In-vivo NMR Metabolomics

Tissues, live animals etc

Major sources of line-broadening of NMR signals in intact cells, tissues, organs and animals

Chemical Shift Anisotropy ($H_{Zeeman} = I.\underline{\boldsymbol{\sigma}}.B_0$)

$\sigma = \sigma_{isotropic} + \sigma_{anisotropic}$

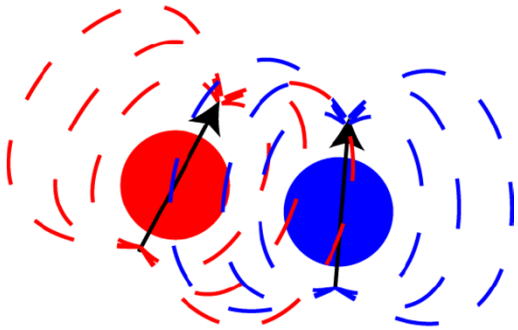
Dipolar Anisotropy ($H_{DD} = I.\underline{\mathbf{D}}.S$)

$D = D_{isotropic} + D_{anisotropic}$

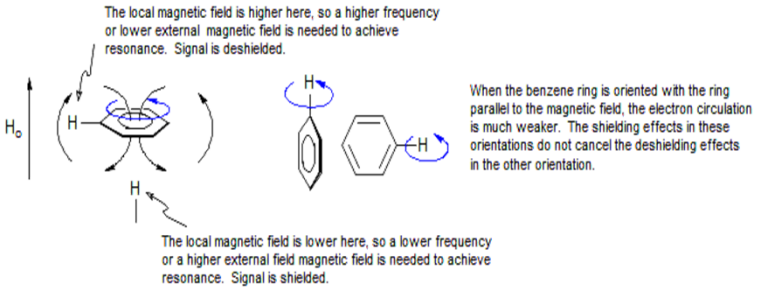
Magnetic Susceptibility Anisotropy ($M = \underline{\boldsymbol{\chi}}.B_0$)

In solutions, because of the rapid tumbling of the molecules (from rotational Brownian diffusion), the anisotropic components are all averaged out, leaving behind only the "isotropic part" that is experimentally observed.

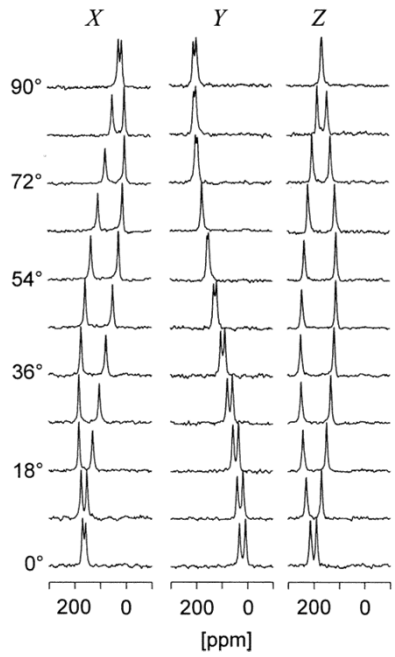
However, in cells, tissues, and intact animals, we will see significant NMR line broadening from incomplete averaging of anisotropic components of the above three tensors. The nuclear spins can generally exist in an environment that is somewhere between free solution and solid state.



Magnetic dipole-dipole interaction



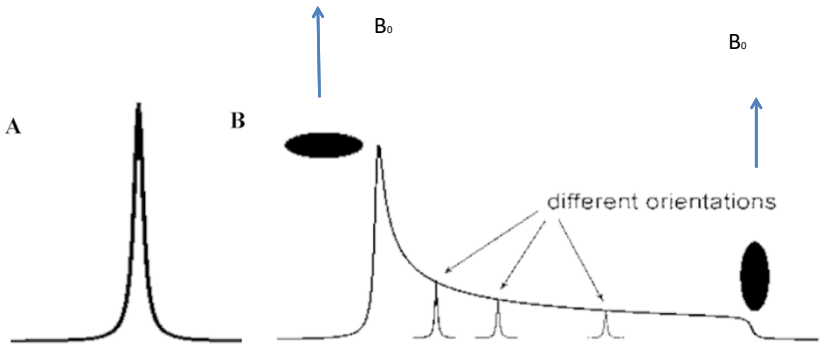
Chemical shift anisotropy



Chemical Shift Anisotropy of 31P in a single crystal.

