NMR Hands On

UAB Metabolomics Training Course
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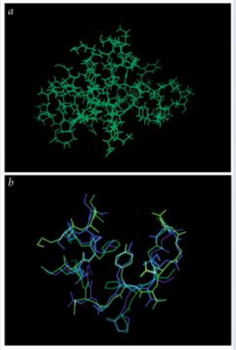
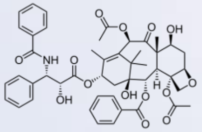
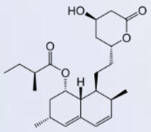
Nuclear Magnetic Resonance (NMR) Spectroscopy

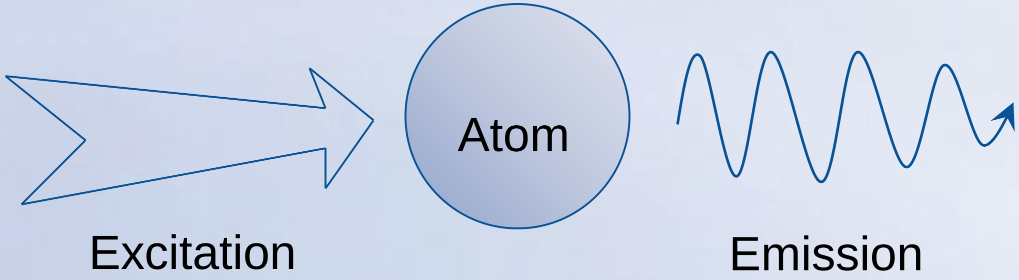
- Detects NMR active nuclei
- Robust and highly reproducible
- Non-destructive
- Quantitative
- Used in
  - Structure elucidation
    - Small molecules
    - Macromolecules (DNA, RNA, Proteins)
  - A number of techniques
    - 1D , 2D, 3D
  - Molecular motion and dynamics
- Similar method used in Imaging (MRI, fMRI)







NMR Spectroscopy



NMR Frequencies

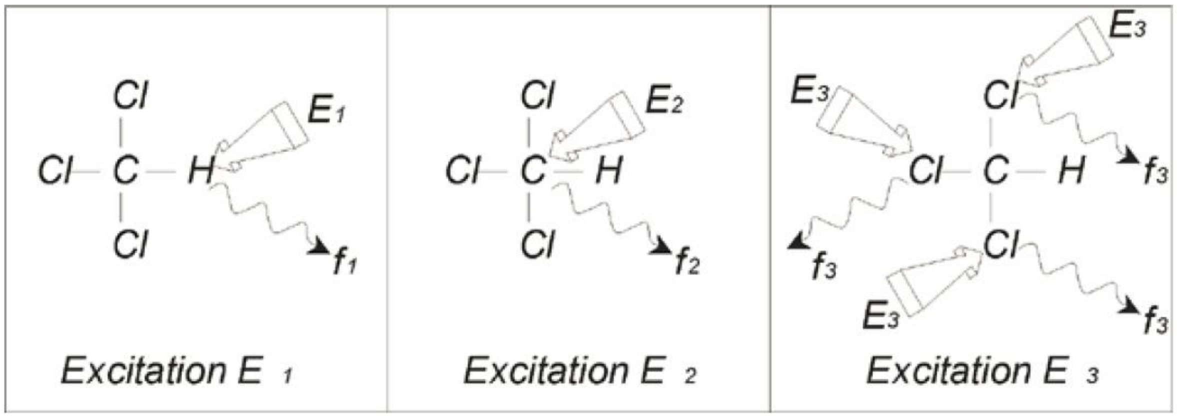


Figure 3.3: NMR Analysis of $CHCl_3$

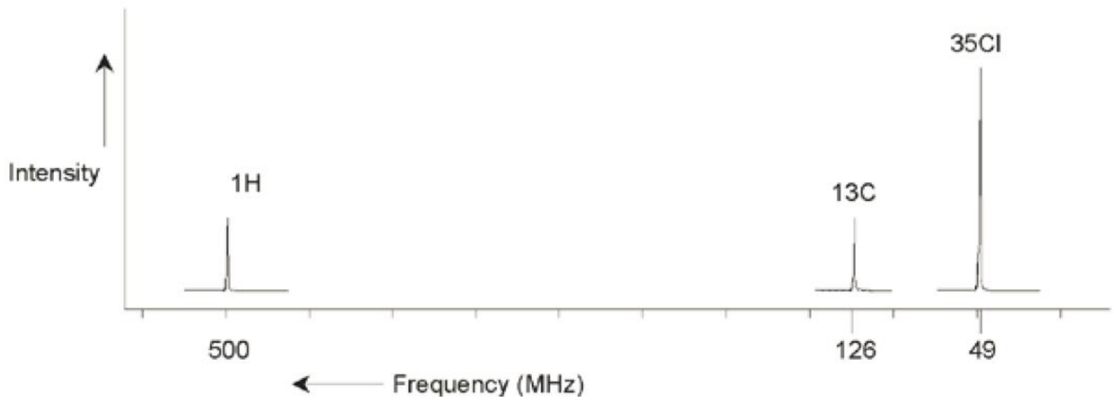


Figure 3.4: NMR Signals Emitted by $CHCl_3$

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NMR Spectroscopy

Frequencies in 11.7T magnet

Nucleus	Basic Frequency (MHz)	Natural Abundance (%)
^{1}H	500	100
^{2}H	77	0.015
^{3}H	533	0.005
^{13}C	126	1.11
^{35}Cl	49	75.53
^{37}Cl	41	24.47
^{15}N	50	0.37
^{19}F	470	100
^{31}P	202.5	100
^{57}Fe	16.25	2.20

