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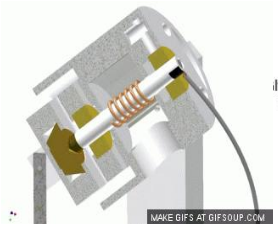
Non-, or Minimally Invasive High Resolution ^{1}H NMR Metabolomics on Intact Biological Objects Using Slow Magic Angle Spinning

Jian Zhi Hu, Mary Hu, Ju Feng, Xiongjie Xiao, Hardeep S. Mehta

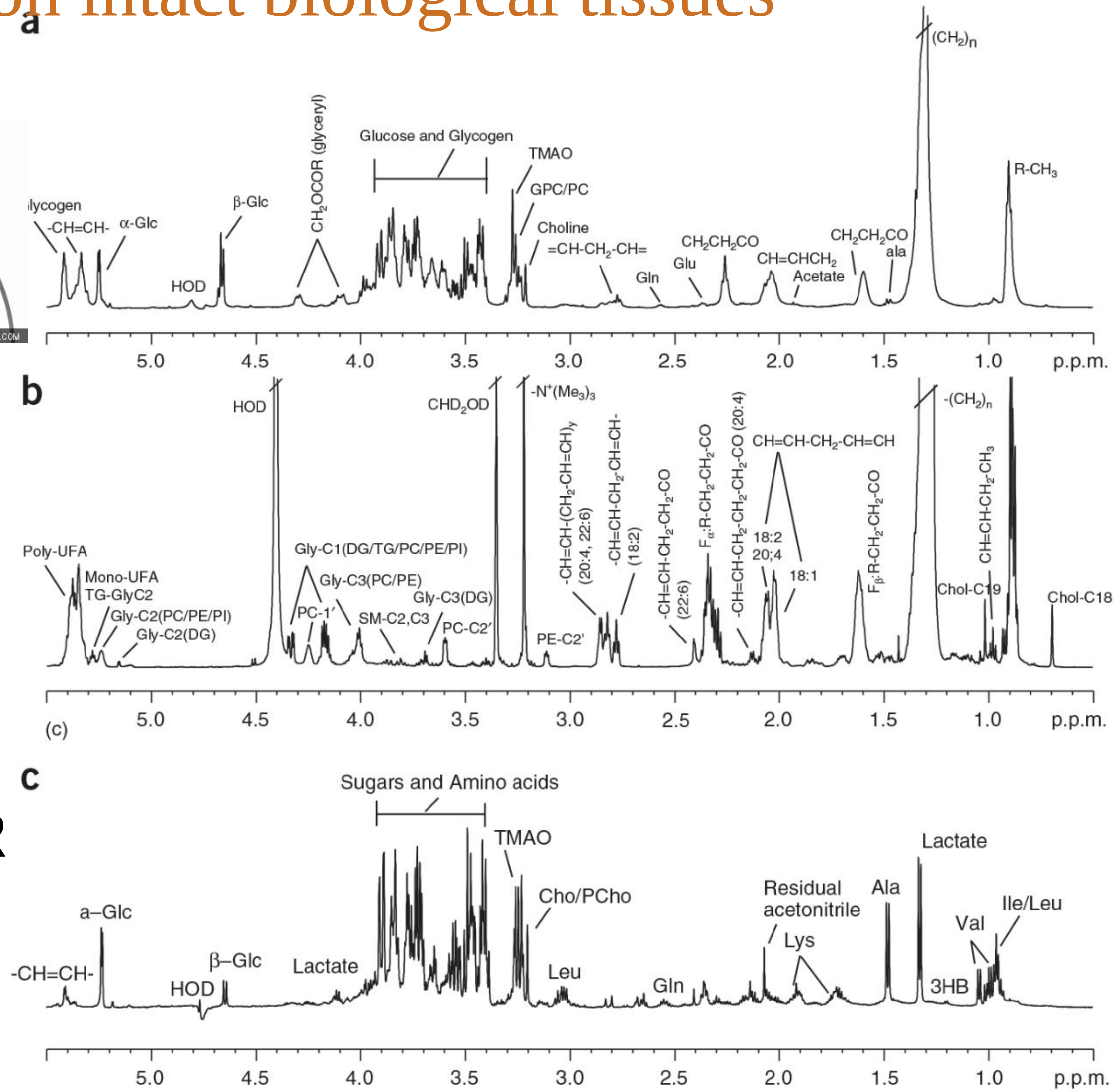
Introduction: ^{1}H HR-MAS NMR is a powerful technique for metabolic profiling on intact biological tissues

(a) ^{1}H HR-MAS NMR of **intact** mouse liver tissue.



(b) Liquid state ^{1}H NMR spectrum of a **lipid soluble** mouse liver tissue **extracts**.

(c) Liquid state ^{1}H NMR of an **aqueous soluble** liver mouse tissue **extracts**.



Beckonert, et al, nature protocols 5(6), 1019-1032 (2010)

HR-MAS is an excellent technique but the followings:

- Production of a 100% leakage free sample rotor for HR-MAS is challenging due to the use of a sample spinning rate of a few kHz or more.

