2018 UF Metabolomics Workshop: *In-vivo* NMR Spectroscopy

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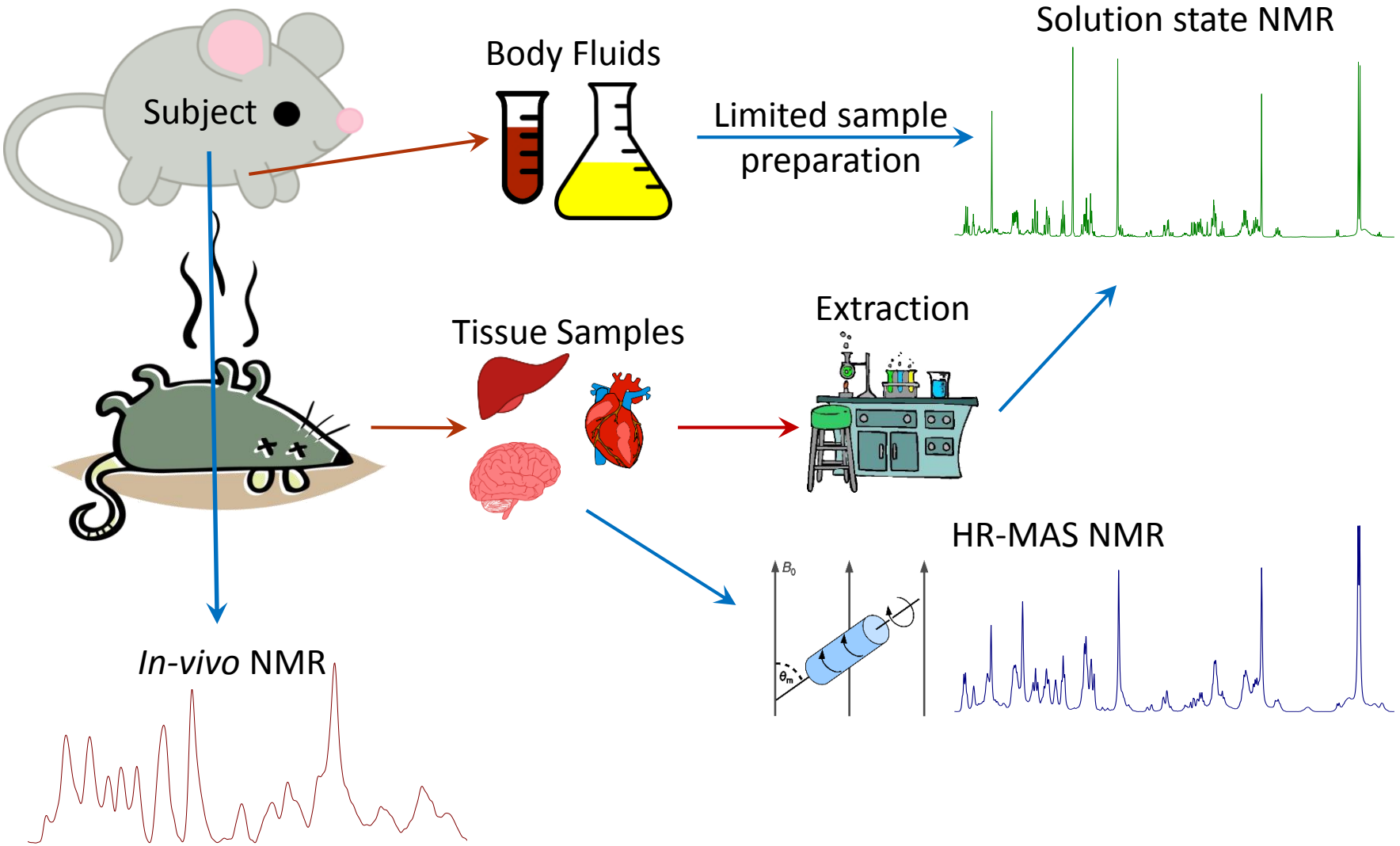
Why *In-vivo* NMR Spectroscopy?



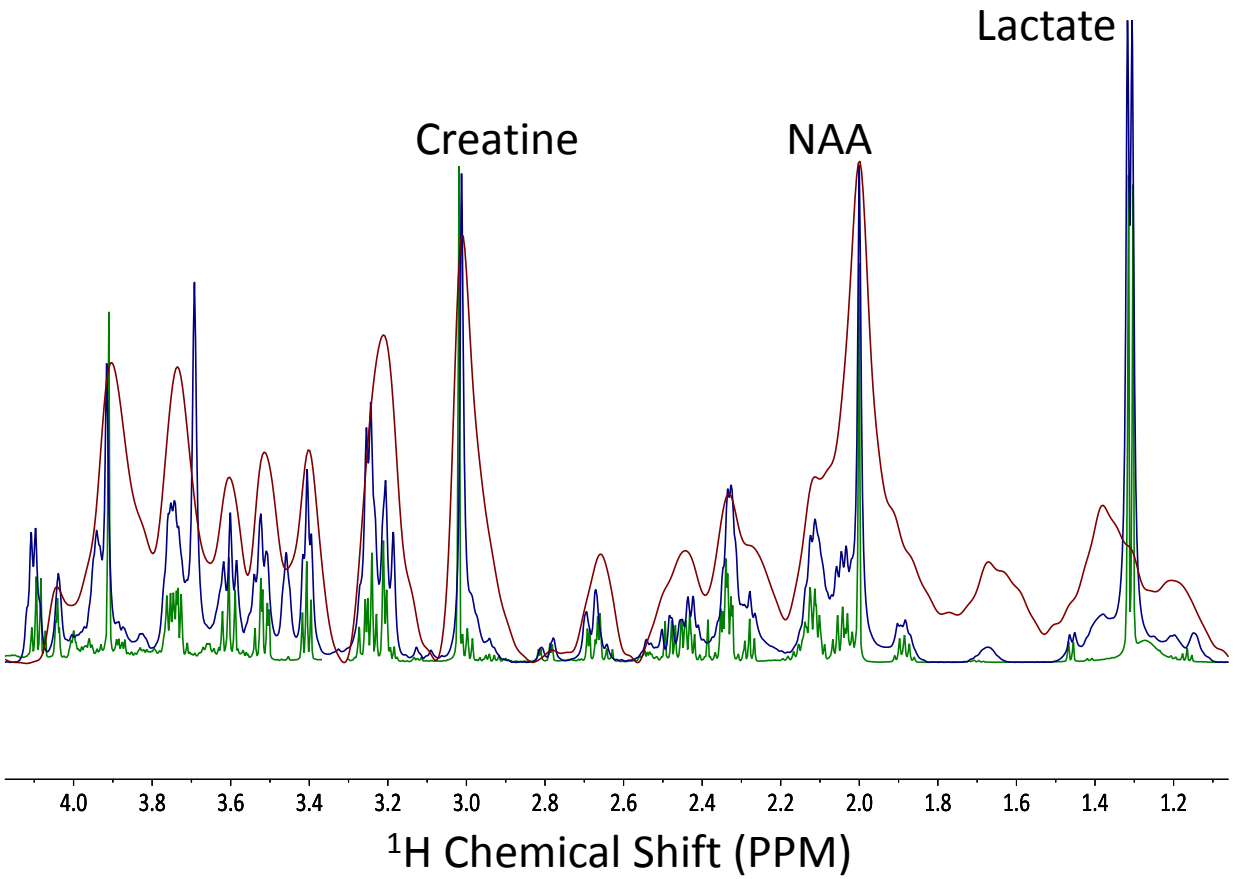
Why *In-vivo* NMR Spectroscopy?

- Example NMR spectra of rat brain tissue

Polar Extract: 600 MHz
150 – 200 Peaks
20 – 25 Metabolites
Heavily processed

HR-MAS: 600 MHz
50 – 100 Peaks
15 – 20 Metabolites
Dead tissue

In-vivo: 470 MHz
10 – 15 Peaks
5 – 10 Metabolites
Living subject



Why *In-vivo* NMR Spectroscopy?

- **In-vivo spectroscopy trades resolution for relevance**

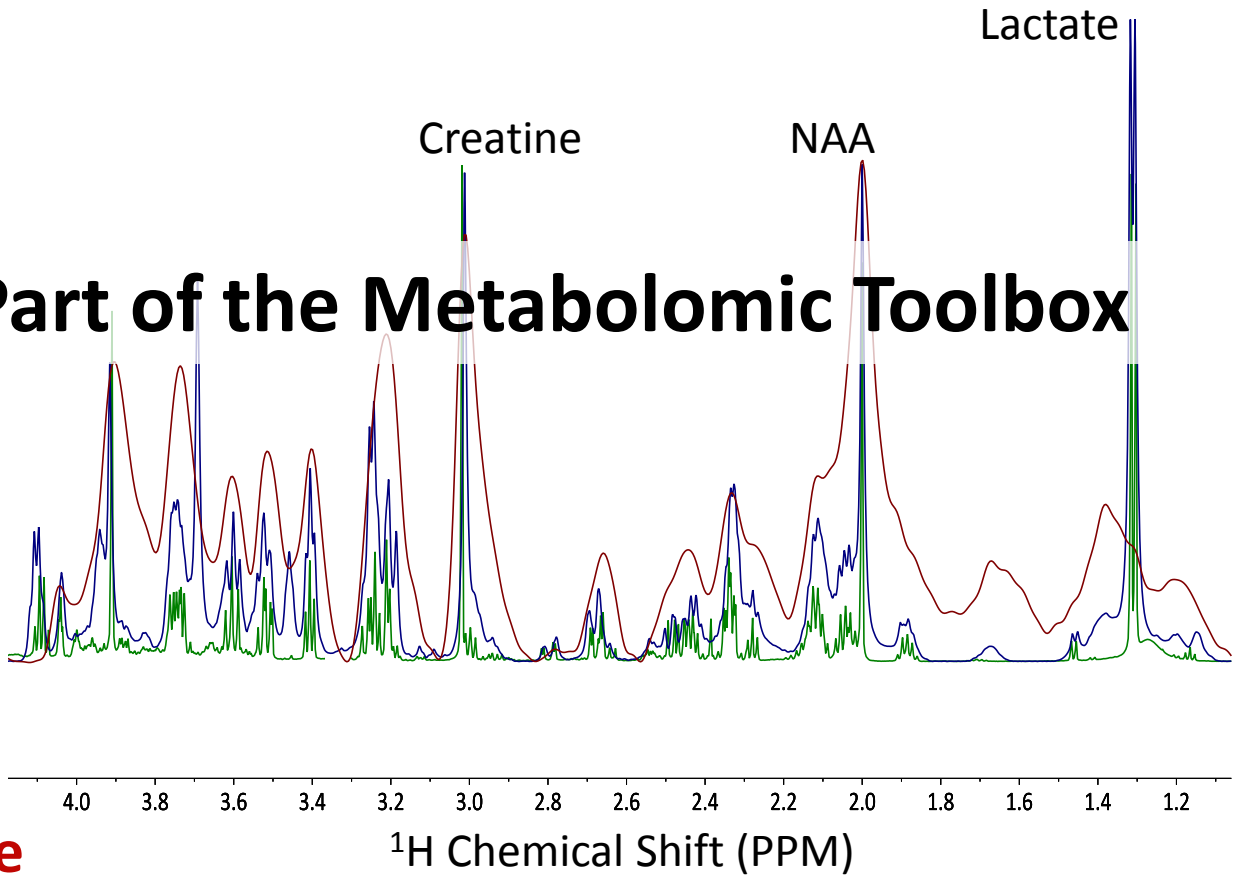
Spectra Quality

Polar Extract

HR-MAS

In-vivo

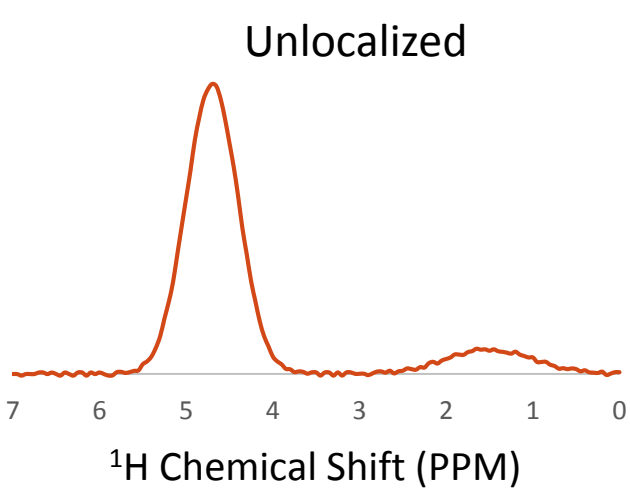
Biological Relevance

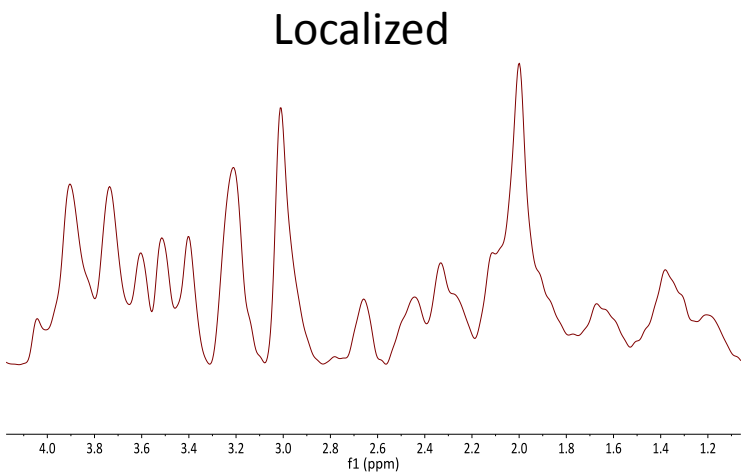


Part of the Metabolomic Toolbox

Why Spatial Localization?

- Acquire data from a specific organ/ region of the subject
- Evolution does not optimize for NMR!
  - B_0 variations across an animal are large, typically we can achieve ~1 PPM linewidth.
  - Local B_0 variations are much smaller, and we can achieve < 0.1 PPM linewidths

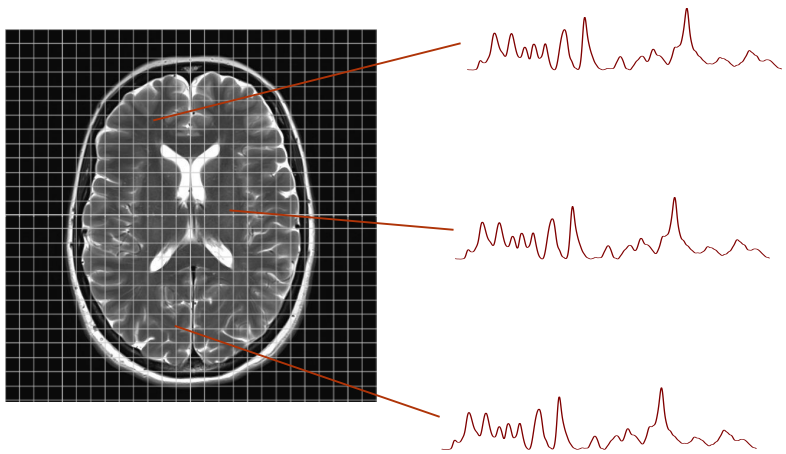




Spatial Localization Strategies

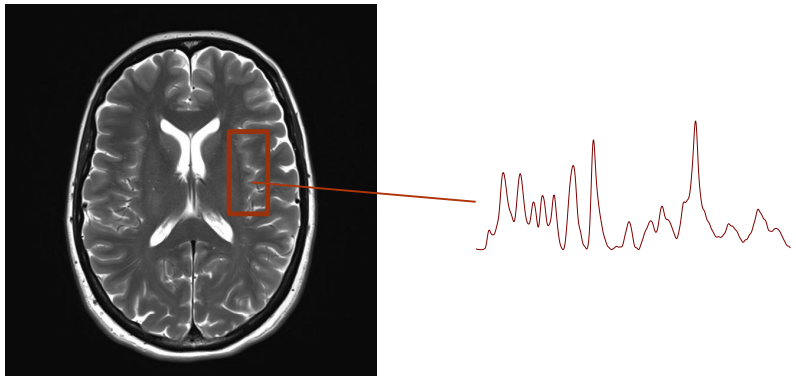
Imaging

- Adapt MR Imaging methods to include spectral data
- Chemical Shift Imaging
  - Single Point Imaging
  - EPSI