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NMR Spectroscopy Explained : Simplified Theory, Applications and Examples for Organic Chemistry and Structural Biology: Neil E Jacobsen, John Wiley & Sons, Inc. 2007, ISBN 978-0-471-73096-5

NMR Spectrometer

NMR Console

Computer

Magnet

Pre-amplifier

Probe and
shim system

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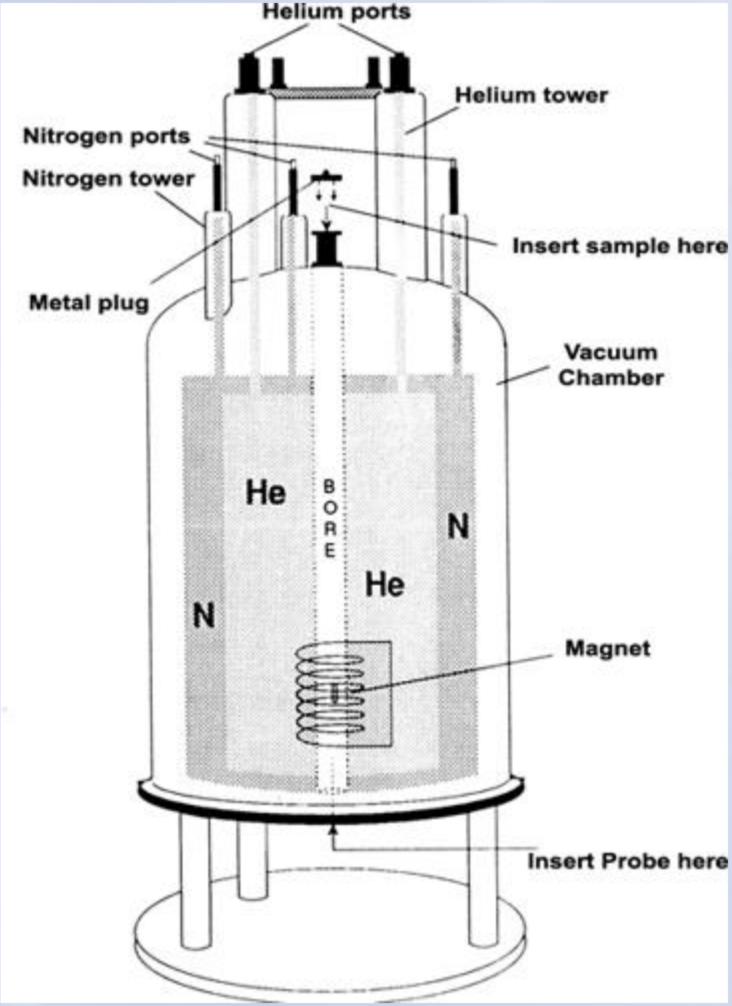
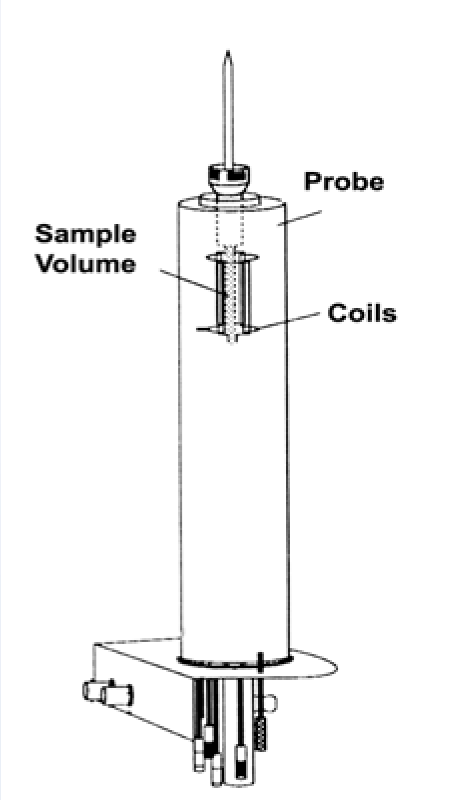
NMR Magnet and the probe





Sample is positioned on the probe using a spinner.