- The large centrifugal force associated with fast sample spinning is destructive to the tissue structure and even some of the cells.

- Difficult to spin a large tissue sample (300 mg or more) to the desired spinning rate required for hr-MAS analysis.

- Work with small volume sample, e.g., ~1.0 μl or less is challenging.

Our Research Objectives

Develop an alternative MAS NMR that

- Provides high resolution, high sensitivity ^{1}H NMR metabolic profiling on biological tissues
- Is capable of non-, or minimally destructive detection
- Can work on samples with size as small as ~0.2 μl (200 nl) to as large as > 1 ml using a single probe.

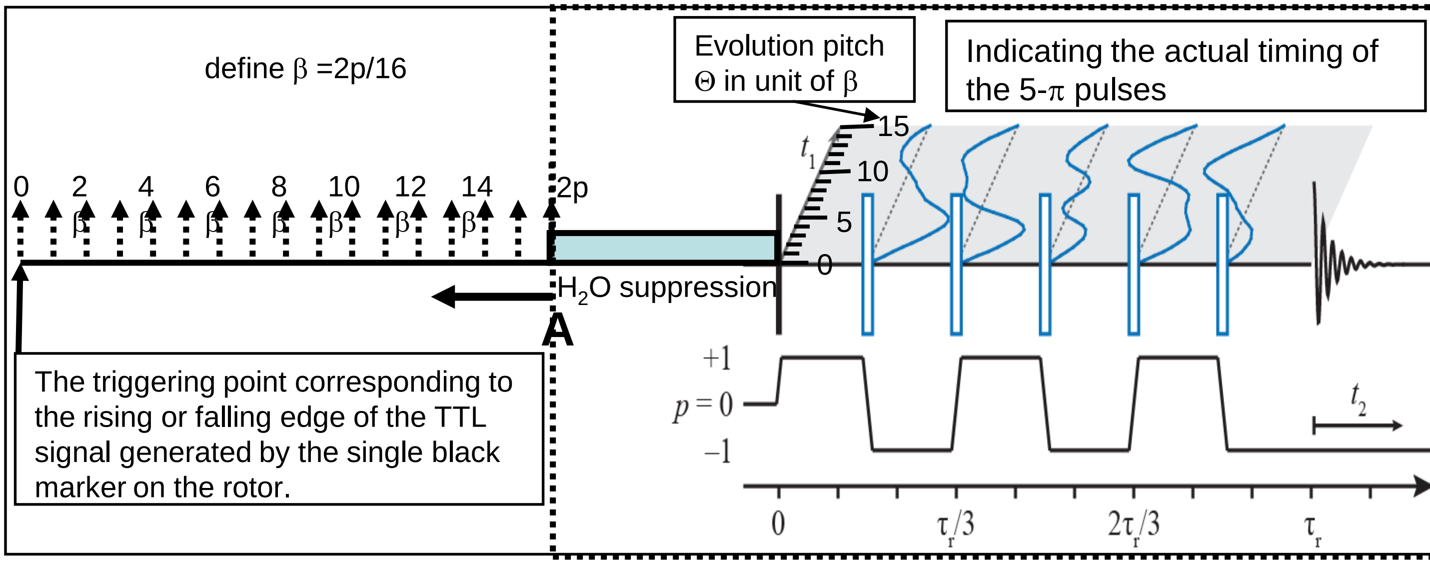
Our Approach

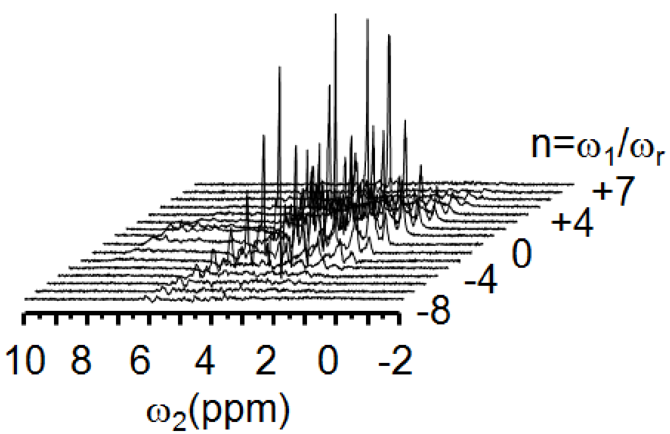
Combining the techniques of

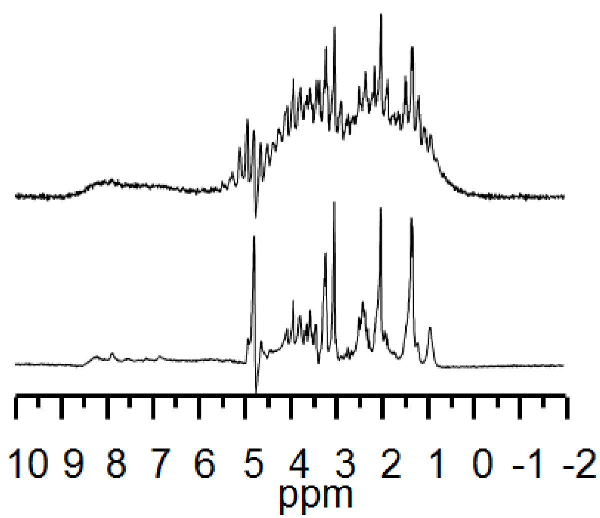
- High resolution slow-MAS ^{1}H NMR technique and
- Switchable inductively coupled static micro-RF coil resonator

New Slow-MAS (40-200Hz) Pulse Sequence for High Resolution ^{1}H NMR

The rotor position synchronized ^{1}H PASS for metabolic profiling of ordered biological samples using PASS-16.