Phosphorus-31 NMR spectra of a single crystal of a compound as a function of crystal orientation in the external magnetic field. Rotations were performed consecutively about the cube X, Y, Z axes. The crystal contains Four P atoms per unit cell in two symmetry-related Pairs that are magnetically equivalent, so two P resonances are observed.

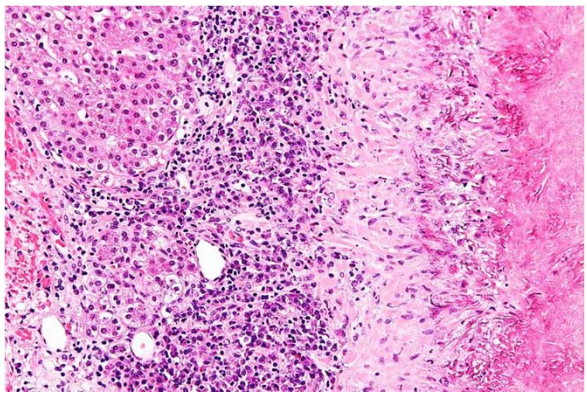
Eichele et al., JACS 124:1541-52 (2002)



Solution
Or isotropic

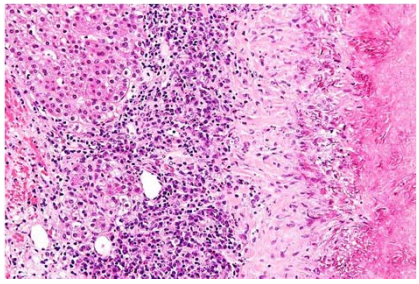
Powder pattern

NMR spectra in solution and powder samples



Slice of liver tissue

The presence of different compartments with boundaries contributes to variations in magnetic susceptibility



Magic Angle Spinning (MAS):

The nuclear spins in a tissue resemble nuclear spins in a powder, i.e., all possible random orientations of molecules (hence the dipolar, CSA, and susceptibility tensors) with respect to the external field are possible. So, if we spin the sample at an angle θ w.r.t. to the external magnetic field, all molecular orientations perpendicular to the rotation axis will be averaged out, thus significantly sharpening the lines. It can be shown that the only remaining anisotropic components (in DD, CSA, and Susceptibility) that are not averaged out are proportional to $(3\cos^2\theta - 1)$.

But, $(3\cos^2\theta - 1) = 0$ when $\cos^2\theta = 1/3$; or $\theta = 54.7^o$.

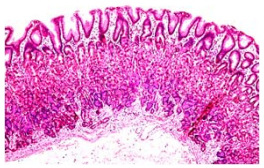
Only isotropic component remains in MAS performed at $\theta = 54.7^o$.

So, MAS experiments are always performed by spinning the samples at $\theta = 54.7^o$ to get the narrowest possible NMR lines.

HR-MAS NMR Spectroscopy in the Characterization of Human Tissues: Application to Healthy Gastric Mucosa

LUISA SCHENETTI,[1] ADELE MUCCI,[1] FRANCESCA PARENTI,[1] RITA CAGNOLI,[1] VALERIA RIGHI,[1, 3] M. RAFFAELLA TOSI,[2] VITALIANO TUGNOLI[3]

Concepts in Magnetic Resonance Part A, Vol. 28A(6) 430–443 (2006)



Gastric mucosa
from stomach lining

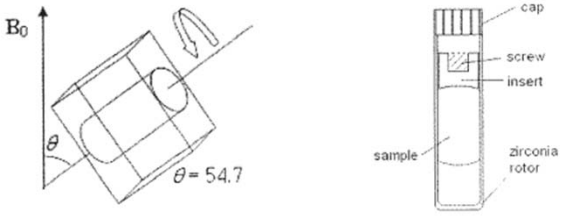


Figure 1 Schematic representation of HR-MAS probe (left); HR-MAS rotor (right).