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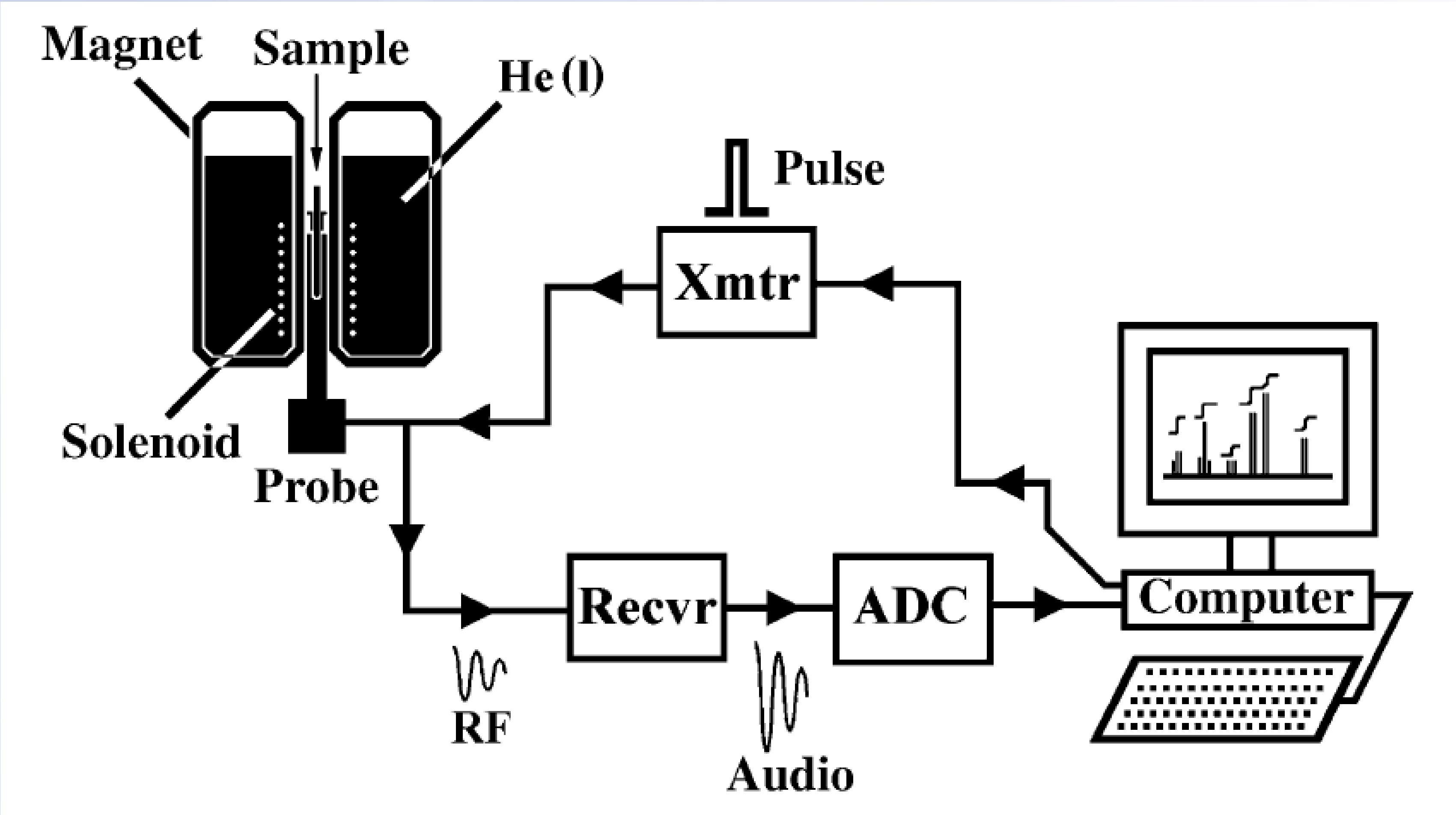
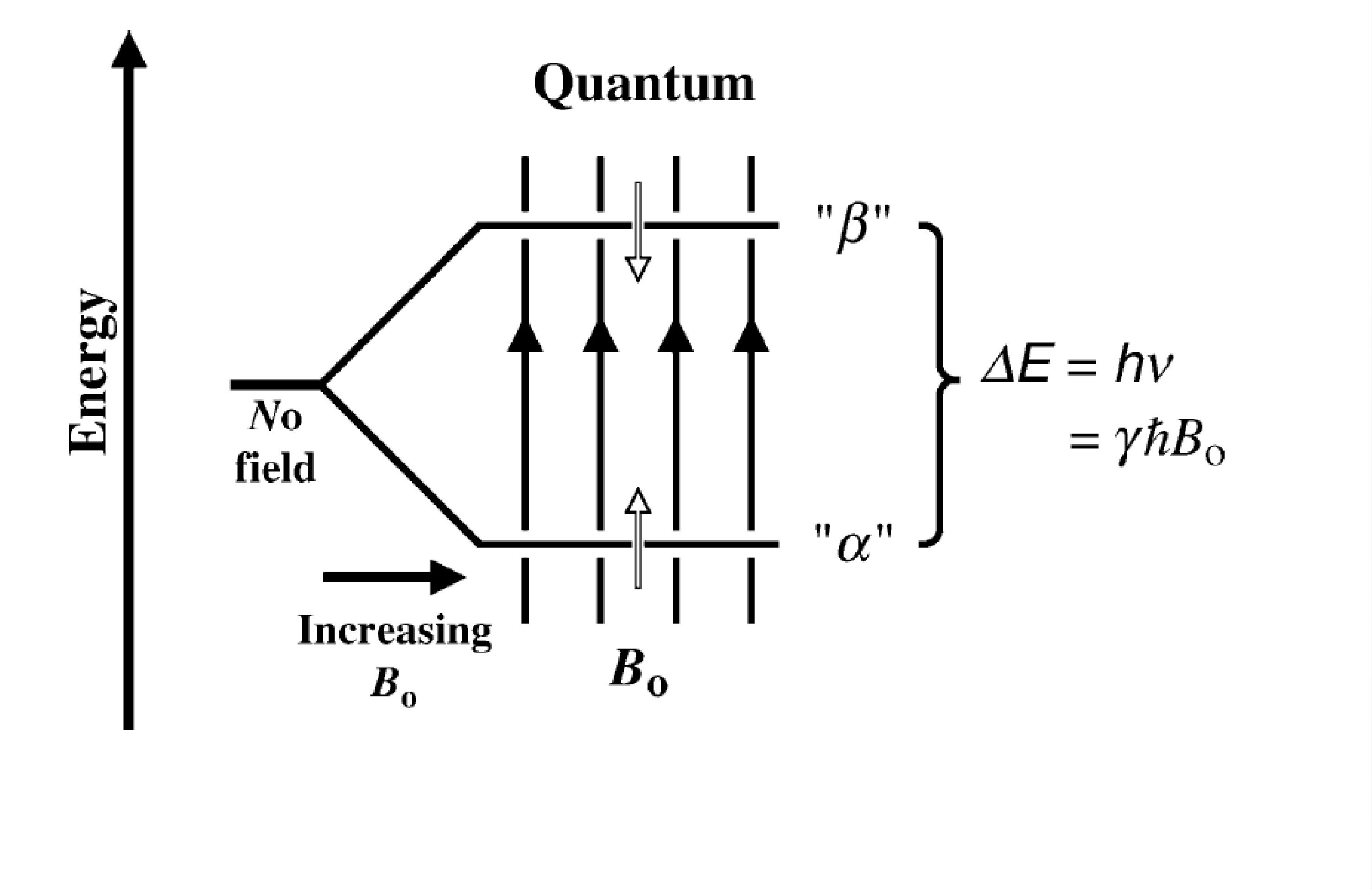
NMR Experiment