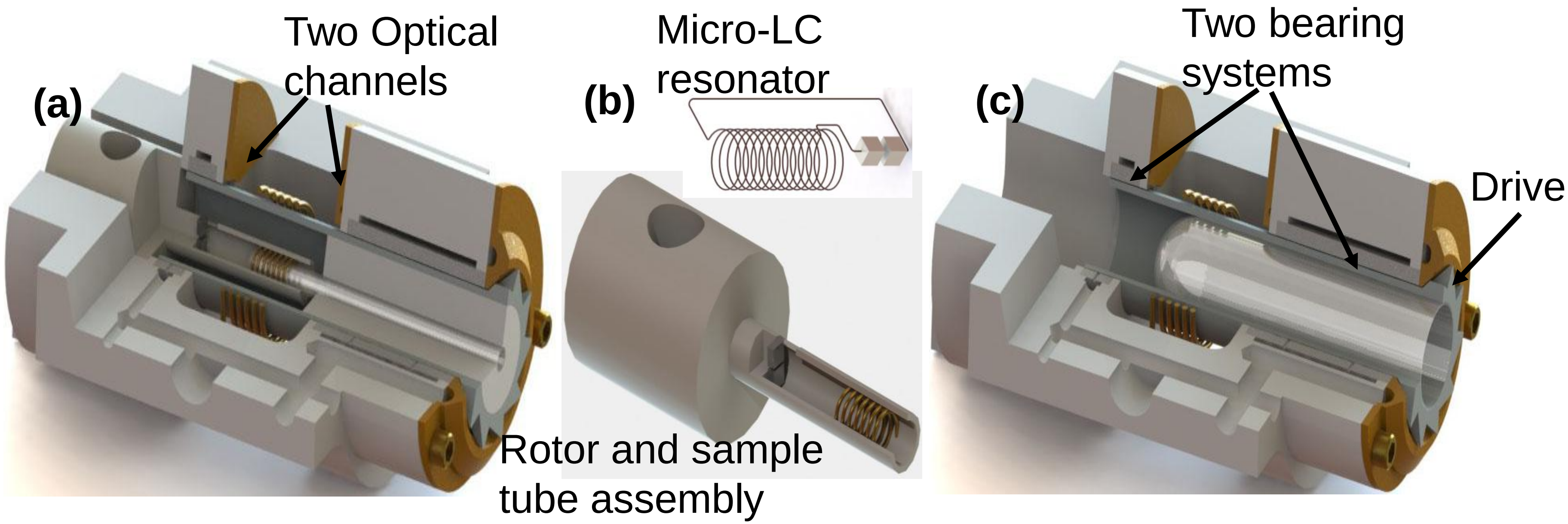






2D 300MHz ^{1}H-PASS of 100 mg fresh mouse brain at sample spinning rate of 43Hz

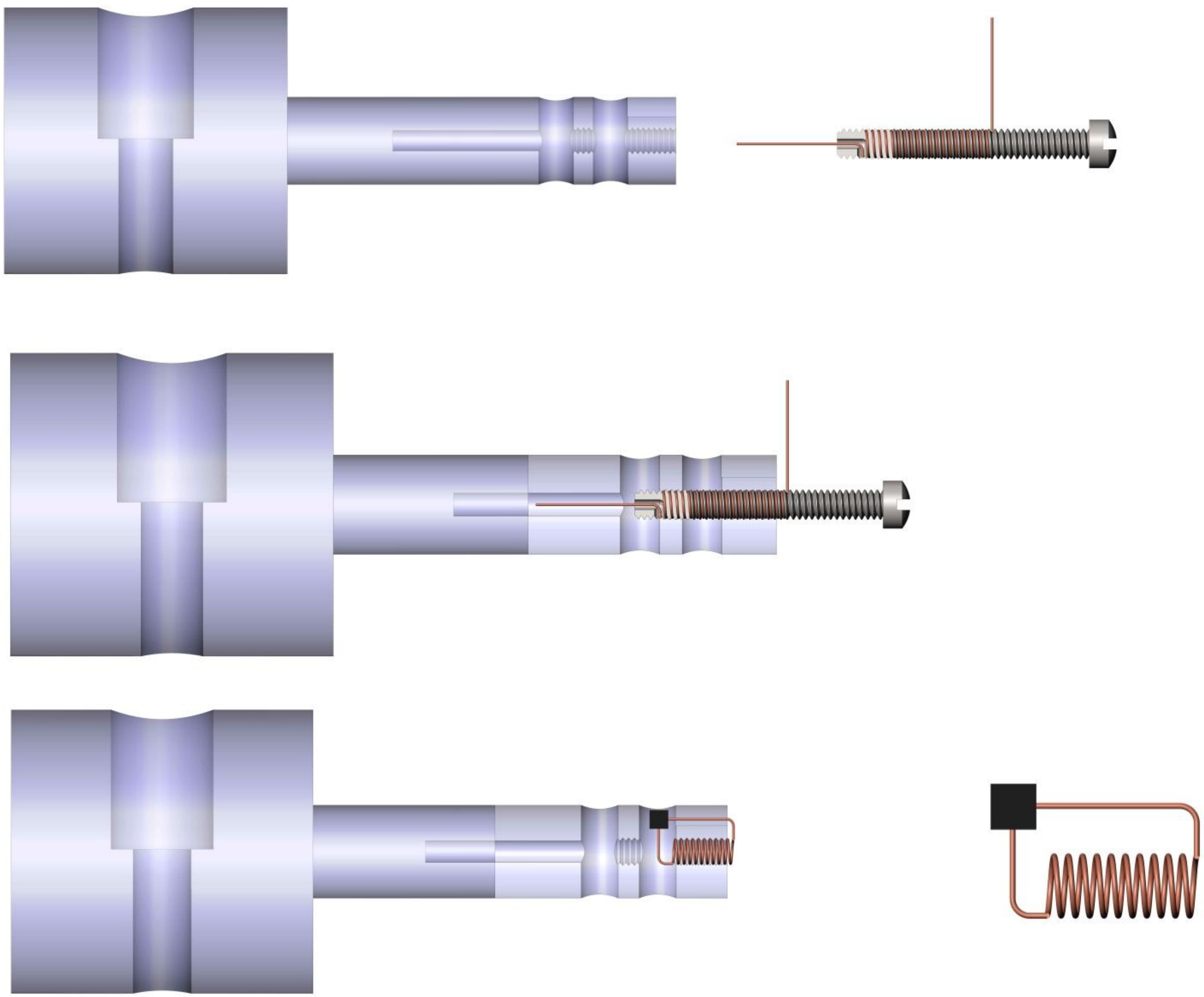
Switchable Micro-RF Coil Slow-MAS Probe



(a) Small samples of variable sizes; **(b)** Switchable plastic plug with a micro-LC resonator for enhanced sensitivity; **(c)** Large biological objects.

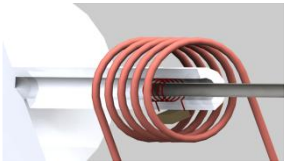
- Provides smooth sample spinning of various sample sizes
- Work with anisotropic biological samples

