History and Applications of the Nano Probe

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Agilent Technologies

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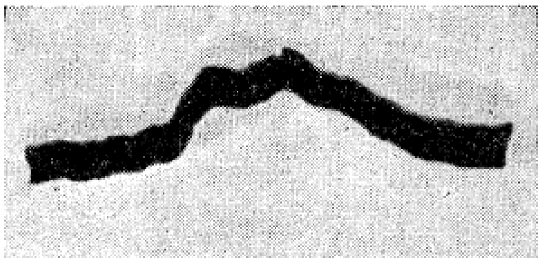
Reducing the linewidth of the resonances

liquid state
- shimming
- spinning the sample along Z
- heteronuclear decoupling

solid state
- MAS
- different types of decoupling

Reducing the linewidth of the resonances

liquid state



spin 20 Hz

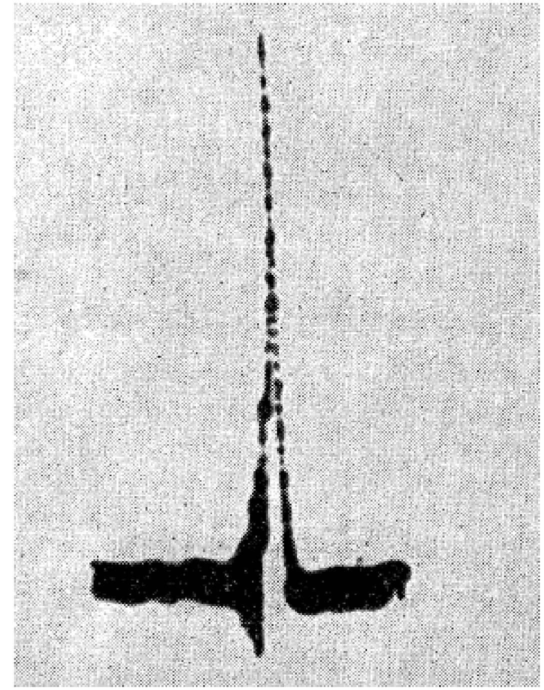
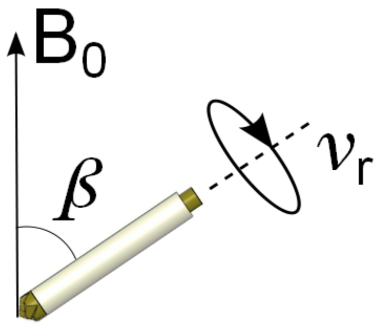


FIG. 1. Line narrowing by rotation. The top figure is a photograph of a proton resonance from distilled water displayed on the cathode-ray screen. The beam was swept from right to left in about 30 seconds. A measure of the external field inhomogeneity is given by the width at half-maximum and was found to be 1.7×10^{-3} gauss. The bottom figure was obtained by rotating the spherical sample at a rate of about 25 times per second, but under otherwise identical conditions. The variation of the field between the two ends of this trace was determined by calibration to be 3.5×10^{-3} gauss, yielding for the width at half-maximum about 10^{-4} gauss. This reduction in half-width is accompanied by an increase of the height by a factor of about 7.

Anderson and Arnorld, Chem Rev 94, 497 (1954)