NMR Spectroscopy



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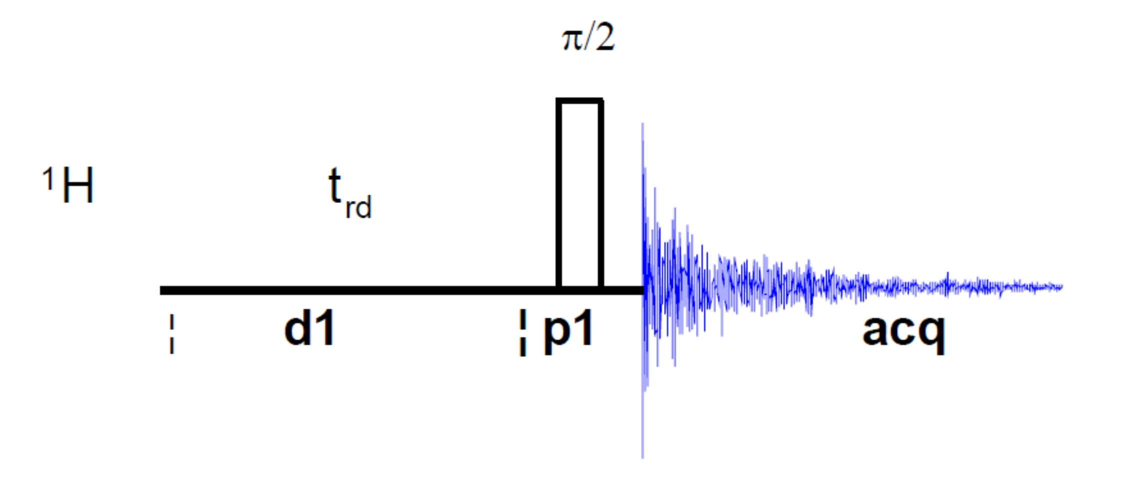
Basic ^1H Experiment