Procedures for constructing the Micro-RF Coil LC resonator

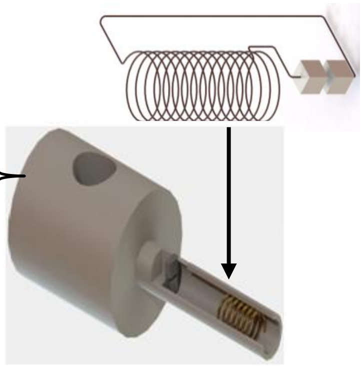


Features of the probe

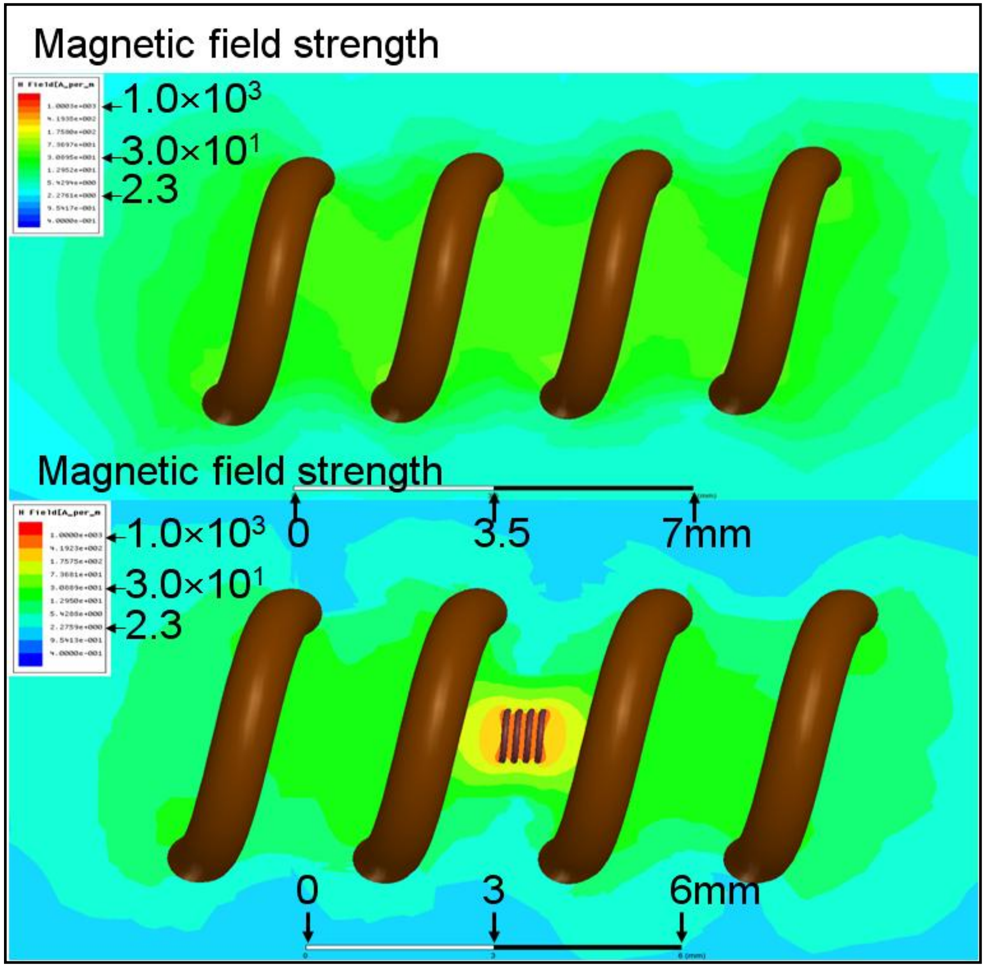
- Static micro RF-Coil is wound inside the coil support for maximizing the filling factor.
- Inductive coupling between the micro coil and the outer coil for increased sensitivity due to increased sample filling factor from the micro coil resonator.
