system/data format, RF channels may need to be combined offline by the user.
- Each channel has different signal and noise amplitudes, and phase offset.
- Need to be combined in a way that preserves SNR and data quality.

Combining multiple RF channels

- Combination is performed in 2 steps
 1. Zero-order phase correction on each channel
 2. Apply amplitude weighting to each channel
- Coil phases and weights are determined using the high-SNR water reference scan (1st point in FID).



Common processing operations

- Apodization
- Phase Correction (0^{th} and 1^{st} order)
- RF coil combination
- Drift correction
- Motion correction
- Eddy current correction
- Alignment of subtraction sub-spectra

B0 frequency drift (and phase drift)

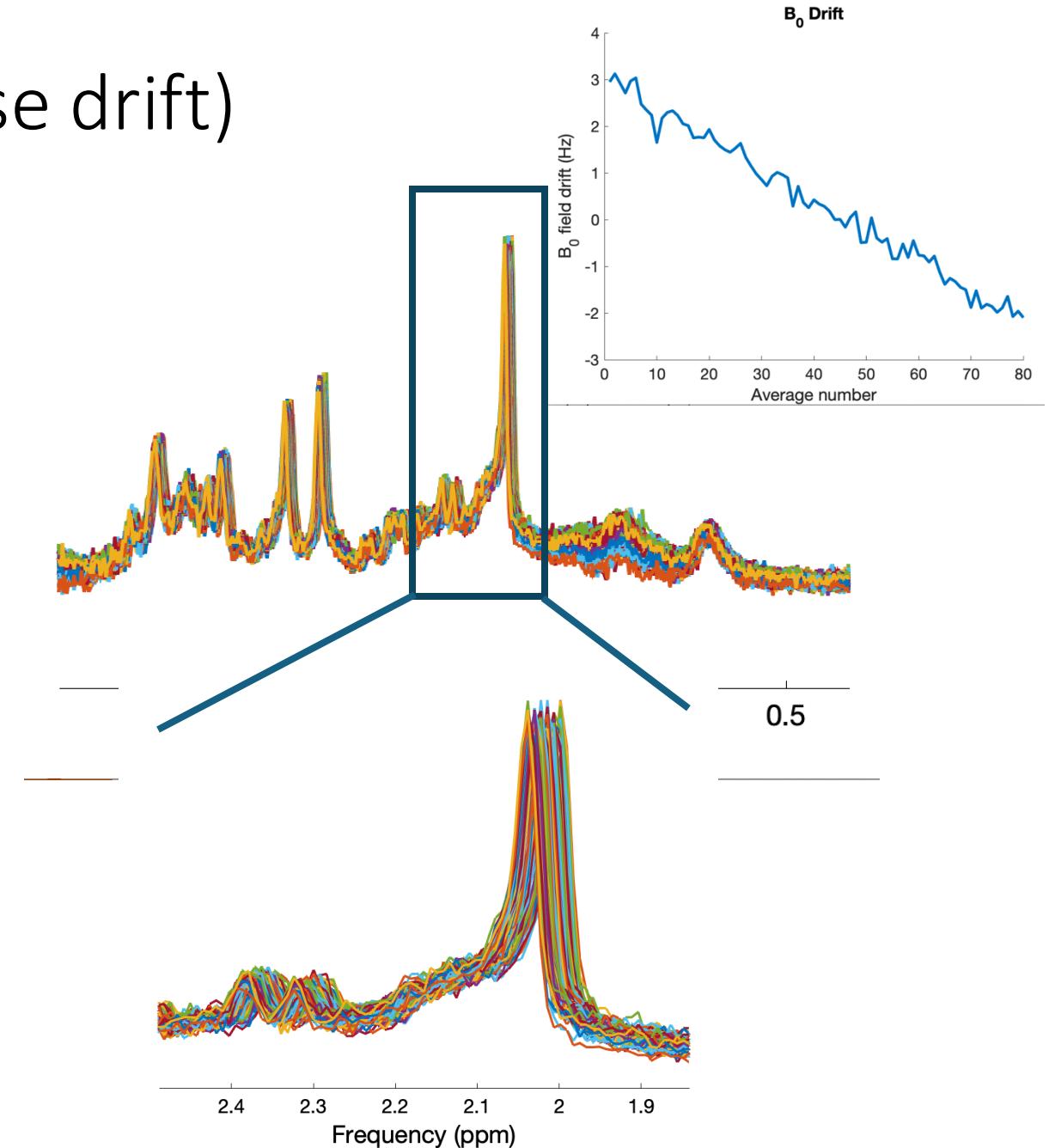
- In MRS, we typically acquire many signal ‘averages’ to increase SNR.
- Ideally, all averages should be identical for coherent signal averaging
- HOWEVER:
 - Scanner B0 field shifts slightly over time

$$\omega = \gamma B_0$$

- Thus, the frequency, ω , changes over time -> frequency drift
- What does this do to our spectrum?

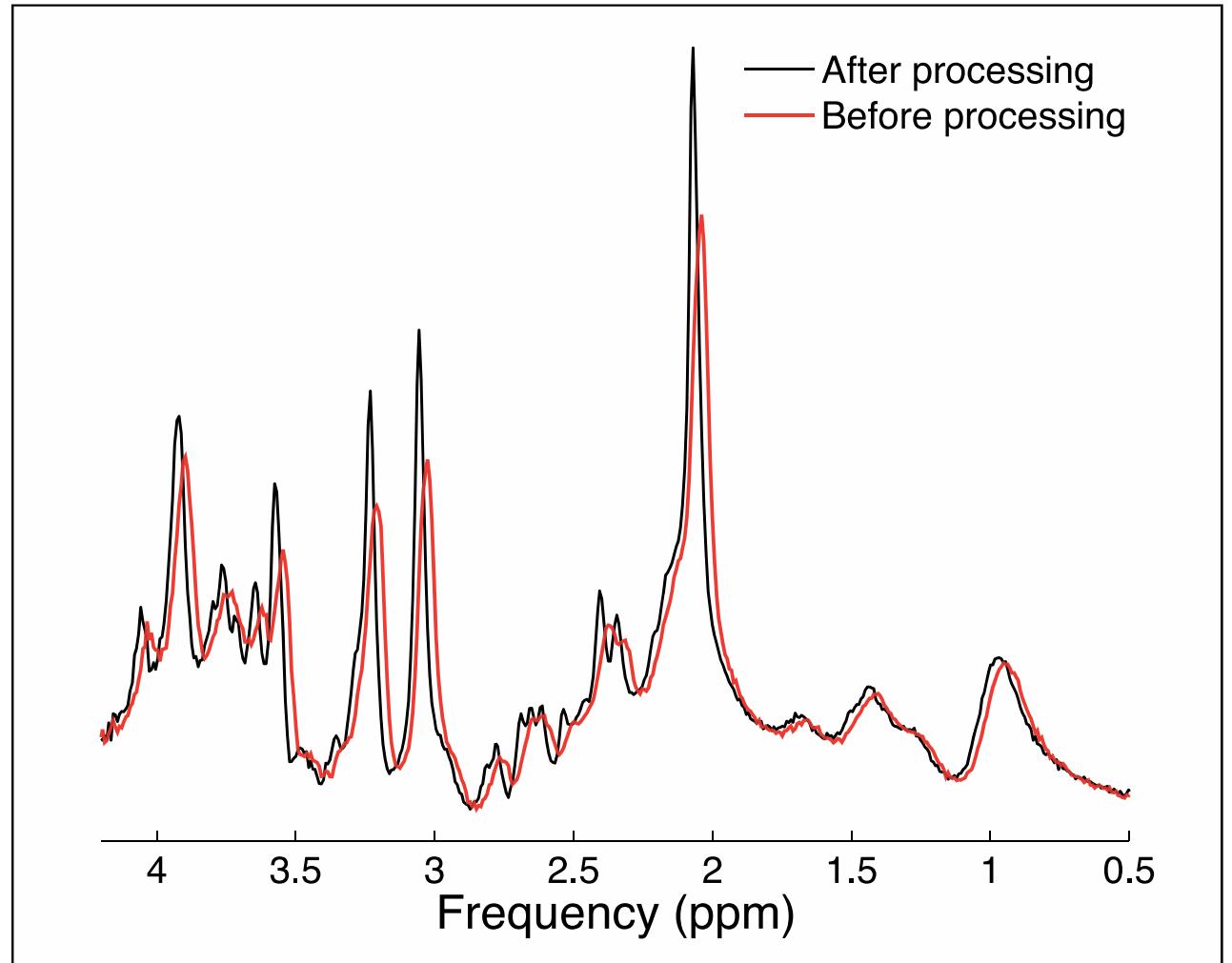
B0 frequency drift (and phase drift)

- Drift causes the signal averages to be shifted
- If combined without correction, this causes smearing/blurring of spectral peaks



Frequency and Phase Drift Correction

- Drift correction can be done retrospectively, provided individual averages are stored
- Several algorithms available
 - “Spectral registration” aligns each average to a reference (1st average in series)
- Can greatly improve spectral quality (linewidth and SNR)



Common processing operations

- Apodization
- Phase Correction (0^{th} and 1^{st} order)
- RF coil combination
- Drift correction
- Motion correction
- Eddy current correction
- Alignment of subtraction sub-spectra

Movement artifacts

Small subject movements:

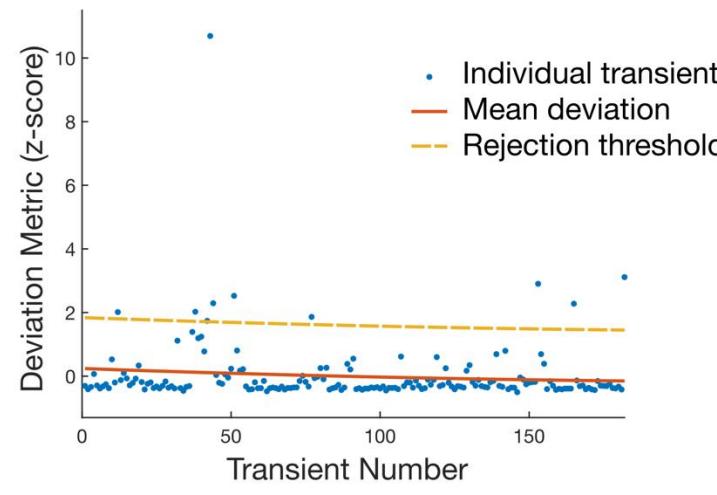
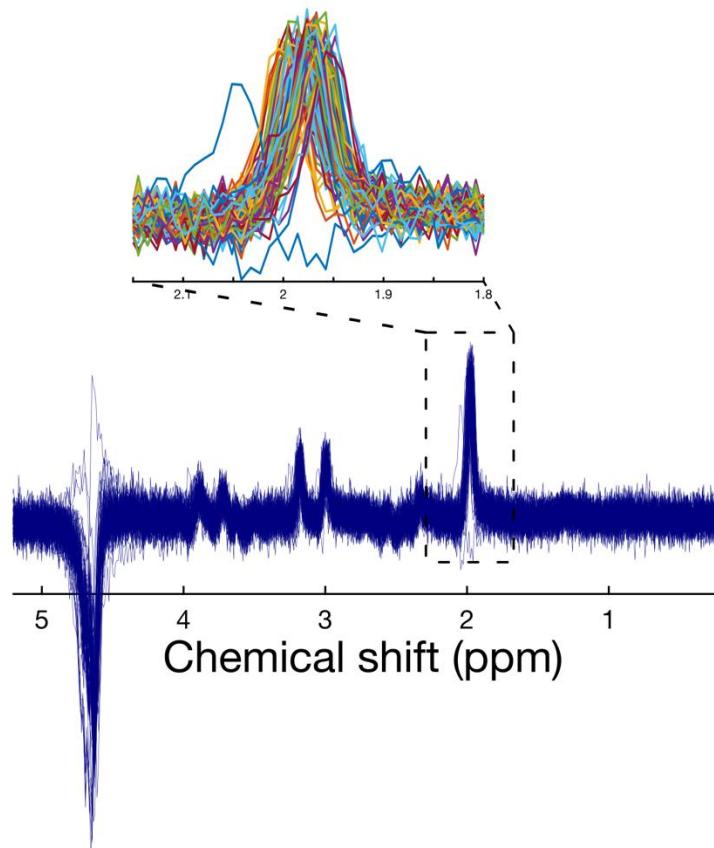
- i.e. normal physiological motion, breathing, cardiac pulsation, etc.
- causes small phase shifts over time.
- Can be corrected retrospectively using drift correction (see previous slides)

Large subject movements:

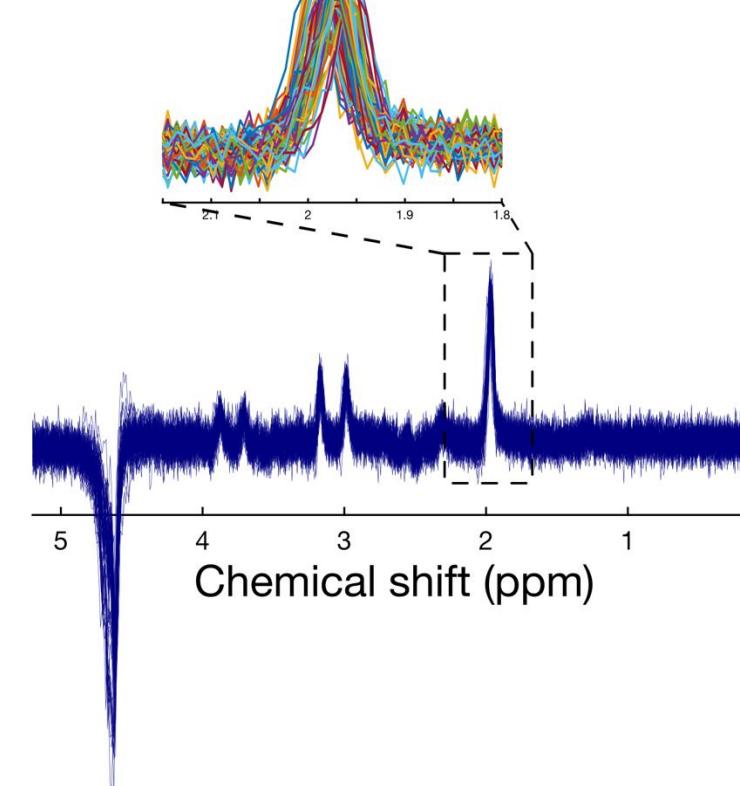
- i.e. patient moving head, sneezing, escaping scanner
- result in badly corrupted signal averages.
- Motion corrupted averages should be removed prior to signal averaging

Removing motion corrupted averages

1. Identify motion corrupted averages (ones that are different from the rest)
2. Remove them



Removal of motion
corrupted averages

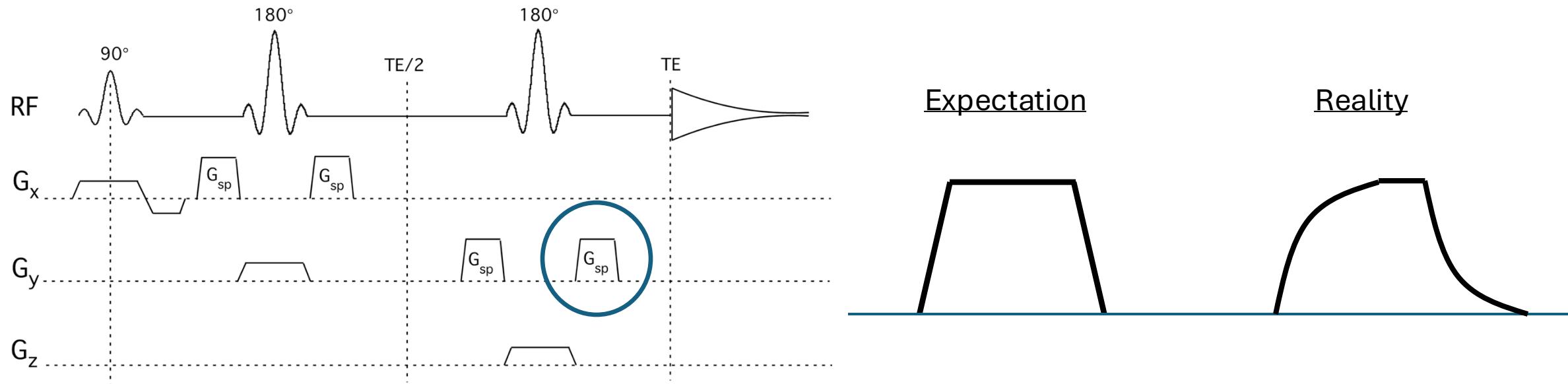


Common processing operations

- Apodization
- Phase Correction (0^{th} and 1^{st} order)
- RF coil combination
- Drift correction
- Motion correction
- Eddy current correction
- Alignment of subtraction sub-spectra

Eddy current effects

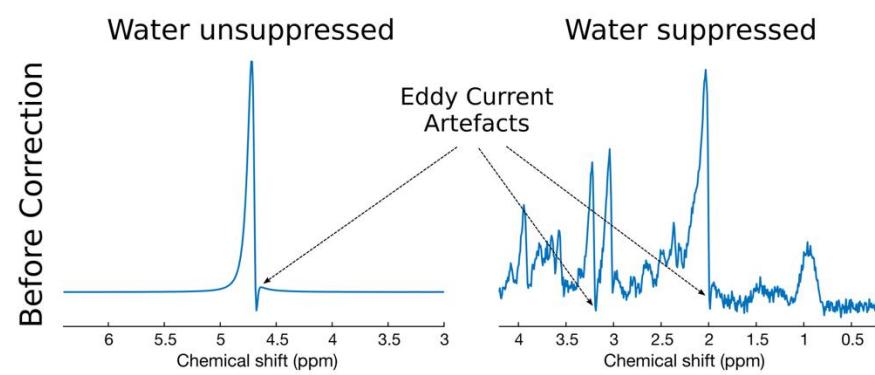
- MRS pulse sequences have pulsed magnetic field gradients:



- Rapid gradient switching gives rise to transient gradient fields that persist beyond the end of the gradient event.
- Causes *time varying B₀* during the FID

Eddy current Correction

- Time varying B_0 during the readout causes unwanted lineshape distortion
- Effect is visible in phase of water FID:
 - Phase should be linear
 - Eddy current effects cause wobbles in FID phase
- To correct: subtract corrected water phase from both the water reference FID and water-suppressed FID.



Common processing operations

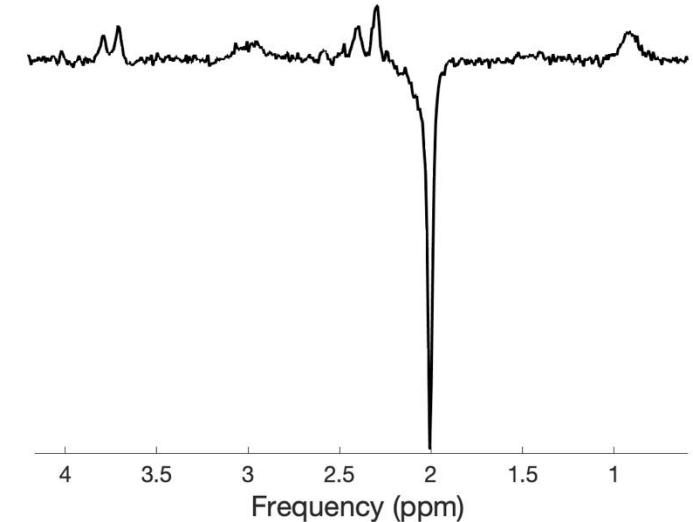
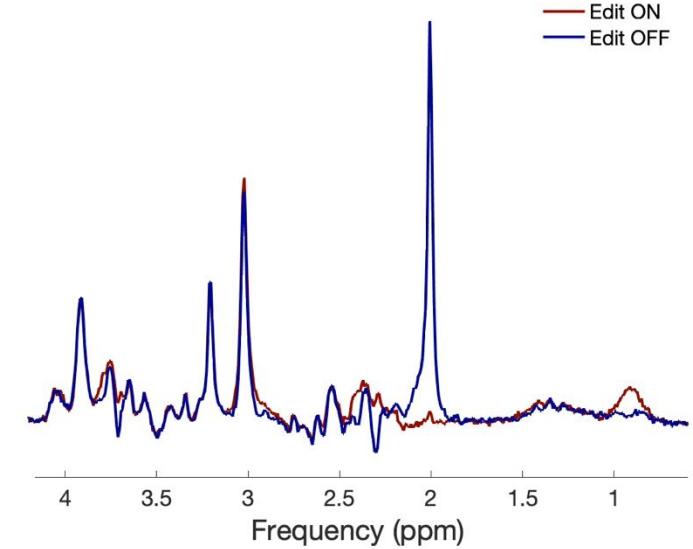
- Apodization
- Phase Correction (0^{th} and 1^{st} order)
- RF coil combination
- Drift correction
- Motion correction
- Eddy current correction
- Alignment of subtraction sub-spectra

Subtraction misalignment

- Some sequences involve the subtraction of two or more sub-spectra, to yield a ‘difference signal’.
- Examples include:
 - ISIS
 - MEGA-PRESS
 - SPECIAL
- Slight misalignment leads to unwanted residual peaks in difference spectrum.