The 1D ^{1}H NMR experiment with suppression of the residual HDO/H_2O generates a spectrum containing signals from metabolites, macromolecules, and lipids. It is necessary to suppress water signal because its high intensity can obscure the resonances of the other components.

$$[d_1\text{-}90^\circ\text{-}(\tau\text{-}180^\circ\text{-}\tau)_n\text{-}AQ]$$

CPMG pulse sequence
To filter out broad signals
From lipids and high MW components

$$[d_1\text{-}90\text{-}g_1\text{-}\delta\text{-}180\text{-}g_2\text{-}\delta\text{-}90\text{-}g_3\text{-}\Delta\text{-}90\text{-}g_1\text{-}\delta\text{-}180\text{-}g_2\text{-}\delta\text{-}90\text{-}g_4\text{-}d_{21}\text{-}90\text{-}AQ]$$

where $g_2 = -g_1$, the diffusion time d_{20} = 90-δ-180-δ-90-Δ and d_{21} is the eddy-current time. The correct selection of d_{20} and the gradient strength permits to filter out signals from fast moving small molecules and to retain only macromolecules.

Diffusion-edited
Pulse sequence
to filter out signals
from low MW components

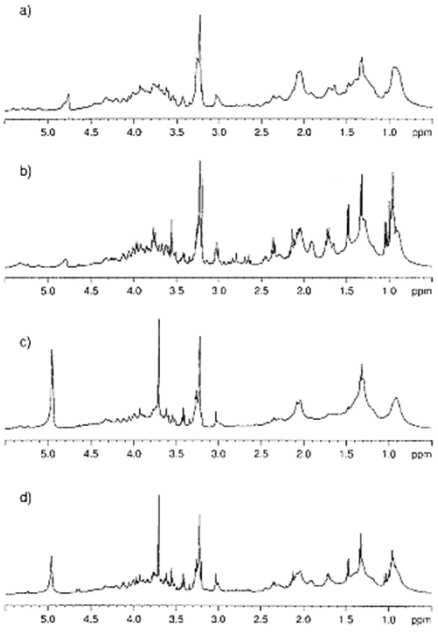


Figure 2 1D water-presaturated ^{1}H NMR spectra of healthy gastric mucosa: (a) sample 1 at 300 K, t = 0 h; (b) sample 1 at 300 K, t = 21 h; (c) sample 2 at 278 K, t = 0 h; (d) sample 2 at 278 K, t = 18 h.

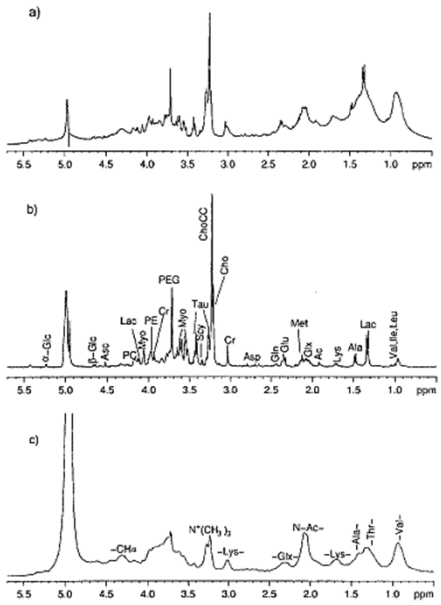


Figure 3 1D proton NMR spectra of healthy gastric mucosa obtained with (a) water-presaturation pulse sequence with composite pulse; (b) CPMG sequence with total spin-echo time $2n\tau = 360$ ms; (c) diffusion-edited spectrum obtained with $\Delta = 200$ ms, $\delta = 4$ ms and gradient strength of 32 G/cm. The metabolites are identified according to Table 1.

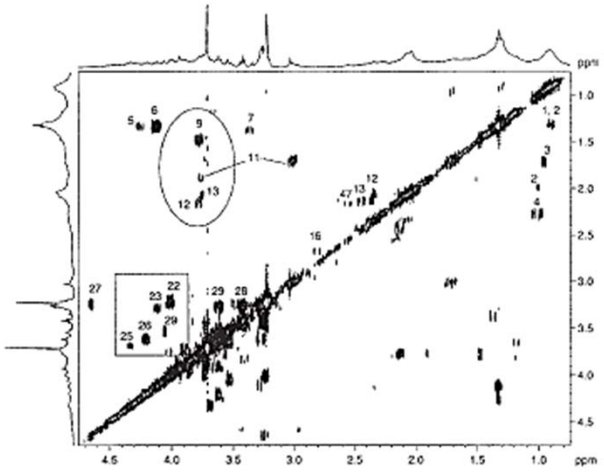


Figure 4 COSY NMR spectrum of healthy gastric mucosa. The identified metabolites are labeled according to Table 1.