Reducing the linewidth of the resonances

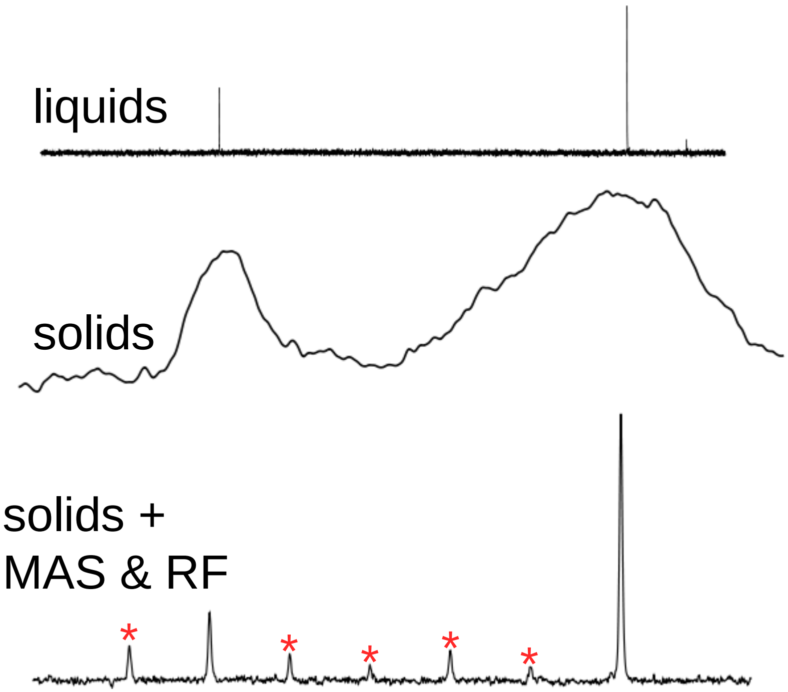
solid state



'Fast' spinning reduces most of anisotropic interactions by factors of

$$\left(3\cos^2\beta - 1\right)/2$$

Anisotropic interactions should completely disappear at β=54.7° (magic angle).



NMR spectra of hexamethylbenzene

Liquid or solid?

a continuum....

First application

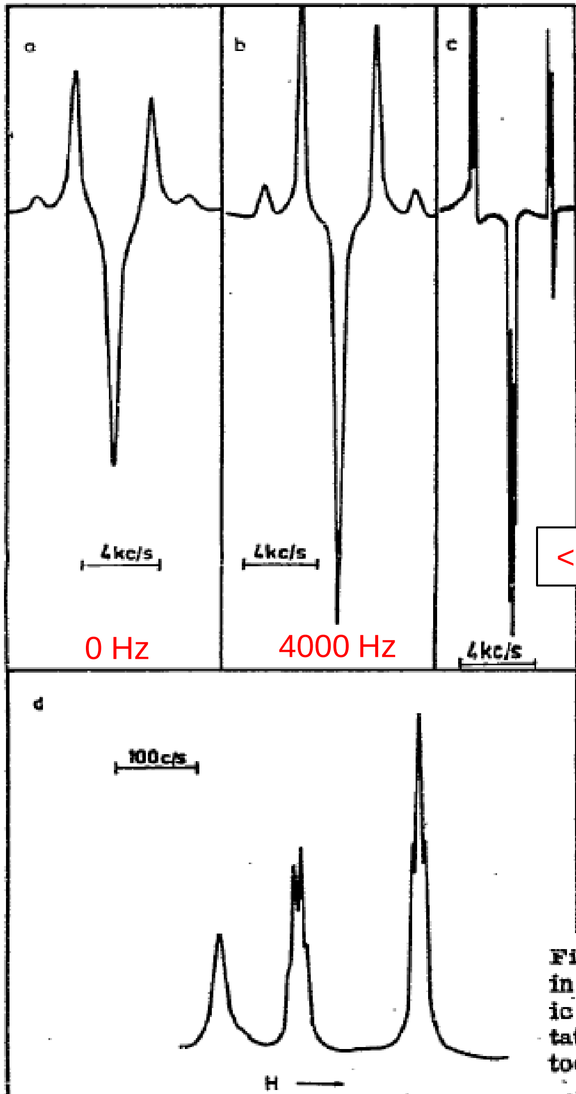


Fig. 2. High resolution NMR spectra of ethanol soaked in diatomaceous earth (2.5 g EtOH/1 g of solid). a) Static sample; b) rotated with ν_r = 4 kHz, θ = 90^o; c, d) rotated with ν_r = 4 kHz, θ = 54.7^o. Spectra a, b, c: saw-tooth sweep with 2 kHz modulation; d: slow sweep with field homogeneity controls adjusted to optimum.