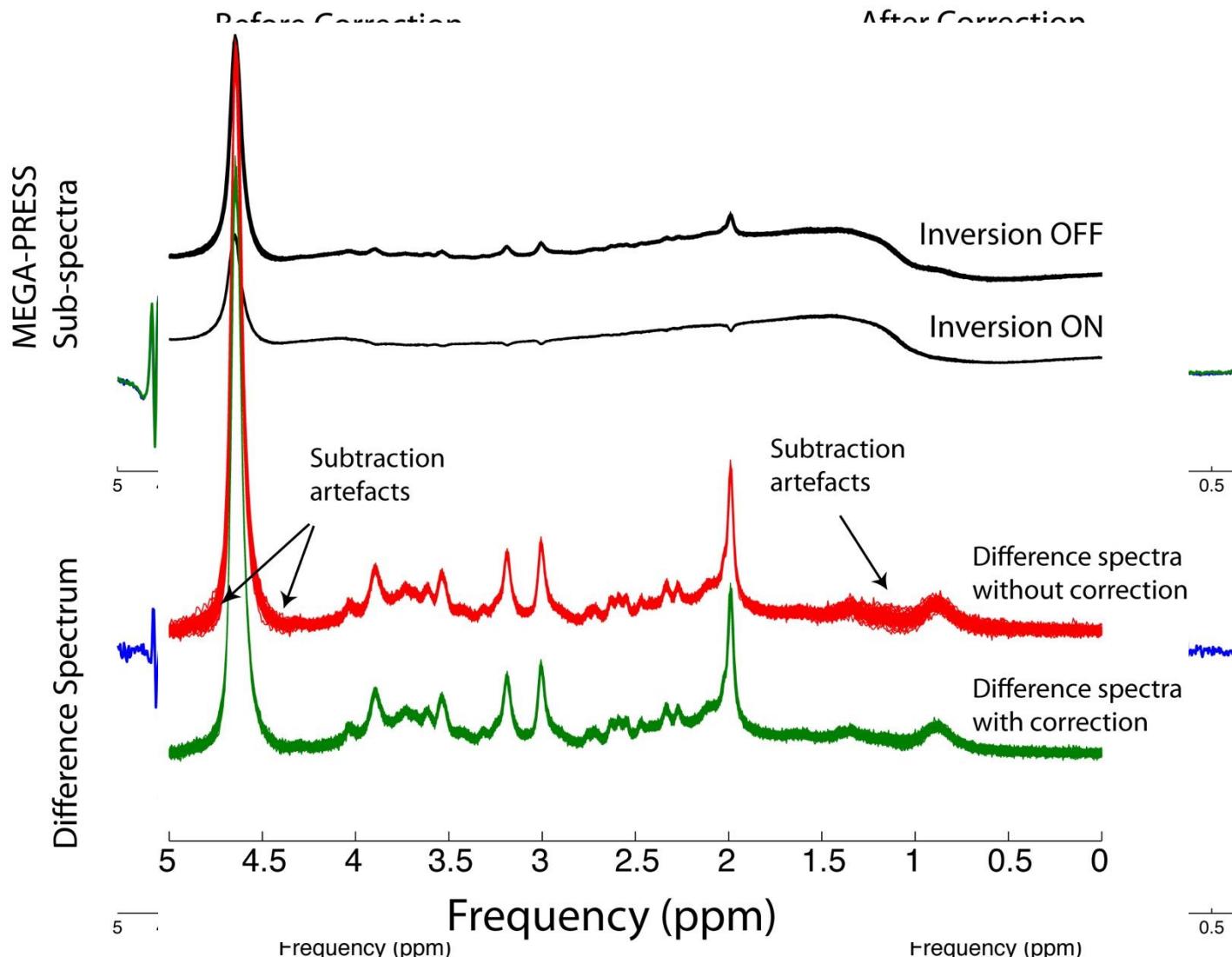
Editing subtraction artefacts

- Edit-ON and Edit-OFF sub-spectra must be aligned before subtraction
- Frequency and/or phase misalignment may cause
 - Subtraction errors (NAA, Ch, Cr)
 - GABA estimation error
- Alignment done automatically in processing pipelines
 - But can fail



Alignment of difference sub-spectra

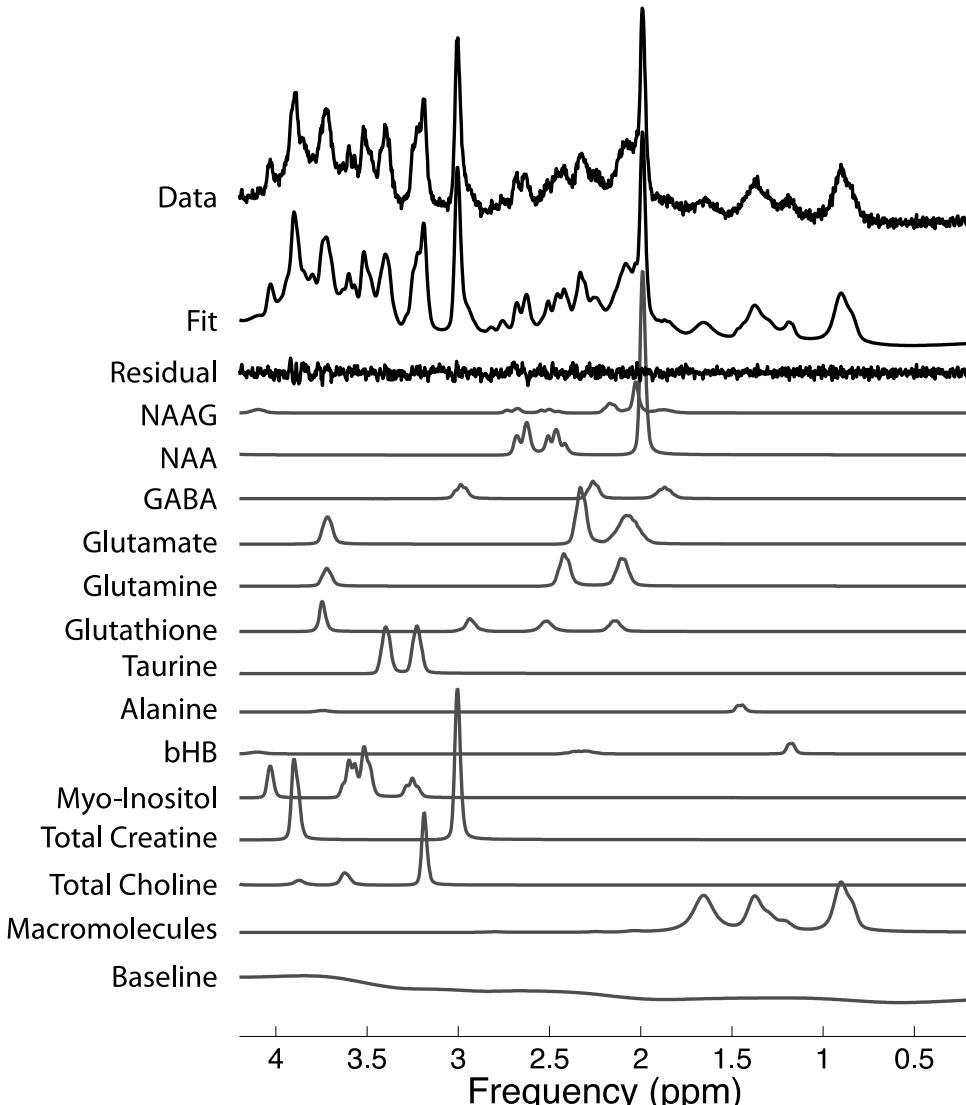
- Example: MEGA-PRESS
 - Automated alignment performed using spectral registration [6]
- Example: SPECIAL
 - Automated alignment performed using spectral registration [6]



MRS Analysis

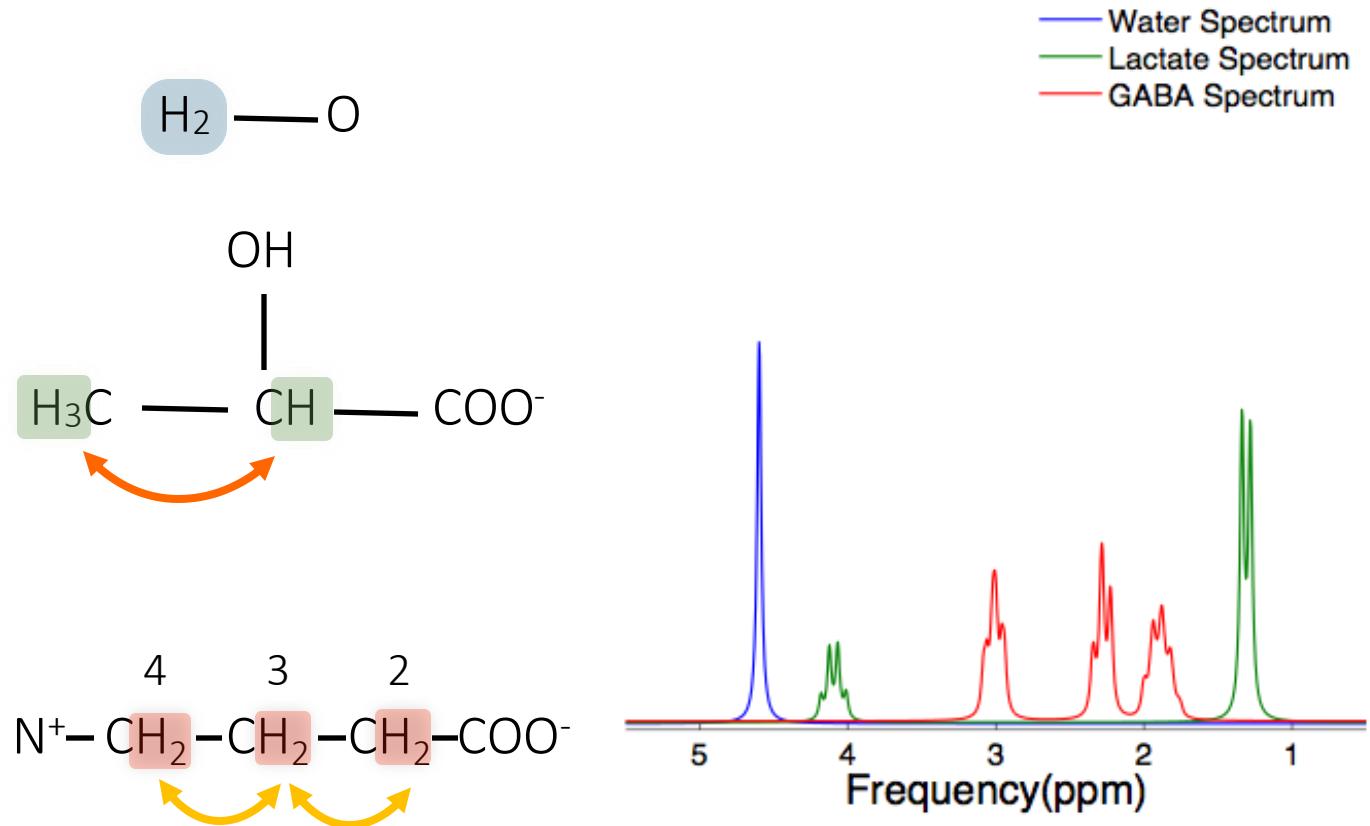
Linear Combination of Signals

- A spectrum is an amalgamation of all the metabolite signals
- Thus, with each metabolite we can fit (or model) its contribution to the spectrum
- Sometimes, we will have multiple peaks overlapping one another
 - With proper modelling they should be taken into account



Scalar Coupling: Singlets, Doublets, Triplets, Multiplets!

- Depending on the structure of the metabolite, its signal could either be simple single peak, or complicated multi-peak
- Due to chemical bonds within the molecule, neighbouring protons will split the signal (coupling)



Govindaraju, NMR Biomed, 2000

“Proton NMR chemical shifts and coupling constants for brain metabolites”

PROTON METABOLITE SHIFTS AND COUPLING CONSTANTS

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Table 1. Proton chemical shift and *J*-coupling values for low molecular weight brain metabolites. Chemical shifts are reported with reference to DSS-trimethyl singlet resonance at 0.000 ppm, and multiplicity definitions are: s, singlet; d, doublet; t, triplet; q, quartet; qu, quintet; m, other multiplet. The multiplicity given here was observed in conventional one-dimensional spectra recorded at 500 or 600 MHz. Multiplet groups having a pH-dependent chemical shift in the physiological range are indicated by an asterisk

Compound	Group	Shift (ppm) in H ₂ O	Shift (ppm) in D ₂ O	Multiplicity	<i>J</i> (Hz)	Connectivity	
<i>Acetate</i>	² CH ₃ *	1.9040	1.9030	s	None		
<i>NAA</i>							
Acetyl moiety	² CH ₃	2.0080	2.0050	s			
Aspartate moiety	² CH	4.3817	4.3823	dd	3.861	2-3	
	³ CH ₂	2.6727	2.6759	dd	9.821	2-3'	
		2.4863	2.4866	dd	-15.592	3-3'	
	NH	7.8205	7.8155	d	6.400	NH-2	
<i>NAAcG^a</i>							
Acetyl moiety	² CH ₃	2.042	s	None			
Aspartyl moiety	² CH	4.607	dd	4.412	2-3		
	³ CH ₂	2.721	dd	9.515	2-3'		
		2.519	dd	-15.910	3-3'		
Glutamate moiety	² CH	4.128	dd				
	³ CH ₂	1.881	m				
		2.049	m				
	⁴ CH ₂	2.190	m				
		2.180					
<i>ATP^b</i>							
Ribose moiety	¹ CH	6.126†	6.129	d	5.7	1'-2'	
	² CH		4.796	t	5.3	2'-3'	
	³ CH		4.616	dd	3.8	3'-4'	
	⁴ CH		4.396	qu	3.0	4'-5'	
	^{5,5'} CH ₂		4.295	m	3.1	4'-5"	
			4.206	m	-11.8	5'-5"	
					1.9	4'-P	
					6.5	5'-P	
					4.9	5"-P	
Adenosine moiety	² CH	8.224†	8.234	s			
	⁸ CH	8.514†	8.522	s			
	NH ₂	6.755†	s				
<i>Alanine</i>							
² CH	3.7746	3.7680	q	7.234	2-3		
	³ CH ₃	1.4667	1.4655	d	-14.366	3-3', 3"	
<i>GABA</i>					-14.366	3'-3"	
² CH ₂	3.0128	3.0082	m	5.372	2-3		
	³ CH ₂	1.8890	1.8888	qu	7.127	2-3'	
					6.982	2'-3'	
	⁴ CH ₂	2.2840	2.2828	t	7.755	3-4	
					7.432	3-4'	
					6.173	3'-4	
					7.933	3'-4'	
<i>Aspartate</i>							
² CH	3.8914	3.8867	dd	3.647	2-3		

Table 1. Continued.

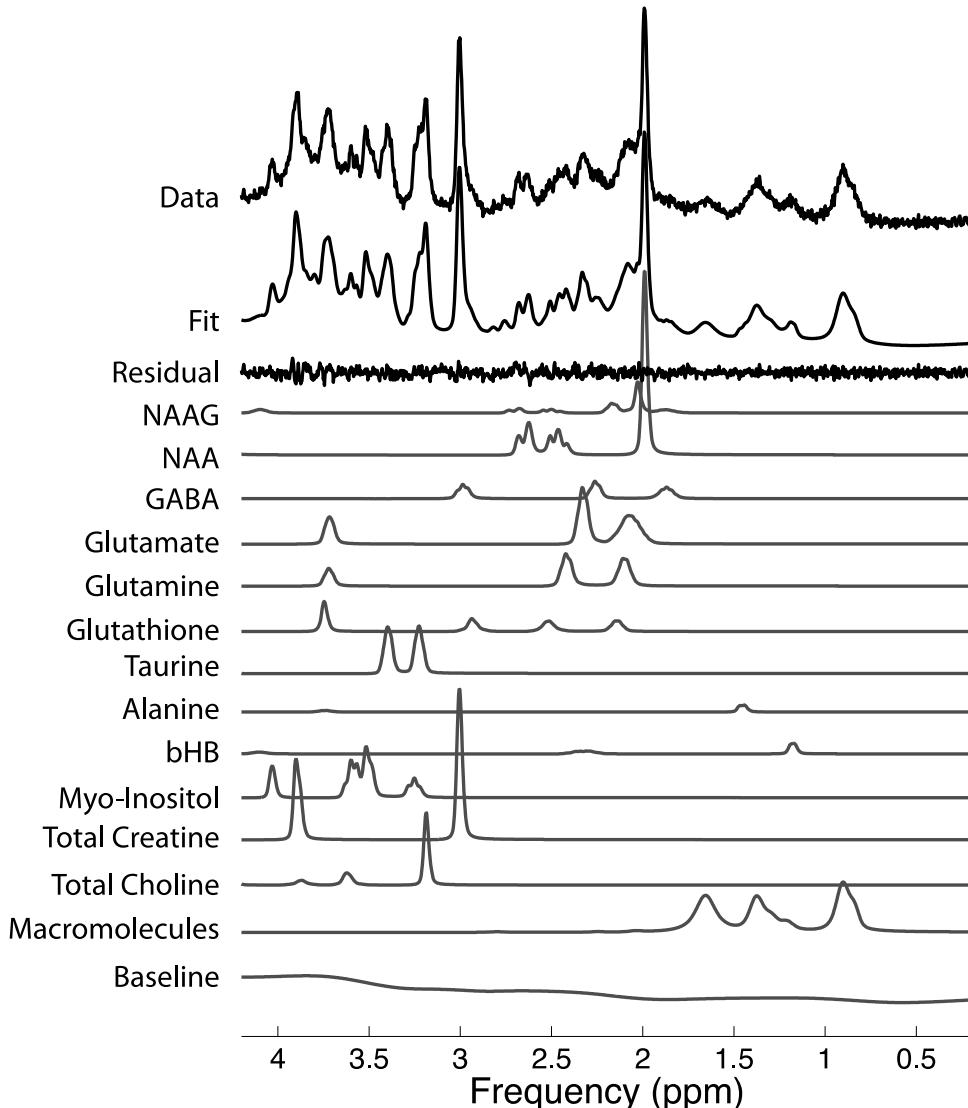
Compound	Group	Shift (ppm) in H ₂ O	Shift (ppm) in D ₂ O	Multiplicity	<i>J</i> (Hz)	Connectivity	
<i>Ethanolamine (cont.)</i>					3.798	1'-2'	
					0.657	1-N	
					0.142	1'-N	
<i>D-Glucose^c</i>							
α -anomer	¹ CH		5.216	d	3.8	1-2	
	² CH		3.519	dd	9.6	2-3	
	³ CH		3.698	t	9.4	3-4	
	⁴ CH		3.395	t	9.9	4-5	
	⁵ CH		3.822	m	1.5	5-6	
	⁶ CH		3.826	dd	6.0	5-6'	
	⁷ CH		3.749	dd	-12.1	6-6'	
	¹ CH		4.630	d	8.0	1-2	
	² CH		3.230	dd	9.1	2-3	
	³ CH		3.473	t	9.4	3-4	
	⁴ CH		3.387	t	8.9	4-5	
	⁵ CH		3.450	m	1.6	5-6	
	⁶ CH		3.882	dd	5.4	5-6'	
	⁷ CH		3.707	dd	-12.3	6-6'	
<i>Glutamate</i>							
² CH	3.7433	3.7444	dd	7.331	2-3		
	³ CH ₂	2.0375	2.0424	m	4.651	2-3'	
			2.1200	2.1206	-14.849	3-3'	
	⁴ CH ₂	2.3378	2.3354	m	8.406	3-4'	
		2.3520	2.3507		6.875	3'-4'	
					6.413	3-4	
					8.478	3'-4	
					-15.915	4-4'	
² CH	3.7530	3.7625	t	5.847	2-3		
	³ CH ₂	2.1290	2.1360	m	6.500	2-3'	
		2.1090	2.1180		-14.504	3-3'	
	⁴ CH ₂	2.4320	2.4350	m	9.165	3-4	
		2.4540	2.4570		6.347	3-4'	
					6.324	3'-4	
					9.209	3'-4'	
					-15.371	4-4'	
NH ₂	6.8160	7.5290	s				
			s				
<i>Glutathione^d</i>							
Glycine moiety	¹⁰ CH ₂	3.769					
	⁹ NH	7.154					
Cysteine moiety	⁷ CH ₂	4.5608	dd	7.09	7-7'		
		2.9264	dd	4.71	7-7"		
		2.9747	dd	-14.06	7-7"		
Glutamate moiety	⁶ NH	8.1770	d				
	² CH	3.769	t	6.34	2-3		
	³ CH ₂	2.159	m	6.36	2-3'		
		2.146	m	-15.48	3-3'		
	⁴ CH ₂	2.510	m	6.7	3-4		
		2.560	m	7.6	3-4'		
				7.6	3'-4		
				6.7	3'-4'		

Basis Sets

- To make life easier for us, we can make basis sets (tutorial 2) which has a library of our metabolites of interest
 - Simulated using known coupling constants and chemical shifts (tutorial 2)
 - Acquired on the MR scanner from a chemical phantom
- Using this basis set is like giving our analysis program a map of what to expect
- It will adjust the amplitudes and line widths according to your basis set and input data

MRS Analysis

- Fit the acquired data to a linear combination of individual metabolite “basis spectra” (LCModel).
- The resulting fitted “signal Intensity” is not a meaningful quantity on its own. Therefore, compare peak intensity to a “reference peak”.
- Common reference peaks include Creatine, NAA, or water.