- Magnetic susceptibility matched wires are used for winding the micro coil for best B_0 field homogeneity.



- Easily switch the plug with micro-RF coil resonator of different ID to accommodate a range of sample sizes using a single probe.



The simulated magnetic field for a given input RF power



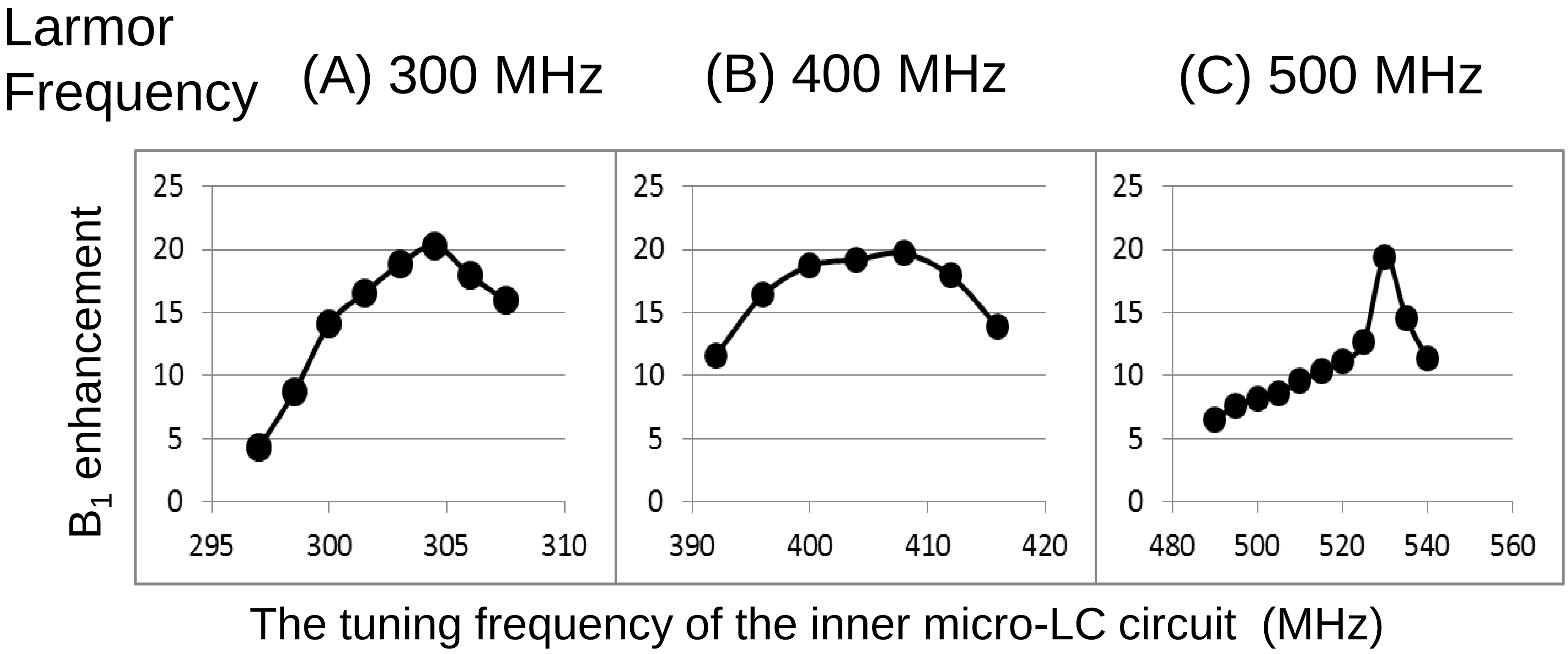
The magnetic field with the inner micro-RF coil resonator is **20 times** that without the inner micro-RF coil resonator.