d1 = delay
p1 = pulse width
acq = acquisition time

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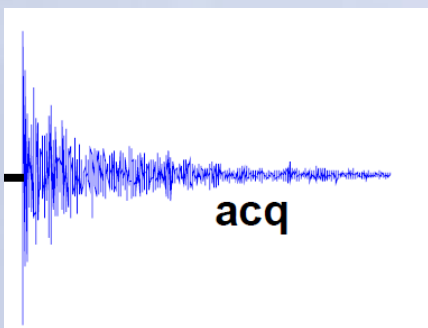
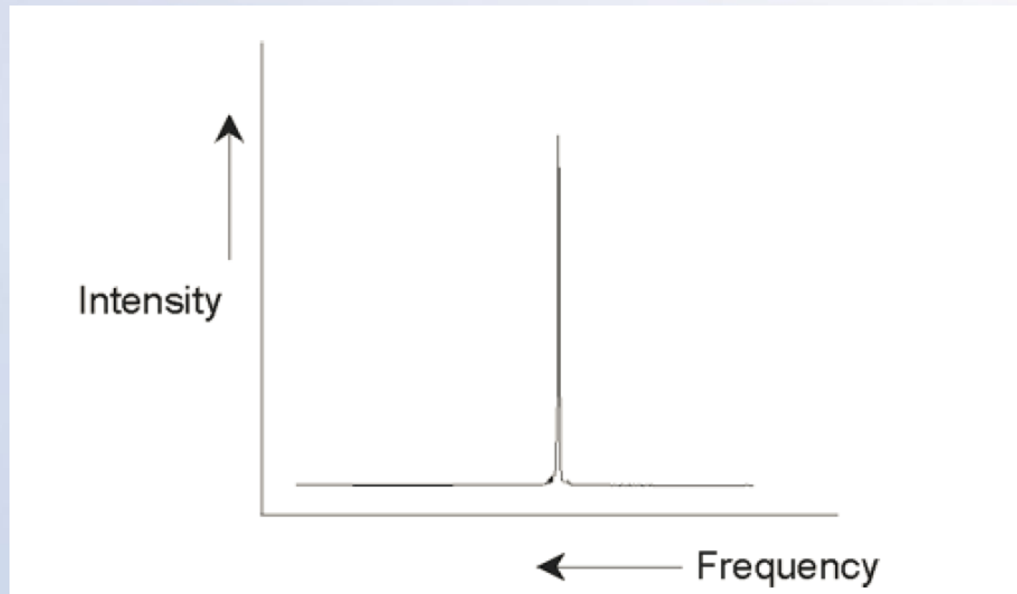
NMR Signal



Fourier
Transform



Chemical shift (ppm scale) = frequency / Spectrometer Frequency (MHz)

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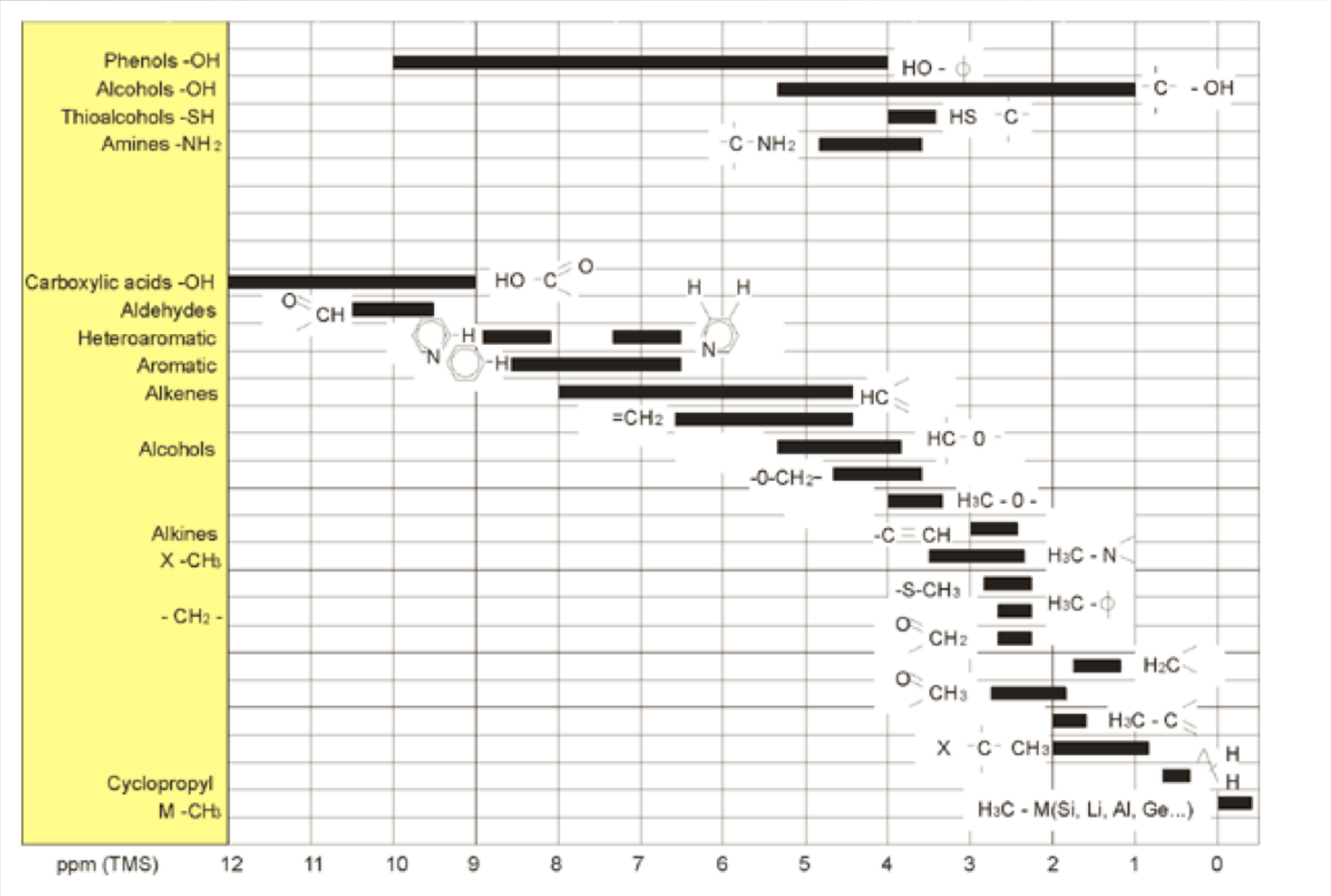
Chemical Shift of molecules