Spectroscopy

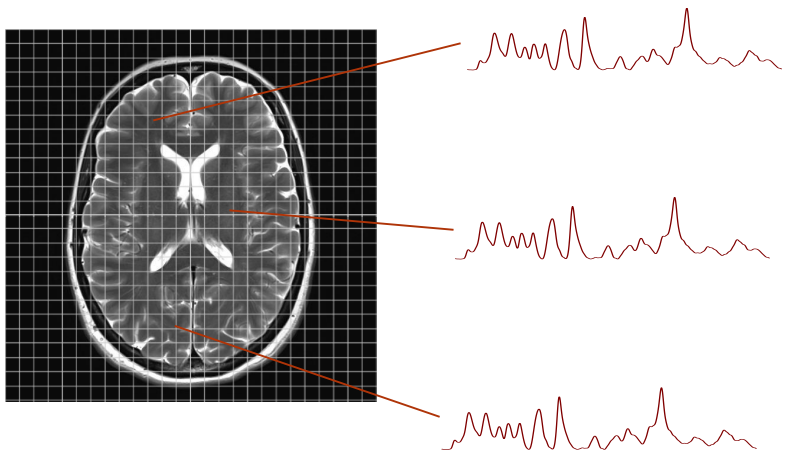
- Use RF and field gradients to localize NMR spectra
- Localized Spectroscopy
  - PRESS, STEAM
  - LASER, semi-LASER
  - ISIS



Spatial Localization Strategies

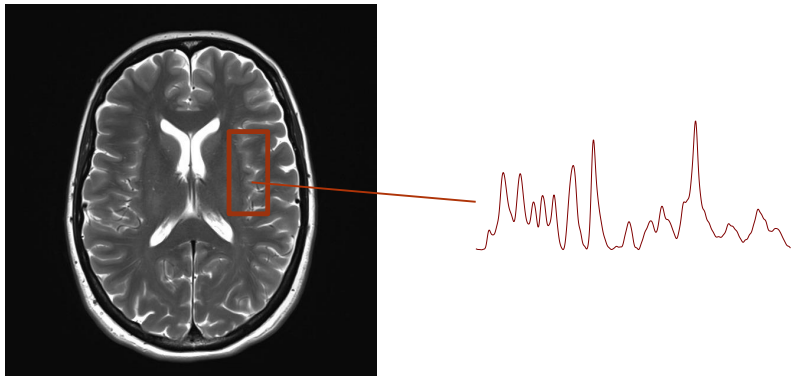
Imaging

- Slower
- Lower SNR
- Hard to shim well enough
- Larger area
- Higher spatial resolution



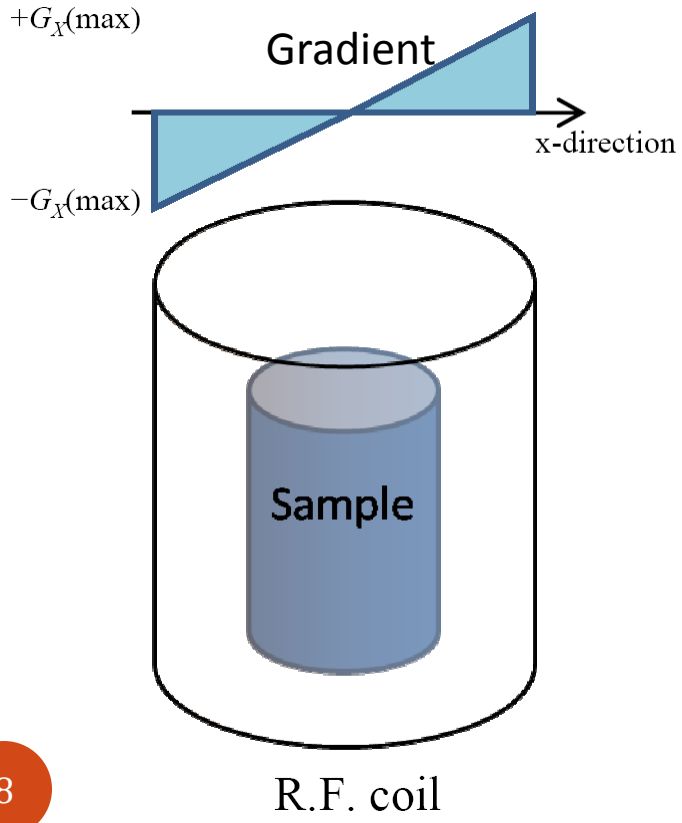
Spectroscopy

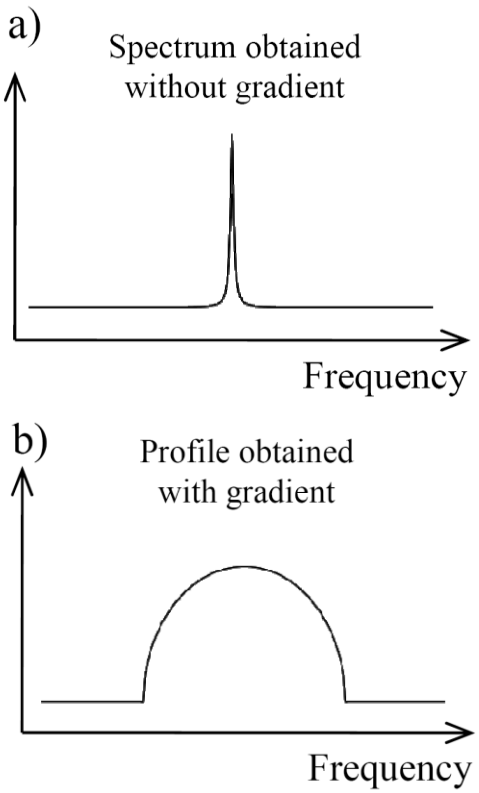
- Lower spatial resolution
- Single arbitrary voxel
- Higher SNR
- Easier to shim
- Complex MT experiments

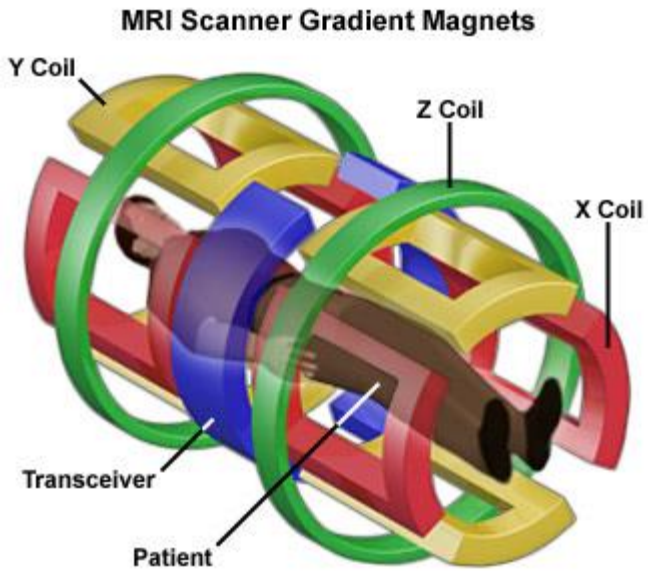


Magnetic Field Gradients: Basics

- **NMR** has (ideally) a homogenous magnetic field such that the recorded frequencies correspond to **Chemical Shift**
- **MRI** deliberately alters the magnetic field such that frequency is now also dependent on **Spatial Position**

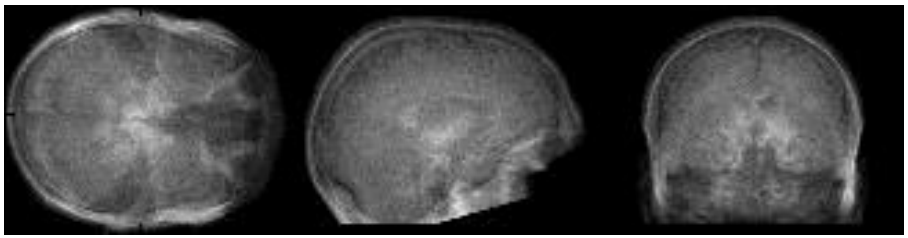




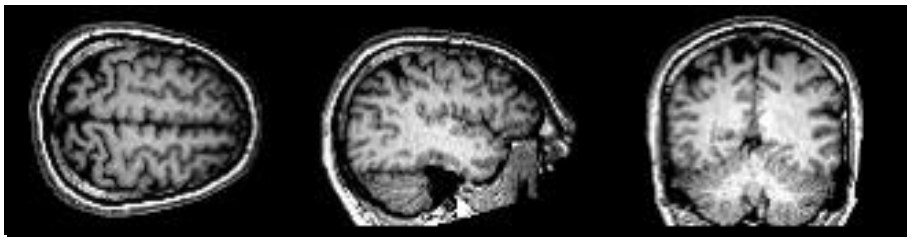


Slice Selective Excitations

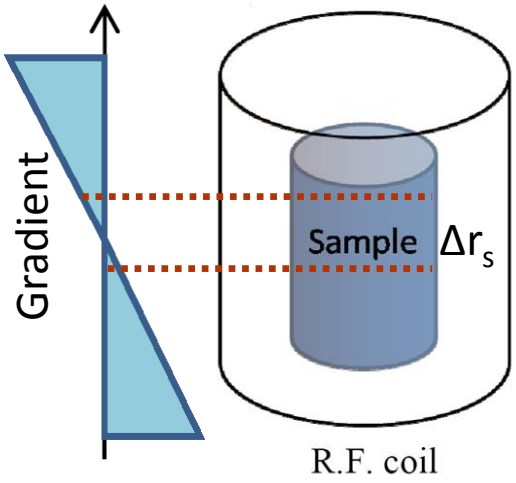
No slice selection



Slice selection

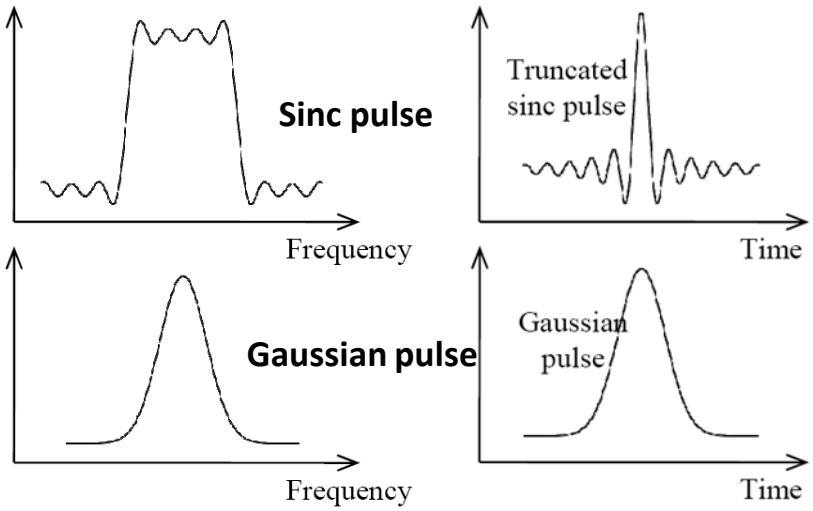


Frequency dependent on position



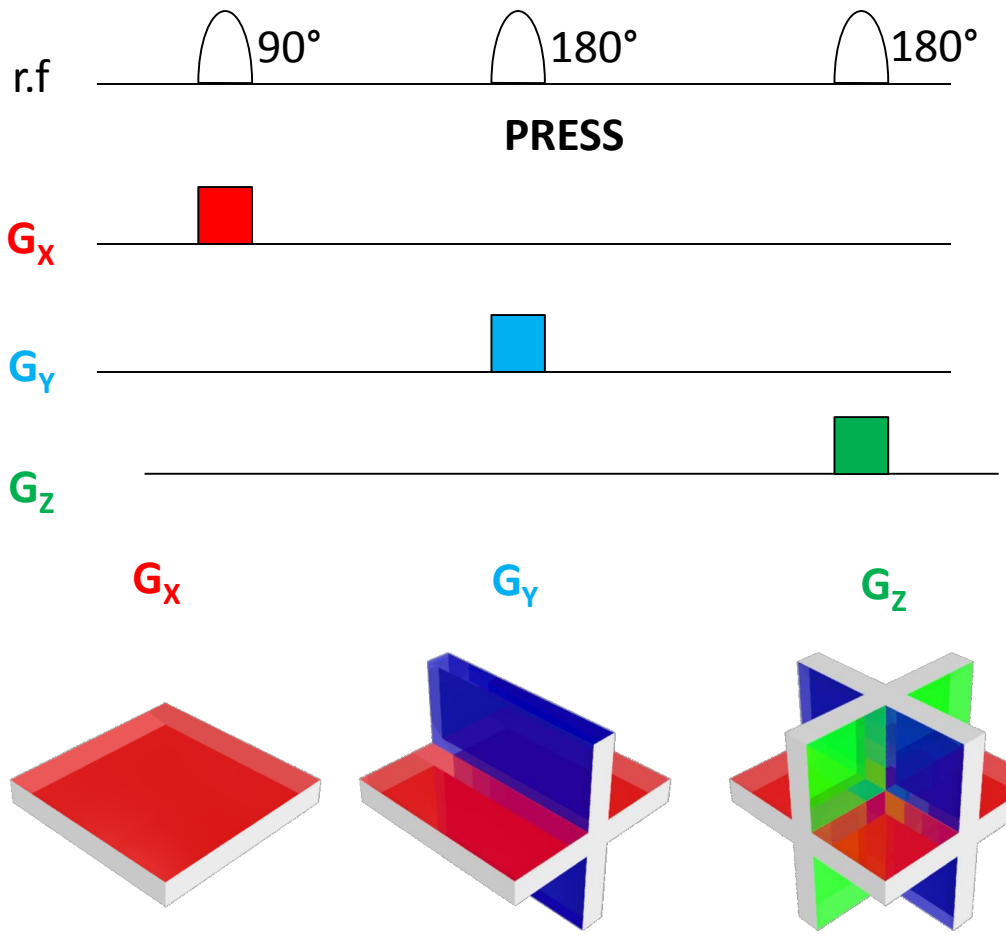
$$\Delta r_s = \frac{\Delta\omega}{\gamma G_{slice}}$$