The effect of turns used for winding the RF coils and the effect of copper wires for winding the RF coils to the B_1 field inside the micro RF

Coil Geometries	Outer Coil Turns	Inner Coil Turns	B_1 Field (A/m)
One large coil only **OD=5 mm, length=12 mm, wire diameter=1 mm**	4	none	18
	5	none	27
	6	none	46
Two RF coils, varying # of turns for inner coil **Outer Coil: OD=5 mm, Length=12mm, wire diameter=1 mm.** **Inner Coil: ID=1mm, length=0.8mm, Wire diameter=0.1 mm**	5	4	343
	5	5	413
	5	6	404
Two RF coils, same # of turns for both outer and inner coil **Outer coil: OD=5mm, length=12mm, wire diameter=1mm** **Inner coil: ID=1mm, Length=0.8mm, wire diameter=0.1mm**	4	4	334
	5	5	413
	6	6	357
	Outer Coil OD (mm)	Inner Coil ID (mm)	B_1 Field (A/m)
Two RF coils: varying the OD of outer, varying ID of inner coil but keep the # of turns the same (i.e., 5) for both coils **Outer Coil: Turns = 5, Length=12mm, Wire Diameter=1mm** **Inner Coil: Turns = 5, Length=0.8mm, Wire Diameter=0.1mm**	5	0.6	591
	5	1	413
	5	1.25	685
	10	0.6	277
	10	1	194
	10	1.25	202

Note that for this table the outer RF coil is at resonance of 300 MHz and the resonant frequency of the inner micro RF coil is set at the optimum conditions so that maximum B_1 field inside the inner micro RF coil is obtained.

Theoretical relationship: B_1(inner coil)/B_1(outer coil) *vs* the resonant frequency of the inner micro-LC circuit



***Outer RF coil** (4 turns, coil length of 12 mm that is wound with copper wire of diameter of 1 mm);*
***Inner micro-RF coil** (4 turns, coil length of 1 mm that is wound with copper wire of diameter of 0.1 mm).*

Performance test results of the Inductively Coupled Micro-coil Resonator (one example)

Outer RF Coil: 3 turns flat; ID: 12.6 mm; OD: 13.8 mm; width: 1.2 mm; Coil length: 9 mm.

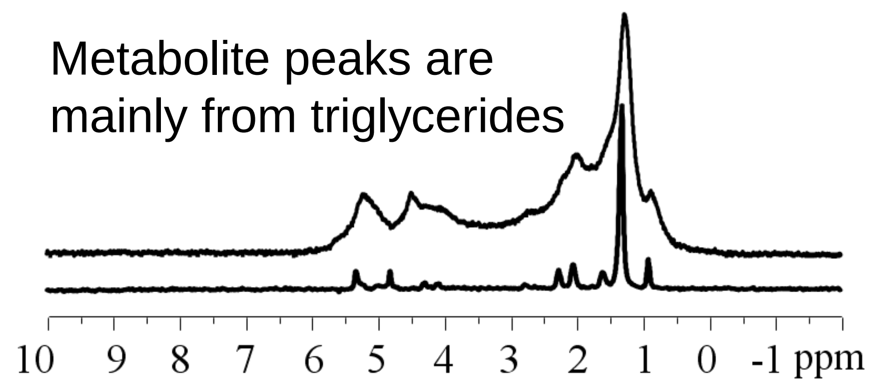
Inner micro RF Coil: 6 turns Pd plated OFHC Cu AWG wire; ID: 1.3 mm; OD: 1.7 mm; Length: ~2.7 mm.

π/2 pulse width for a given RF power at the probe
Without micro-coil: 12.5 μs at 28 W
With micro-coil: 3.75 μs at 0.6 W
Estimated S/N enhancement by using micro-coil: ~22