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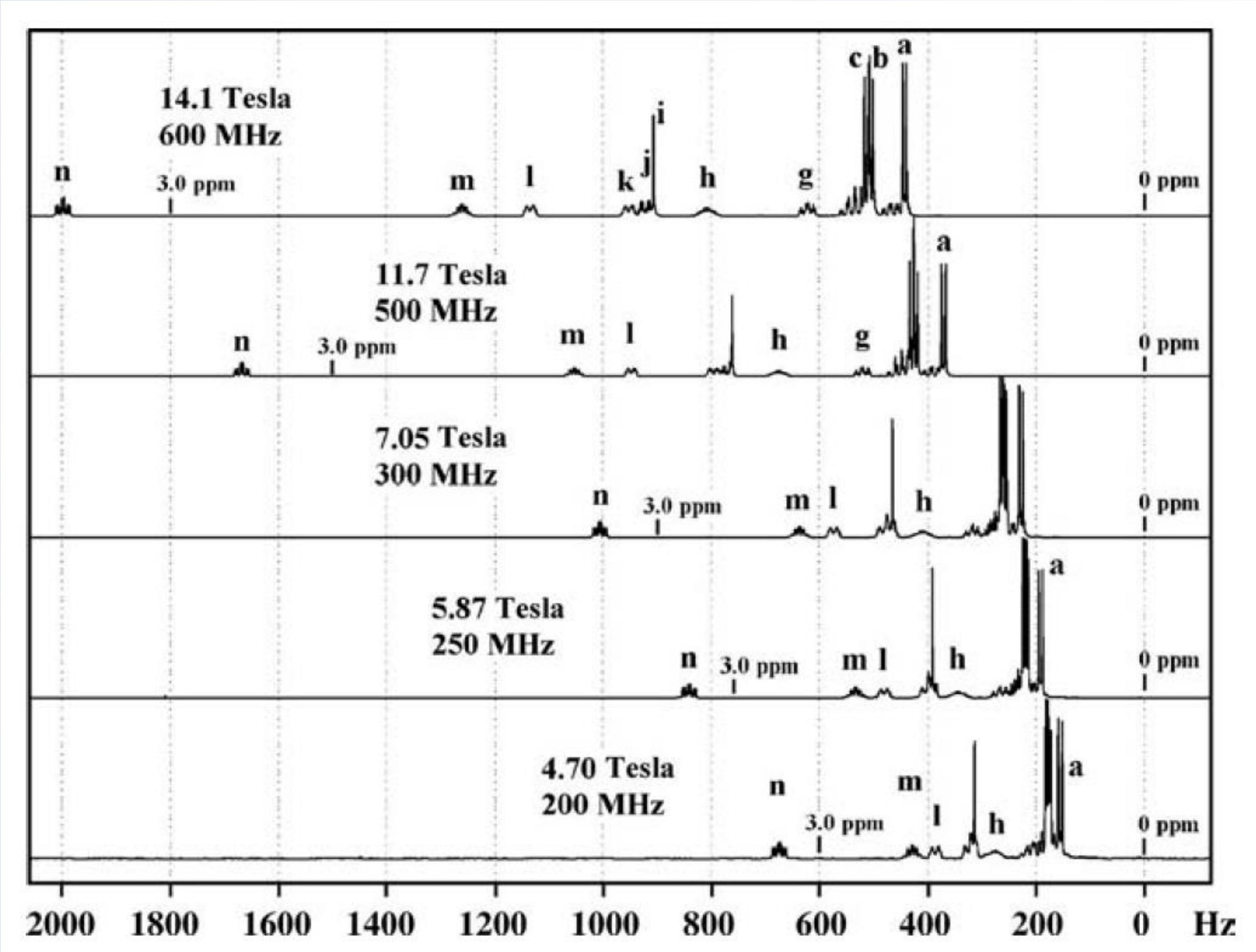
Static Magnetic field strengths



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Sample Preparation for metabolomics

- Balance and calibration check
- Prepare samples on ice, Minimize freeze thaw cycles
- Dilution
  - Using D_2O or Buffer (0.2M Phosphate)
- Extraction
  - MeOH or MeOH/ Water
  - MeOH/ $CHCl_3$/ H_2O (Folch Method)
  - 50% Acetonitrile in Water
  - Dry the sample
  - Reconstitute in D_2O or 0.2M Phosphate Buffer
- Internal standards
  - Chemical shift reference (DSS, also for line shape)
  - pH reference (Imidazole)
- Pooled QC Samples
- Consistency across the whole study is very important