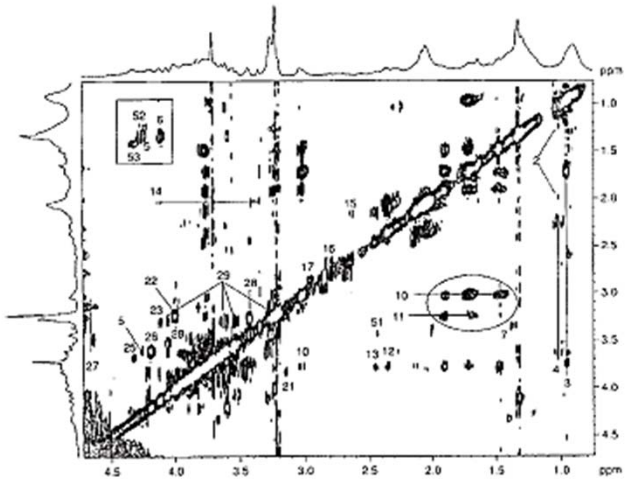


Figure 5 TOCSY NMR spectrum of healthy gastric mucosa. The identified metabolites are labeled according to Table 1.

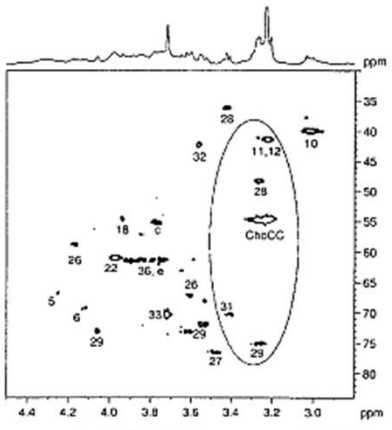


Figure 6 Partial $^{1}H,^{13}C$-HSQC spectrum of healthy gastric mucosa. The identified metabolites are labeled according to Table 1.

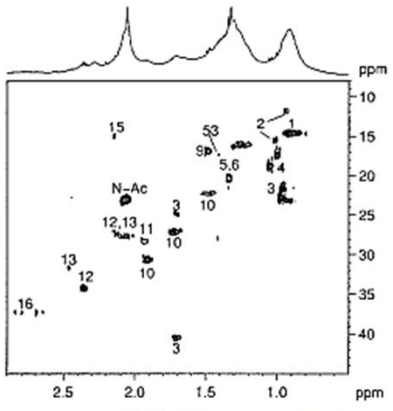


Figure 7 Partial $^{1}H,^{13}C$-HSQC spectrum of healthy gastric mucosa. The identified metabolites are labeled according to Table 1.

Table 1 List of ^{1}H and ^{13}C Chemical Shift (δ, ppm) of Metabolites and Exogenous Molecules Found in HR-MAS Spectra of Healthy Gastric Mucosa[a,b]

Entry	Metabolite	δ ^{1}H	δ ^{13}C	
1	Fatty acids	0.89	14.13–14.17	CH_3
		1.31	29.4–32.2	$(CH_2)_n$
		1.59–1.60	25.2	$CH_2CC{=}O$
		2.02	27.8	$CH_2C{=}$
		2.24	34.2	$CH_2C{=}O$
		2.78	26.2	$=CCH_2C=$
		5.30–5.32	130.2; 128.4	$CH{=}CH$
2	Isoleucine	0.94 (t)	11.7	δ-CH_3
		1.02 (d)	15.5	γ-CH_3
		1.29, 1.48	25.1	γ-CH_2
		1.97		β-CH
		3.69		α-CH
3	Leucine	0.95 (d)	21.5	δ-CH_3
		0.97 (d)	22.8	δ-CH_3
		1.70	24.8	γ-CH
		1.72	40.4	β-CH_2
		3.74		α-CH
4	Valine	0.99 (d)	17.3	γ-CH_3
		1.04 (d)	18.7	γ-CH_3
		2.25		β-CH
		3.61	d	α-CH
5	Threonine	1.33 (d)	20.3	γ-CH_3
		4.26	66.6	β-CH
		3.60	61.2	α-CH
6	Lactate	1.33 (d)	20.3	CH_3
		4.11	69.1	CH
7	Lidocaine chlorohydrate	1.37 (t)	9.2	CH_3
		3.37 (q)	50.6	CH_2
		2.20 (s)	17.8	2,6-CH_3
8	β-Alanine	2.56	32.1	CH_2
		3.18	40.0	CH_2
9	Alanine	1.48 (d)	16.8	β-CH_3
		3.78	51.1	α-CH
10	Lysine	3.02 (t)	39.9	ε-CH_2
		1.71	27.1	δ-CH_2
		1.48	22.6	γ-CH_2