Using a Reference Peak

- It is important to have peak which we can reference between different people/timepoints
- This reference peak is ideally a metabolite that is stable within/between people
 - H₂O
 - Cr
- Using a reference peak with our metabolite peak (as a ratio) also helps remove experimental imperfections, such as RF inhomogeneity, coil loading, transmit gain, etc.
- Depending on research interests there might be some specific metabolite:reference ratios
 - For tumours you might use Cho/NAA ratio to peer into tumour activity

MRS Quantification

MRS Quantification in Practice

- From our metabolite fits, we only get the area under the curve
- The area under the curve is proportional to the number of moles (along with number of protons, volume of interest, and experimental imperfections)

- $S_{met} \propto \frac{moles_{met} * \#H_{met} * f(experiment)}{Volume_{met}}$
- $\frac{moles_{met}}{Volume_{met}} \propto \frac{S_{met}}{\#H_{met} * f(experiment)}$

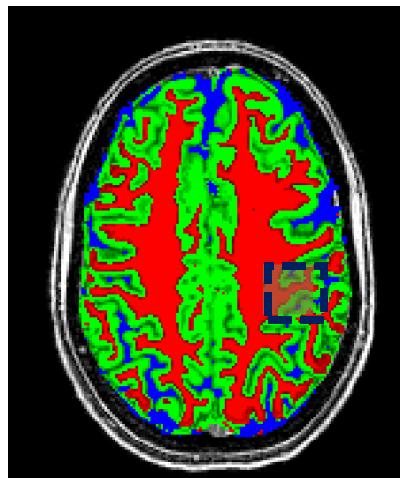
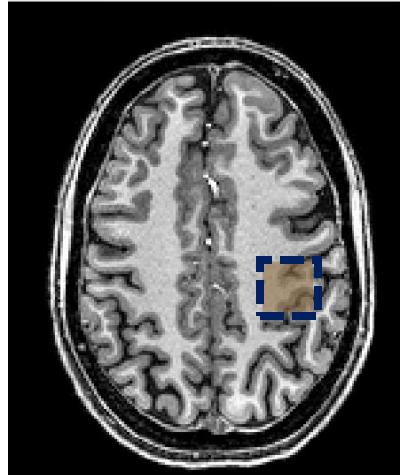
- However, as mentioned in the last slide, we are using ratio to the unsuppressed water reference! This cancels out the $f(experiment)$ term, as it's equal for metabolites and unsuppressed water scans

- $$\frac{moles_{met}/Volume_{met}}{moles_{H_2O}/Volume_{H_2O}} = \frac{S_{met}}{\#H_{met}} * \frac{\#H_{H_2O}}{S_{H_2O}} * \frac{f(experiment)}{f(experiment)}$$
- $$\frac{moles_{met}}{Volume_{met}} = \frac{S_{met}}{S_{H_2O}} * \frac{\#H_{H_2O}}{\#H_{met}} * \frac{moles_{H_2O}}{Volume_{H_2O}}$$

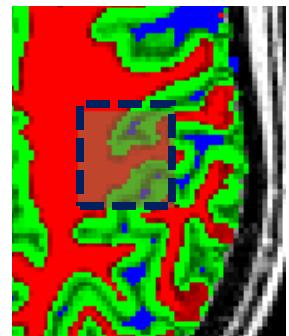
Moles to Molar

- We can go from moles to molar concentration (mol of solute/L of solvent) and since we are dealing with the same volume of tissue, $\text{Volume}_{\text{met}} = \text{Volume}_{\text{H}_2\text{O}}$
 - $$[\text{met}]_{\text{molar}} = \frac{s_{\text{met}}}{s_{\text{H}_2\text{O}(\text{GM},\text{WM})}} * d_{\text{H}_2\text{O}(\text{CSF},\text{GM},\text{WM})} * \frac{\#H_{\text{H}_2\text{O}}}{\#H_{\text{met}}} * [\text{H}_2\text{O}]_{\text{molar}}$$
- $[\text{H}_2\text{O}]_{\text{molar}} = 55.51 \text{ mol/L}$ for pure water at 25°C
- $d_{\text{H}_2\text{O}(\text{CSF},\text{GM},\text{WM})}$ is the relative water density, we need to introduce this term as it corrects the water concentration specific to our volume of interest
 - This value is not known, since we don't know its tissue composition, but it can be calculated
 - From pure tissue: $d_{\text{CSF}} = 0.97$; $d_{\text{GM}} = 0.78$; $d_{\text{WM}} = 0.65$
(Ernst, J Magn Reson, 1993)
- We are only interested in the signal coming from the tissue. Thus, we use $s_{\text{H}_2\text{O}(\text{GM},\text{WM})}$ for the signal intensity from tissue water! However, the water signal we fitted have comes from the CSF, GM, and WM.
- Tissue segmentation is needed!

Tissue Segmentation:



- You can get tissue fractions from your voxel using a few different programs:
 - FSL + FSLMRS
 - SPM + Osprey/Gannet
- Within your volume of interest:
 - $1 = f_{vol(CSF)} + f_{vol(GM)} + f_{vol(WM)}$



Correcting for Tissue Composition

- $[met]_{molar} =$

$$\frac{S_{met}}{S_{H_2O(GM,WM)}} * d_{H_2O(CSF,GM,WM)} * \frac{\#H_{H_2O}}{\#H_{met}} * [H_2O]_{molar}$$

$$\begin{aligned} \bullet S_{H_2O(GM,WM)} &= S_{H_2O} * (f_{vol(GM)} + f_{vol(WM)}) \\ &= S_{H_2O} * (1 - f_{vol(CSF)}) \end{aligned}$$

$$\begin{aligned} \bullet d_{H_2O(CSF,GM,WM)} &= \\ d_{H_2O(CSF)} * f_{vol(CSF)} + d_{H_2O(GM)} * f_{vol(GM)} + d_{H_2O(WM)} * f_{vol(WM)} \end{aligned}$$

$$d_{CSF} = 0.97; d_{GM} = 0.78; d_{WM} = 0.65$$

(Ernst, J Magn Reson, 1993)

Correcting for T1 and T2 Relaxation

- Additionally, the previous equations assume that the signal is fully relaxed ($TR \gg T1_{met,H2O}$, $TE \ll T2_{met,H2O}$), but this is not the case in most experiments

$$[met]_{molar} = \frac{S_{met}}{S_{H_2O(GM,WM)}} * d_{H_2O(CSF,GM,WM)}$$

$$* \frac{R_{H_2O(CSF,GM,WM)}}{R_{met}} * \frac{\#H_{H_2O}}{\#H_{met}} * [H_2O]_{molar}$$

- Where:

$$R_x = \exp\left(-\frac{TE}{T2_x}\right) * \left(1 - \exp\left(-\frac{TR}{T1_x}\right)\right)$$

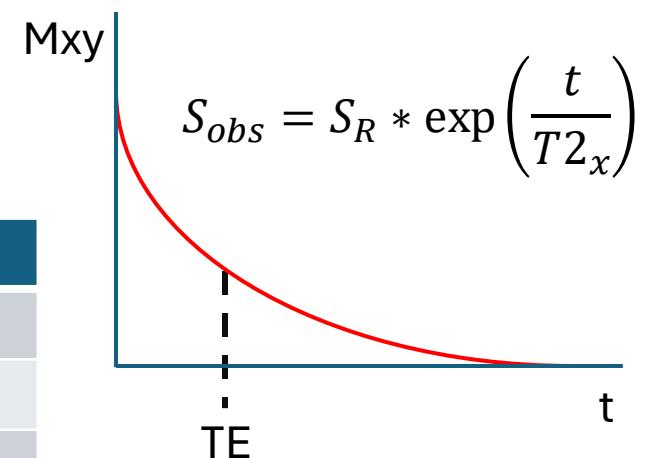
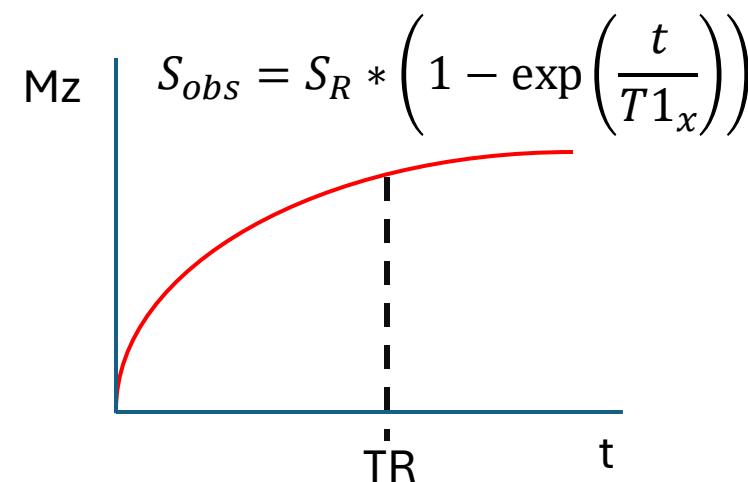
x being either met or $H_2O(CSF, GM, or WM)$

T2 Correction

T1 Correction

Water	T1	T2
CSF	4s	2.55s
GM	1.304s	0.11s
WM	0.83s	0.08s

*Gasparovic, MRM, 2011



T₁ and T₂ Values for Metabolites

JOURNAL OF MAGNETIC RESONANCE IMAGING 9:531-538 (1999)

Original Research

NMR IN BIOMEDICINE
NMR Biomed. 2001;14:325-331
DOI:10.1002/nbm.713

NMR Relaxation Times in the Human Brain at 3.0 Tesla

Janaka P. Wansapura, PhD,¹ Scott K. Holland, PhD,^{2*} R. Scott Dunn, RT,² and William S. Ball, Jr., MD²

Proton T₁ and T₂ relaxation times of human brain metabolites at 3 Tesla

Vladimír Mlynárik,¹ Stephan Gruber¹ and Ewald Moser^{1,2*}

¹NMR Group, Institute of Medical Physics, University of Vienna, A-1090 Vienna, Austria
²Department of Radiology, University of Vienna, A-1090 Vienna, Austria

Received 13 October 2000; revised 31 January 2001; accepted 8 May 2001

JOURNAL OF MAGNETIC RESONANCE IMAGING 19:537-545 (2004)

Original Research

¹H Metabolite Relaxation Times at 3.0 Tesla: Measurements of T₁ and T₂ Values in Normal Brain and Determination of Regional Differences in Transverse Relaxation

Frank Träber, PhD,^{1*} Wolfgang Block, PhD,¹ Rolf Lamerichs PhD,² Jürgen Gieseke, MSc,² and Hans H. Schild, MD¹

Final Equation

Tissue
Correction

T1/T2 Relaxation
Correction

$$\begin{aligned}
 \bullet [met]_{molar} &= \frac{s_{met}}{s_{H_2O} * (1 - f_{vol(CSF)})} \\
 &\times \frac{f_{vol(GM)} * d_{GM} * R_{H_2O(GM)} + f_{vol(WM)} * d_{WM} * R_{H_2O(WM)} + f_{vol(CSF)} * d_{CSF} * R_{H_2O(CSF)}}{R_{met}} \\
 &\times \frac{\#H_{H_2O}}{\#H_{met}} * [H_2O]_{molar}
 \end{aligned}$$

- Where:
 - $[H_2O]_{molar} = 55.51 \text{ mol/L}$
 - $d_{CSF} = 0.97; d_{GM} = 0.78; d_{WM} = 0.65$
 - $R_x = \exp\left(-\frac{TE}{T2_x}\right) * \left(1 - \exp\left(-\frac{TR}{T1_x}\right)\right)$
 x being either met or $H_2O(GM, WM, CSF)$

Gasparovic, MRM, 2011

Water	T1	T2
CSF	4s	2.55s
GM	1.304s	0.11s
WM	0.83s	0.08s

Quantification confounds

- Whether using metabolite referencing (Cr, NAA) or absolute quantification, potential confounds are practically unavoidable
- With metabolite referencing, observed effects may be driven by the reference metabolite content or relaxation.
- Likewise, with absolute quantification, observed effects may be driven by tissue water content or relaxation.
- Most published MRS studies are susceptible to such confounds.

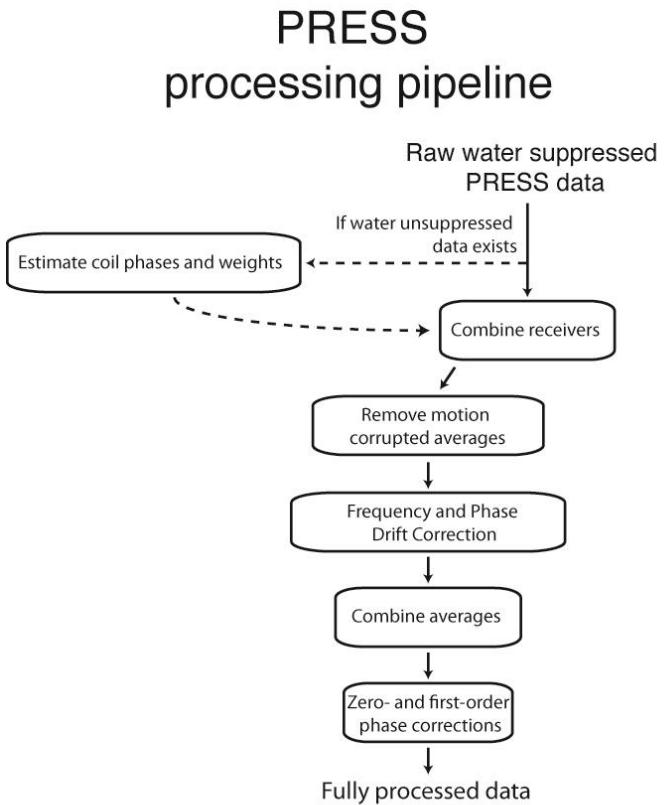
Processing/analysis Software packages

Automation

- Automated processing methods are generally preferred because:
 - they are faster
 - they are reproducible
 - they reduce user error/bias

Processing Pipelines

- Processing operations can be chained together to make a “pipeline”
- “Order of operations” matters!!



Quality Assurance Measures

- The following quality assurance metrics can be used to assess the quality of your data:
 - Signal-to-noise ratio
 - Spectral linewidth
 - Artifacts
 - Unsuppressed water
 - Lipid contamination
 - Unspoiled coherences
 - Baseline distortion
 - Eddy current artefacts

Practical aspects

- Growing number of toolkits available for MRS postprocessing:
 - FID-A (<https://github.com/CIC-methods/FID-A>)
 - OSPREY (<https://github.com/schorschinho/osprey>)
 - FSL-MRS (https://open.win.ox.ac.uk/pages/fsl/fsl_mrs/)
 - Gannet (www.gabamrs.com)
 - jMRUI/Spectrlm (www.jmrui.eu)
 - TARQUIN (<https://tarquin.sourceforge.net>)
 - INSPECTOR (<http://juchem.bme.columbia.edu/research/software-and-tools>)
 - VeSPA (<https://scion.duhs.duke.edu/vespa>)
 - SUSPECT (<https://github.com/openmrslab/suspect>)
 - LCModel
 - <https://s-provencher.com/lcmodel.shtml> (for installation on Linux-based systems)
 - <https://github.com/schorschinho/LCModel> (for installation for Windows, Mac, & Linux)
 - Some functionality no longer works

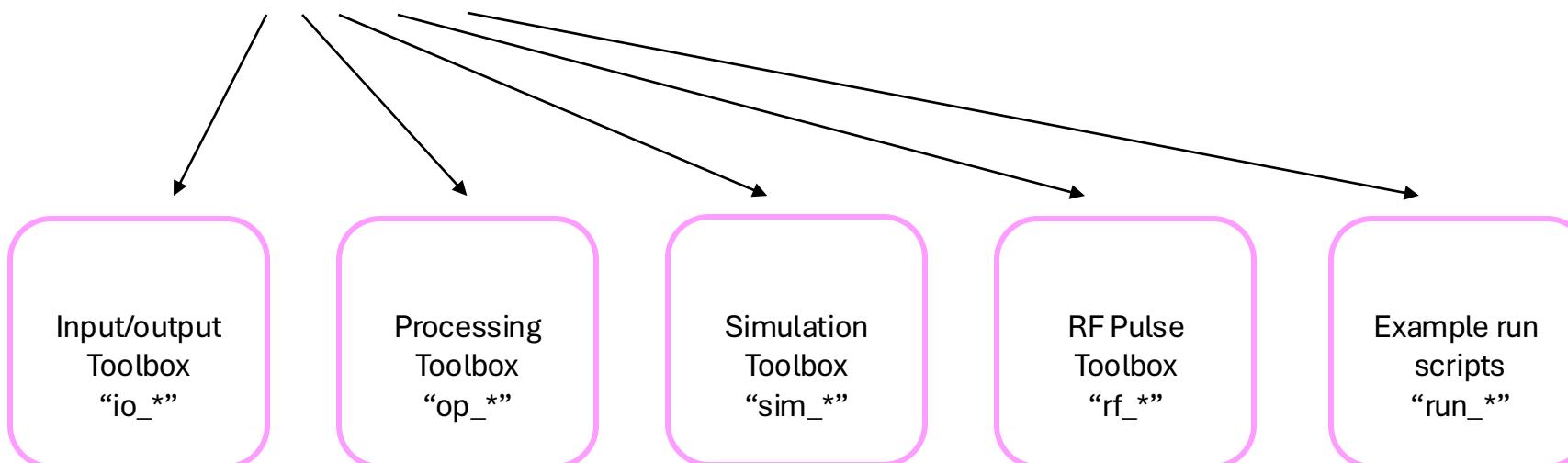
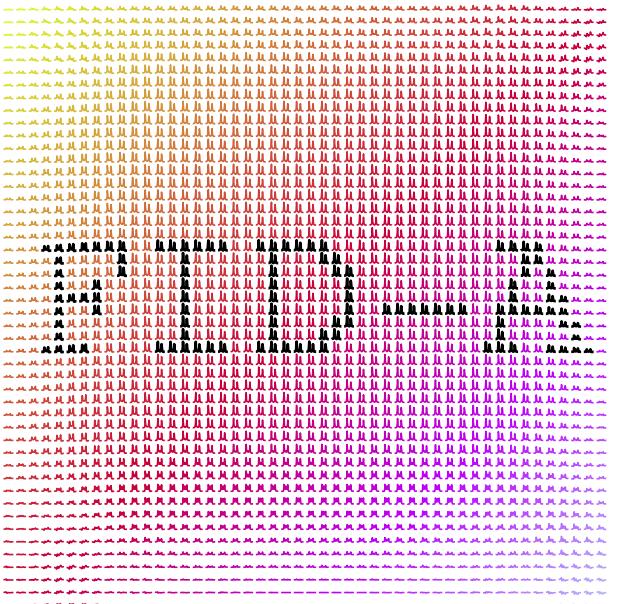
Outline: Afternoon practical session

- Brief intro to FID-A.
 - Downloading
 - Adding to path
 - Toolboxes (input/output, processing, RF, simulation, exampleRunScripts)
 - Load a dataset (`io_loadspec_twix`);
 - Show the FID-A data structure (fields of structure, dims, etc.)
 - Show a few manual processing steps (`op_averaging`, `op_combineCoils`)
- Processing a single voxel MRS dataset in FID-A
 - PRESS (`run_pressproc_auto.m`)
 - MEGA-PRESS (`run_megapressproc_auto.m`)
 - SPECIAL (`run_specialproc_auto.m`)

FID-A Slides

What is FID-A?

- The FID appliance (FID-A) is an open-source, MATLAB-based software toolkit for advanced MRS simulation and data processing, as well as basic RF pulse design and analysis.
- A framework to access, manage, and visualize all aspects of your MRS data
- This toolkit is freely available from www.github.com/CIC-methods/FID-A.



Features

- Written in MATLAB, mostly command line based
- Most common operations done with one-line command
- Maximum control with minimal user input

Features (Cont'd):

- Designed for raw datasets with multiple dimensions
 - RF coil channels
 - Averages
 - Subtraction sub-spectra, etc.
- Large library of processing tools, simulation tools
- Data structure format contains all relevant header information
- Contains example "run scripts" with pre-implemented:
 - Processing pipelines
 - Simulation of common MRS sequences (both ideal and shaped-pulse versions)
 - Basis set generation
- Users with MATLAB experience can easily modify code to suit their needs.

Full list of FID-A input/output tools:

Siemens

- `io_loadRFwaveform.m`
- `io_loadspec_IMA.m`
- `io_loadspec_twix.m`
- `io_loadspec_rda.m`
- `io_readpta.m`
- `io_writepta.m`

Bruker

- `io_loadRFwaveform.m`
- `io_loadspec_bruk.m`
- `io_loadspec_brukNMR.m`
- `io_loadspec_irBruk.m`
- `io_readRFBruk.m`

GE

- `io_loadspec_GE.m`

Philips

- `io_loadspec_data.m`
- `io_loadspec_sdat.m`

Varian/Agilent

- `io_loadRFwaveform.m`
- `io_loadspec_varian.m`
- `io_readRF.m`
- `io_writeRF.m`

jMRUI

- `io_loadjmrui.m`
- `io_readjmrui.m`
- `io_writejmrui.m`

LCModel

- `io_loadlcmdetail.m`
- `io_readlcmcoord_getBackground.m`
- `io_readlcmcoord.m`
- `io_readlcmraw_basis.m`
- `io_readlcmraw_dotraw.m`
- `io_readlcmraw.m`
- `io_readlcmtab.m`
- `io_writelcm.m`
- `io_writelcmraw.m`

VeSPA

- `io_readRFtxt.m`
- `io_writeRFtxt.m`

Full list of FID-A processing operations:

Dimensionality manipulation:

- op_ISIScombine.m
- op_addrvrs.m
- op_averaging.m
- op_combineRcvrs.m
- op_combinesubspecs.m
- op_concatAverages.m
- op_concatFreq.m
- op_concatSubspecs.m
- op_downsamp.m
- op_median.m
- op_takeaverages.m
- op_takesubspec.m
- op_takeextras.m

Phase/Frequency shifting:

- op_addphase.m
- op_addphaseSubspec.m
- op_autophase.m
- op_freqshift.m
- op_freqshiftSubspec.m
- op_makeFreqDrift.m
- op_makePhaseDrift.m
- op_movef0.m
- op_ppmref.m

Filtering:

- op_filter.m
- op_matchLW.m
- op_unfilter.m

Maths operations:

- op_addScans.m
- op_ampScale.m
- op_complexConj.m
- op_integrate.m
- op_subtractScans.m

Truncation:

- op_fqrange.m
- op_leftshift.m
- op_timerange.m
- op_zeropad.m
- op_zerotrim.m

Basic peak fitting:

- op_crefit.m
- op_gaussianPeak.m
- op_integrate.m
- op_lorentz.m
- op_lorentz_linbas.m
- op_lorentzianPeak.m
- op_peakFit.m

Quality assurance:

- op_arsos.m
- op_getLW.m
- op_getSNR.m
- op_relyTest.m
- op_rmbadaverages.m
- op_rmNworstaverages.m
- op_rmworstaverage.m

Spectral Registration:

- op_alignAllScans.m
- op_alignAllScans_fd.m
- op_alignAverages.m
- op_alignAverages_fd.m
- op_alignISIS.m
- op_alignMPSubspecs.m
- op_alignMPSubspecs_fd.m
- op_alignScans.m
- op_alignScans_fd.m
- op_alignrvrs.m
- op_freqAlignAverages.m
- op_freqAlignAverages_fd.m
- op_getcoilcombos.m
- op_getcoilcombos_specReg.m
- op_phaseAlignAverages.m
- op_phaseAlignAverages_fd.m

Plotting:

- op_plotfid.m
- op_plotspec.m

Other:

- op_addNoise.m
- op_dccorr.m
- op_ecc.m
- op_fddccorr.m
- op_getPeakHeight.m
- op_getcoilcombos.m
- op_getcoilcombos_specReg.m
- op_removeWater.m

Full list of FID-A simulation tools:

Basic Spin Operators

- sim_Hamiltonian.m
- sim_evolve.m
- sim_excite.m
- sim_excite_arbPh.m
- sim_gradSpoil.m
- sim_readout.m
- sim_rotate.m
- sim_rotate_arbPh.m
- sim_shapedRF.m
- sim_spoil.m
- sim_dAdd.m
- sim_dDiv.m
- sim_dMul.m

Pulse Sequences (Ideal)

- sim_laser.m
- sim_lcmrawbasis.m
- sim_megapress.m
- sim_onepulse.m
- sim_onepulse_arbPh.m
- sim_onepulse_delay.m
- sim_press.m
- sim_spinecho.m
- sim_spinecho_xN.m
- sim_steam.m
- sim_steam_gradSim.m

Pulse Sequences (shaped)

- sim_make2DSimPlot.m
- sim_megapress_shaped.m
- sim_megapress_shapedEdit.m
- sim_megapress_shapedRefoc.m
- sim_megaspecial_shaped.m
- sim_onepulse_shaped.m
- sim_press_shaped.m
- sim_sLASER_shaped.m
- sim_spinecho_shaped.m

Full list of FID-A RF pulse tools:

Bloch Simulation

- rf_blochSim.m
- rf_refocusedComponent.m

RF Pulse Design

- rf_addGrad.m
- rf_dualBand.m
- rf_freqshift.m
- rf_gauss.m
- rf_goia.m
- rf_hs.m
- rf_plotWaveform.m
- rf_resample.m
- rf_scaleGrad.m
- rf_sinc.m
- rf_verse.m

Full list of FID-A example run scripts:

Processing

- `run_megapressproc_auto.m`
- `run_megapressproc_GEauto.m`
- `run_megapressproc.m`
- `run_pressproc_auto.m`
- `run_pressproc_brukAuto.m`
- `run_pressproc_GEauto.m`
- `run_pressproc.m`
- `run_specialproc_auto.m`
- `run_specialproc_fmrs_slidingWindow.m`
- `run_specialproc_fmrs.m`
- `run_specialproc.m`

Simulation

- `run_make2DSimPlot.m`
- `run_simExampleBasisSet.m`
- `run_simMegaExTEShaped.m`
- `run_simMegaPressShaped.m`
- `run_simMegaPressShapedEdit.m`
- `run_simMegaPressShapedRefoc.m`
- `run_simMegaPressShapedRefoc_fast.m`
- `run_simMegaPressShaped_fast.m`
- `run_simMegaSpecialShaped.m`
- `run_simPressShaped.m`
- `run_simPressShaped_fast.m`
- `run_simsLaserShaped.m`
- `run_simsLaserShaped_fast.m`
- `run_simSpinEchoShaped.m`

Quality Assurance

- `run_getLWandSNR.m`

What doesn't FID-A do:

- Does not have its own graphical user interface.
- Does not support MRSI / CSI data. Currently single voxel MRS data only.
 - Support for MRSI development in progress.
- Does not support X-nuclei. Currently proton only.
 - Support for x-nuclei development in progress.
- Not a standalone piece of software
 - Requires a MATLAB license
 - Some functions require certain MATLAB toolboxes:
 - Statistics and Machine Learning Toolbox (A.K.A. Statistics Toolbox in older MATLAB Versions)
 - Parallel Computing Toolbox
 - Newly updated to run in Octave, though not all functions/operating systems have been tested
 - Requires the following packages: statistics; optim; and image

Practical examples:

- Downloading/installing the software
- Loading and manually processing an MRS dataset
- Automated processing pipelines
- Data quality assurance
- Loading and analyzing an RF pulse waveform
- Simulating MRS experiments

Downloading and Installing:

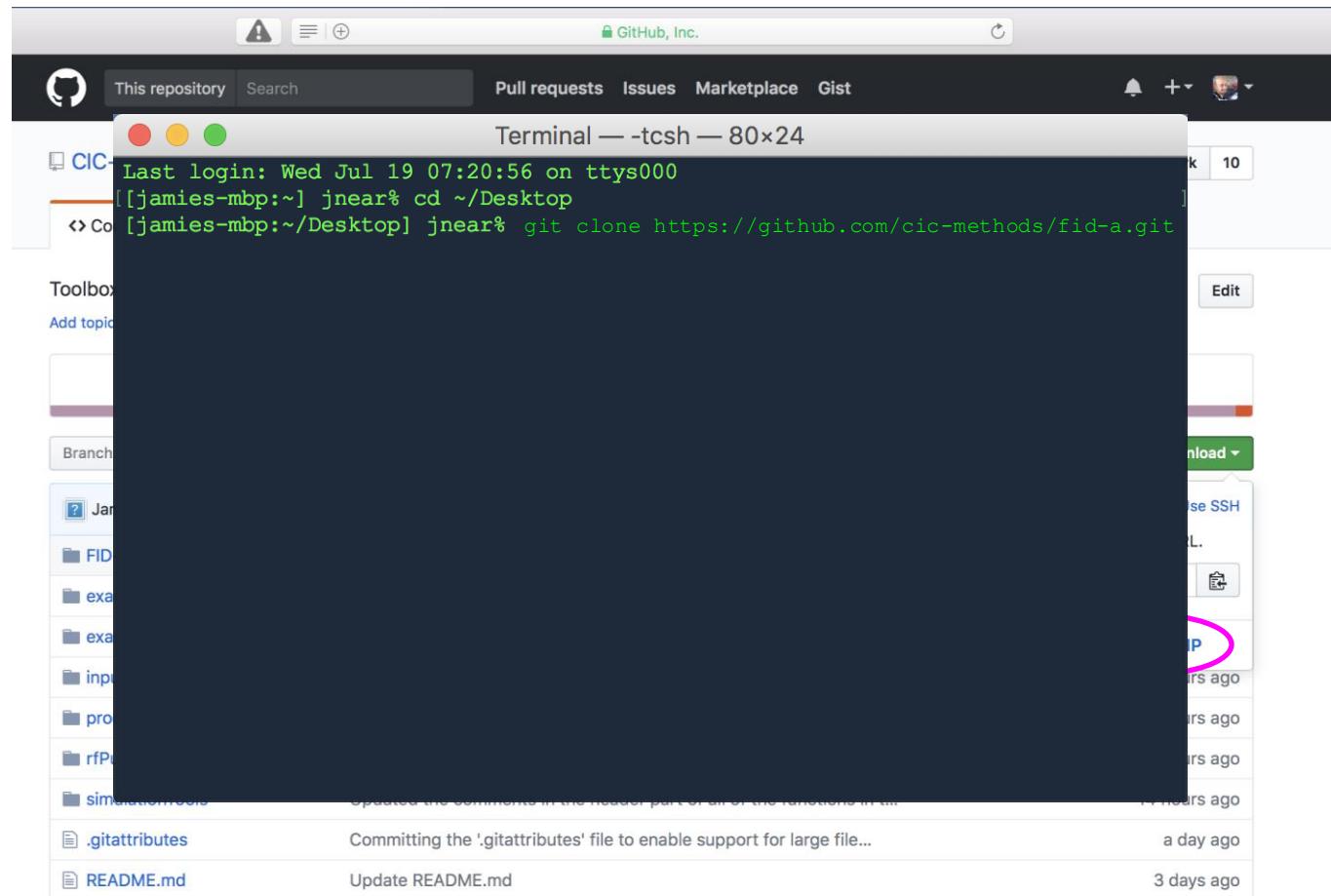
Basic Installation:

1. Go to github.com/cic-methods/fid-a
2. Click on the “Clone or Download” button
3. Click on “Download ZIP”
4. Unzip the ‘FID-A-master.zip’ file and move the resulting folder into the desired location.

Advanced Installation (Requires git and git-lfs to be installed):

1. Open linux terminal
2. Navigate to the desired directory
3. Type:

```
git clone https://github.com/cic-methods/fid-a.git
```



A screenshot of a GitHub repository page for 'fid-a'. The repository has 10 stars. A terminal window is open in the sidebar, showing the command 'git clone https://github.com/cic-methods/fid-a.git' being run. The terminal output shows the user's last login information and the command being executed. The main repository page shows several files and their commit history, including '.gitattributes' and 'README.md'.

File	Commit Message	Time Ago
.gitattributes	Committing the '.gitattributes' file to enable support for large file...	a day ago
README.md	Update README.md	3 days ago

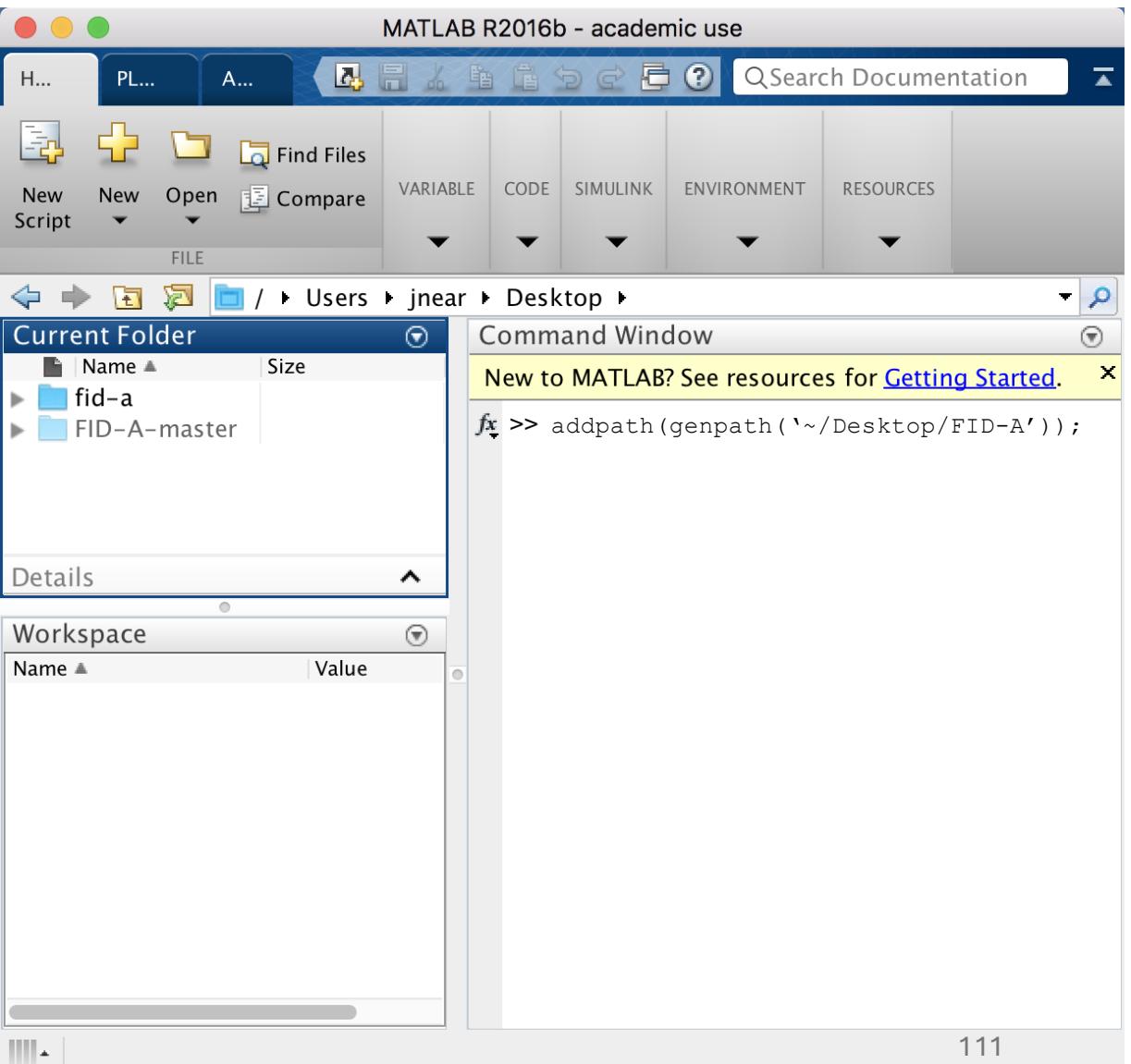
Downloading and Installing (cont'd):

Next:

1. Open MATLAB
2. Add the FID-A directory and all its subdirectories to path using the path tool, or by typing:

```
addpath (genpath ('~/Desktop/FID-A')) ;
```

3. DONE!!



Practical examples:

- Downloading/installing the software
- Loading and manually processing an MRS dataset
- Automated processing pipelines
- Data quality assurance
- Loading and analyzing an RF pulse waveform
- Simulating MRS experiments

Load example data

(Siemens MEGA-PRESS data)

1. Go to ‘exampleData’ directory containing Siemens raw twix (.dat) dataset:

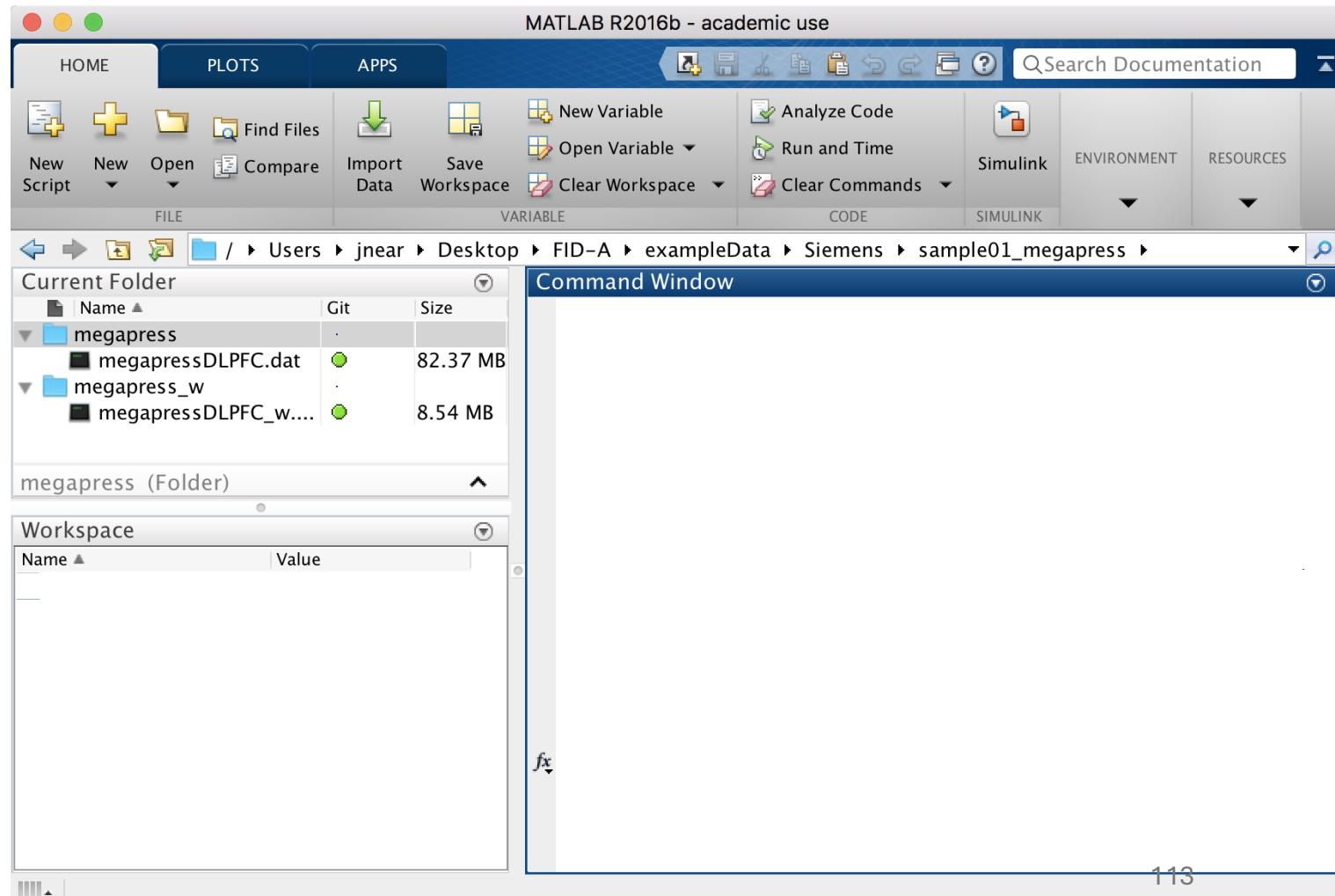
```
cd FID-A/exampleData/Siemens/sample01_megapress
```

2. Load water suppressed data using io_loadspec_twix:

```
raw=io_loadspec_twix('megapress/megapressDLPFC.dat');
```

3. Load water suppressed data using io_loadspec_twix:

```
raw_w=io_loadspec_twix('megapress_w/megapressDLPFC_w.dat');
```



View the data structure

To view the structure, type the variable name at the command line:

```
raw
```

Data structures contain both data, and header information.

Same format is used for both experimental and simulated data.

Note size of data arrays, raw.fids and raw.specs (2080 x 32 x 80 x

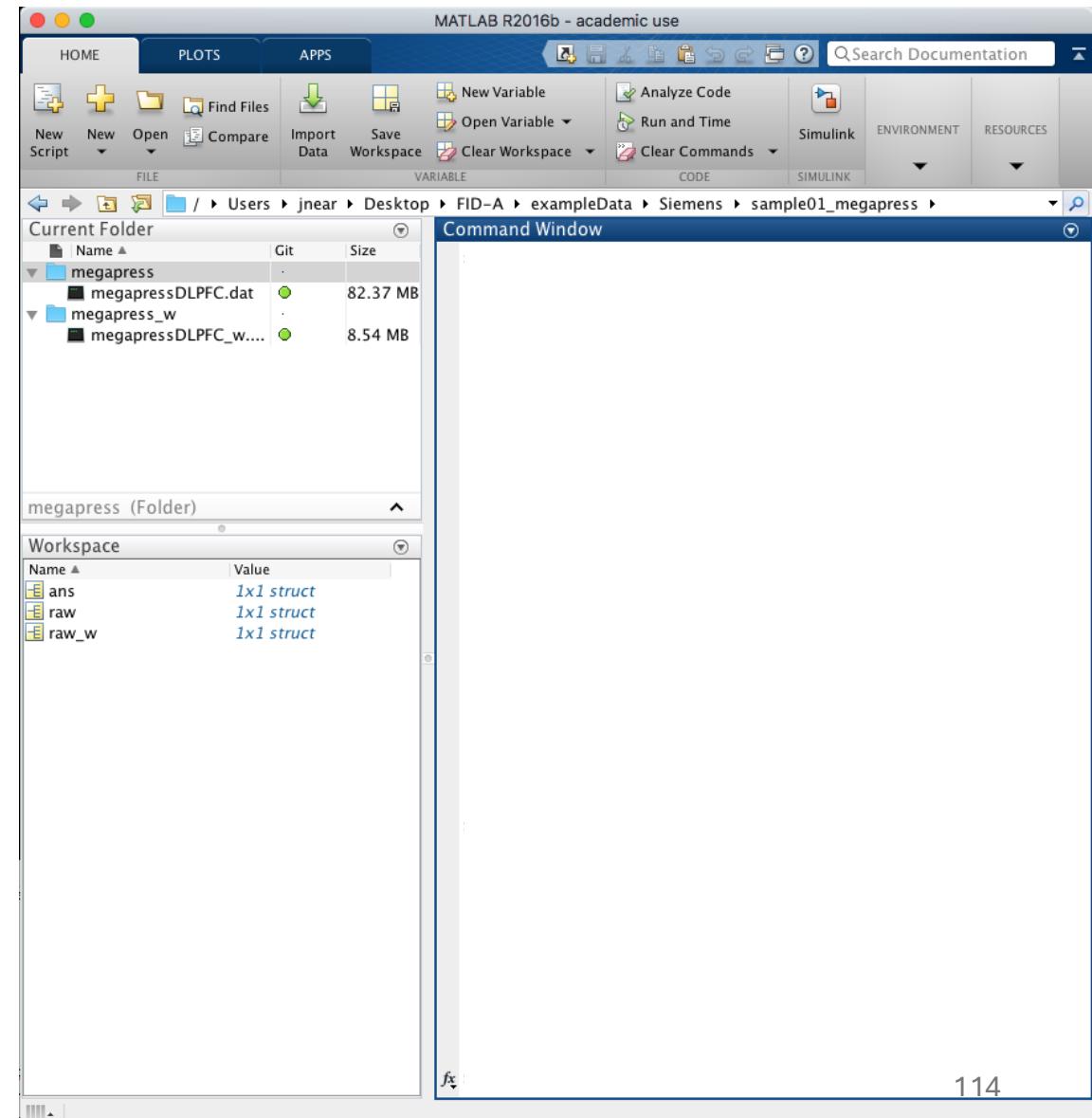
2). To find out what those dimensions mean, type:

```
raw.dims
```

The raw.dims structure indicates:

- 1st dimension is the “time” dimension (2080 points),
- 2nd dimension is the “coils” dimension (32 channels),
- 3rd dimensions is the “averages” dimension (80 averages)
- 4th dimension is the “subSpecs” dimension (2 subspectra).

Next step: combine the rf channels.