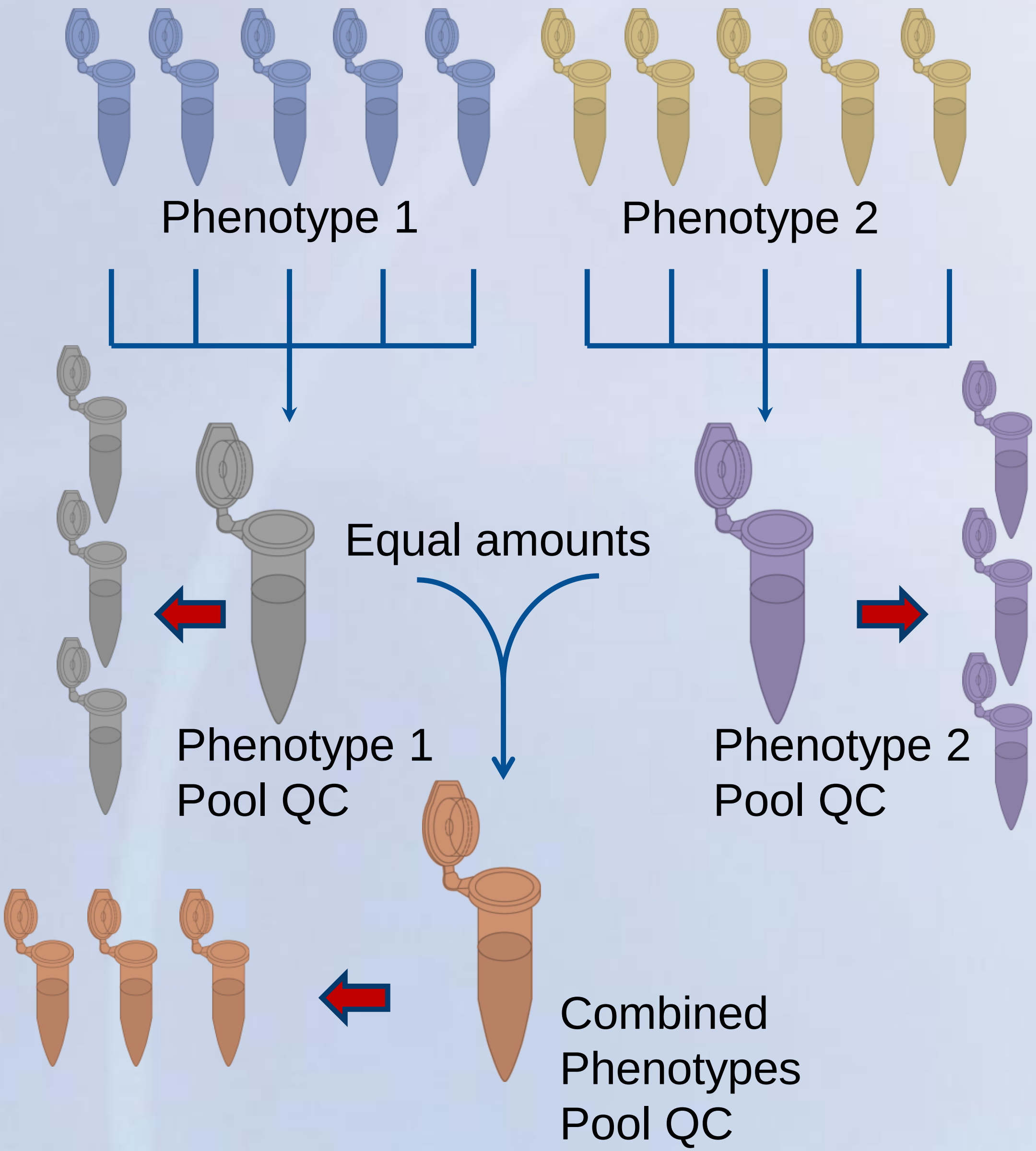
Sample Preparation for Metabolomics Analysis

Current sample preparation practices (in brief)

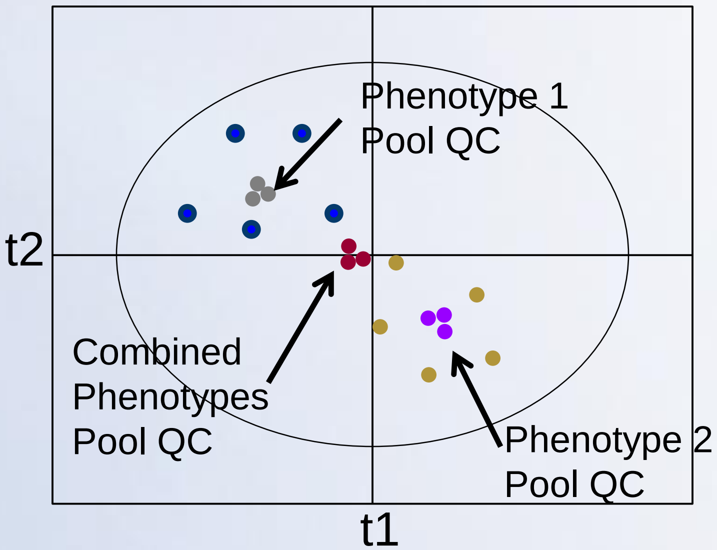
- **Biofluids**
  - Dilute with D_2O/ buffer/ 0.9% Saline
  - Add internal standard (ISTD, eg. Chenomx) solution or formate (for serum).
  - Centrifuge and transfer an aliquot into NMR tube
- **Tissue and Cells**
  - Homogenization performed in ice cold 50/50 acetonitrile/water
  - Supernatant dried down (lyophilized)
  - Reconstituted in D_2O and ISTD (eg. Chenomx) solution
- **Pooled QC Samples (Sample Unlimited)**
  - Mix equal volume of study samples to get pooled QC samples
  - 10% QC samples
- **Pooled QC Samples (Sample Limited)**
  - Use independent pool of similar samples
  - 10% QC samples
- **Daily balance and pipette check**