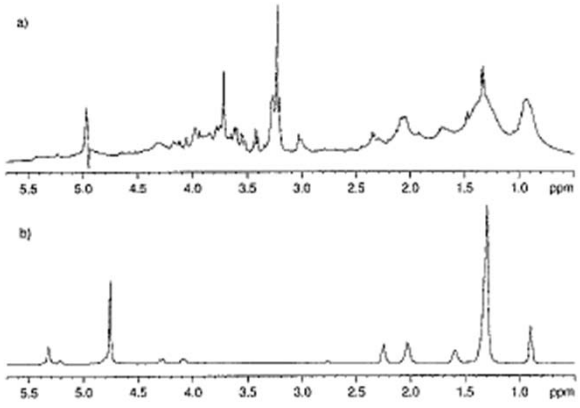


Figure 8 1D ^{1}H NMR spectra of healthy gastric mucosa (a) and adenocarcinoma (b).

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

Jian Zhi Hu

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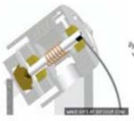
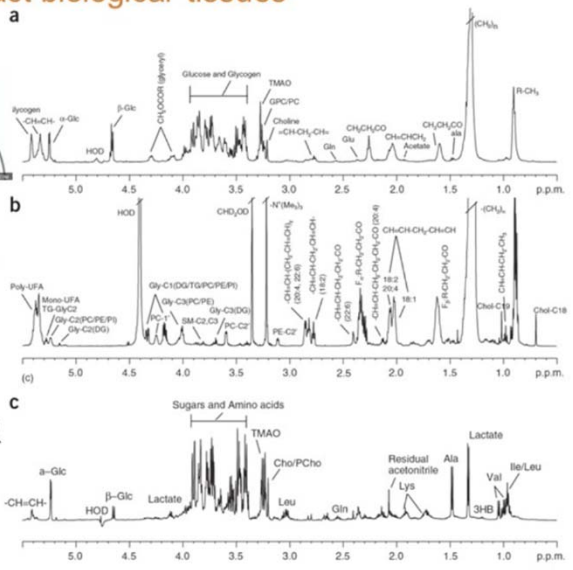
Introduction: ^{1}H HR-MAS NMR is a powerful technique for metabolic profiling on intact biological tissues

(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 **water soluble** liver mouse tissue **extracts**.

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

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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.

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Our Approach

- **Aproach-1:** Slow-MAS using a sample spinning rate of 40-300 Hz. Non-destructive to intact biological tissues.
- **Aproach-2:** Ultra-slow-MAS using a sample spinning rate of 1 to < 6 Hz. Non-destructive to live small biological objects

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Approach-1: Slow-MAS (40-300 Hz)

Objectives

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

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Key Concepts Used

Combining the techniques of

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

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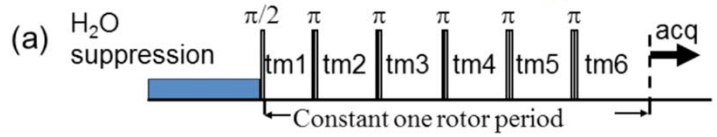
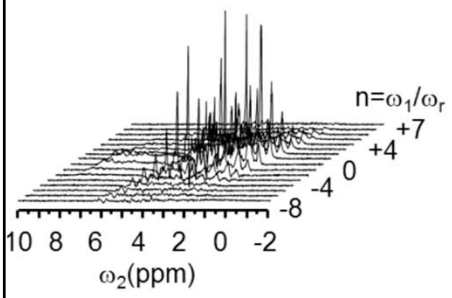
Slow-MAS using ^{1}H-PASS



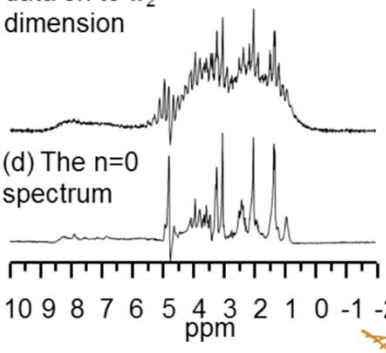
Wind RA, Hu JZ, and Rommereim DN. *Magn. Reson. Med.* 2001; 46: 213-218.