Shaped or 'soft' pulses excite only a narrow range of frequencies

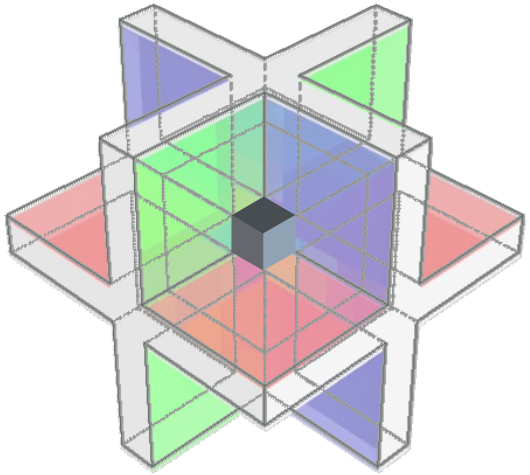


Single Voxel Spectroscopy

- Localization requires a minimum of three RF pulses

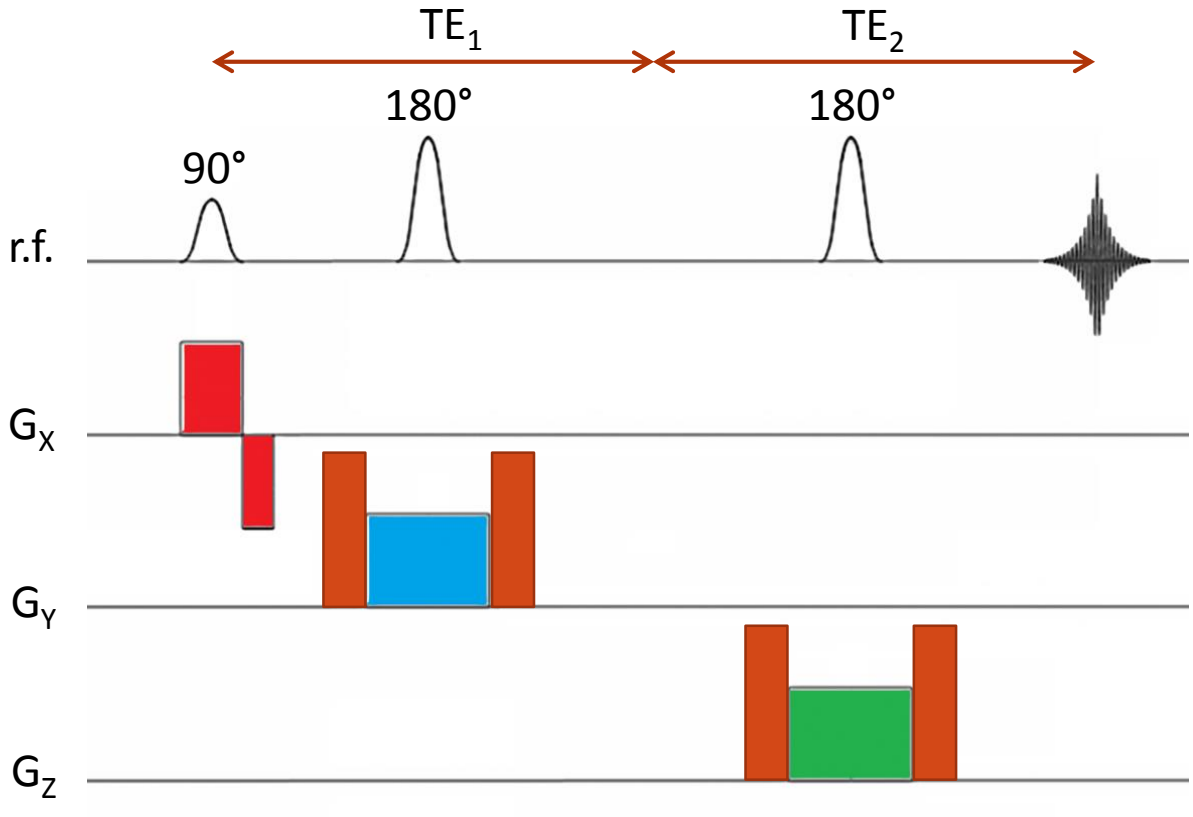


NMR spectrum from voxel



PRESS

- Point REsolved Spectroscopic Sequence
- Double Spin Echo Sequence



Minimum TE when $TE_1 = TE_2$
Typically > 30 ms

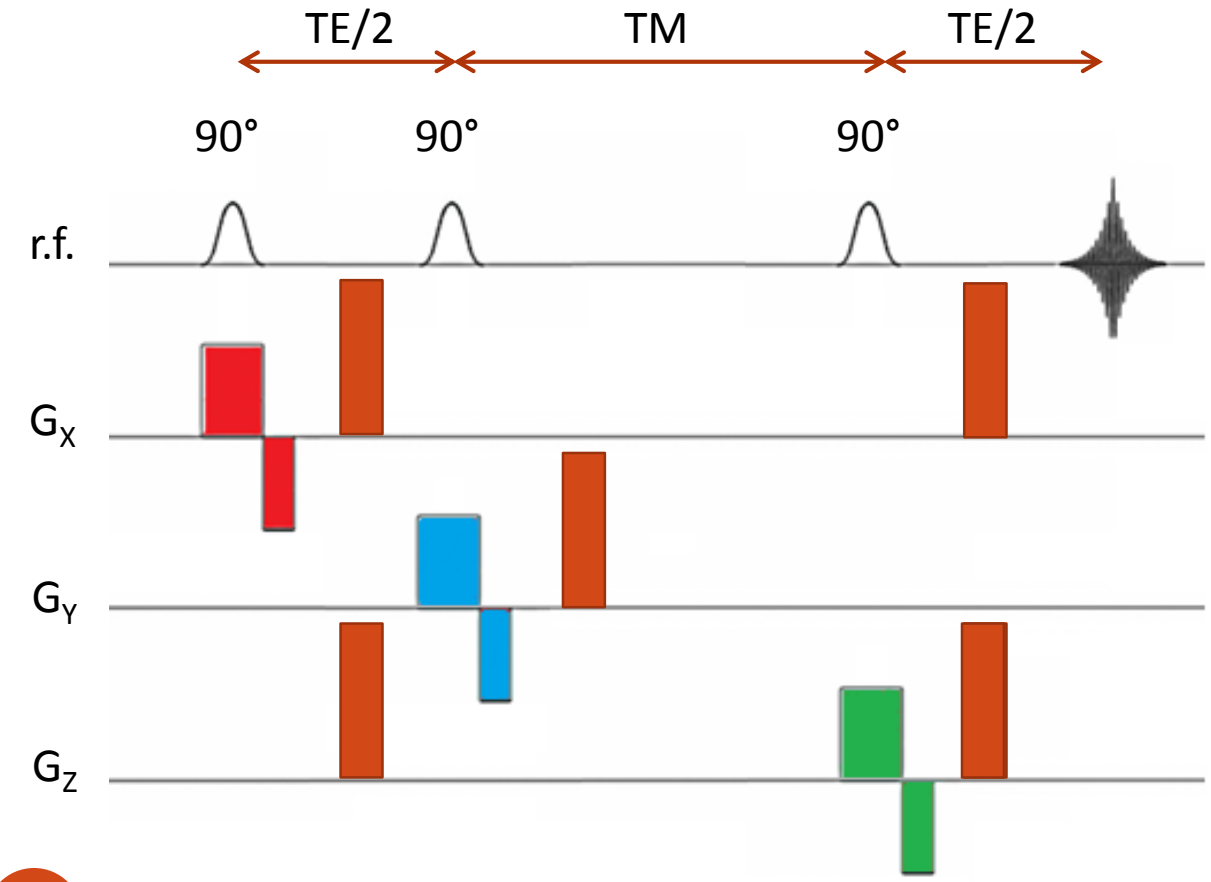
Signal contrast due to T_2

$$\frac{M_Z}{M_0} = e^{-\frac{T_E}{T_2}}$$

Spoiler gradients to remove signal from imperfect pulses

STEAM

- STimulated Echo Acquisition Mode



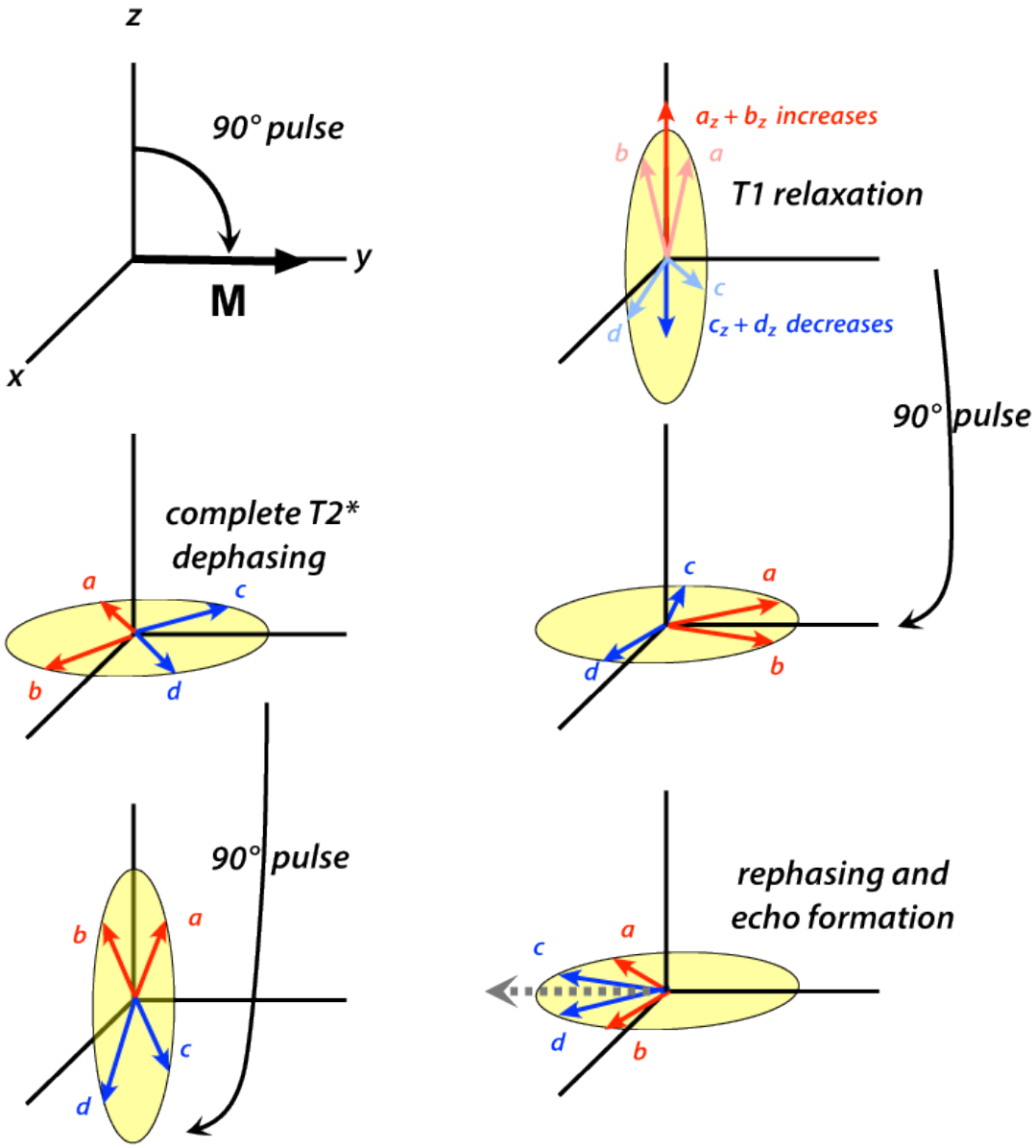
Minimum TE < 10 ms
T_1 relaxation during TM

Stimulated echos produce only half maximum signal compared to a spin echo

$$\frac{M_Z}{M_0} = \frac{1}{2} e^{-(\frac{T_E}{T_2}+\frac{T_M}{T_1})}$$

Spoiler gradients to remove signal from imperfect pulses and unwanted coherences

Stimulated Echo



PRESS vs. STEAM: SNR

- In principle SNR for a given volume dependent on relaxation properties and sequence timings
- If $T_1 >> T_2$ and $T_2 \approx TE_{PRESS}$, then STEAM gives better SNR

PRESS

$$\frac{M_Z}{M_0} = e^{-\frac{T_E}{T_2}}$$

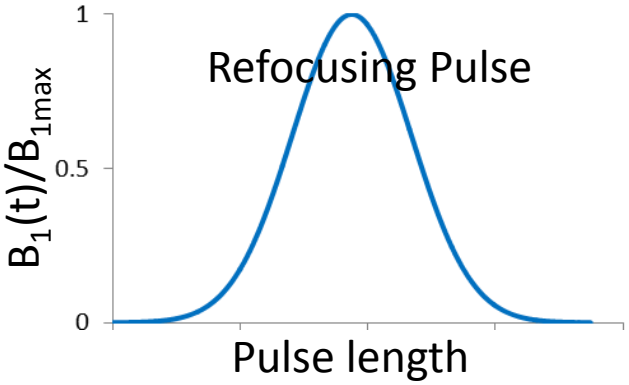
STEAM

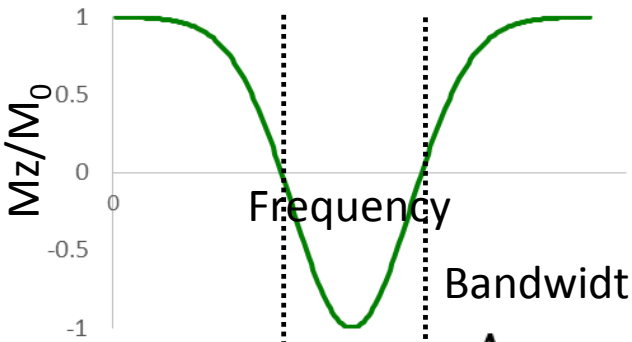
$$\frac{M_Z}{M_0} = \frac{1}{2} e^{-(\frac{T_E}{T_2}+\frac{T_M}{T_1})}$$

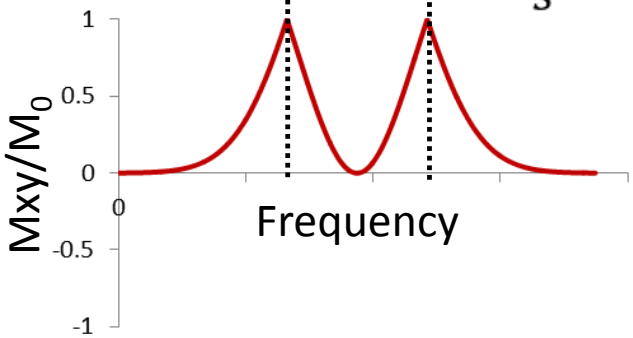
- **However there are other considerations….**

Partial Excitation of Slices

Refocusing pulses in PRESS cause partial slice excitation



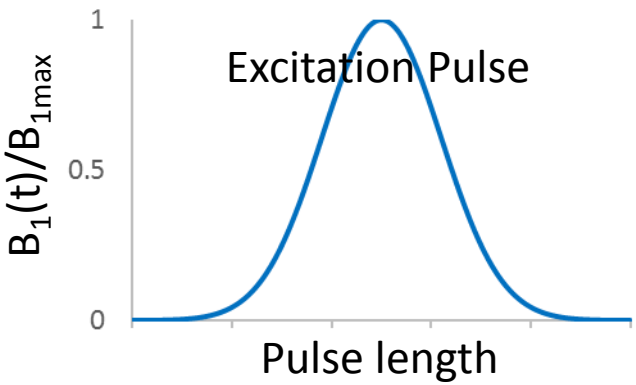


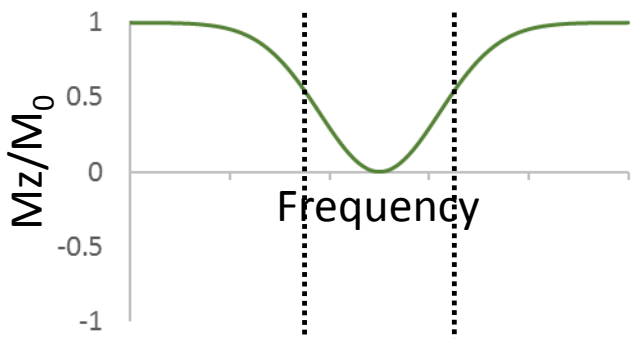


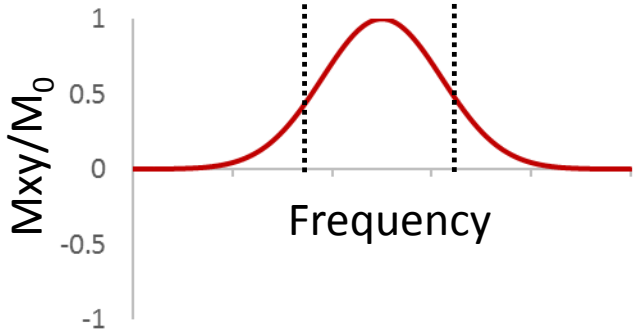
Bandwidth

$$\Delta r_s = \frac{\Delta\omega}{\gamma G_{slice}}$$

Excitation pulses also have partial slice excitation

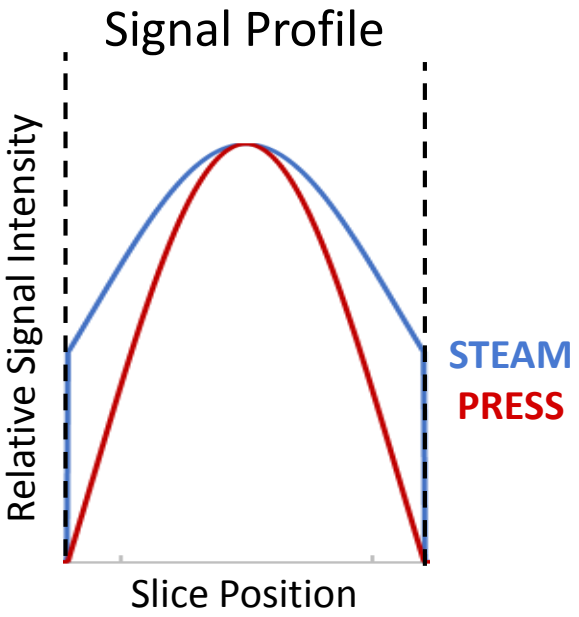






Slice Signal Profile

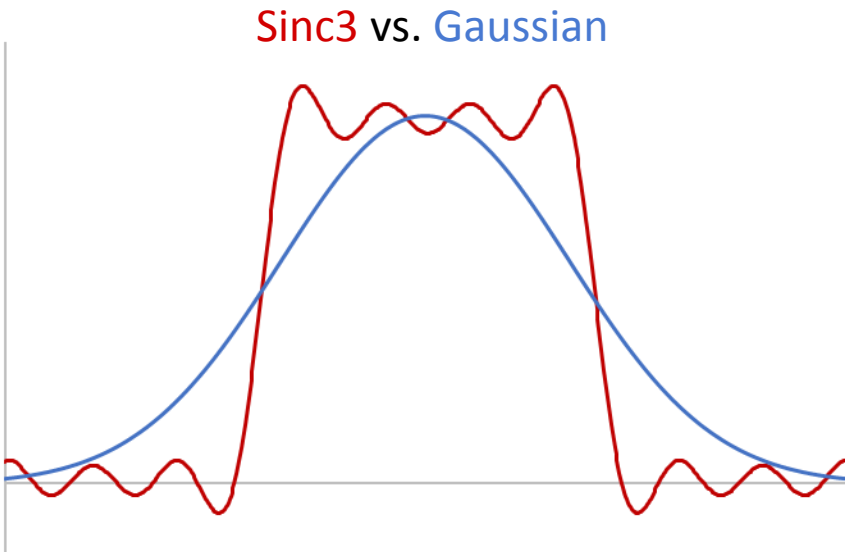
- RF pulse shapes affect signal profile across slice, as well as unwanted excitation outside of slice selected regions
- For a given pulse shape, excitations (hence, STEAM) have a 'better' slice signal profile