Samples are randomized for preparation and data acquisition

Pooled QC Samples



- Aliquots from each sample in the study phenotype are pooled (phenotypic pool)
- Equal amount of each phenotypic pools are pooled (Combined phenotypic pool)
- Replicates of pools are prepared
- Pool samples are prepared along with the study samples

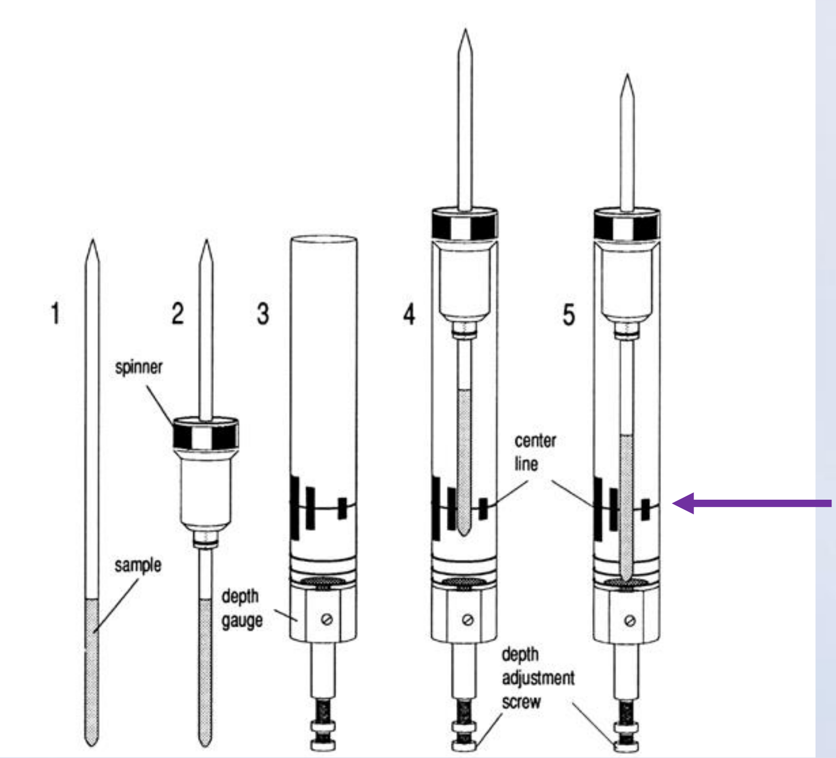


Pooled samples should cluster tightly

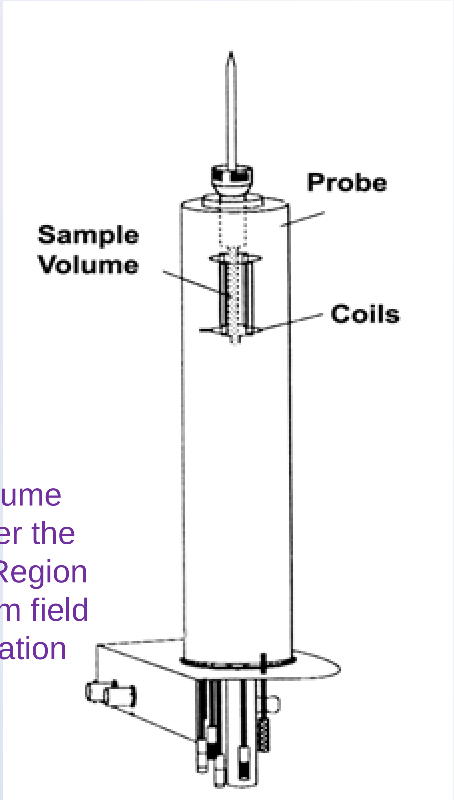
NMR Data Acquisition

- 1D NMR
  - 1st increment of NOESY
    - noesyprid (Bruker)
  - CPMG (serum or plasma)
    - cpmgpr1d (Bruker)
    - To remove broadening of signals due to macromolecules (eg. Proteins and lipids)
- 2D NMR (for structure elucidation)
  - 2D J-Resolved
  - COSY
  - TOCSY
  - HSQC
  - HMBC

Sample Amount in NMR tube



Sample volume should cover the NMR Coil Region For optimum field homogenization



- At least 10% D_2O in the sample
- Optimum volume
  - 550 – 600 uL (5mm tube)
  - 200 uL (3 mm tube)
- Sample gauge is used

For very small sample amounts, a NMR with a microcoil probe is an option.

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Steps in Data Acquisition

- Place the sample in the spinner
  - Use sample gauge
- Tune and match the probe
  - Automatic in new instruments
- Lock and shim the instrument
  - Gradient shimming
- Create and set up NMR parameters
- Acquire data
- Process the NMR spectrum

ERCMRC at UNC Chapel Hill

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DHMRI

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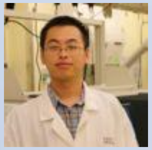

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LC-TOF-MS

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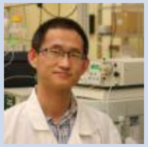

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