Antzutkin ON, Shekar SC, and Levitt MH. *J. Magn. Reson.* 1995; A115: 7-19.

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



(c) Projection of the data on to ω_2 dimension



16 evolutions steps using 52 minutes and a commercial 7.5 mm MAS probe.

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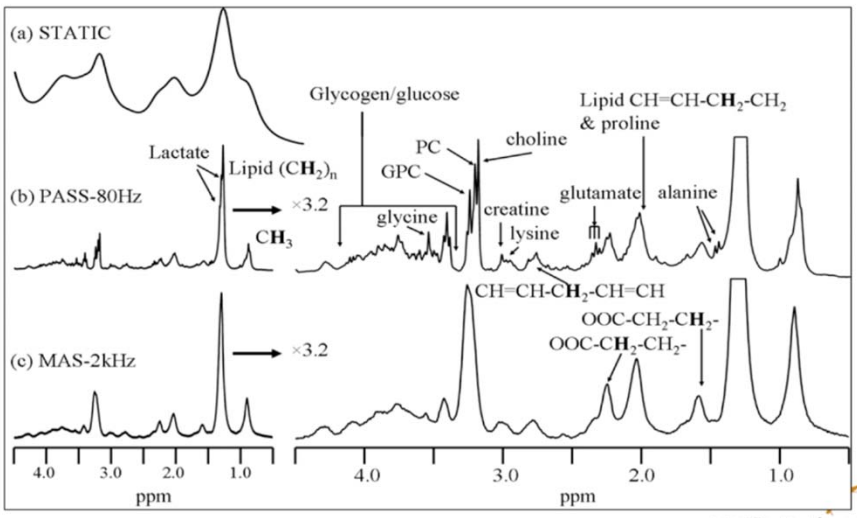
Slow-MAS ^{1}H NMR offers improved spectral resolution than fast-MAS on a same entire intact left mouse lung lobe (~70 mg)



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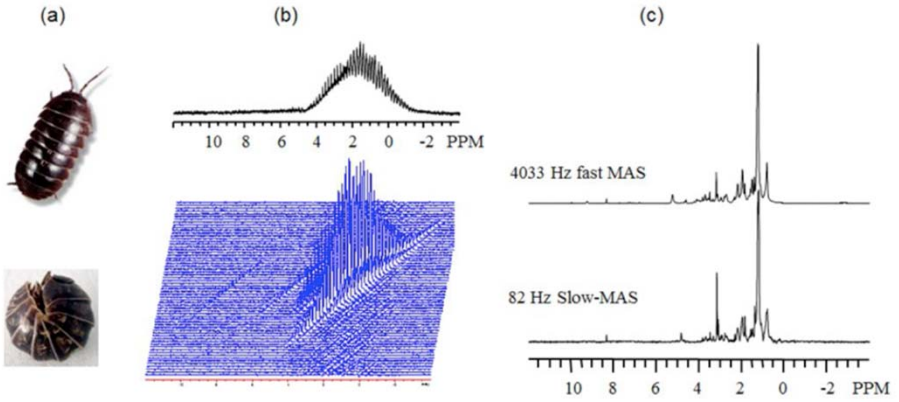
Non, or minimal destructive slow-MAS ^{1}H NMR metabolomics on a live pill bug at a sample spinning rate of 82 Hz compared with fast-MAS of 4033 Hz



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

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Aproach-1: 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.

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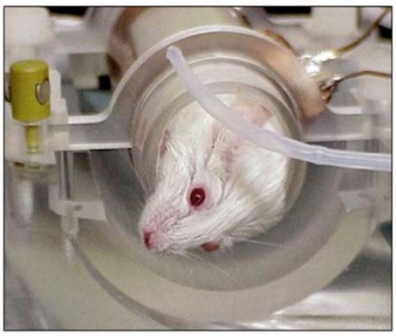
Approach-2: Ultra-Slow MAS (1-6 Hz)