Combining the RF channels

Combine RF channels for both the water suppressed and unsuppressed datasets using:

```
[out1,out1_w]=op_combineRcvrs(raw,raw_w);
```

Look at the result by querying variable name:

```
out1
```

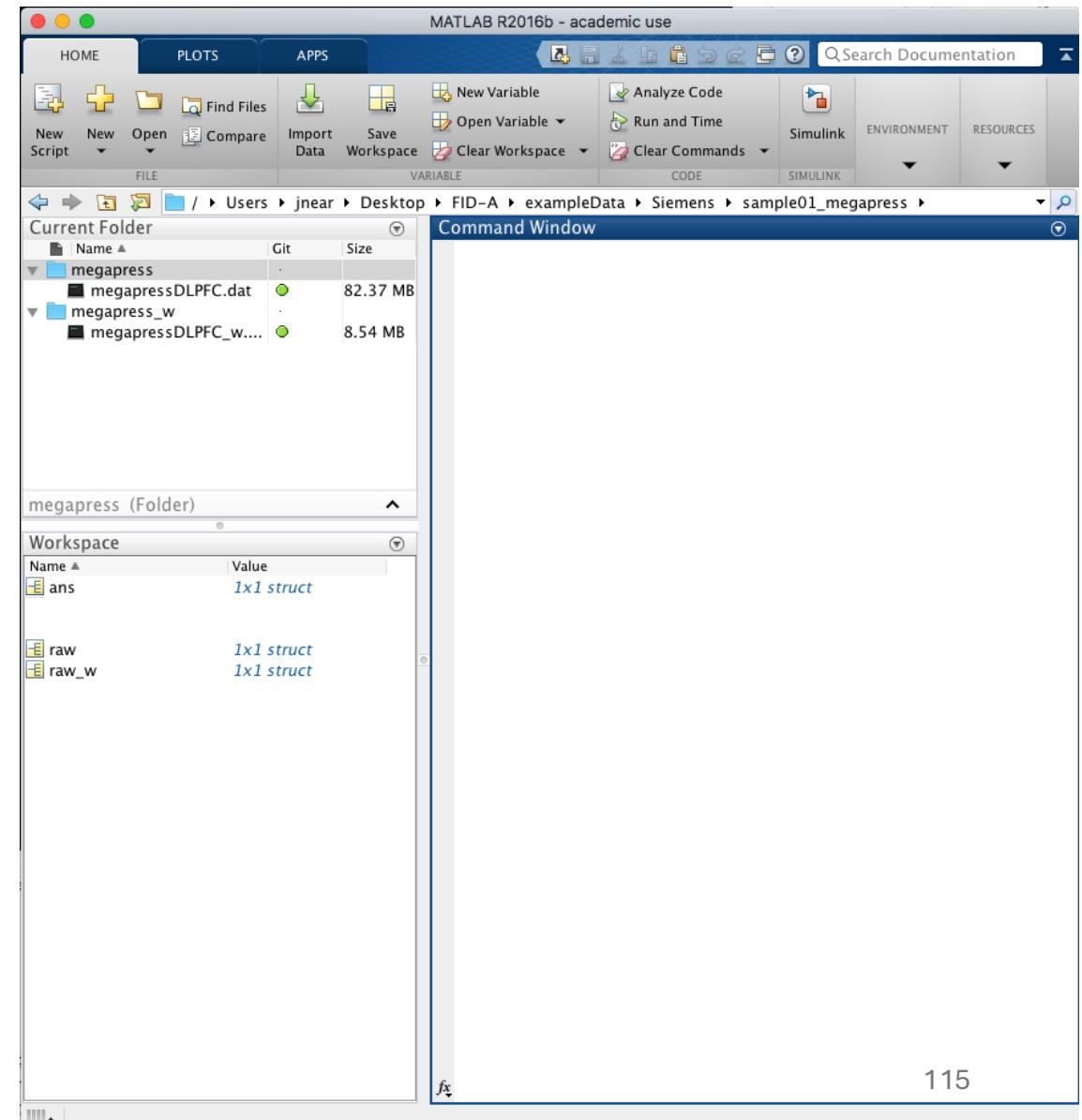
Note the dimensionality of the data arrays, raw.fids and raw.specs has been reduced to 2080 x 80 x 2. The Coils dimension has been removed:

```
out1.dims
```

The out1.dims structure now indicates:

- 1st dimension is the “time” dimension (2080 points),
- 2nd dimension is the “averages” dimension (80 averages),
- 3rd dimensions is the “subSpecs” dimension (2 subSpecs)

Now plot the spectra...



Plotting the data

Plot the time domain data using:

```
op_plotfid(out1);
```

Which dimension would we like to view?

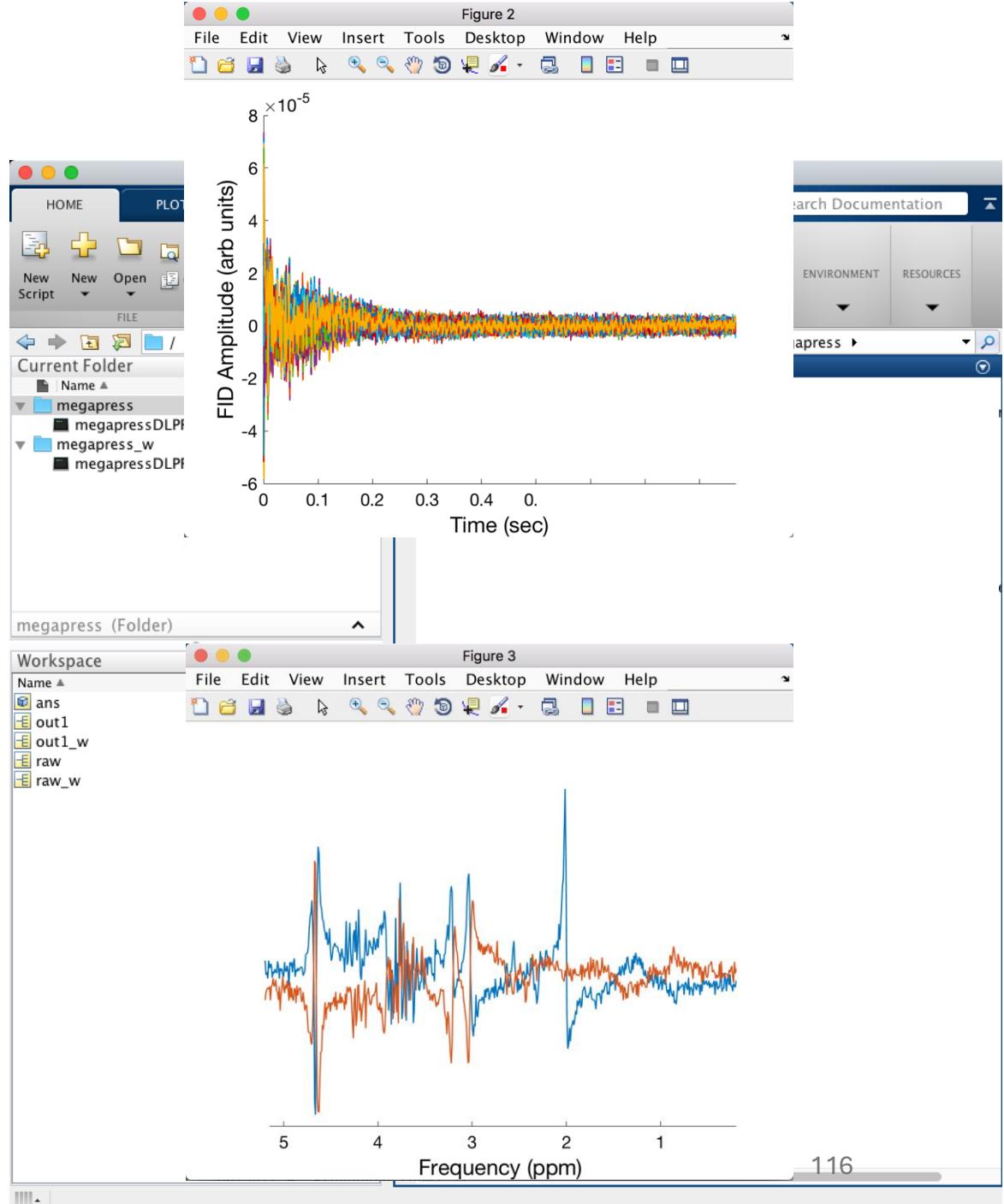
Choose '2' to view all averages (will display only the 1st subspectrum).

We can plot the frequency domain spectra using:

```
op_plotspec(out1);
```

Again, which dimension would we like to view?

This time, choose '3' to view both subspectra (will display only the 1st average).



More processing steps:

Do spectral registration (and plot result):

```
out2=op_alignAverages(out1);  
op_plotspec(out2);
```

Combine averages:

```
out3=op_averaging(out2);
```

Align MEGA-PRESS subspectra :

```
out4=op_alignMPSubspecs(out3);
```

Subtract MEGA-PRESS subspectra :

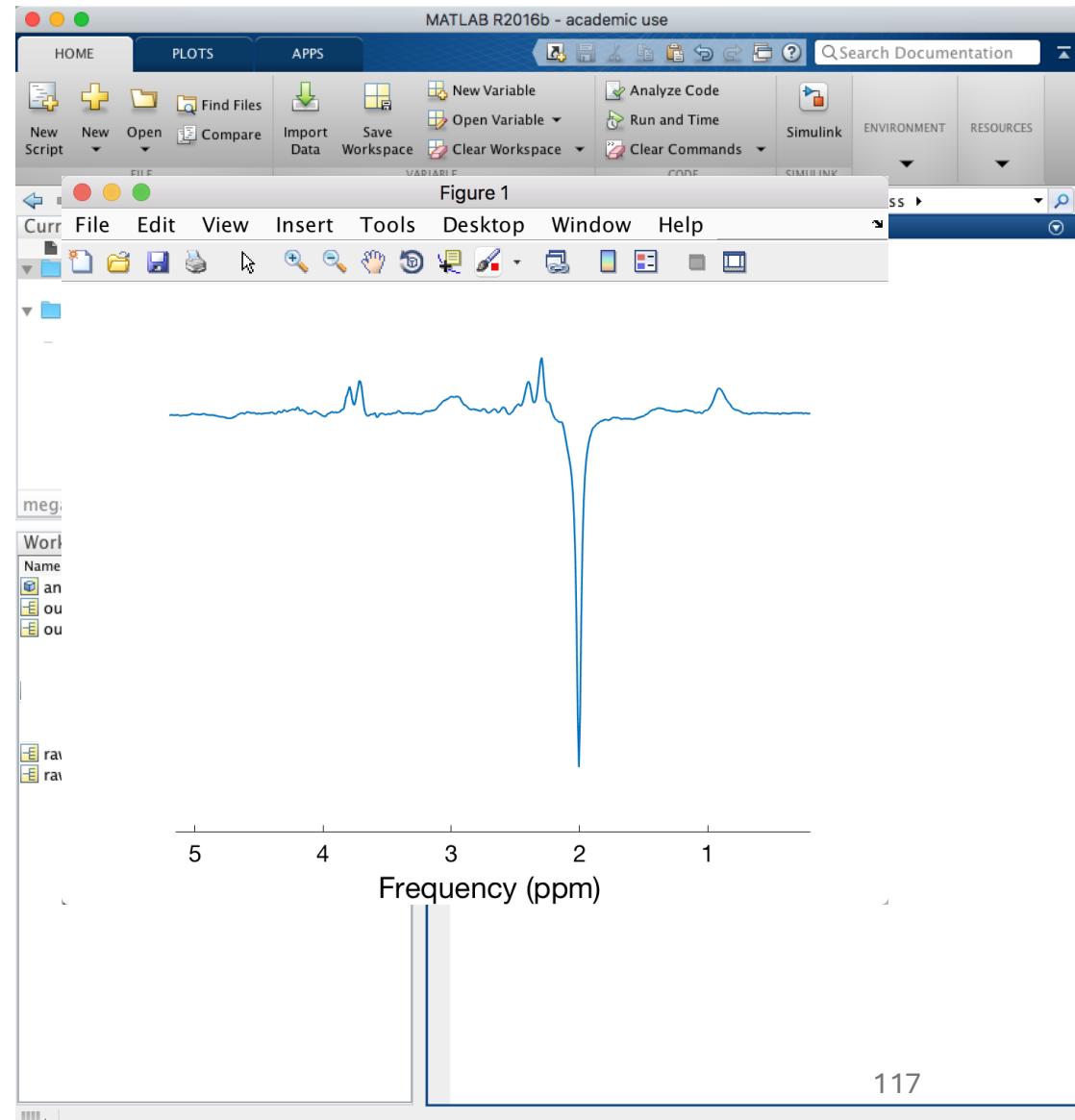
```
out5=op_combinesubspecs(out4,'diff');
```

Zero-order phase, filter (and plot result):

```
out6=op_filter(op_autophase(out5,1.8,2.2,180),2);  
op_plotspec(out6);
```

Finally, write result into LCModel text format for analysis:

```
io_writelcm(out5,'megapress_lcm',68);
```



Practical examples:

- Downloading/installing the software
- Loading and manually processing an MRS dataset
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- Data quality assurance
- Loading and analyzing an RF pulse waveform
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Automated processing pipeline:

Processing steps can be combined into a single automated script for speed and efficiency.

Some example processing pipelines are provided in the “exampleRunScripts” directory.

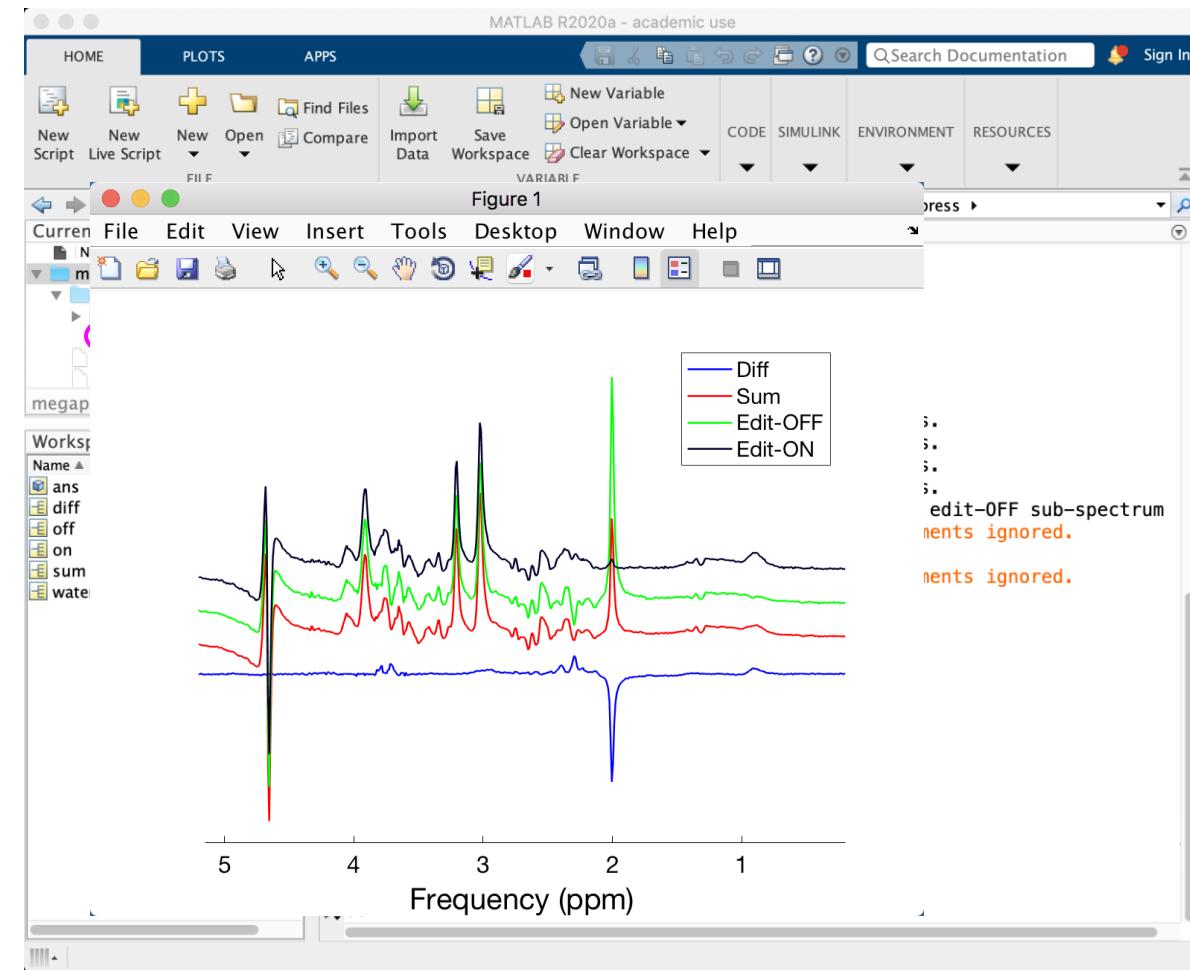
Let's use the “run_megapressproc_auto.m” script to process the same MEGA-PRESS data as before:

```
[diff,sum,off,on,water]=run_megapressproc_auto('megapress');
```

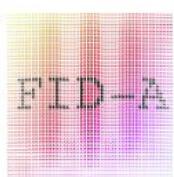
The full processing pipeline takes less than one minute. Now plot all resulting spectra on a single plot using op_plotspec.m:

```
op_plotspec({diff,sum,off,on});  
legend('Diff','Sum','Edit-OFF','Edit-ON');
```

Notice the new “report” directory that appears inside the ‘megapress’ directory. Let's look inside at the ’report.html’ file.



report.html file



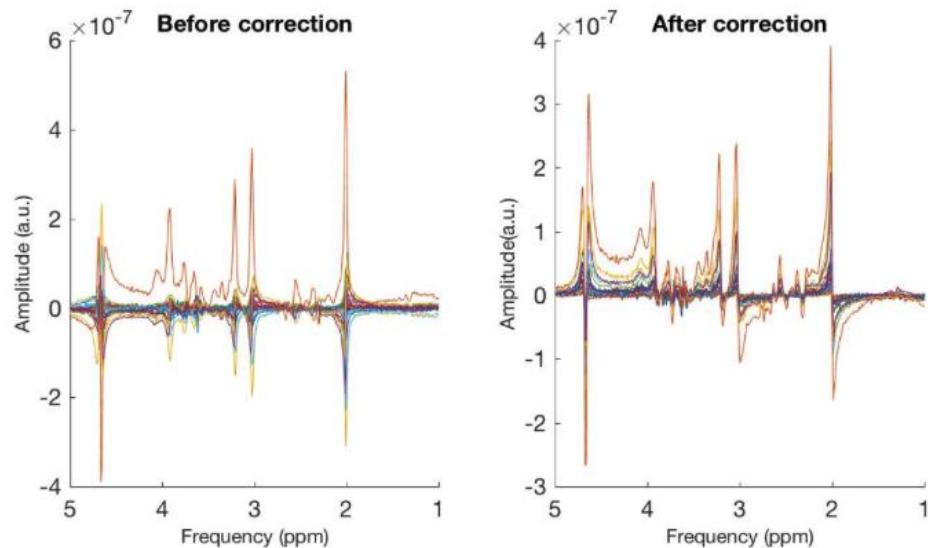
FID-A Processing Report

Processing pipeline applied to MEGA-PRESS data using run_megapressproc.m

FILENAME: /Users/jnear/Desktop/FID-A/exampleData/Siemens/sample01_megapress/megapress/megapressDLPFC.dat

DATE: 19-Jul-2017

Results of multi-coil combination:



report.html file

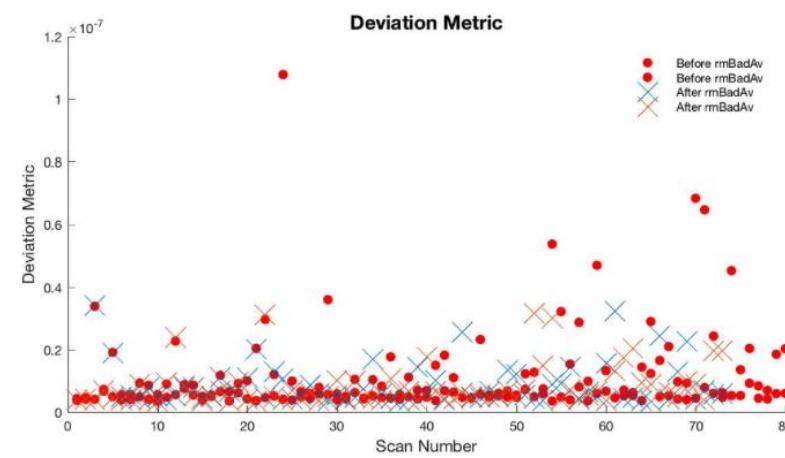
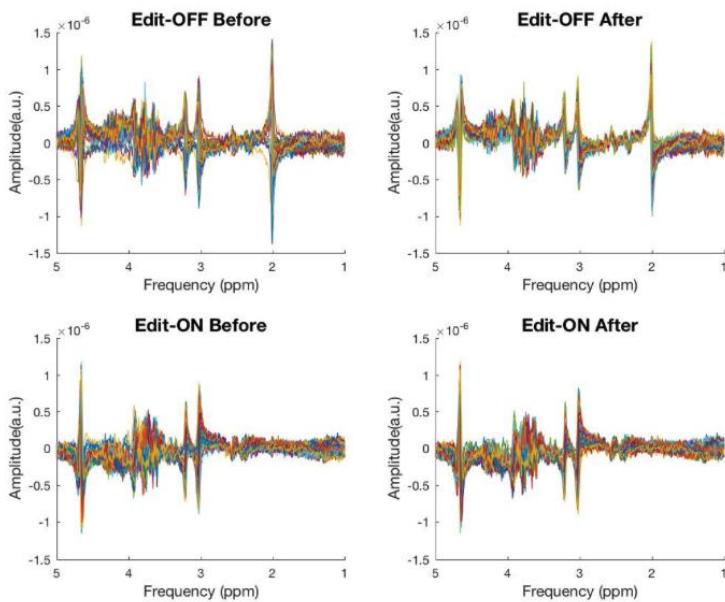
Results of removal of bad averages:

Original number of averages: 160.000000

Number of bad Averages removed: 14.000000

Number of remaining averages in processed dataset: 146.000000

Bad Averages Removal Threshold was: 4.00

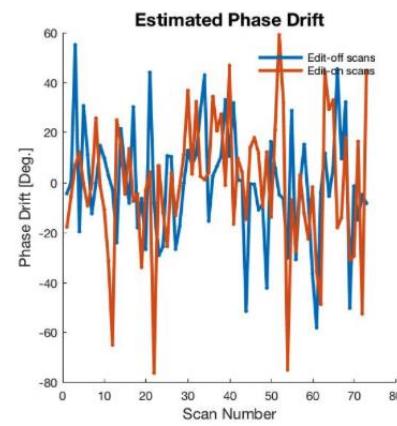
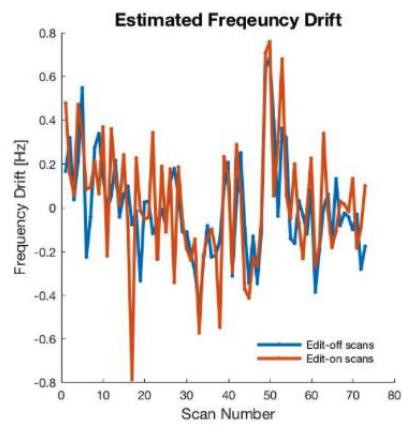
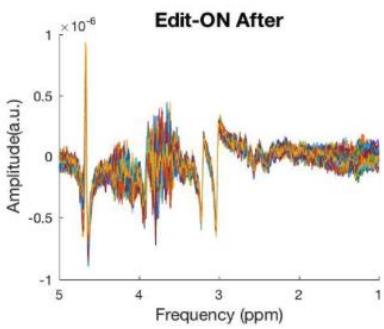
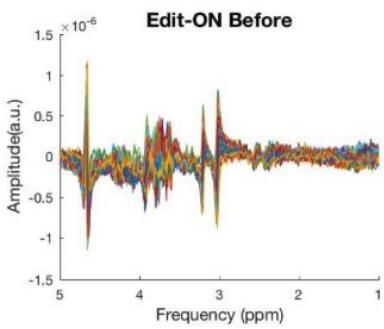
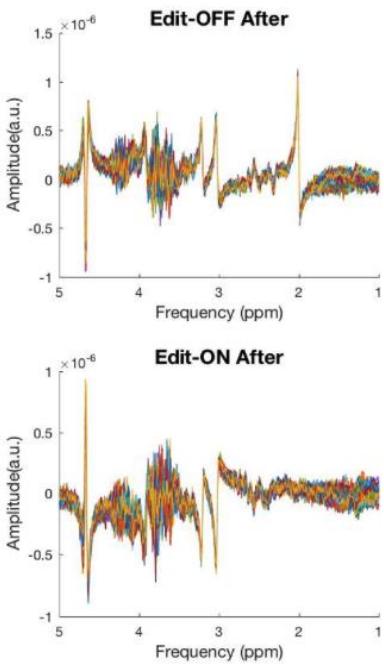
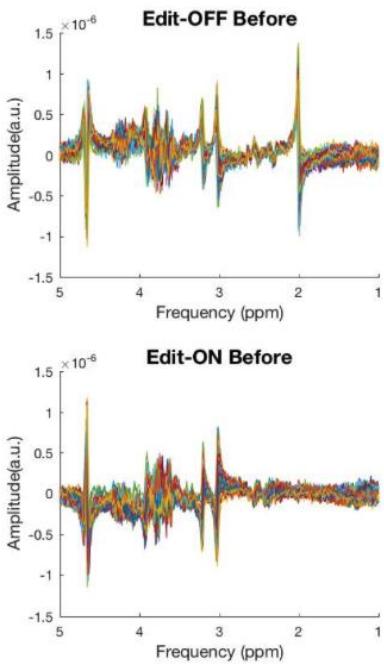


report.html file

Results of spectral registration:

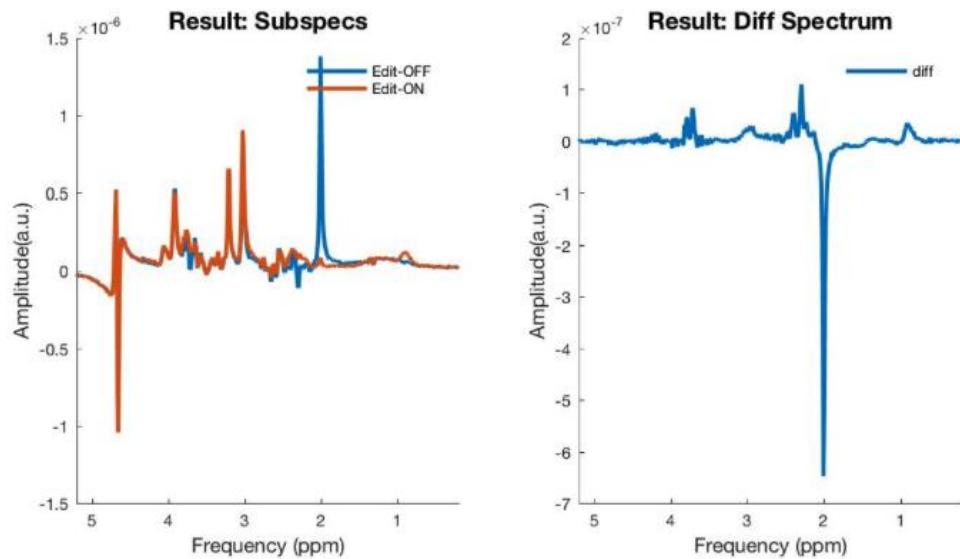
Total frequency drift was: 1.350786

Total phase drift was: 124.315027



report.html file

Final Result:



Practical examples:

- Downloading/installing the software
- Loading and manually processing an MRS dataset
- Automated processing pipelines
- Data quality assurance
- Loading and analyzing an RF pulse waveform
- Simulating MRS experiments

Data quality assurance

After processing, it is a good idea to check spectral quality.

The two most important and unbiased spectral quality metrics are:

- **Linewidth**, as measured by the full-width at half-maximum (FWHM) of a given peak
- **Signal-to-noise ratio (SNR)**, as measured by the peak height divided by the standard deviation of the noise.

Calculate the linewidth using the ‘op_getLW’ function. You can measure the linewidth of the water peak as follows:

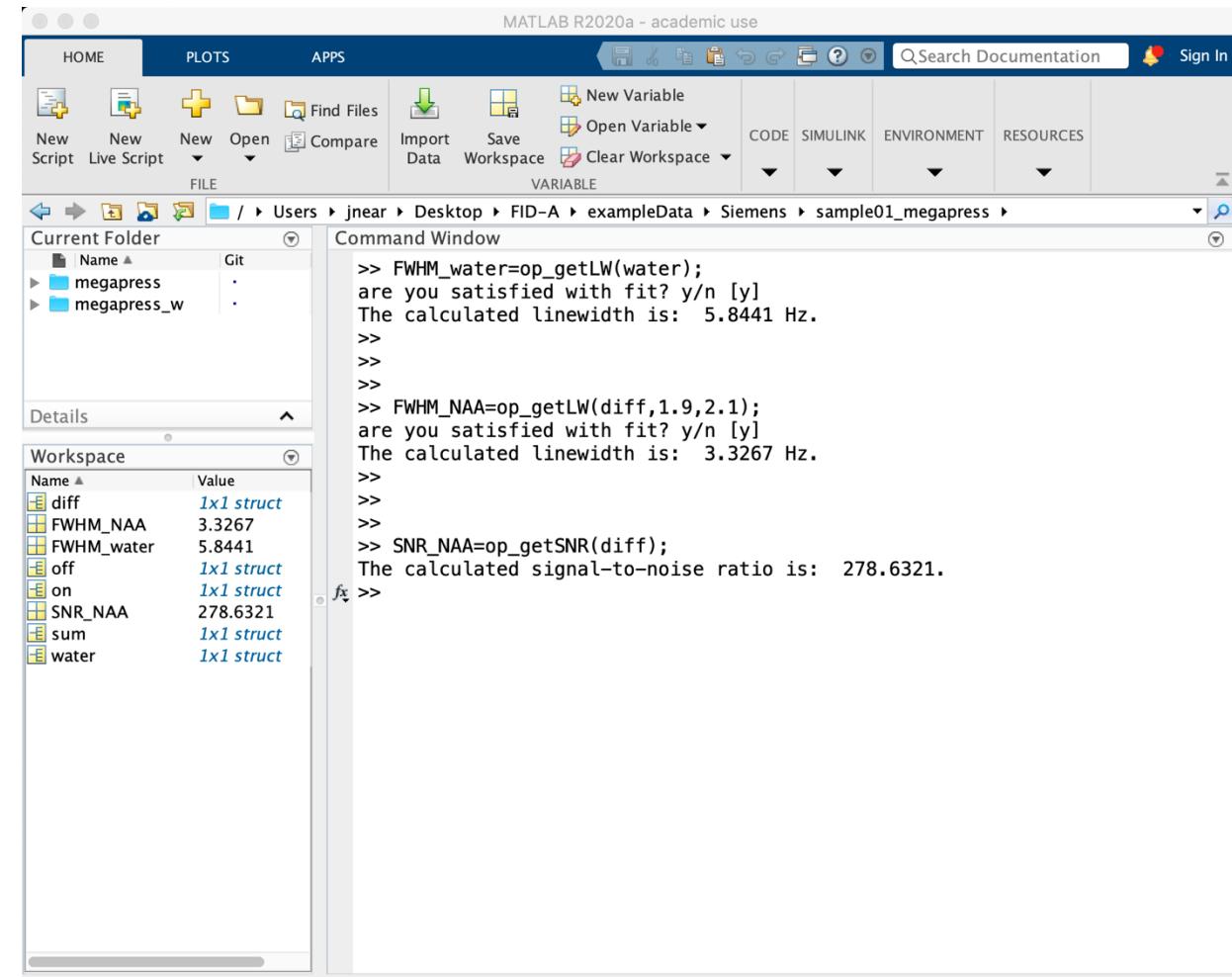
```
FWHM_water=op_getLW(water);
```

Or of a the NAA peak (for example) as follows:

```
FWHM_NAA=op_getLW(diff,1.9,2.1);
```

Lastly, calculate the SNR using ‘op_getSNR’ as follows:

```
SNR_NAA=op_getSNR(diff);
```



Practical examples:

- Downloading/installing the software
- Loading and manually processing an MRS dataset
- Automated processing pipelines
- Data quality assurance
- Loading and analyzing an RF pulse waveform
- Simulating MRS experiments

Loading and simulating

Some sample RF pulses are located in:

FID-A/rfPulseTools/rfPulses/

Load an RF pulse:

```
refoc=io_loadRFwaveform('sampleRefocPulse.pta','ref');
```

View the RF pulse structure by querying the RF pulse variable name:

```
refoc
```

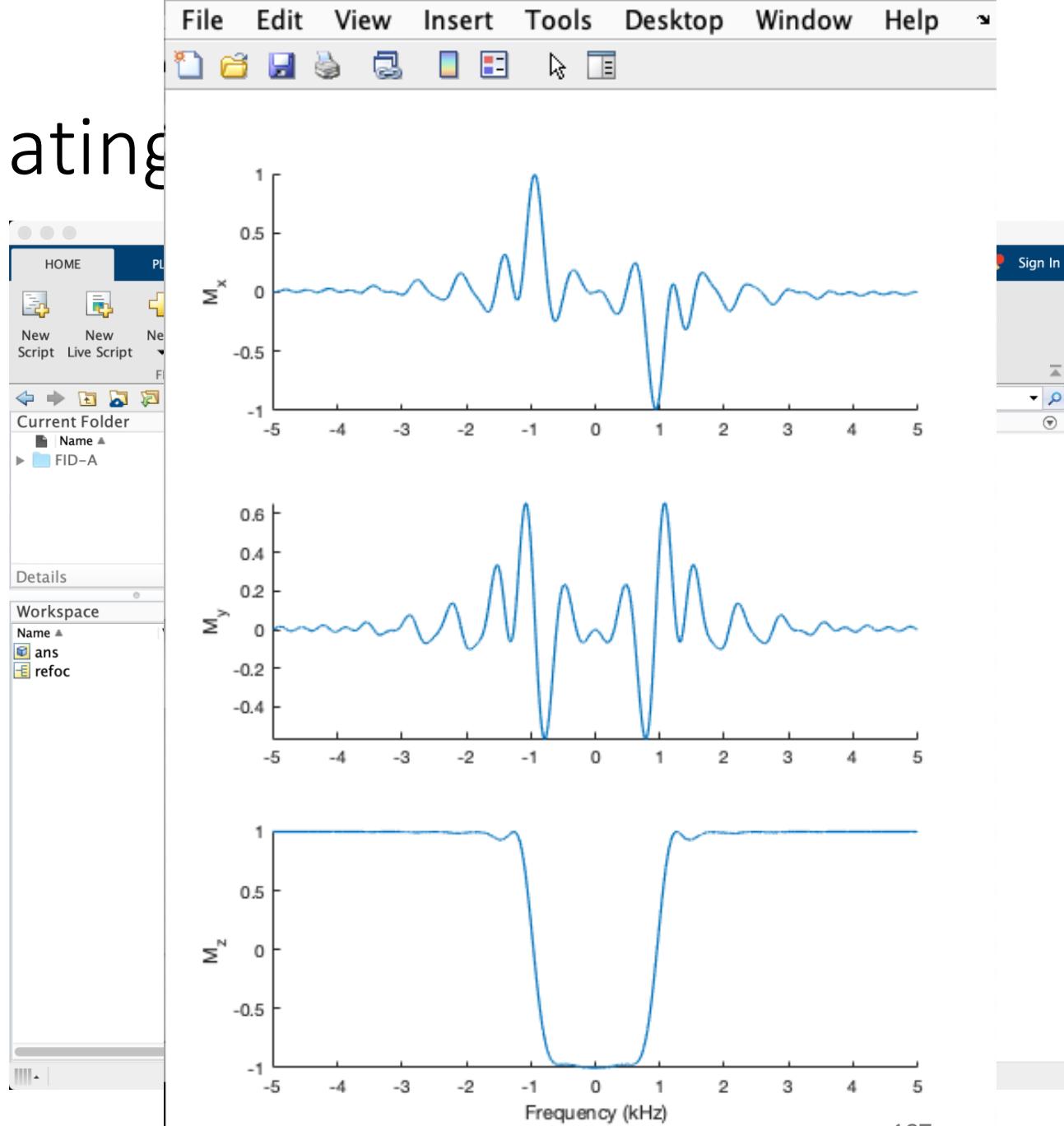
RF pulse structure contains both the waveform, and header information.

Plot the amplitude and phase waveforms using:

```
rf_plotWaveform(refoc);
```

Do bloch simulation to determine the inversion profile (3 ms pulse duration):

```
[mv, sc]=rf_blochSim(refoc, 3);
```

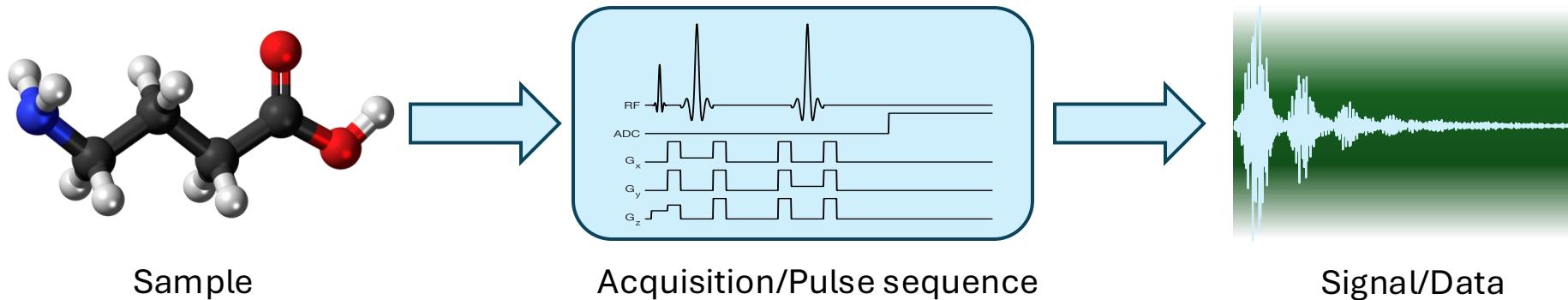


Practical examples:

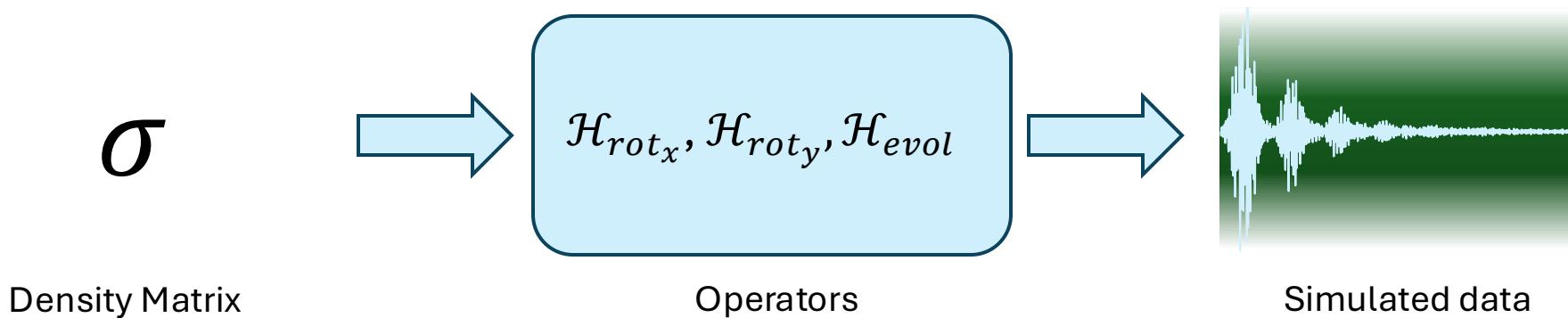
- Downloading/installing the software
- Loading and manually processing an MRS dataset
- Automated processing pipelines
- Data quality assurance
- Loading and analyzing an RF pulse waveform
- Simulating MRS experiments

Simulation Overview

Experiment:



Density Matrix Simulation:



Spin systems in FID-A

- Many spin system definitions are included:

- 2-hydroxyglutarate
- Alanine
- Ascorbate
- Aspartate
- Beta-hydroxybutyrate
- Citrate
- Creatine
- GABA
- Glucose
- Glutamine
- Glutamate
- Glycine
- Glycerophosphocholine
- Glutathione
- Lactate
- Myo-inositol
- NAA
- NAAG
- Phosphocholine
- Phosphocreatine
- Phosphorylethanolamine
- Phenylalanine
- Scyllo-inositol
- Serine
- Taurine
- Tyrosine

FID-A spin system definitions

Metabolite spin system definitions are stored in:

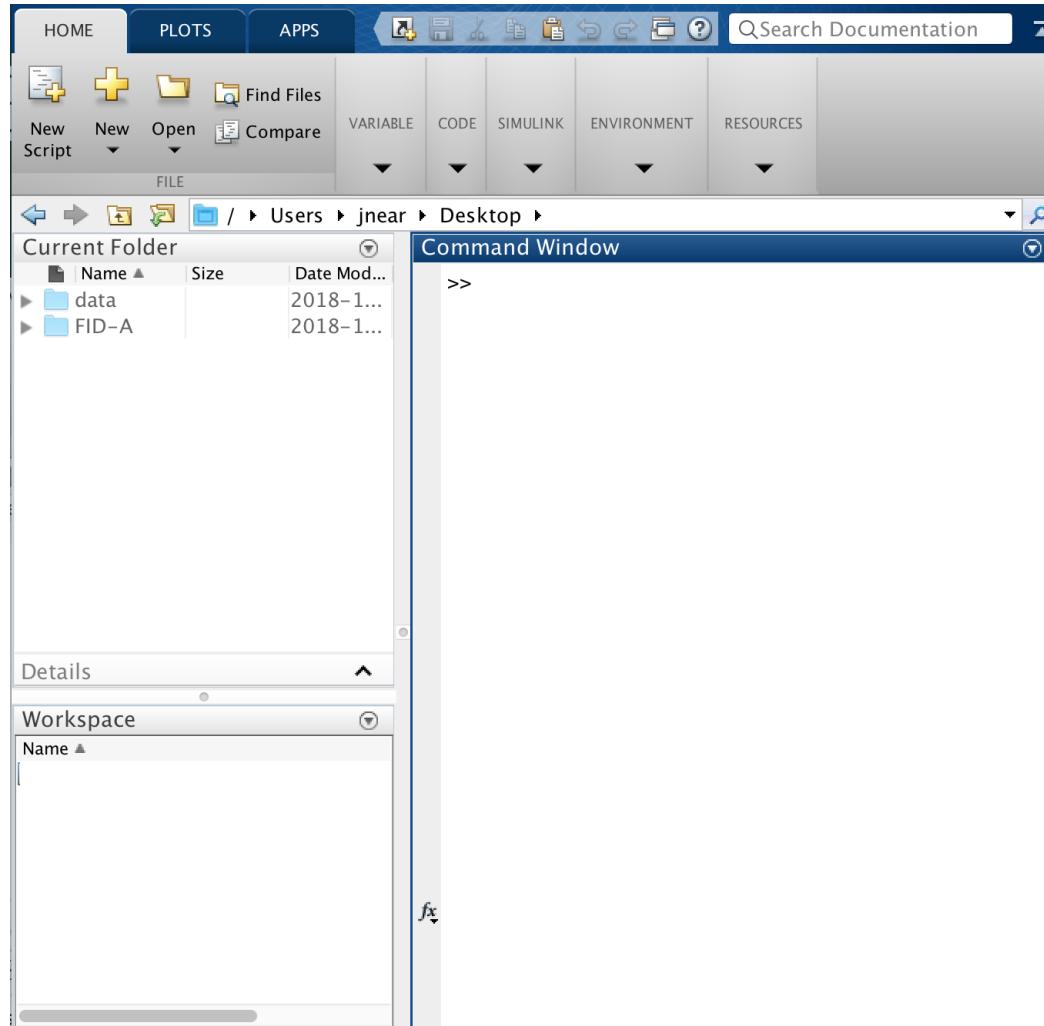
FID-A/simulationTools/Metabolites/

Load the Lactate spin system:

```
load Lac
```

The resulting variable “sysLac” is a structure array
with 4 fields:

- J
- shifts
- name
- scaleFactor

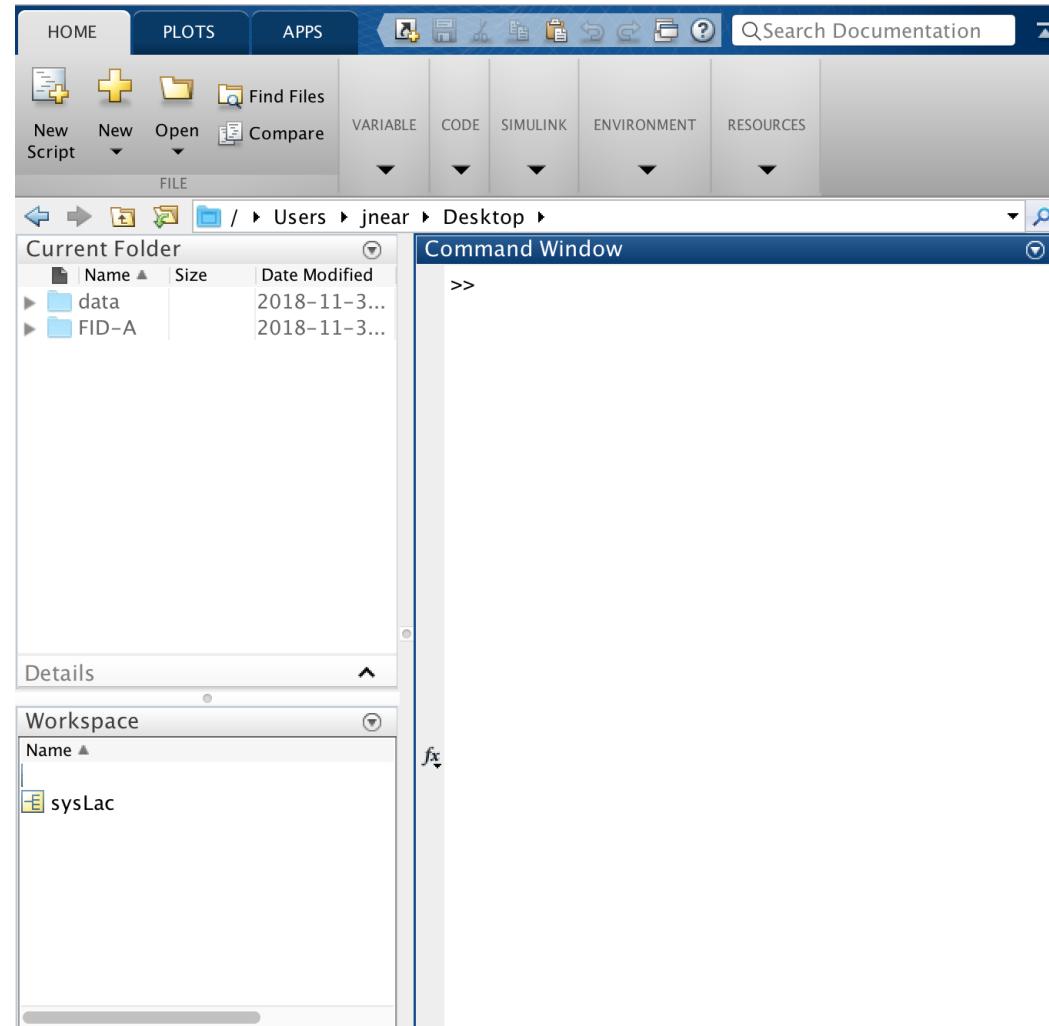


FID-A spin system definitions

Let's have a look at the values of the chemical shifts and coupling constants:

```
sysLac(1).shifts
```

```
sysLac(1).J
```



Full list of FID-A simulation tools:

Basic Spin Operators

- sim_dAdd.m
- sim_Hamiltonian.m
- sim_evolve.m
- sim_excite.m
- sim_excite_arbPh.m
- sim_gradSpoil.m
- sim_readout.m
- sim_rotate.m
- sim_rotate_arbPh.m
- sim_shapedRF.m
- sim_spoil.m

Pulse Sequences (Ideal)

- sim_laser.m
- sim_lcrawbasis.m
- sim_megapress.m
- sim_onepulse.m
- sim_onepulse_arbPh.m
- sim_press.m
- sim_spinecho.m
- sim_steam.m
- sim_steam_gradSim.m

Pulse Sequences (shaped)

- sim_make2DSimPlot.m
- sim_megapress_shaped.m
- sim_megapress_shapedEdit.m
- sim_megapress_shapedRefoc.m
- sim_megaspecial_shaped.m
- sim_onepulse_shaped.m
- sim_press_shaped.m
- sim_spinecho_shaped.m

The **Basic Spin Operators** form the building blocks for all of the **Pulse Sequences**.



Example simulation CODE:

Pulse-and-acquire sequence (sim_onepulse.m):

Usage:

```
out=sim_onepulse(np, sw, B0, lw, sys);
```

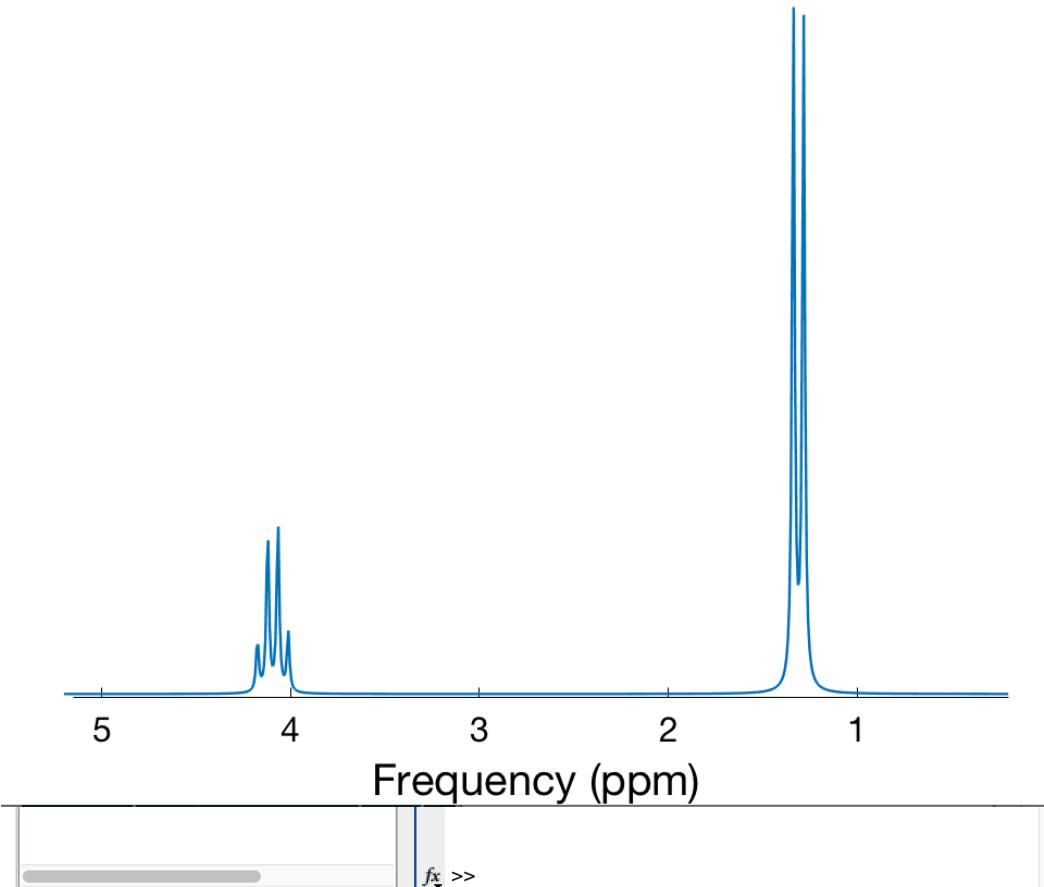
```
%Calculate Hamiltonian matrices and starting density matrix.  
[H,d]=sim_Hamiltonian(sys,Bfield);  
|  
%BEGIN PULSE SEQUENCE*****  
d=sim_excite(d,H,'x'); %EXCITE  
[out,dout]=sim_readout(d,H,n,sw,linewidth,90); %Readout along y (90 degree phase);  
%END PULSE SEQUENCE*****
```

Pulse-and-acquire Lactate simulation



Let's simulate a basic pulse-and-acquire sequence on the Lactate spin system:

And plot the result:



Example simulation CODE:

PRESS sequence (sim_press.m):

Usage:

```
out=sim_press(np, sw, B0, lw, sys, te1, te2);
```

```
%Calculate Hamiltonian matrices and starting density matrix.  
[H,d]=sim_Hamiltonian(sys,Bfield);  
  
%BEGIN PULSE SEQUENCE*****  
d=sim_excite(d,H,'x'); %EXCITE  
d=sim_evolve(d,H,tau1/2); %Evolve by tau1/2  
d=sim_rotate(d,H,180,'y'); %First 180 degree refocusing pulse about y' axis.  
d=sim_evolve(d,H,(tau1+tau2)/2); %Evolve by (tau1+tau2)/2  
d=sim_rotate(d,H,180,'y'); %second 180 degree refocusing pulse about y' axis.  
d=sim_evolve(d,H,tau2/2); %Evolve by tau2/2  
[out,dout]=sim_readout(d,H,n,sw,linewidth,90); %Readout along y (90 degree phase);  
%END PULSE SEQUENCE*****
```

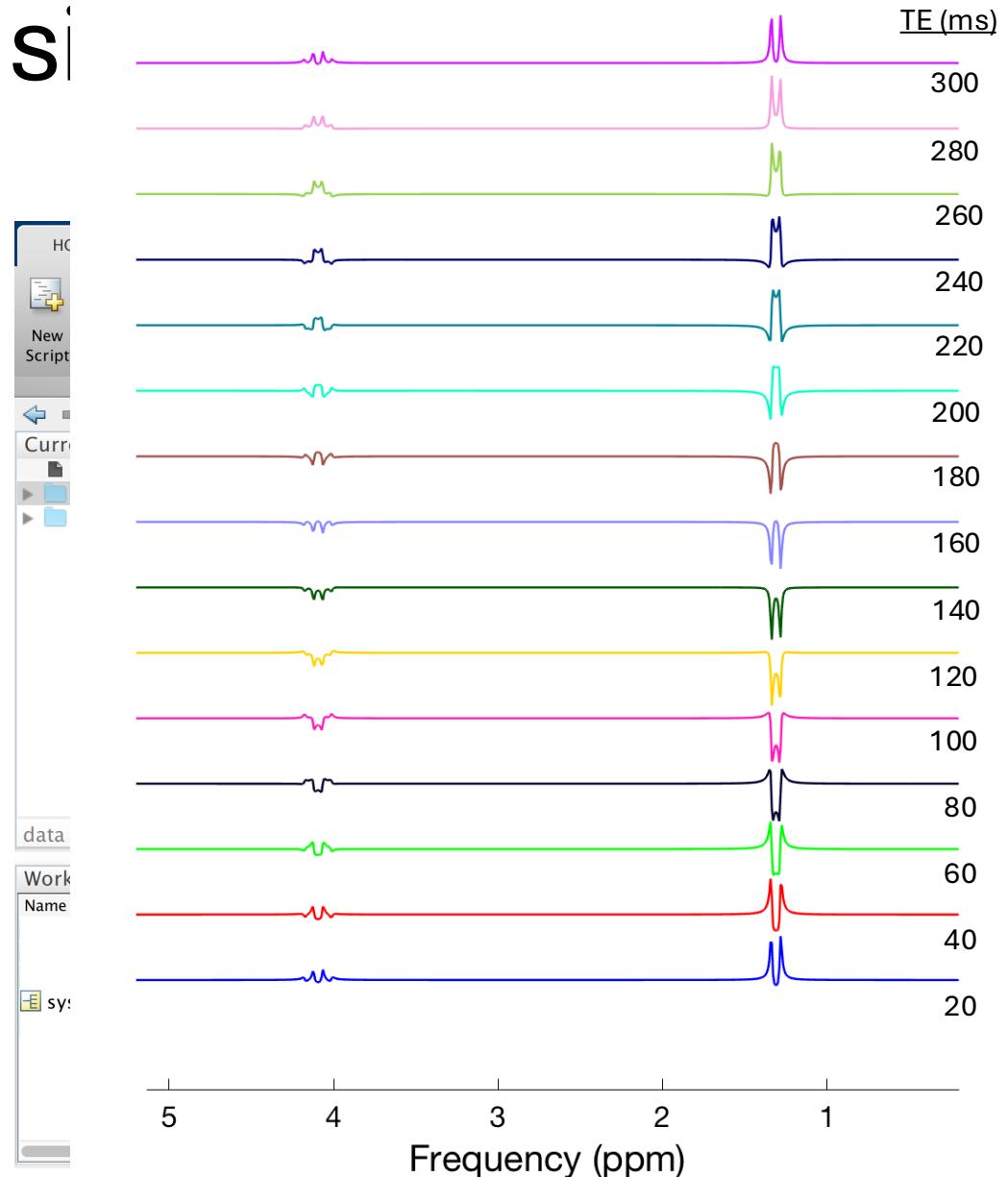
PRESS Lactate simulation

Now let's quickly generate some PRESS spectra of Lactate with a series of different echo times:

And plot the results:

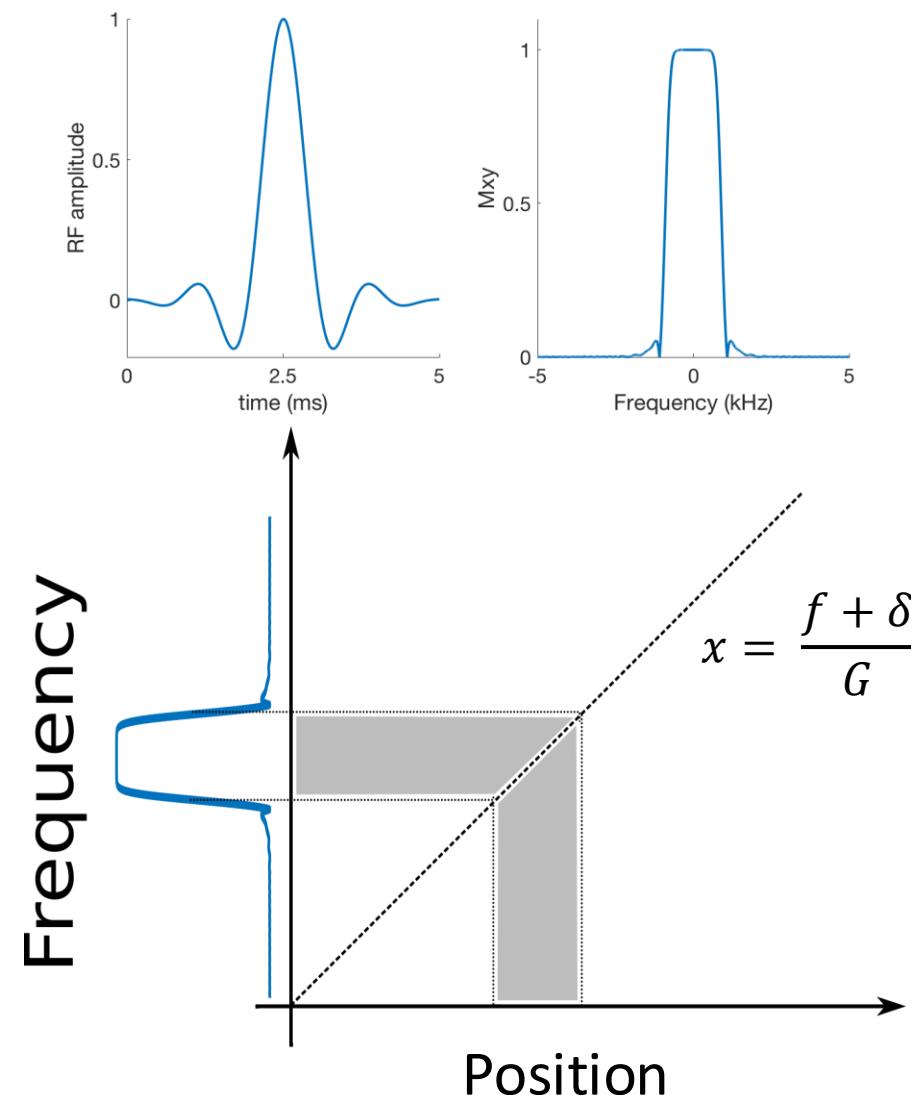
Problem: Simulations using ideal (instantaneous) RF pulses do not agree well with in-vitro experiments

Solution: Take into account chemical shift effects



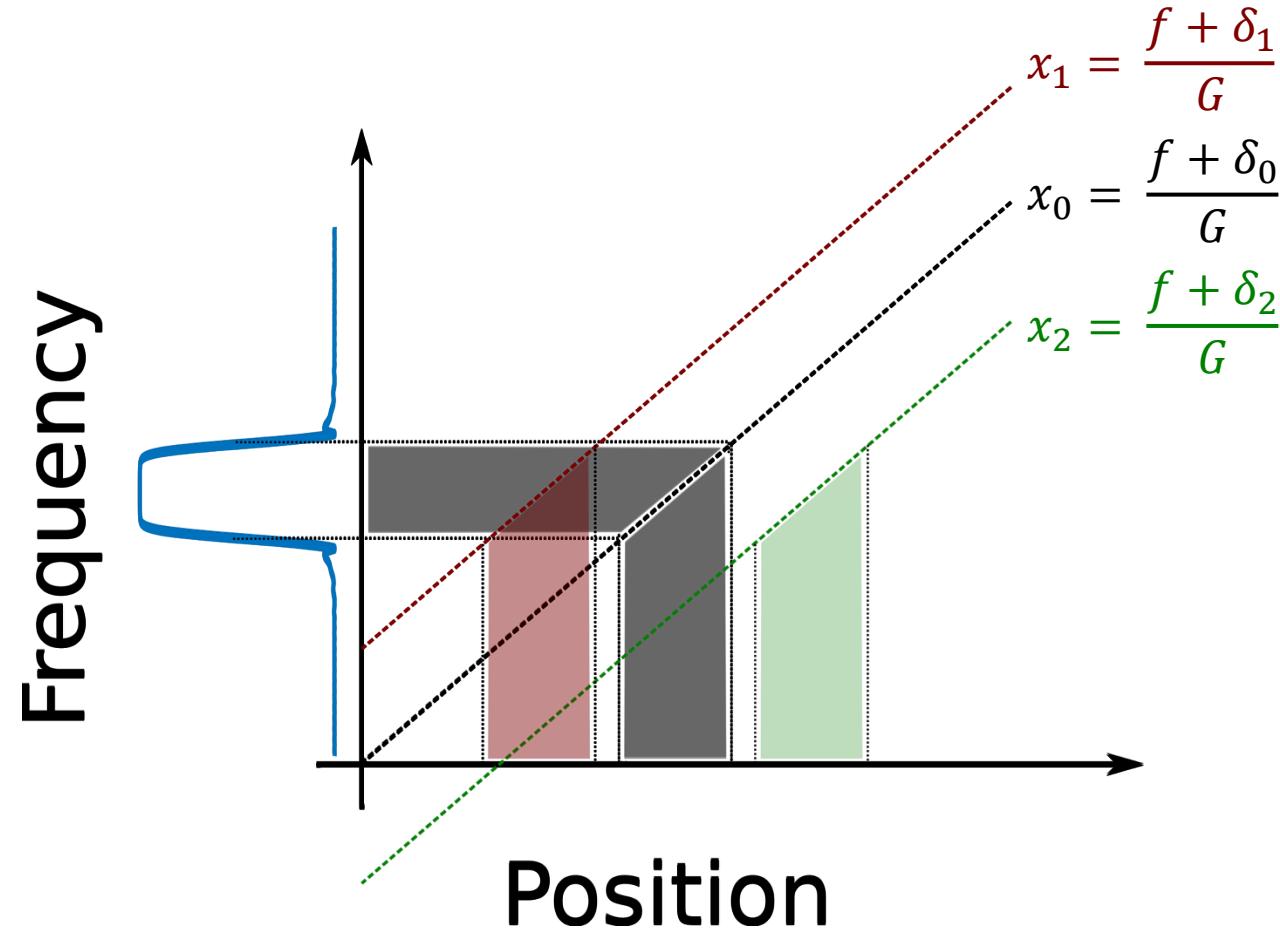
Slice selective excitation

- Spatial localization is performed using “slice selective excitation”:
 - Frequency selective RF pulse + gradient.
- Gradient creates a mapping (M) between position and frequency.
- Problem: This mapping depends on chemical shift, δ !