- In a gaussian pulse the difference in SNR from one slice is approx. 30%
- The SNR difference due to partial slice excitation may cancel the ½ SNR penalty from the stimulated echo
- 'better' pulse shapes reduces the difference in signal profiles

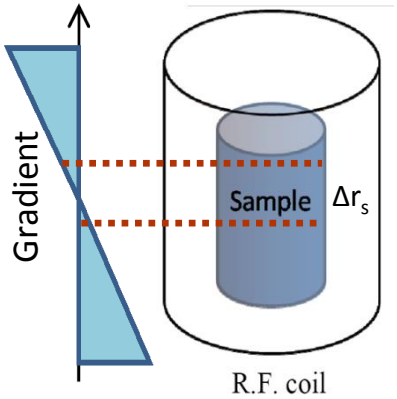
Typical RF Pulse Shapes

- Pulses such as sinc3, and sinc7 give improved slice profiles, at the expensive of pulse length
- Shinar-LeRoux (SLR) pulses are often used, PV6 calculates them on the fly (default ‘calculated’ pulse), and give good slice excitation & refocusing performance
- Gaussian is less used, but has a few advantages. It is one of the shortest shaped pulses for a given power & bandwidth



Importance of Pulse Bandwidth

- To decrease voxel size, either increase gradient strength or decrease slice bandwidth
- Pulse bandwidth inversely proportional to pulse length for a given pulse shape
- Minimum pulse time (max bandwidth) dictated by the maximum RF power available
- RF bandwidth is proportional to the chemical shift offset…

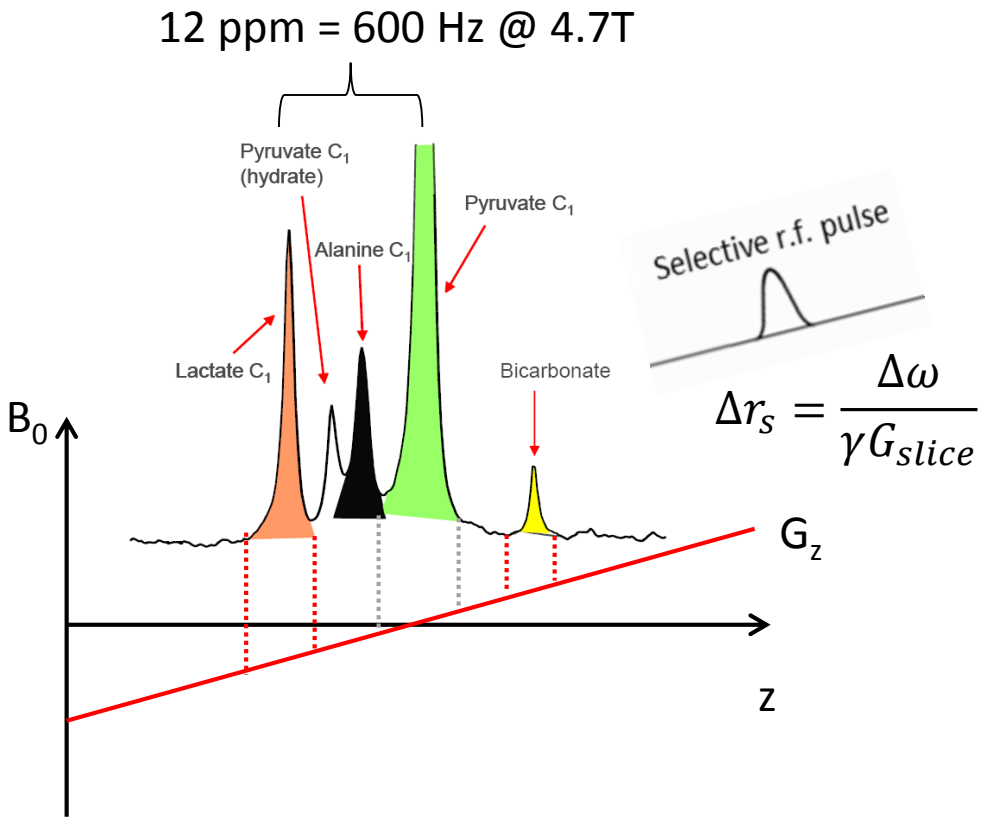


$$\Delta r_s = \frac{\Delta\omega}{\gamma G_{slice}}$$

$$\Delta\omega \propto \frac{1}{Pulse\ length}$$

Chemical Shift Offset

- Position of slice is dependent on frequency of excitation
- ^{13}C example



$$\Delta r_s = \frac{\Delta\omega}{\gamma G_{slice}}$$

$$\frac{\Delta r_{Chemical\ Shift}}{\Delta r_{slice}} = \frac{\Delta\omega_{Chemical\ Shift}}{\Delta\omega_{slice}}$$

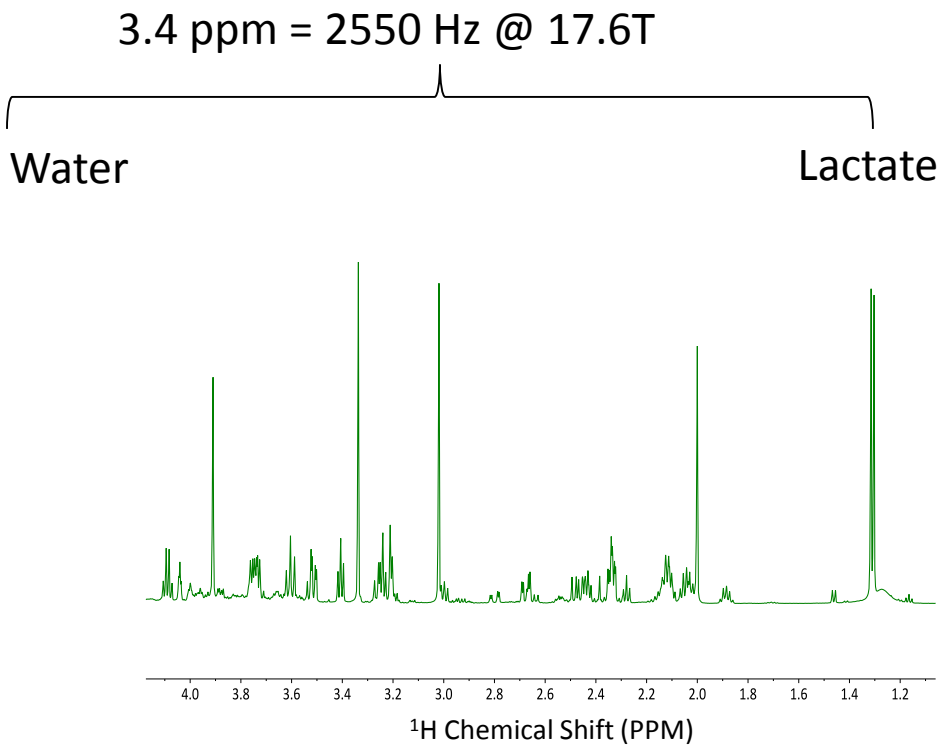
$$\frac{\Delta r_{Chemical\ Shift}}{15\ mm} = \frac{600\ Hz}{3000\ Hz}$$

$$\Delta r_{Chemical\ Shift} = 3\ \text{mm}$$

- Only 51% of coincident volume between
- High bandwidth r.f pulses reduce the issue

Chemical Shift Offset

- Position of slice is dependent on frequency of excitation
- ^{1}H example



$$\frac{\Delta r_{Chemical\ Shift}}{\Delta r_{slice}} = \frac{\Delta \omega_{Chemical\ Shift}}{\Delta \omega_{slice}}$$

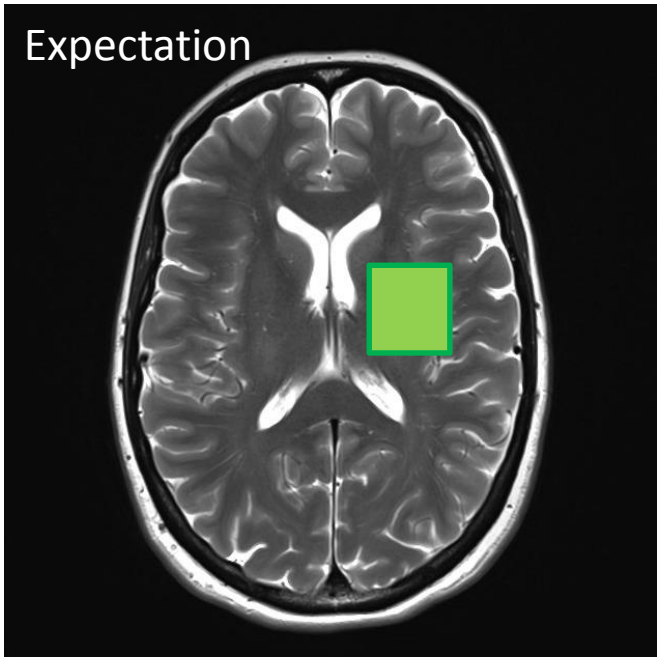
$$\frac{\Delta r_{Chemical\ Shift}}{2\ mm} = \frac{2550\ Hz}{6000\ Hz}$$

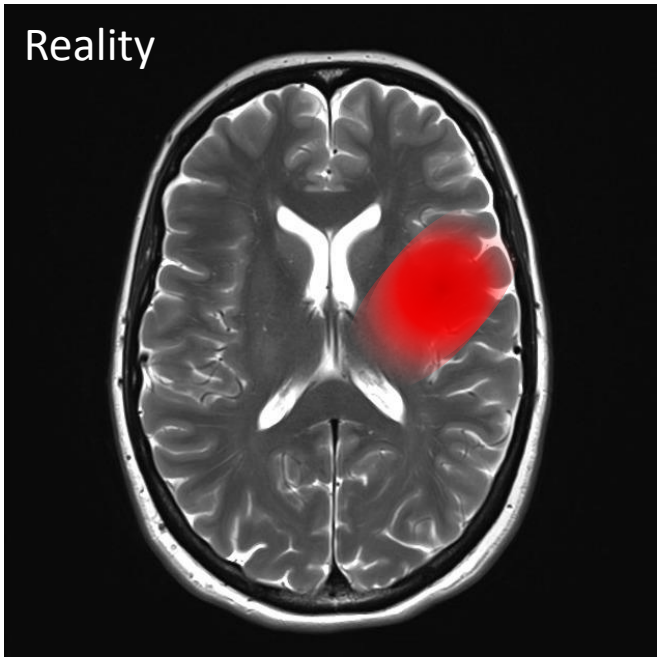
$$\Delta r_{Chemical\ Shift} = 0.85\ \text{mm}$$

- <20% of coincident volume
- High bandwidth r.f pulses reduce the issue

Expectation vs. Reality

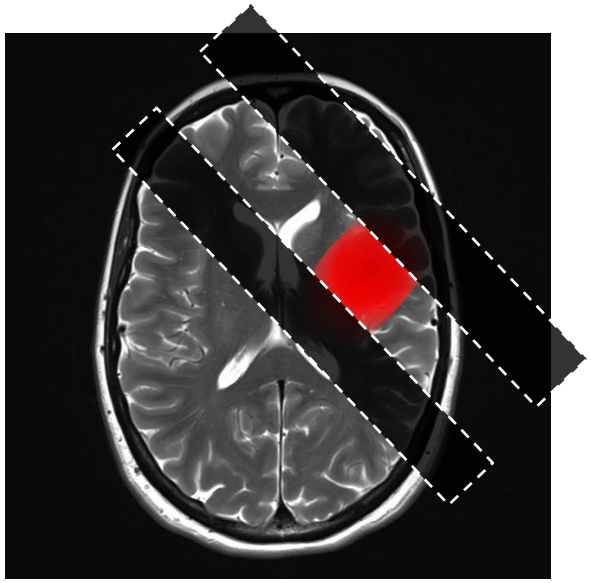
- Chemical shift offset is always present, worse at higher fields and for nuclei with large chemical shift ranges
- Can generate higher excitation bandwidths than refocusing ones, thus STEAM can reduce CSO compared to PRESS
- **Not the sharply defined region we would like!**



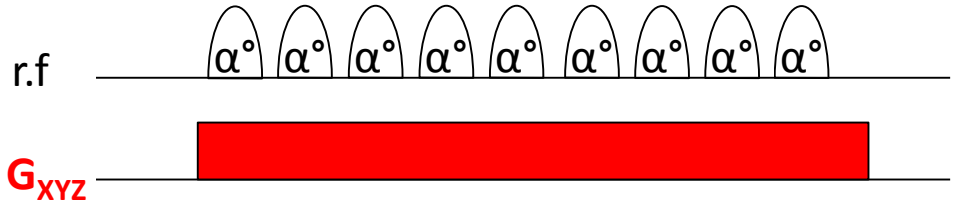


Outer Volume Suppression

- Localisation sequences are ‘modular’, allows the easy use of signal ‘preconditioning’ such as OVS and water suppression
- OVS suppresses signal from bands near the desired voxel



- Saturating a slice of the sample
- Simplest is a train of pulses



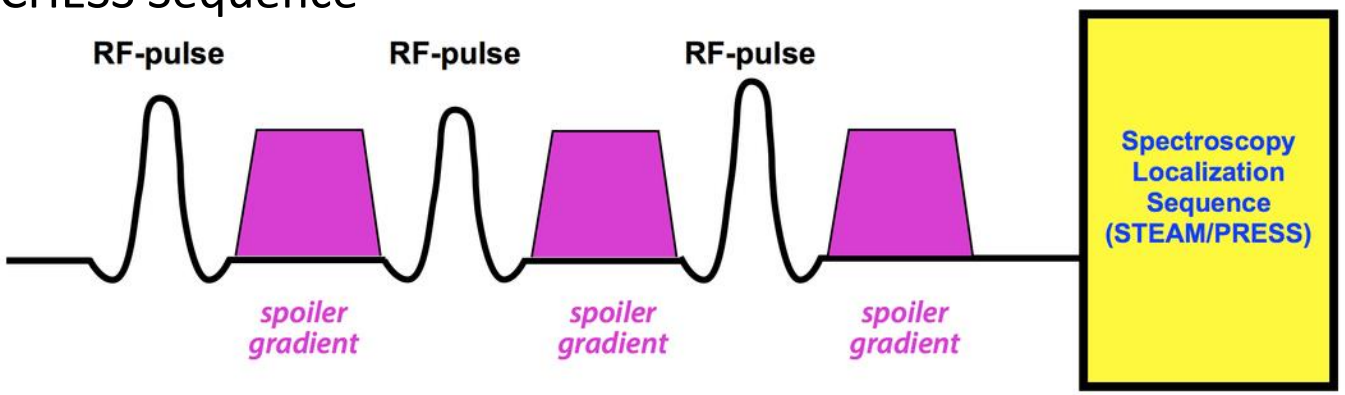
- Reduces signal from outside desired region....
- However, we compromise SNR and therefore quantitation of metabolites based on their chemical shift.

Water Suppression

- Water suppression is required, *in-vivo* approx. 40 M
- PV6 has CHESS and VAPOR as default
- Other derivatives include WET, SWAMP, MOIST
- Need to match suppression bandwidth to water linewidth
- Sequences can be further modified with intra-sequence WS, but PV6 doesn't as standard

Basic CHESS Sequence

Basic CHESS Sequence



STEAM vs. PRESS

- Which is 'better' ? ¯_(ツ)_/¯ it depends…
- PRESS is preferred at lower fields, STEAM at higher fields
- Many sequences based on these such as DRYSTEAM and MEGA-PRESS, which introduce spectral editing
- Can we do 'better'?