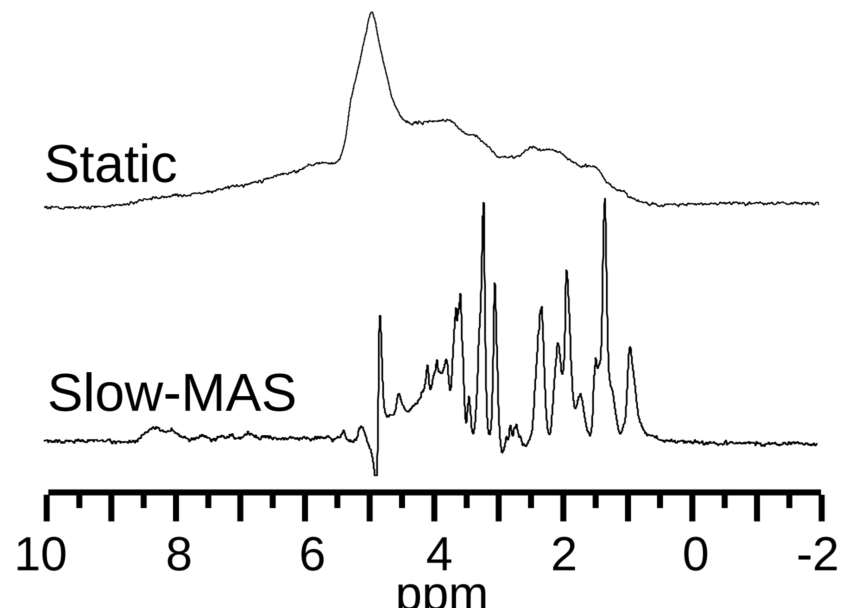
Slow-MAS Preliminary Results

Small volume biological tissue: 2.6 minutes ^{1}H slow-MAS NMR spectra of **200 nanoliter** (~0.2 mg) muscle from an obese mouse acquired at a sample spinning rate of 147 Hz (bottom trace) compared with spectrum acquired using standard method (top trace). Significant spectral resolution enhancement is obtained with slow-MAS.

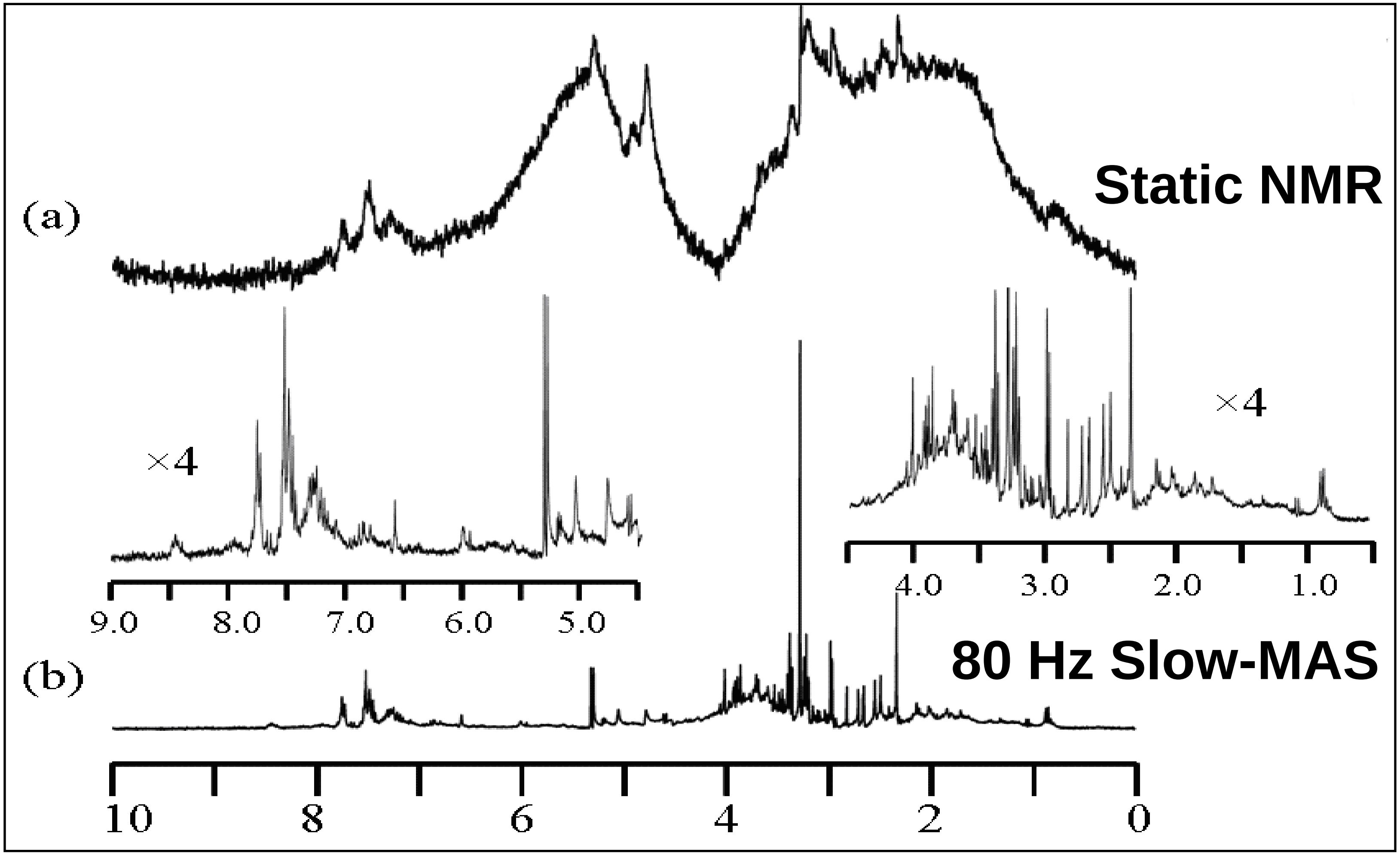
Metabolite peaks are mainly from triglycerides