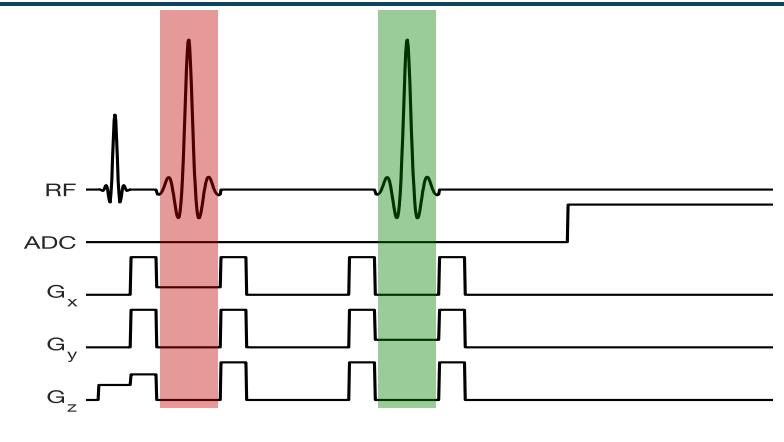
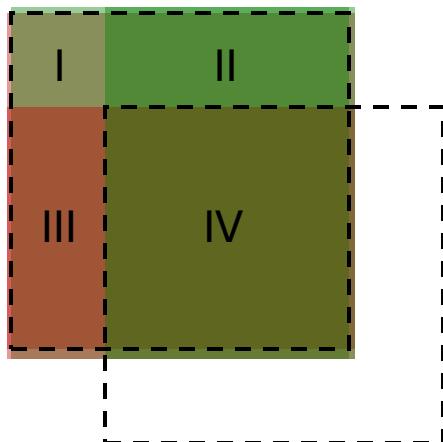
Chemical shift displacement (CSD)

- Result: spins with different chemical shifts are excited in different locations



CSD in localized MRS for coupled spin systems

- I – Spin X experiences neither refoc pulse
- II – Spin X experiences only the 2nd refoc pulse
- III – Spin X experiences only the 1st refoc pulse
- IV – Spin X experiences both refoc pulses

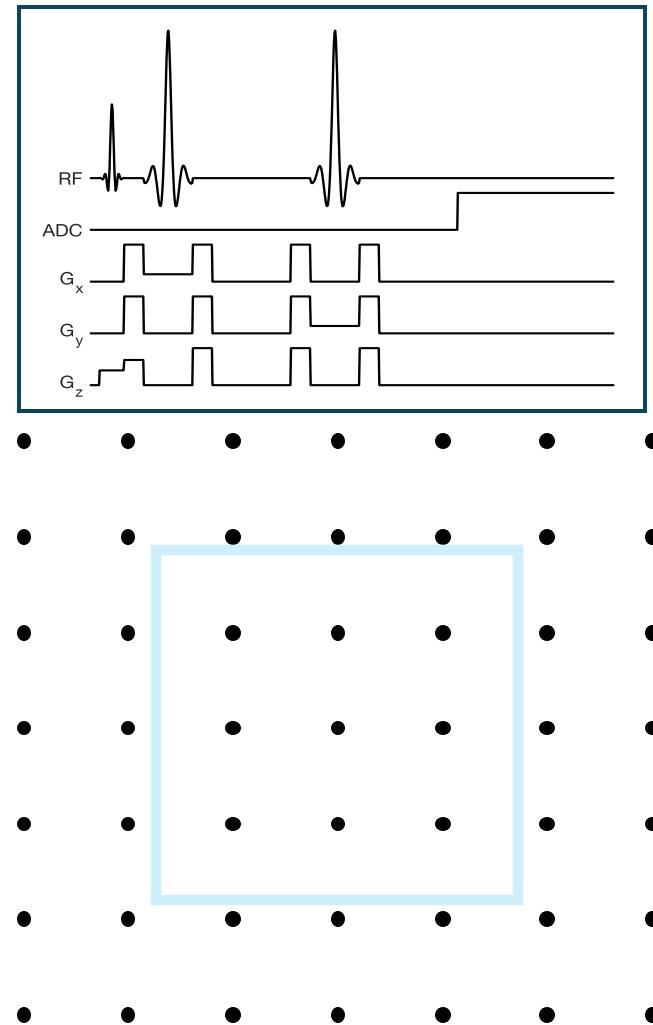


This phenomenon has a few consequences:

- The spectral shape varies within the region of interest (true for both standard PRESS and MEGA-PRESS experiments)
- Accurate simulations must take into account the rf pulse shapes and chemical shift displacement

Accounting for CSD

- Chemical shift displacement is accounted for by repeating the simulation at an array of spatial positions.
- The exact pulse waveform and gradient strength are taken into account.
- Outer volume signals removed either by phase cycling or spoiler gradients.



Advanced simulation with shaped RF waveforms:

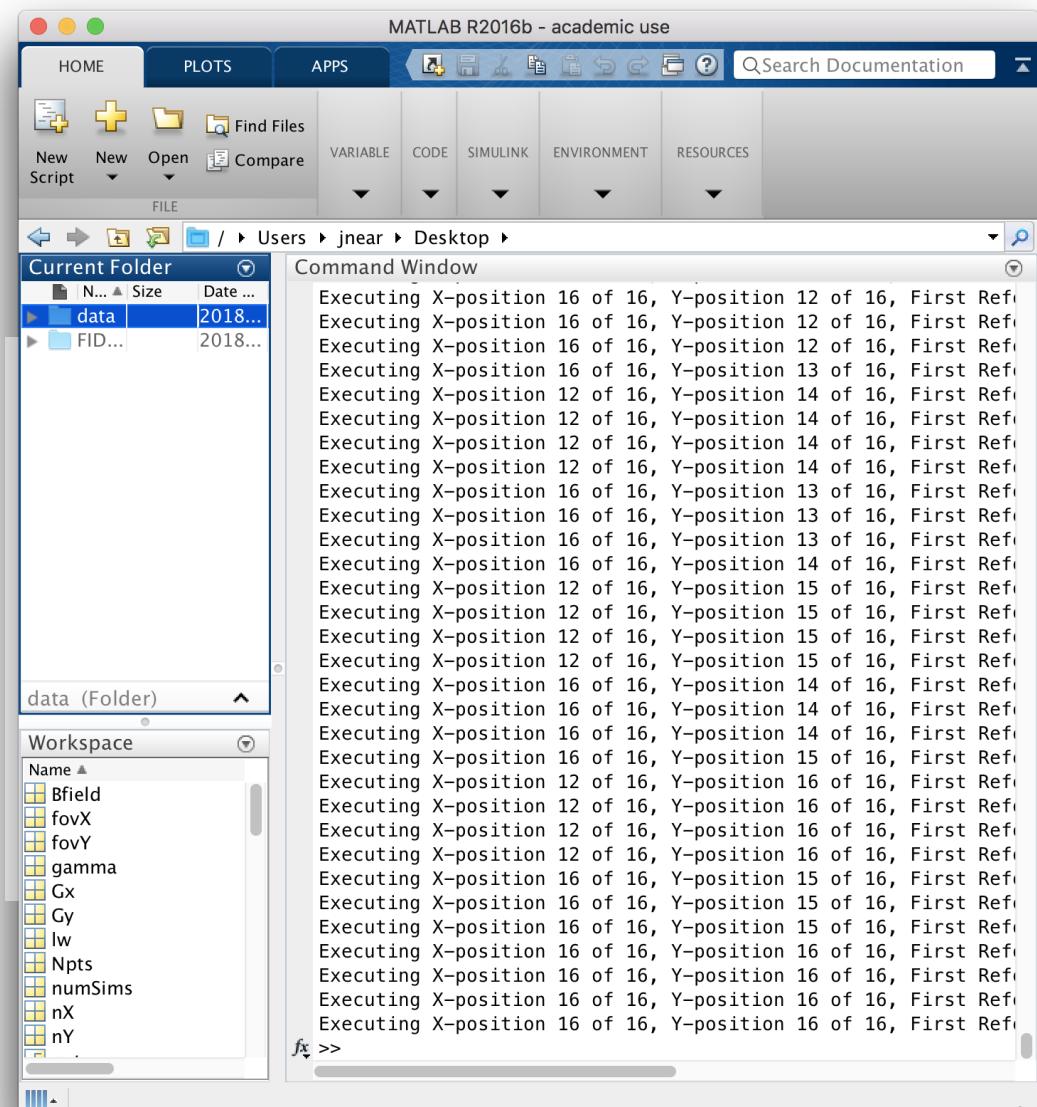
FID-A has example scripts for such spatially resolved simulations. To view the example script for shaped PRESS simulations, type:

```
edit run_simPressShaped.m
```

To run, first adjust input parameters to desired values. We will now run a 3T PRESS simulation for Lactate with TE=135 ms, and a 16 x 16 spatial grid. To run, type the name of the script at the command line:

```
run_simPressShaped
```

Run time < 3 minutes with two-fold parallelization. Two main outputs of interest: the spatially resolved simulated spectra ('out_posxy'), and the result after summing over all space ('out'). Plot both:



Plotting the simulation I

Plot spatially resolved simulation results using sim_make2DSimPlot.m. First, focus on the 1.3 ppm Lactate resonance:

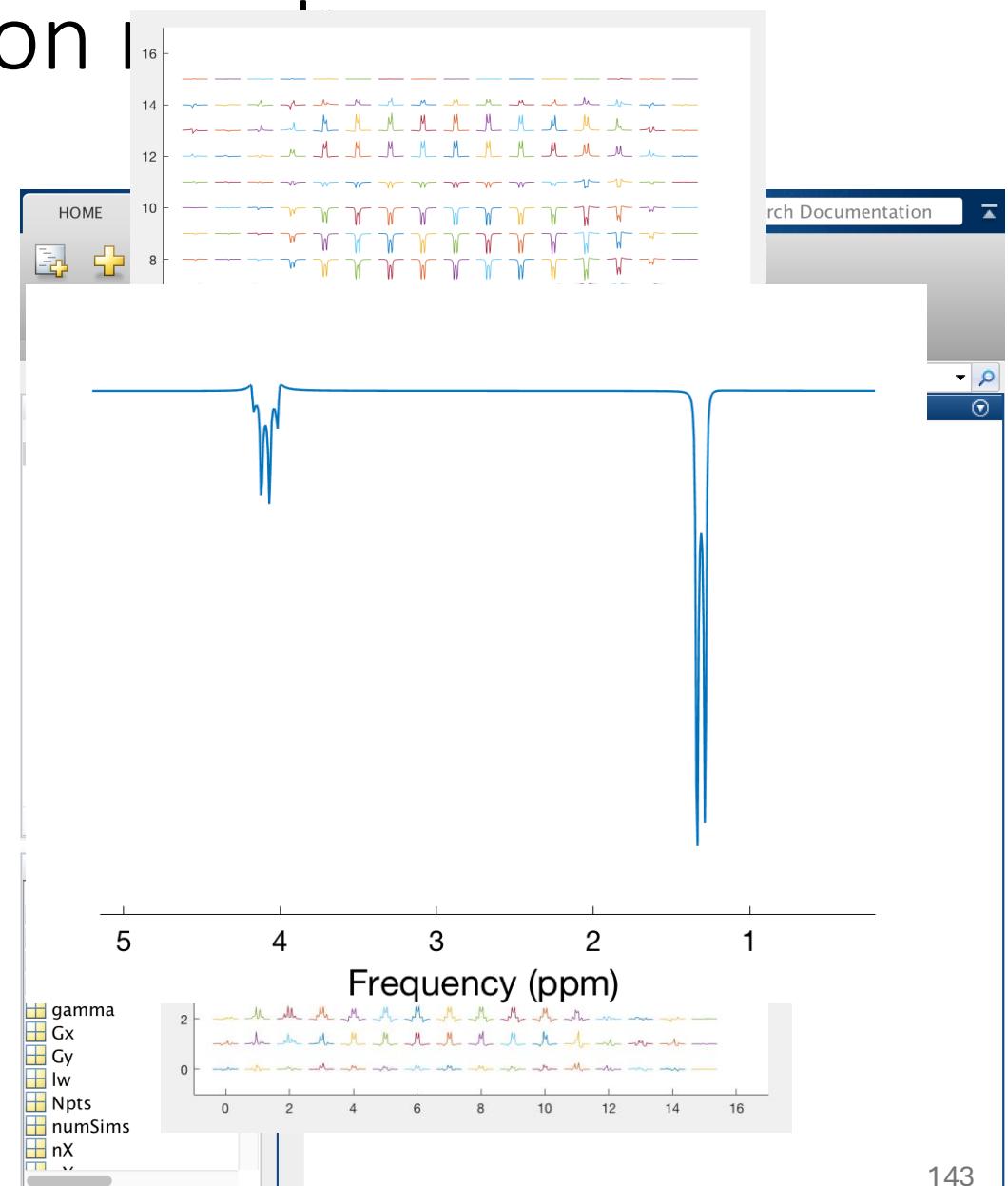
```
sim_make2DSimPlot(out_posxy,1.1,1.5);
```

Now view the 4.1 ppm Lactate resonance:

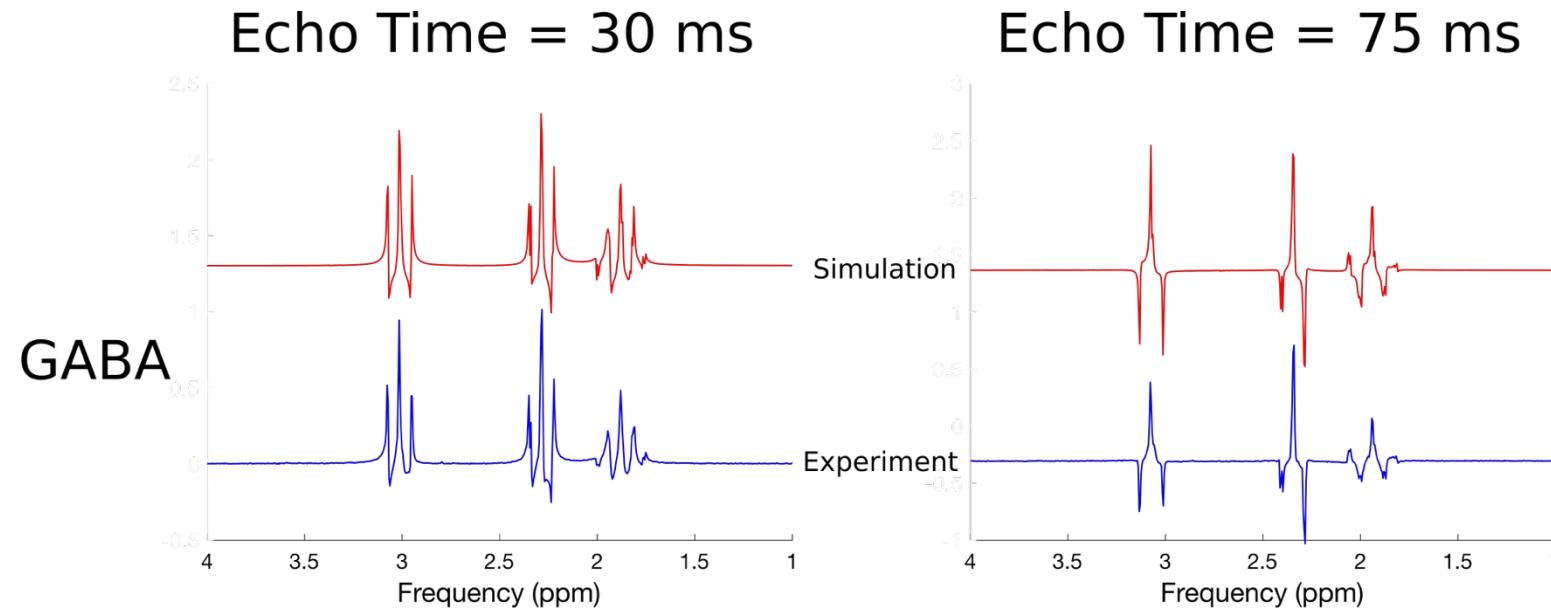
```
sim_make2DSimPlot(out_posxy,3.9,4.3)
```

Finally, view the result after summing over all space:

```
op_plotspec(out);
```



Simulation accuracy:



Simulating MEGA-PRESS

- Requires subtraction of an edit-off and an edit-on simulation.
- FID-A has a few scripts for MEGA-PRESS simulations.
- For non-spatially-resolved simulations:
 - `sim_megapress.m`
 - `run_simMegaPressShapedEdit.m`
- For spatially-resolved simulations:
 - `run_simMegaPressShaped.m`
 - `run_simMegaPressShaped_fast.m`
 - `run_simMegaPressShapedRefoc.m`

Simulating mega-press

run_simMegaPressShapedEdit.m:

```
% *****INPUT PARAMETERS*****
editWaveform='sampleEditPulse.pta'; %name of editing pulse waveform.
editOnFreq=1.88; %frequency of edit on pulse[ppm]
editOffFreq=7.4; %frequency of edit off pulse[ppm]
editTp=20; %duration of editing pulses[ms]
Npts=2048; %number of spectral points
sw=2000; %spectral width [Hz]
Bfield=3; %magnetic field strength [Tesla]
lw=2; %linewidth of the output spectrum [Hz]
taus=[5,... %Time from excitation to 1st refoc pulse [ms]
      17,... %Time from 1st refoc pulse to 1st editing pulse [ms]
      17,... %Time from 1st editing pulse to 2nd refoc pulse [ms]
      17,... %Time from 2nd refoc pulse to 2nd editing pulse [ms]
      12]; %Time from 2nd editing pulse to ADC onset [ms]
spinSys='GABA'; %spin system to simulate
centreFreq=3.0; %Centre Frequency of MR spectrum [ppm];
editPhCyc1=[0 90]; %phase cycling steps for 1st editing pulse [degrees]
editPhCyc2=[0 90 180 270]; %phase cycling steps for 2nd editing pulse [degrees]
% *****END OF INPUT PARAMETERS*****
```

sim_megapress_shapedEdit.m:

```
%BEGIN PULSE SEQUENCE*****
d=sim_excite(d,H,'x');
d=sim_evolve(d,H,delays(1)/1000);
d=sim_rotate(d,H,180,'y');
d=sim_evolve(d,H,delays(2)/1000);
d=sim_shapedRF(d,H,editPulse,editTp,180,90+editPh1);
d=sim_evolve(d,H,delays(3)/1000);
d=sim_rotate(d,H,180,'y');
d=sim_evolve(d,H,delays(4)/1000);
d=sim_shapedRF(d,H,editPulse,editTp,180,90+editPh2);
d=sim_evolve(d,H,delays(5)/1000);
[out,dout]=sim_readout(d,H,n,sw,linewidth,90); %Readout along y (90 degree phase);
%END PULSE SEQUENCE*****
```

%EXCITE
%Evolve by delays(1)
%1st instantaneous 180 degree refocusing pulse :
%Evolve by delays(2)
%1st shaped editing pulse rotation
%Evolve by delays(3)
%2nd instantaneous 180 degree refocusing pulse :
%Evolve by delays(4)
%2nd shaped editing pulse rotation
%Evolve by delays(5)

Simulating MEGA-PRESS

Let's run a basic MEGA-PRESS simulation using `run_simMegaPressShapedEdit`. This function runs a quick MEGA-PRESS simulation with instantaneous refocusing pulses, and shaped editing pulses.

As before, adjust the parameters in the script as desired, and then type the name of the script at the command line:

```
run_simMegaPressShapedEdit;
```

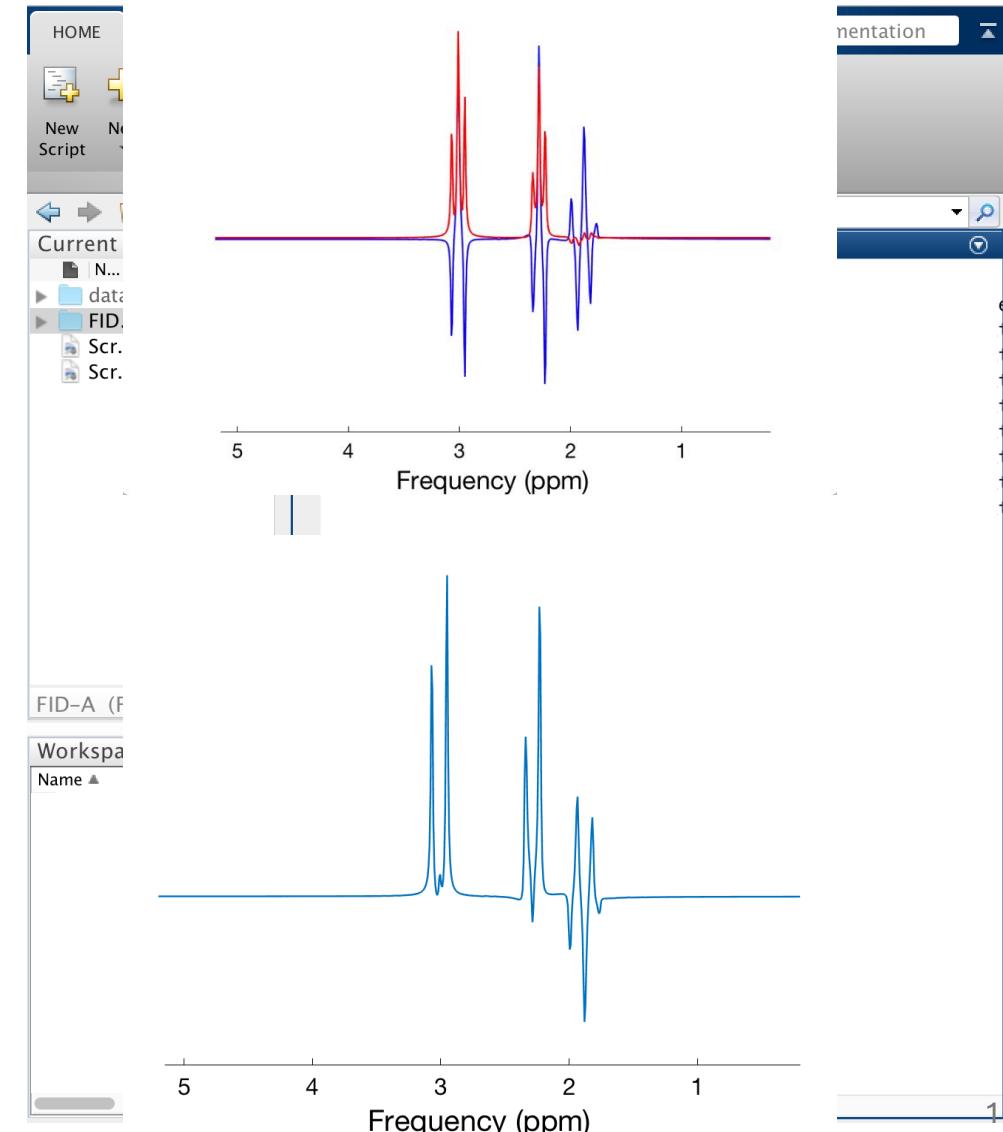
Run time < 1 minute. Three main outputs of interest: the edit-off spectrum ('outOFF'), the edit-on spectrum ('outON'). Plot both:

```
op_plotspec({outOFF,outON});
```

We can also now generate the MEGA-PRESS difference spectrum by subtracting the OFF spectrum from the ON spectrum:

```
diff=op_subtractScans(editON,editOFF);
```

```
op_plotspec(diff);
```



Generating LCModel basis spectra

- For basis sets, use the most accurate possible simulation given your time constraints.
 - Spatially resolved with high-resolution (at least 32 x 32)
 - Using the exact pulse waveforms and timings to be used experimentally
- Once you have generated a simulated spectrum that you are happy with, you can write it into LCModel “.RAW” format using:

```
[~]=io_writelcmraw(diff,'GABA.RAW','GABA');
```

- Repeat the above process for every metabolite of interest. Then, collect the .RAW files on your LCModel server, and run the LCModel command ‘makebasis’ to convert the .RAW files into an LCModel .basis file.

Summary

- The FID-A Toolkit provides a command line environment for MRS data processing, simulation and basic RF pulse design.
- User has high degree of control, flexibility and efficiency.
- Open source provides a platform for users to build upon.