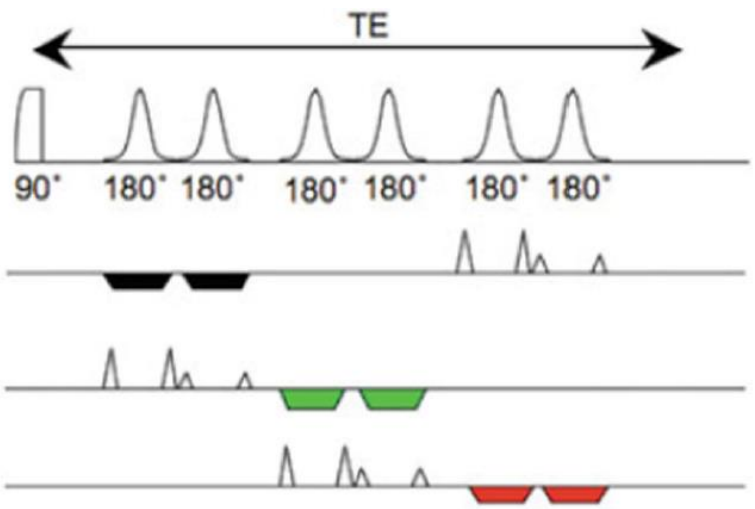
	STEAM	PRESS
Echo Time	Short (< 10ms)	Long (>30 ms)
CSO	OK	Bad at high fields
SNR	Factor of ½ due to STE	OVS can affect it
Slice Profile	Excitations are better	SLR reduces difference
Flow Effects	Spoilers can cause issues	Usually better

LASER & SEMI-LASER

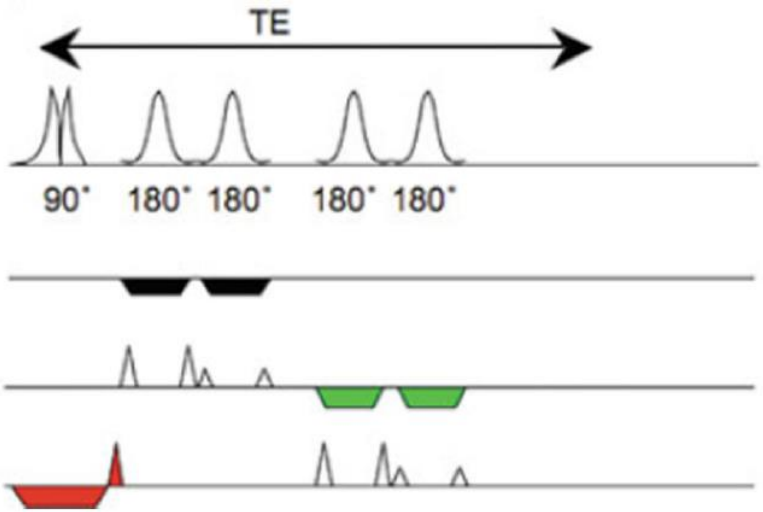
- Maybe….
- Paired adiabatic pulses help correct for phase dispersion across the slice that can occur with certain adiabatic pulses
- LASER excites whole sample, using refocusing to localise

LASER

(localisation by adiabatic selective refocusing)



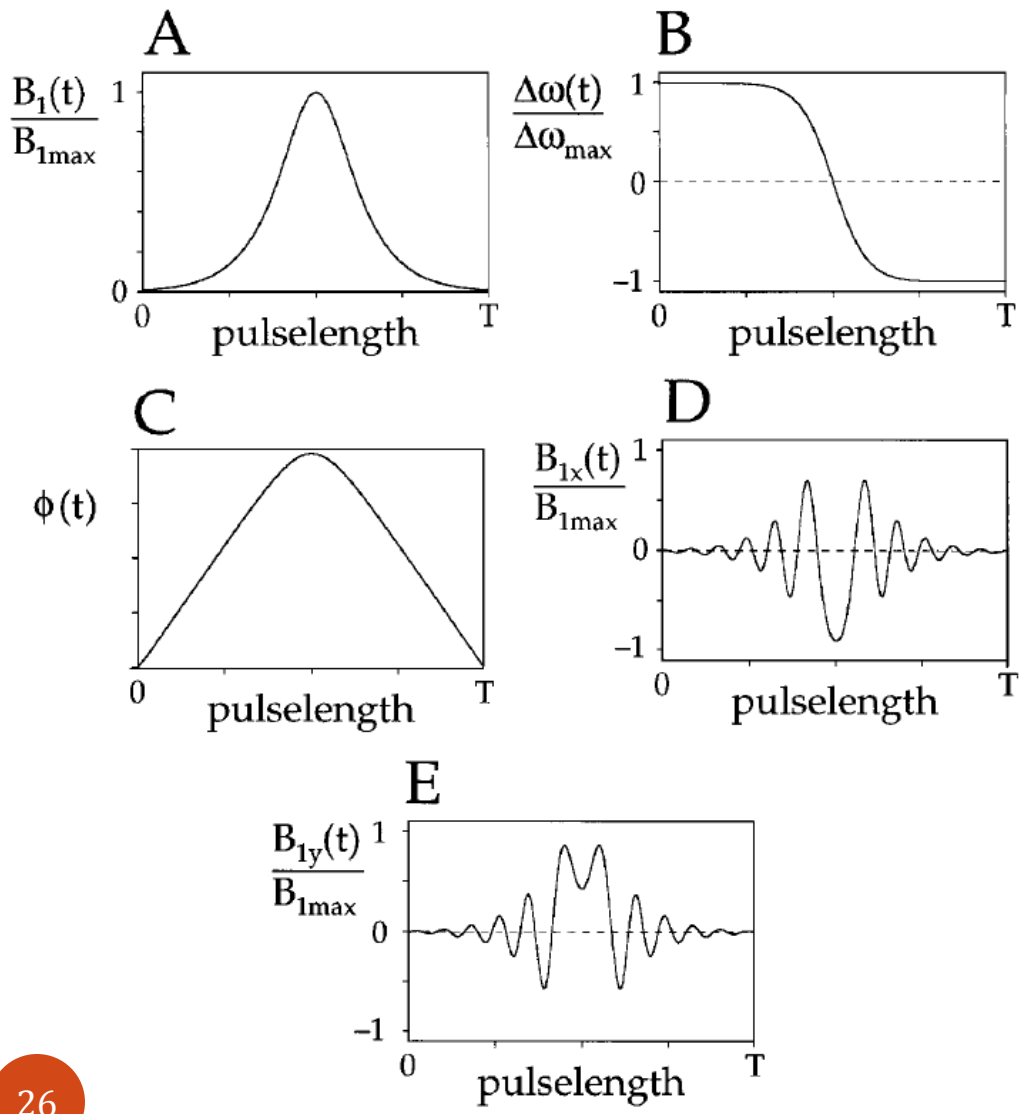
Semi-LASER



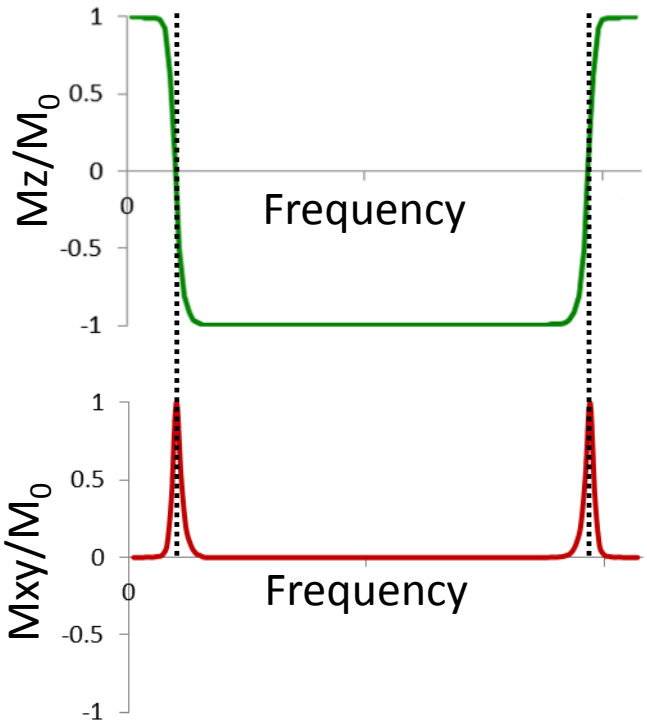
Zhu H., & Barker, P.B., *Methods Mol. Biol.* **711**, 203–226. (2011)

Adiabatic Pulses: SECH pulse

Hyperbolic Secant Refocusing Pulse



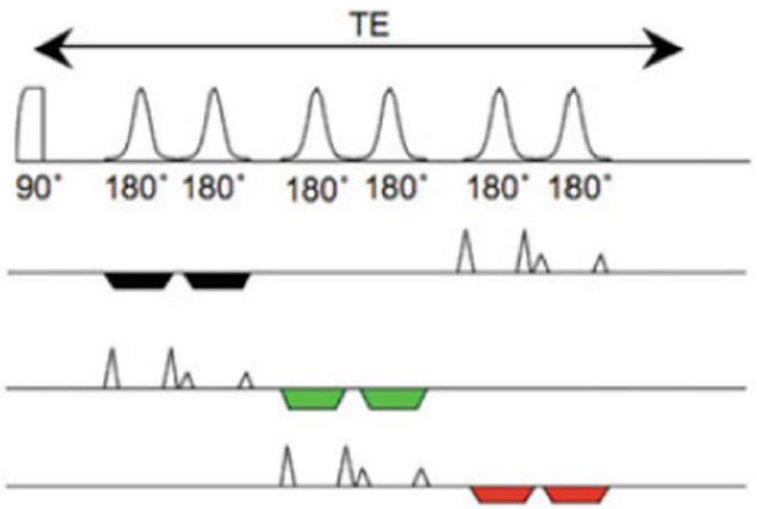
- High bandwidth > 5 kHz
- Reduced chemical shift offset
- Reduced in slice excitation



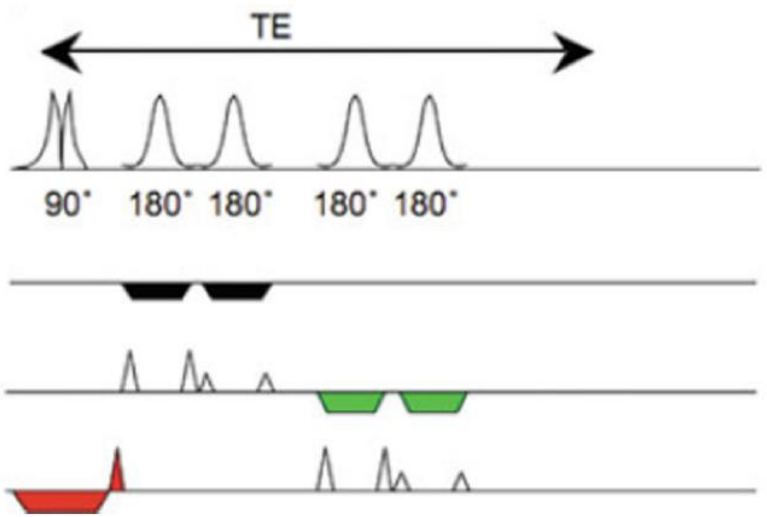
LASER & SEMI-LASER

- Better slice refocusing than PRESS
- Long echo time due to adiabatic pulses
- Relatively high bandwidth excitations reduce CSO issues
- High RF deposition, which is an issue for human subjects

LASER
(localization by adiabatic selective refocusing)



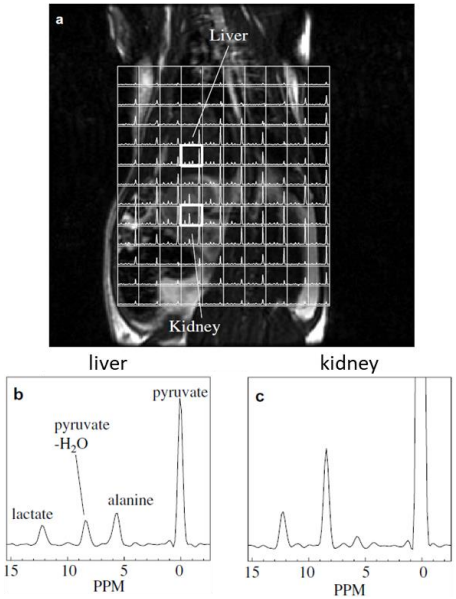
Semi-LASER

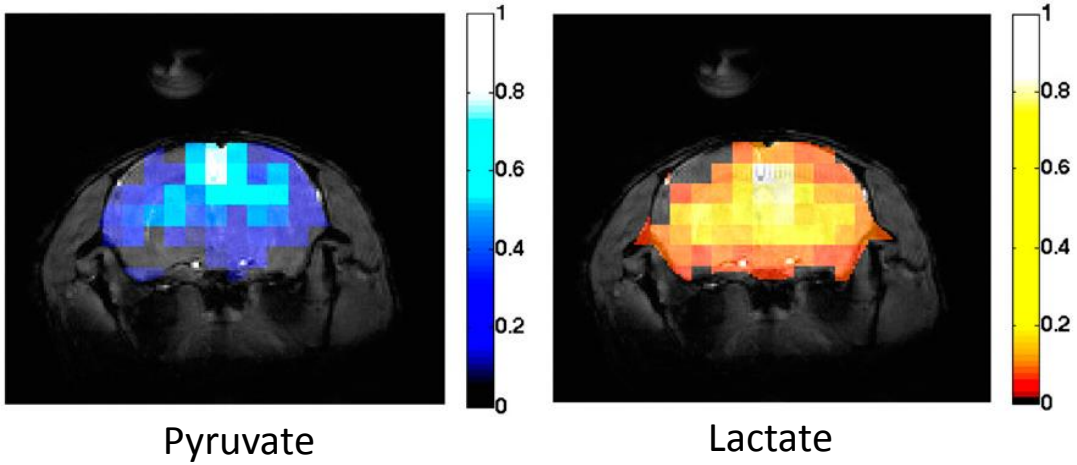


Zhu H., & Barker, P.B., *Methods Mol. Biol.* **711**, 203–226. (2011)

^{13}C *In-vivo* Spectroscopy

- ^{13}C is too low abundance to obtain good spectra
  - Inject labelled metabolites, such as ^{13}C glucose
    - Use of slice selective excitation and OVS bands to select regions
    - Requires decoupling, and CSO artefacts are a major issues
  - Hyperpolarized metabolites such as ^{13}C pyruvate
    - Typically done using CSI type image sequences including EPSI
    - Direct images of metabolites using bSSFP or Spectral-Spatial pulses



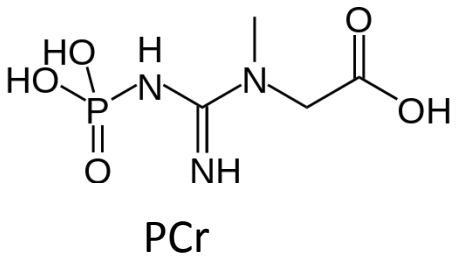


Cunningham CH *et al*., J. Mag. Res. Med., **187**: 357–362 (2007)

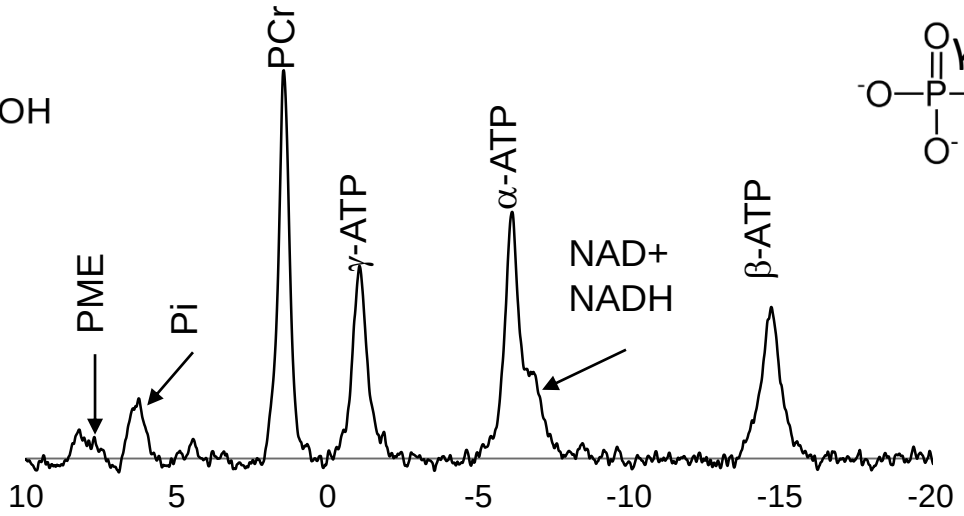
Eichhorn T., *et al.*, PNAS., **45**, 18064-18069,(2013)

^{31}P *In-vivo* Spectroscopy

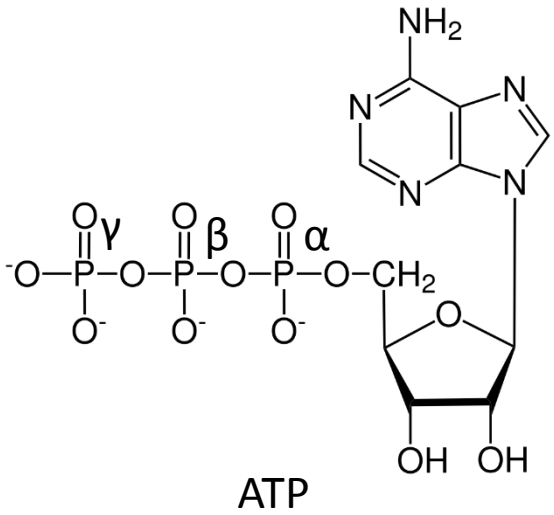
- ^{31}P spectra provide useful metabolic data
- Naturally 100% abundant spin-½ nuclei
  - ATP, NAD,PCr, and inorganic phosphates at >mM concentrations
- ATP T_2 is short, < 20 ms, and decrease with B_0
- Short echo time needed!