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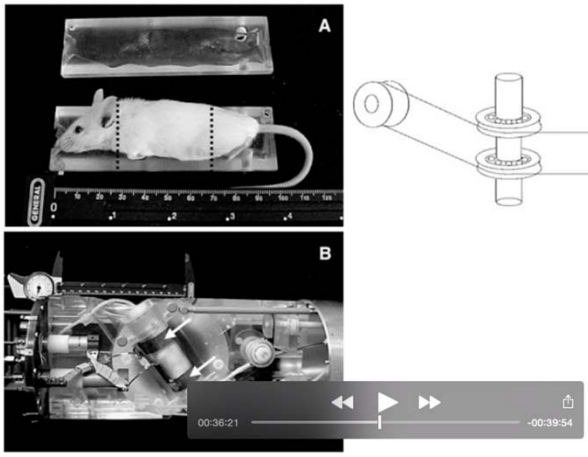
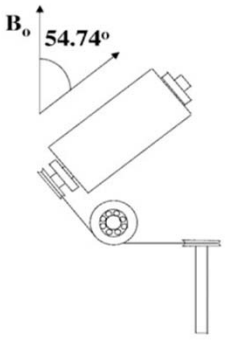
In vivo 85 MHz, 1.5 Hz MAS, ^{1}H PHORMAT on a live mouse*)

Below some pictures of the mouse-MAS probe are shown





*)Wind, and Hu et al, MRM 150,1113 (2003)

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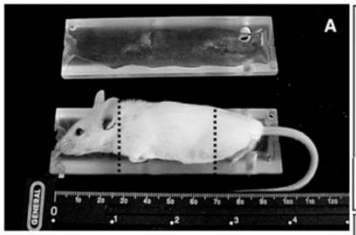
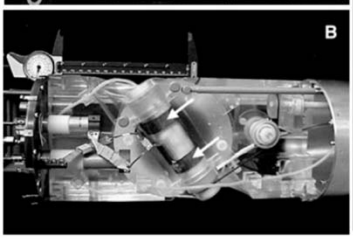
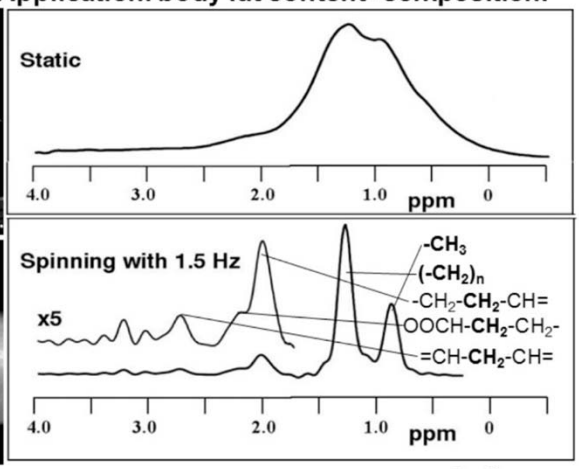
In vivo 85 MHz, 1.5 Hz MAS, ^{1}H PHORMAT on a live mouse*)

Below some pictures of the mouse-MAS probe are shown, and proton NMR spectra are given obtained on a stationary mouse and a spinning live (anesthetized) female BALBc mouse in a 2 T magnet. Even in this relatively low field a significant spectral resolution enhancement is obtained. Application: body fat content+composition.







Proudly Operated by Battelle *Since 1965*

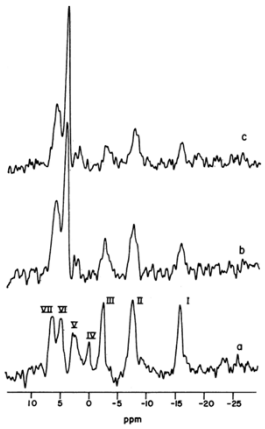


FIG. 1. ^{31}P NMR (80.96 MHz) of a subcutaneously implanted mammary 16/C adenocarcinoma in an anesthesized C3H/He mouse at various times after implantation. Spectra: a, 9 days, 2.0 g, pH = 7.4; b, 10 days, 3.5 g, pH = 7.0; c, 11 days, 5.0 g, pH = 6.8. Peak assignments are: I, ATP_α; II, ATP_γ + ADP_γ + NAD; III, ATP_β + ADP_β; IV, PCr; V, unidentified; VI, inorganic phosphate; VII, sugar phosphate.

Proc Natl Acad Sci U S A. 1983, 80(2):334-8. Work done at UAB without MAS.

Alternative Aproach-2: Rotate the B_o field or both the subject and the field

The same spectral resolution enhancement can also be obtained by rotating the magnetic field instead of the subject. This might make the slow-MAS approach even amenable for humans.