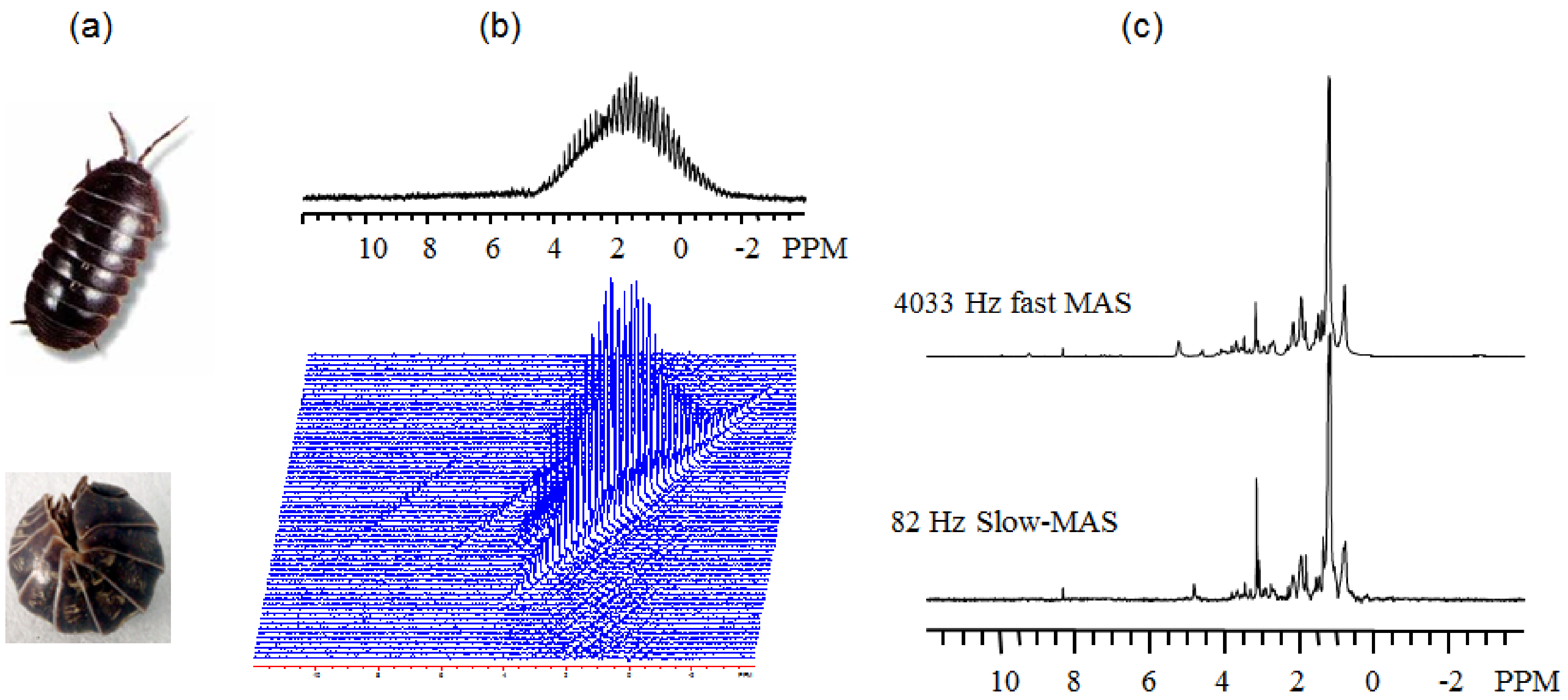
Large volume biological tissue: 1005 mg mouse brain ^{1}H-PASS at 83Hz. 16 evolution steps; total experimental time of 38 minutes



300 MHz ^{1}H NMR spectra obtained from a 2.5 uL mouse urine sample.



Non, or minimal destructive slow-MAS ^{1}H NMR metabolomics on a live pill bug at a sample spinning rate of 82 Hz compared with fast-MAS of 4033 Hz



Note that slow-MAS is performed first before fast MAS experiments.

Summary

- A slow-MAS metabolomic technique is under developmet that allows high resolution ^{1}H NMR metabolic profiling on samples with volume as small as 0.2μl (200 nanoliters) to larger than 1 cm^3 investigated using a single probe.
- The nanoliter capability has the potential to follow the metabolic changes through a continued investigation on a single small laboratory animal over a long period of time using minimally invasive blood and tissue biopsy samples.
- The milliliter capability would allow minimally destructive studies of intact biological object with size as large as >1 cm^3.
- Slow-sample spinning avoids fluid leakage and keeps the integrity of the biological sample. It is a non-, or minimally invasive method and is also a safe method for working with hazardous biological samples.

Acknowledgement

NMR

Mary Hu
Ju Feng
Xiongjie Xiao
Xuan Wang
Xuchu Deng
Zhanchao Zhao
Hardeep S. Mehta

Biologists

Richard A. Corley
Karla D. Thrall

NIH/NIEHS Grant: 1R01ES022176
NIH/NCRR Grant: 1R21RR025785

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