PCr



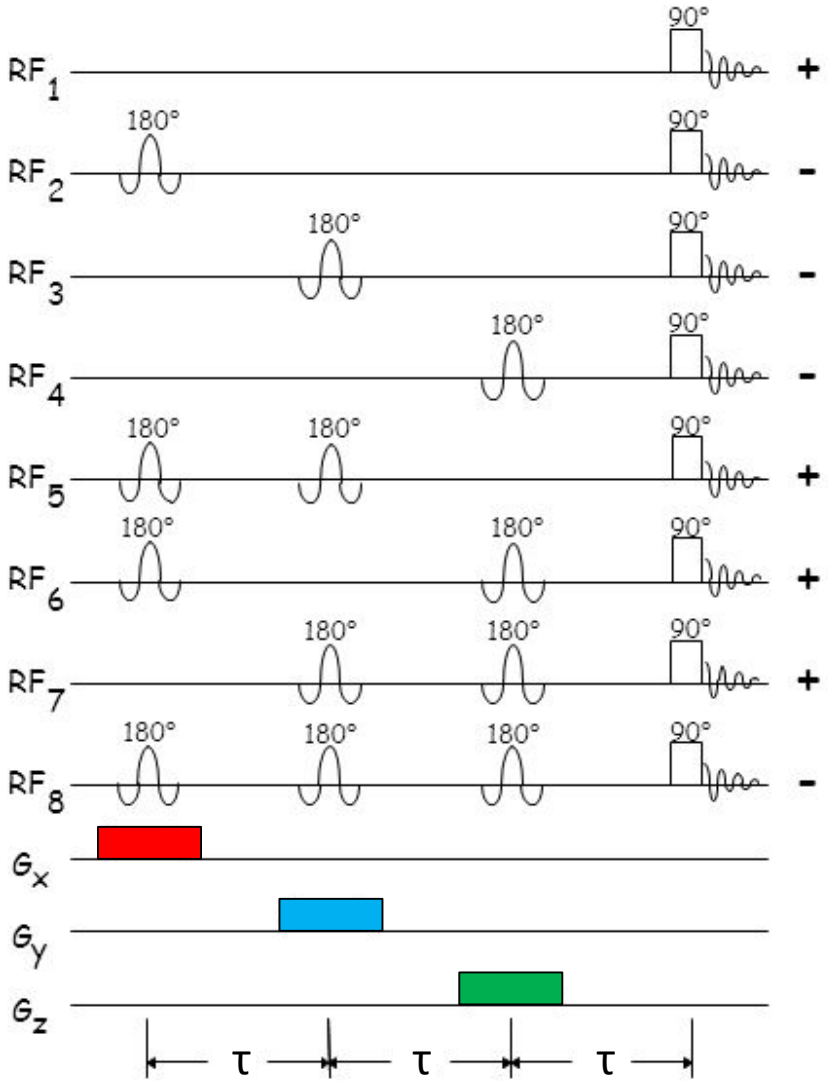
Chemical Shift (ppm)



ATP

ISIS

- Image Selected In-vivo Spectroscopy



No Echo Time, as only FID's are acquired

No T_2 contrast

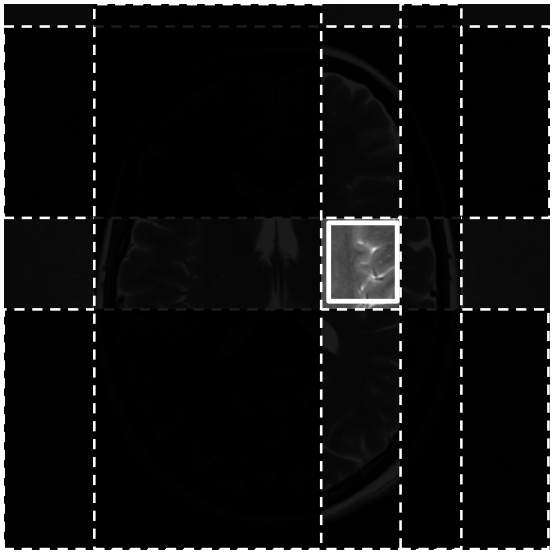
8 Step 'Phase cycle' to localize a voxel, hence 8 times slower than STEAM or PRESS

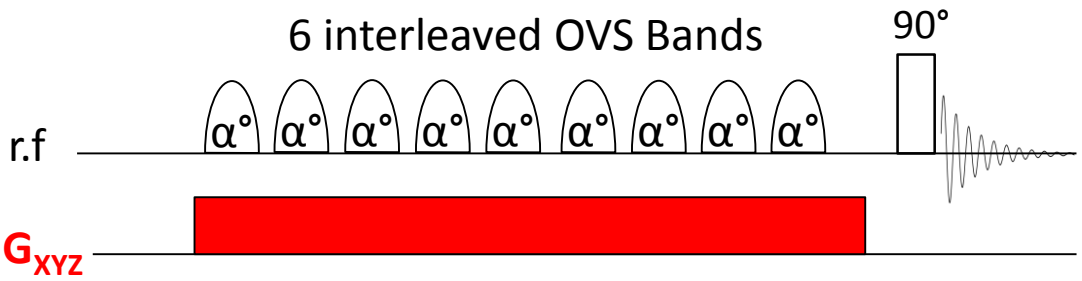
Minimize τ to reduce T_1 contrast

Motion during acquisition significantly affects the spectra

OVS Selected Region

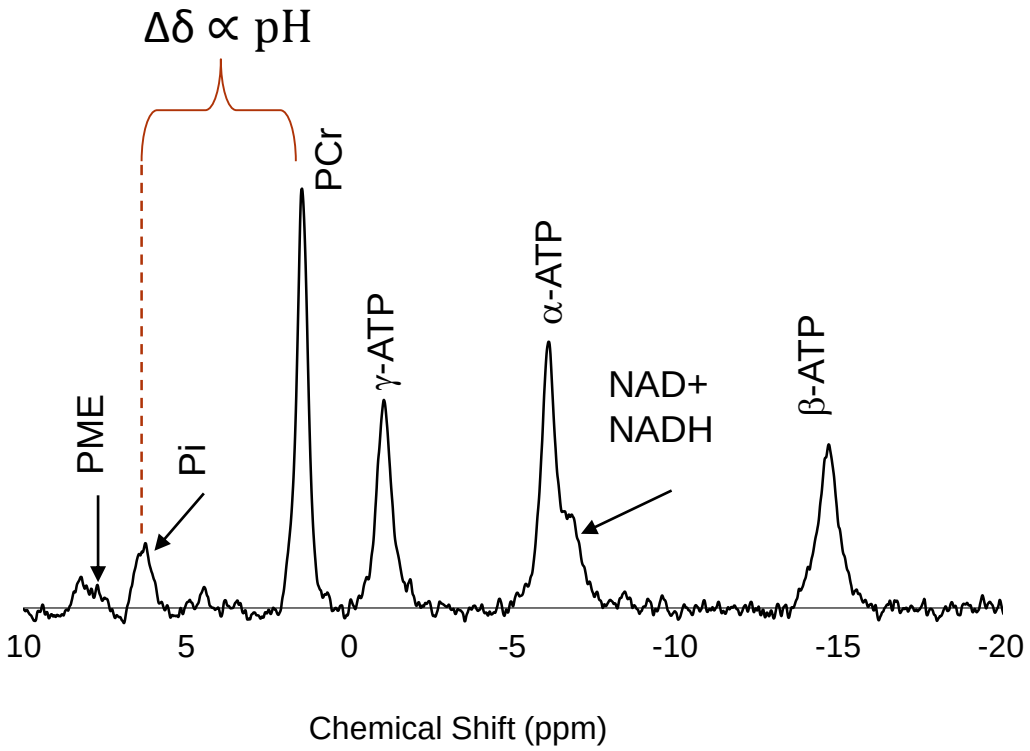
- Another Potential method is to use multiple OVS bands to ‘select’ a localized region
- Rather than directly select the region of interest, we suppress all of the signal from outside this region
- Quicker than ISIS and also acquires an FID, hence no T_2 issues





^{31}P Spectroscopy: pH Estimate

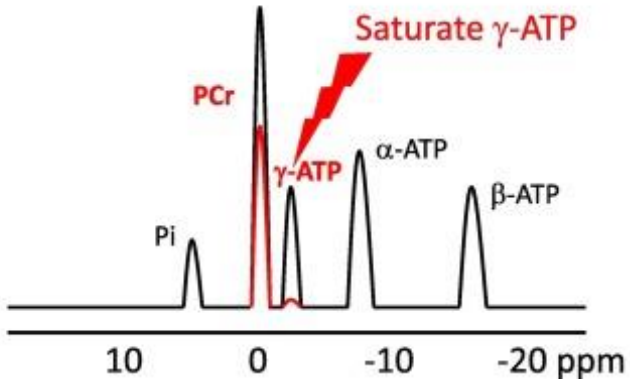
- pH of tissue is related to the chemical shift of the inorganic phosphate compared to phosphocreatine



^{31}P Spectroscopy: Magnetisation Transfer

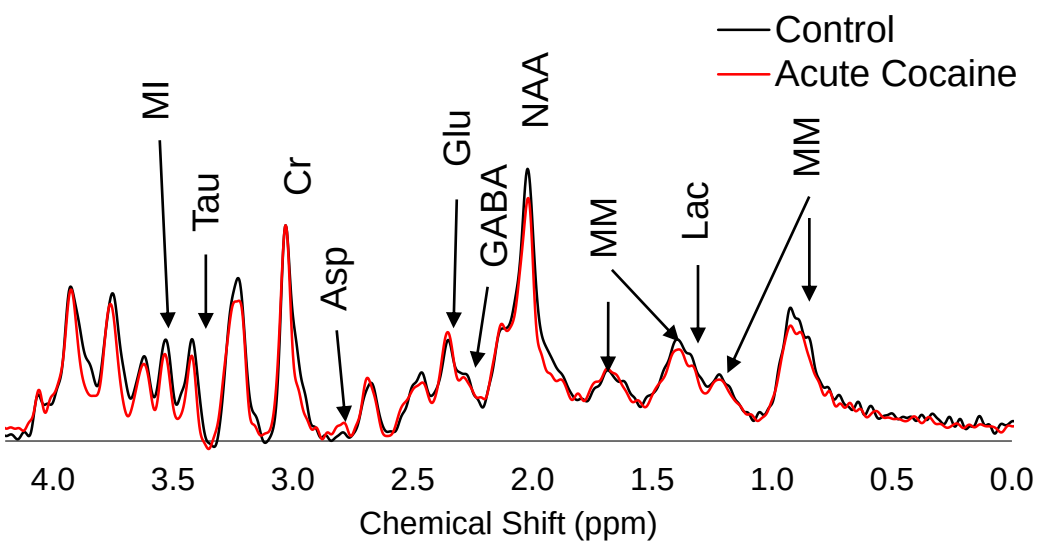
- Saturating the γ-ATP resonance causes a change in signal intensity of the PCr peak (as well as in the other ATP peaks)
- The change in PCr signal is related to conversion rate of ATP to PCr
- Narrow bandwidth RF pulses used to saturate γ-ATP resonance

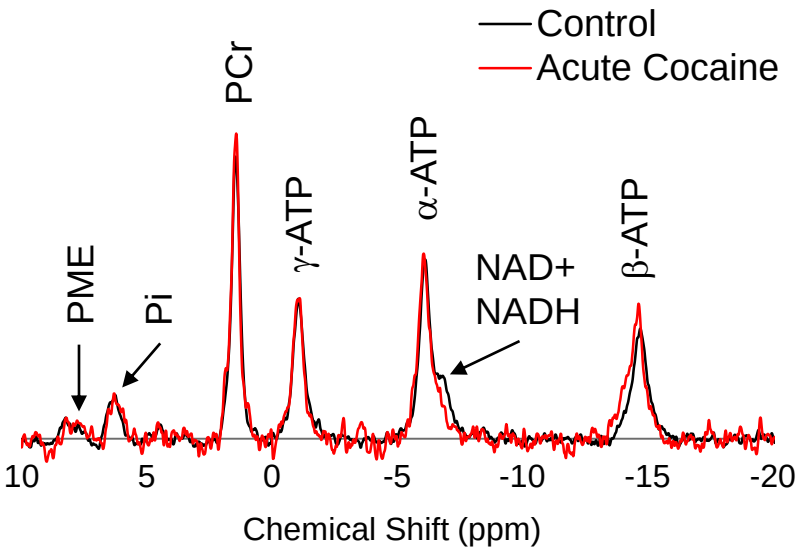
$$PCr + ADP + H^+ \underset{k_{ATP \rightarrow PCr}}{\overset{k_{PCr \rightarrow ATP}}{\rightleftharpoons}} Cr + ATP$$

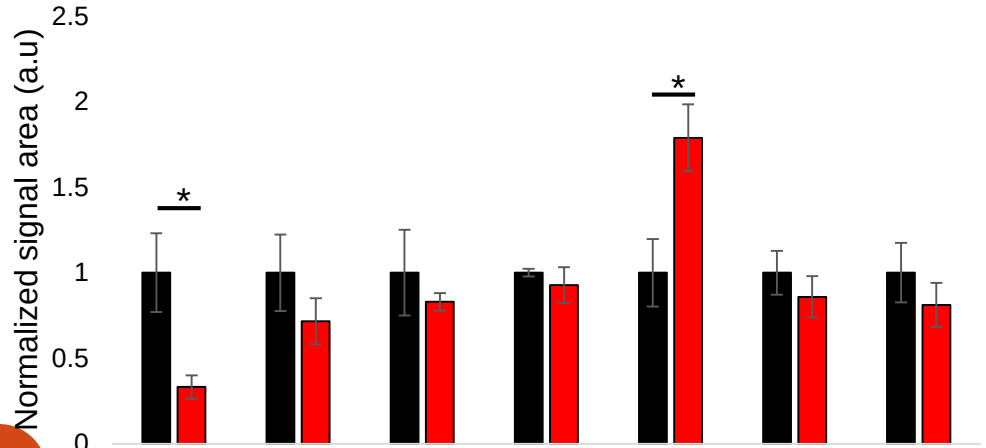


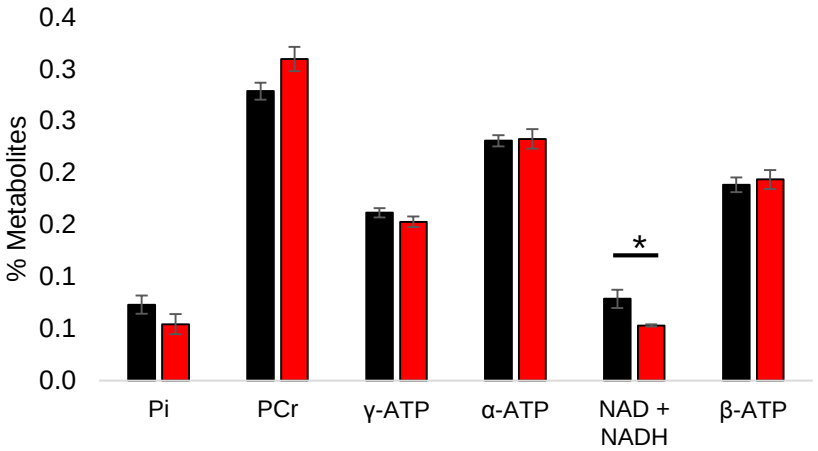
In-vivo measures of Cocaine effects

- Changes in spectra can be subtle, but statistically significant
- Data collected at 11.1 T



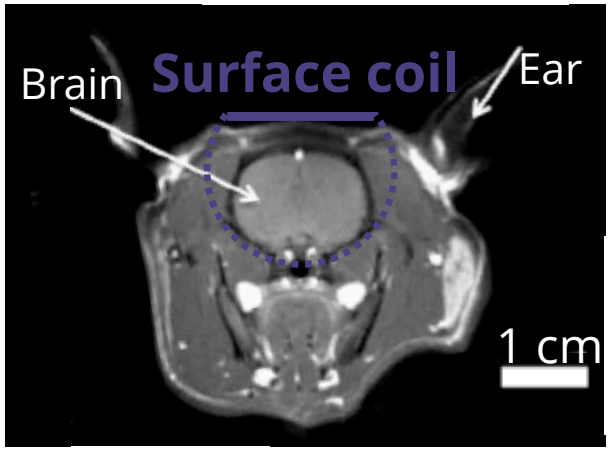






RF Coil Choices: Surface Coil

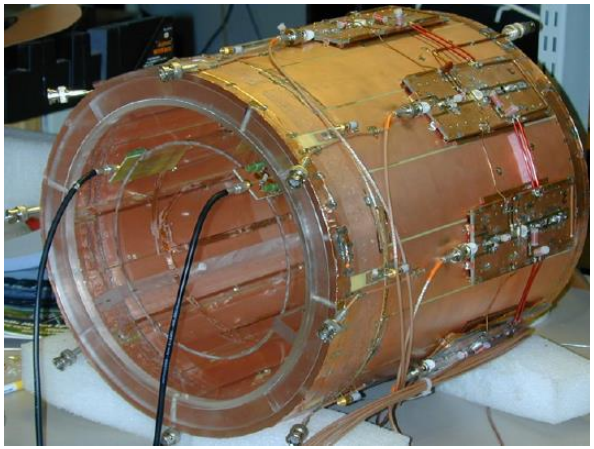
- Coil choice is important and can dictate sequence decisions:
  - Surface coils vs. volume coils

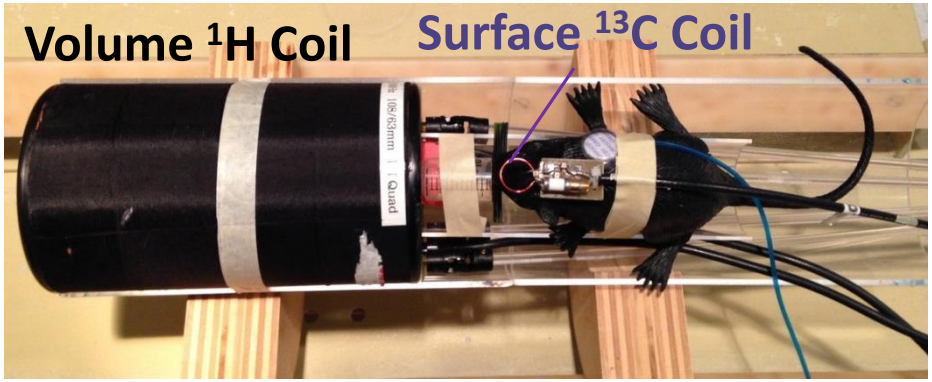


  - Surface coils give better SNR at the expense of poor RF homogeneity. Adiabatic pulses help with this
  - Provide some signal localization, as they have a limited RF range
  - Practically they can be hard to tune accurately, can be susceptible to animal motion depended how they are mounted, and must be accurately placed on the animal

RF Coil Choice: Volume Coil

- Coil choice is important and can dictate sequence decisions:
  - Surface coils vs. volume coils

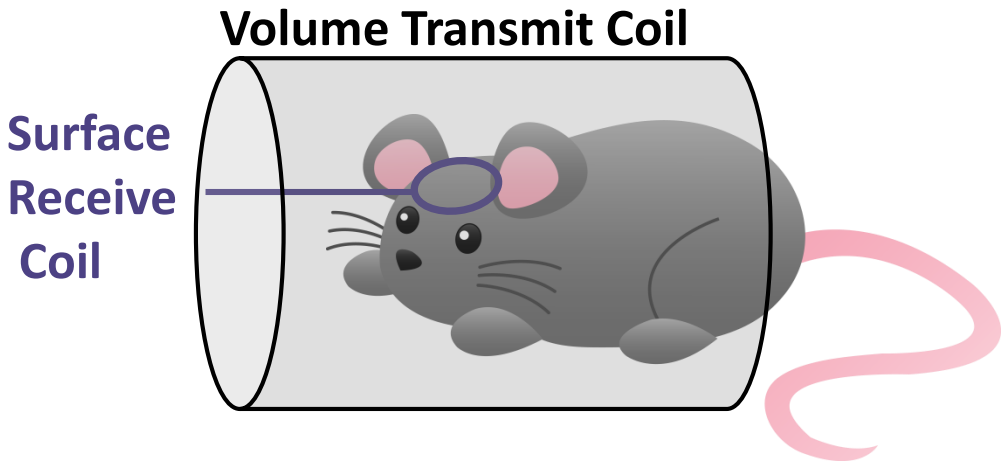




  - Volume coils give much better RF homogeneity, but sacrifice SNR
  - Can be paired with a heteronuclear surface coil relatively easily
  - Usually can be tuned in situ, less coil positioning issues

RF Coil Choice: VT/SR

- Coil choice is important and can dictate sequence decisions:
  - Surface coils vs. volume coils



  - Volume transmit/ Surface receive combines the higher SNR with the good RF performance, but require more complex coil designs and are harder to pair with heteronuclear coils.

Practical Aspects

- So far covered a lot of theory, but there are a number of practical aspects that need to be considered

- Animal cradle design:
  - Needs to keep animal still, and prevent motion from breathing

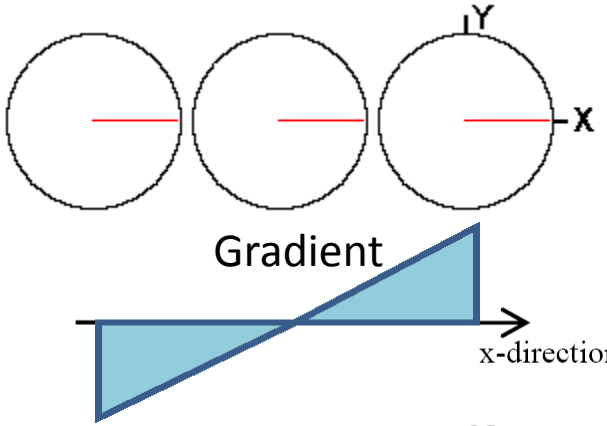
- Shimming is much harder *in-vivo!* Automatic shimming algorithms do exist.
  - PV6 uses a mapshim technique, that maps the B_0 field and then attempts to calculate the required shims. It works great… in a phantom.
  - Iterative local shimming of 1^{st} order shims is also required
  - Manual shimming is harder in PV6 then previous versions or Varian systems, but can be done to a limited extent

Additional Slides

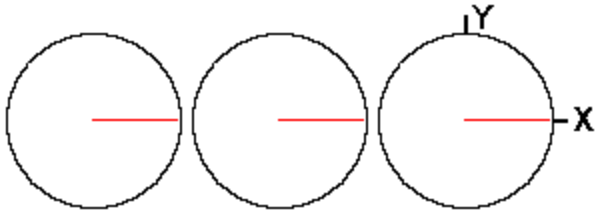
Phase and Frequency Encoding

- MR imaging uses a combination of ‘Phase’ and ‘Frequency’ (also called ‘Read’) encoding magnetic field gradients

‘spins’ precess at the Larmor frequency

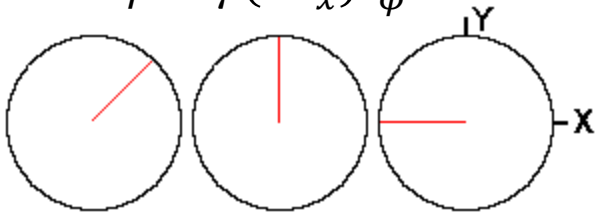


$$\upsilon = \gamma(B_0 + xG_x)$$



Frequency Encoding

$$\phi = \gamma(xG_x)t_\phi$$

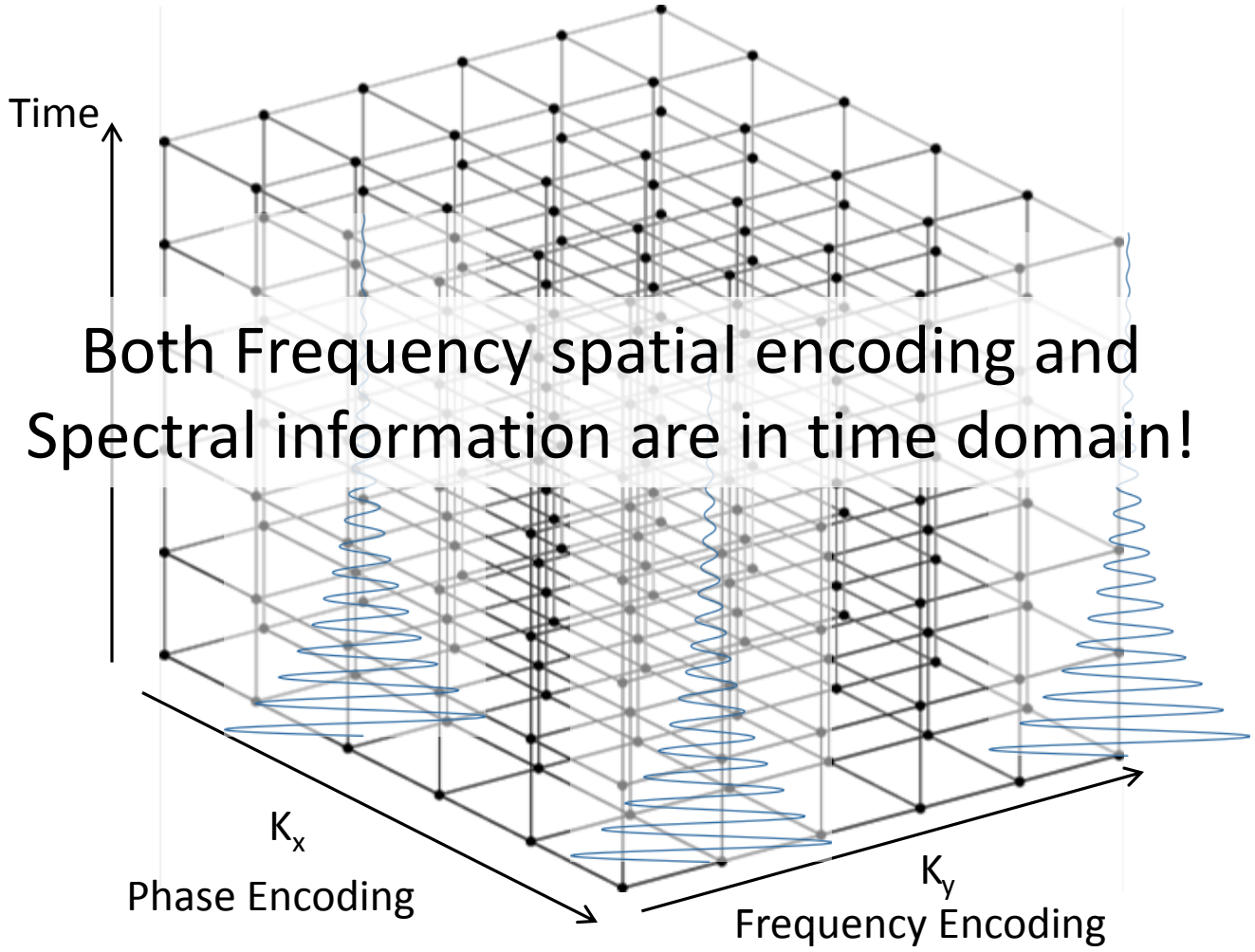


Phase Encoding

Leupold J., *et al.*, Mag. Res. Med., **55**, 557-565,(2006)

Spectral-Spatial: K-t space

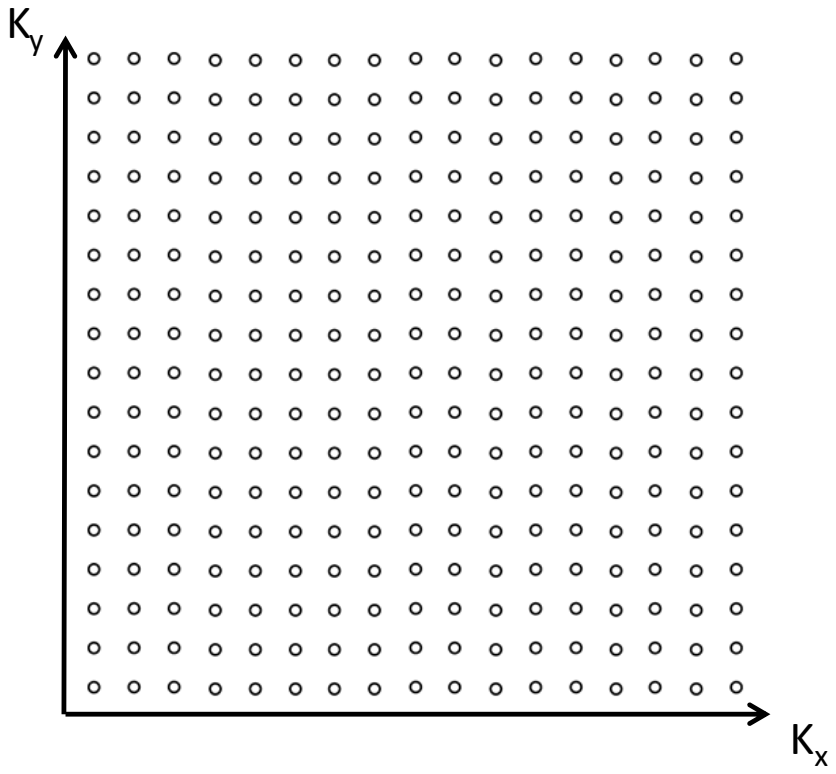
- How do we image spectral & spatial information?



Single Point Imaging: Basic CSI

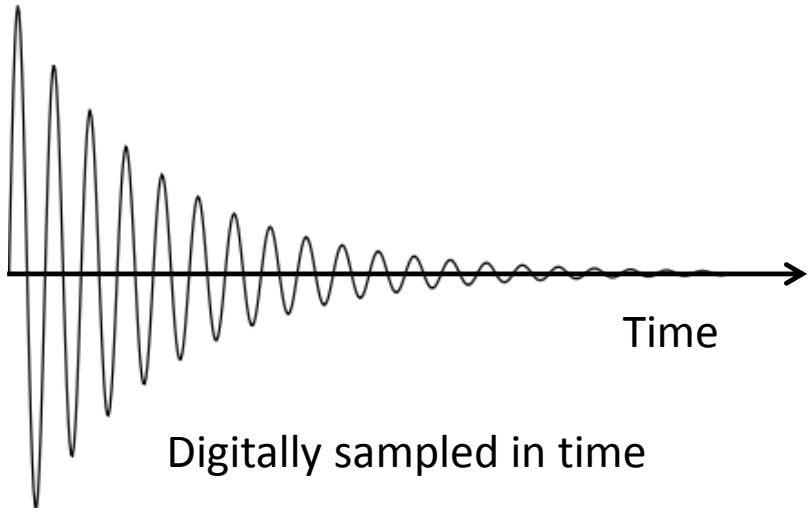
- How do we image spectral & spatial information?

Image recorded in k-space



Phase Encoding

Spectra recorded in time domain

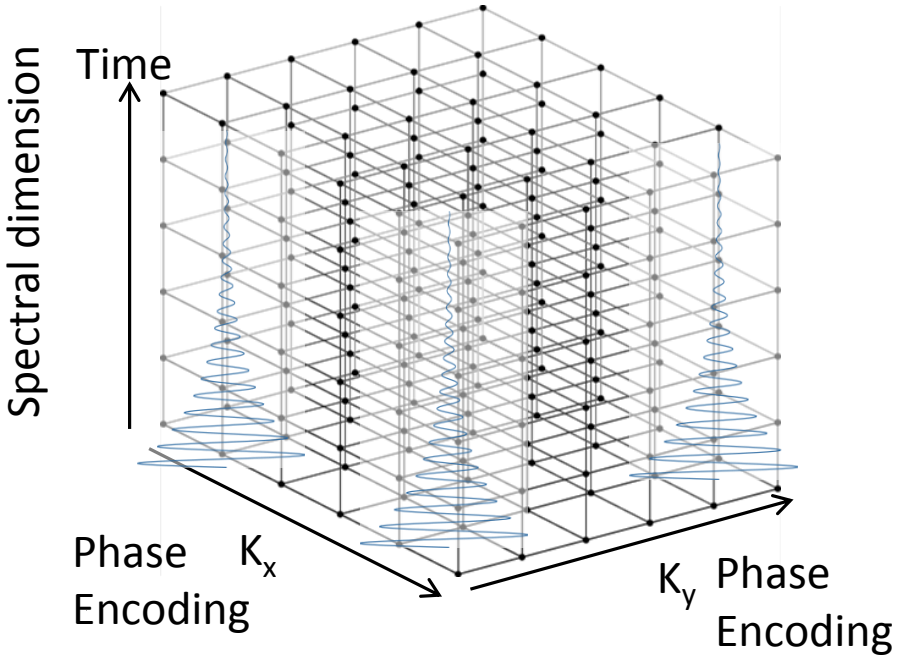


Digitally sampled in time

Frequency Encoding

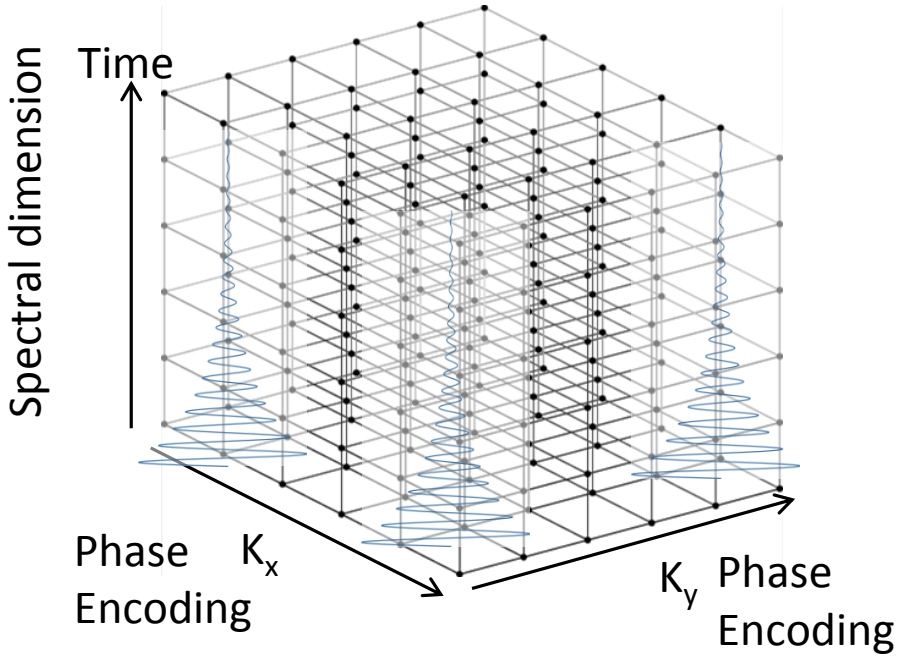
Single Point Imaging

- Using a combination of ‘Phase’ and ‘Frequency’ (also called ‘Read’) is problematic for CSI
- The simplest method is perform Single Point Imaging, where both dimensions are phase encoded
- The time (frequency) dimension is then reserved for spectral information



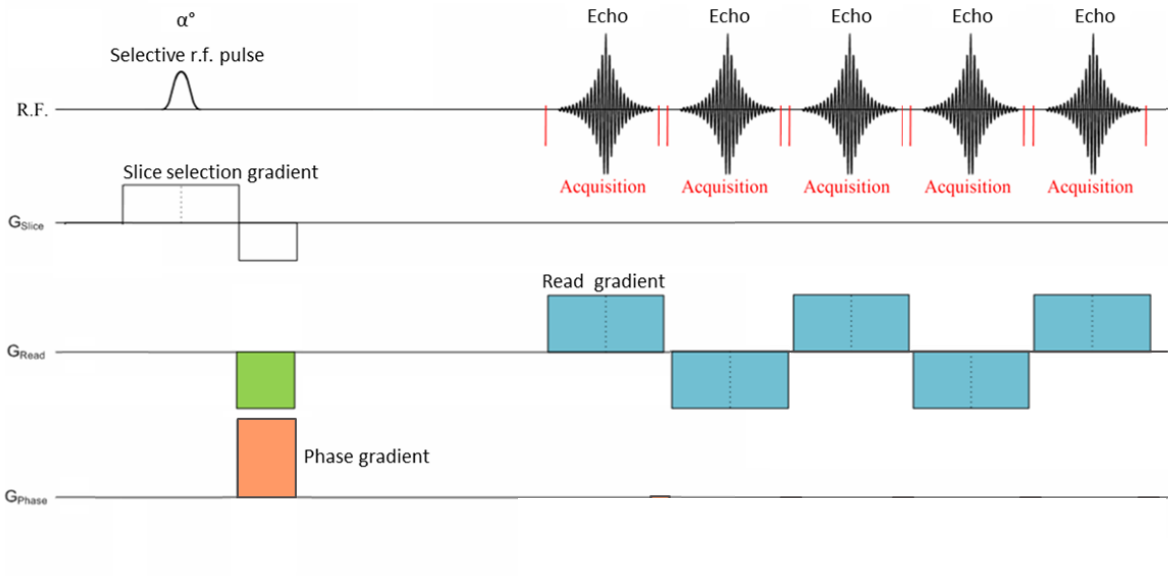
Single Point Imaging

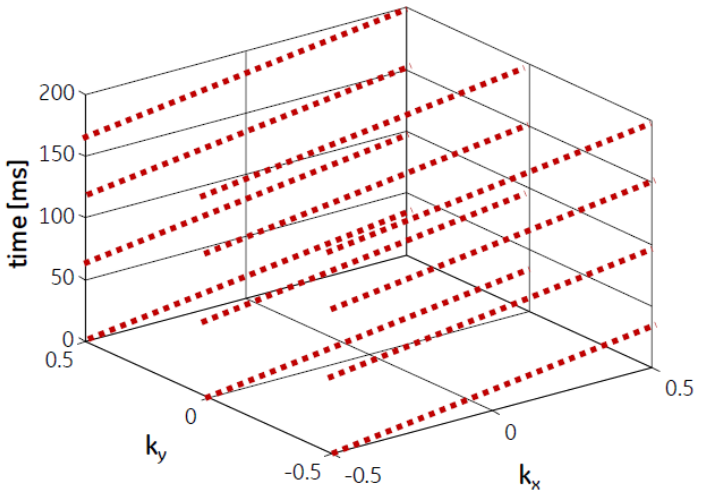
- Each point in k-space will require a separate acquisition
- Each voxel will have a full spectra associated with it
- A 64x64 image is thus 4096 acquisitions, with a fairly short acquisition time of 1 s, that is still > 1hr for a single average
- Many methods to speed up acquisition, but often at the expense of spectral quality



Echo Planar Spectroscopic Imaging

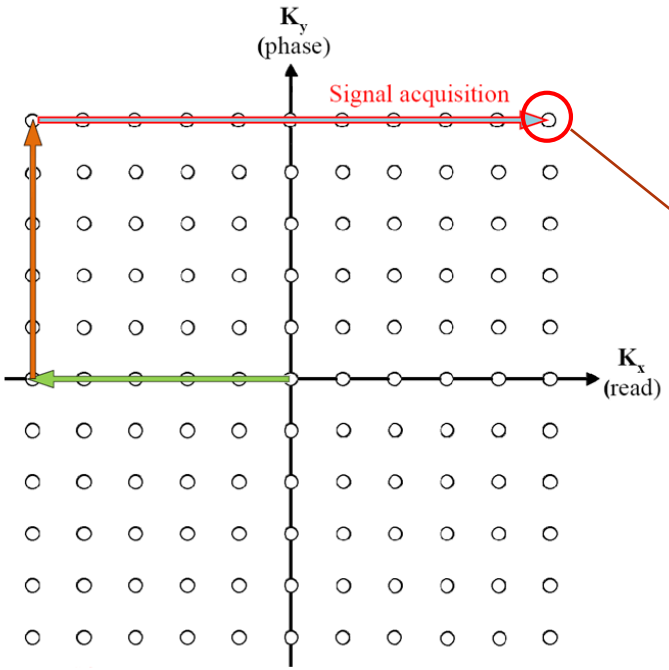
- EPSI is a modification of Echo Planar Imaging to give spectroscopic as well as spatial information
- A sequence of gradient echoes are recorded from a single excitation
- These are used to deconvolve **spectral** information





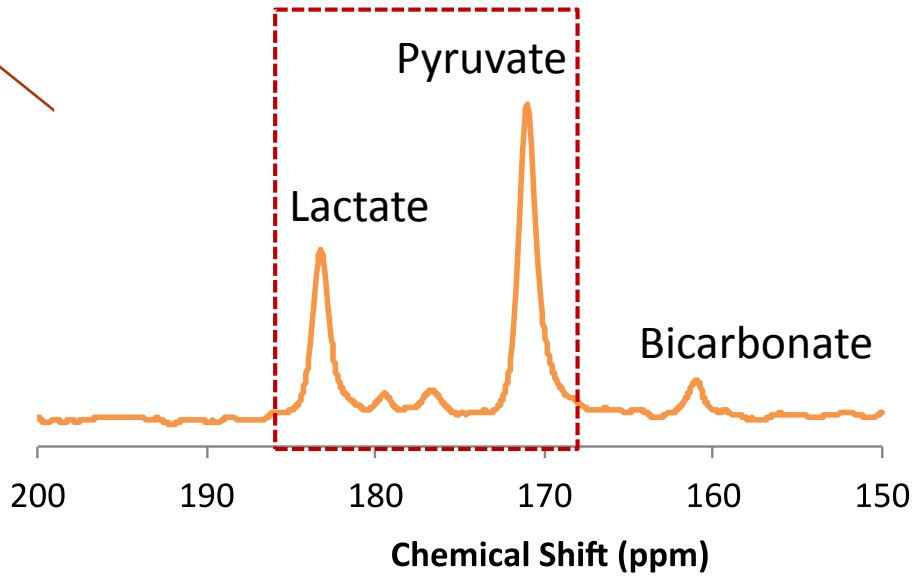
EPSI: Spectral/Spatial Compromise

- In EPSI the spectral sweepwidth is limited by the spatial resolution and field of view
- Increasing the sweepwidth requires either a bigger FOV or less points acquired in the frequency encoding direction
- On 11T we are using a sweepwidth of 2100 Hz (18 ppm)



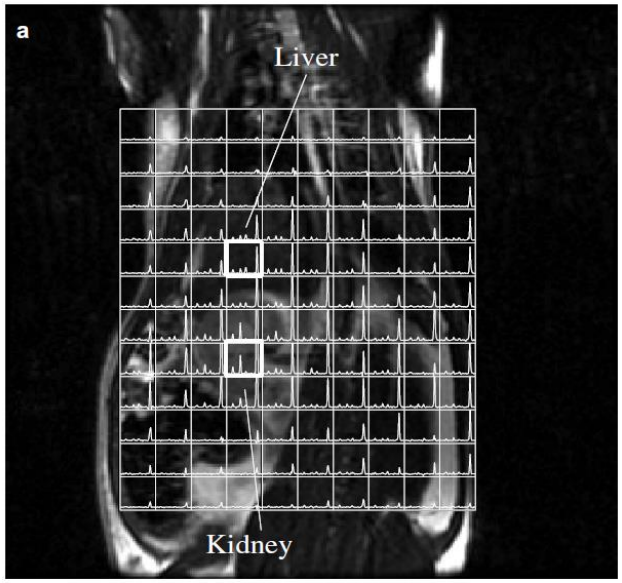
$$FOV \propto {}^{1}/_{t_{DI}}$$

$$SW \propto \frac{1}{K_x t_{DI}}$$

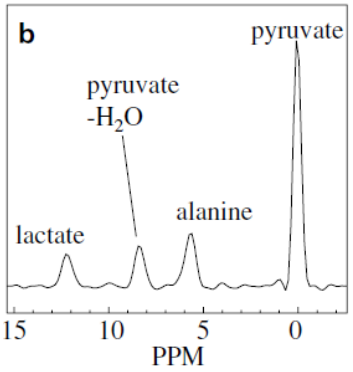


UCSF EPSI Data

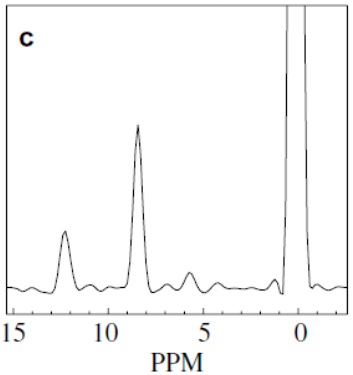
- Data presented as spatially resolved spectra



liver

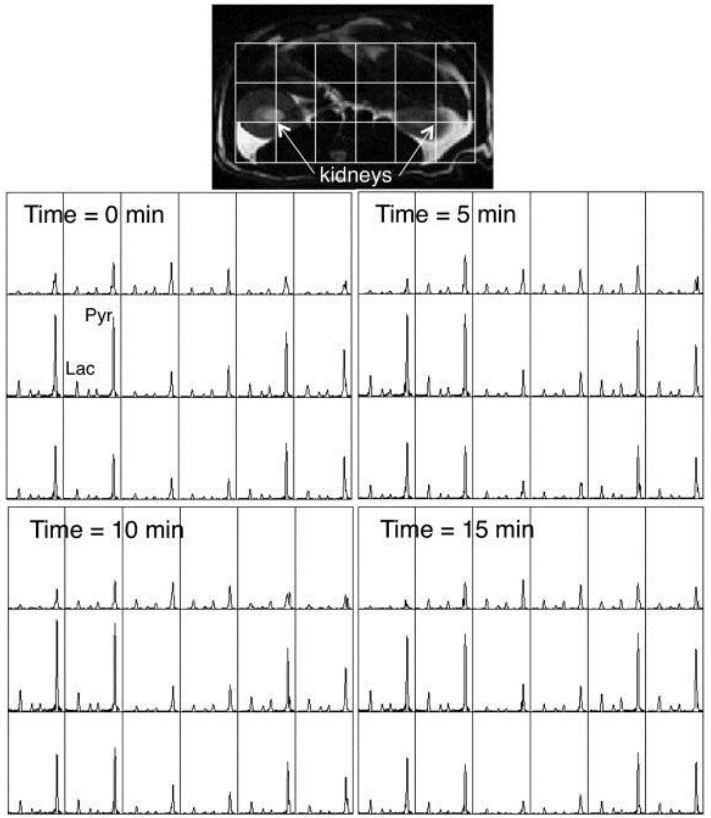


kidney



Cunningham CH *et al.*, J. Mag. Res. Med., **187**: 357–362 (2007)

32 averages

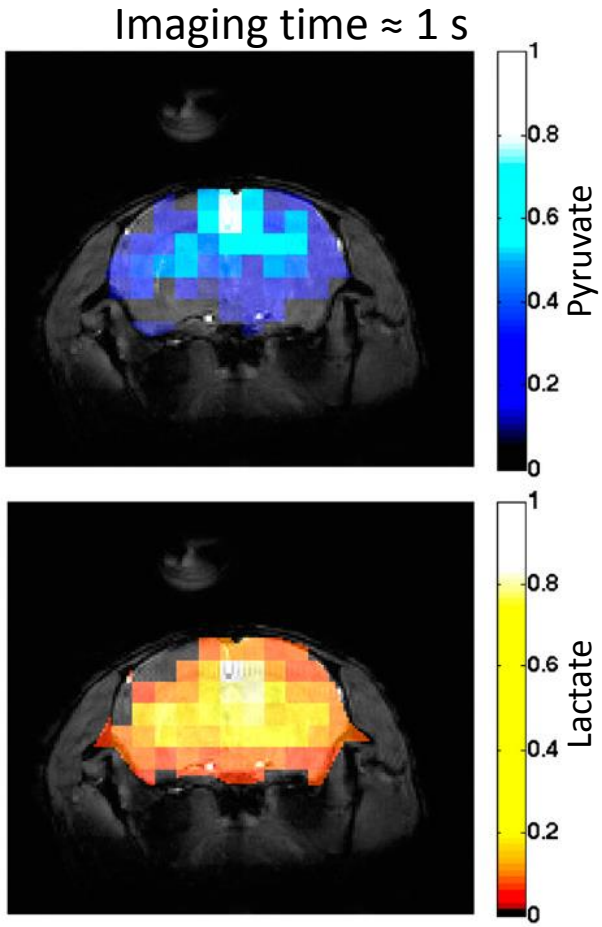


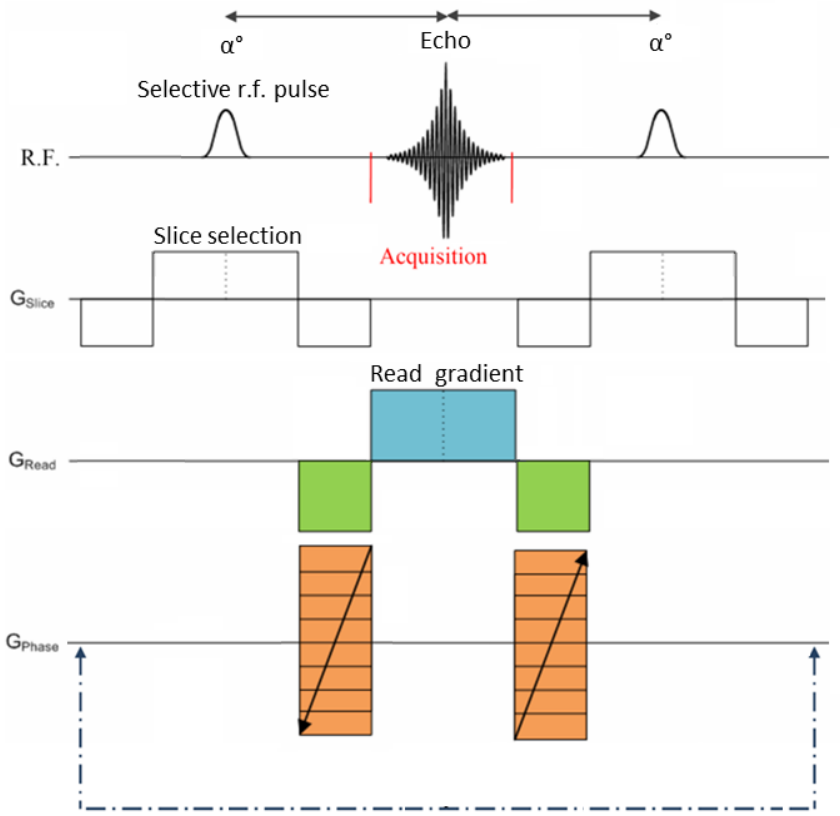
4 separate injections

Hu S *et al.*, J. Mag. Res. Imag., **31**: 490–496 (2013)

Single Compound Images

- Instead of Spectral/Spatial images, directly image each metabolite in interleaved acquisitions
- balanced Steady State Free Precession





Eichhorn T., *et al.*, PNAS., **45**, 18064-18069,(2013)