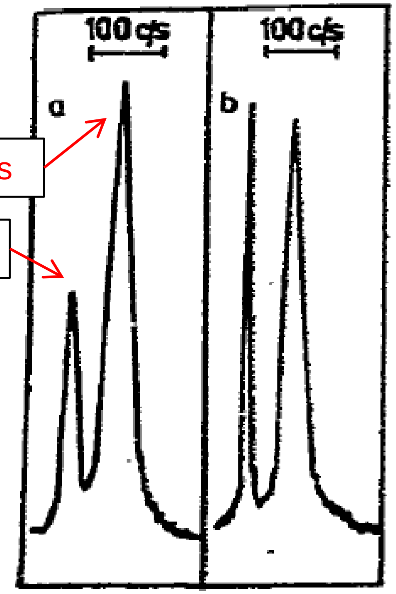
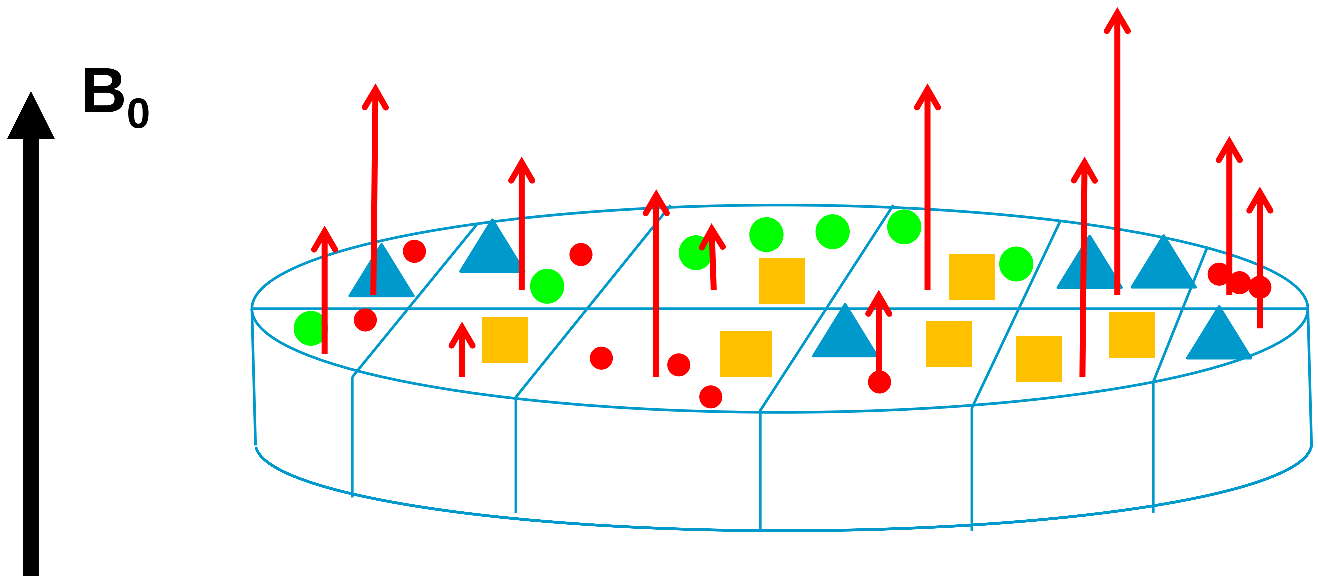


Fig. 3. High resolution spectra of a styrene-divinylbenzene cation exchanger in hydrogen form swollen in water rotated with ν_r = 4 kHz. a) θ = 90^o; b) θ = 54.7^o.

Doskočilova and Schneider, Chem Phys Lett 6, 381 (1970)

When the sample is heterogeneous

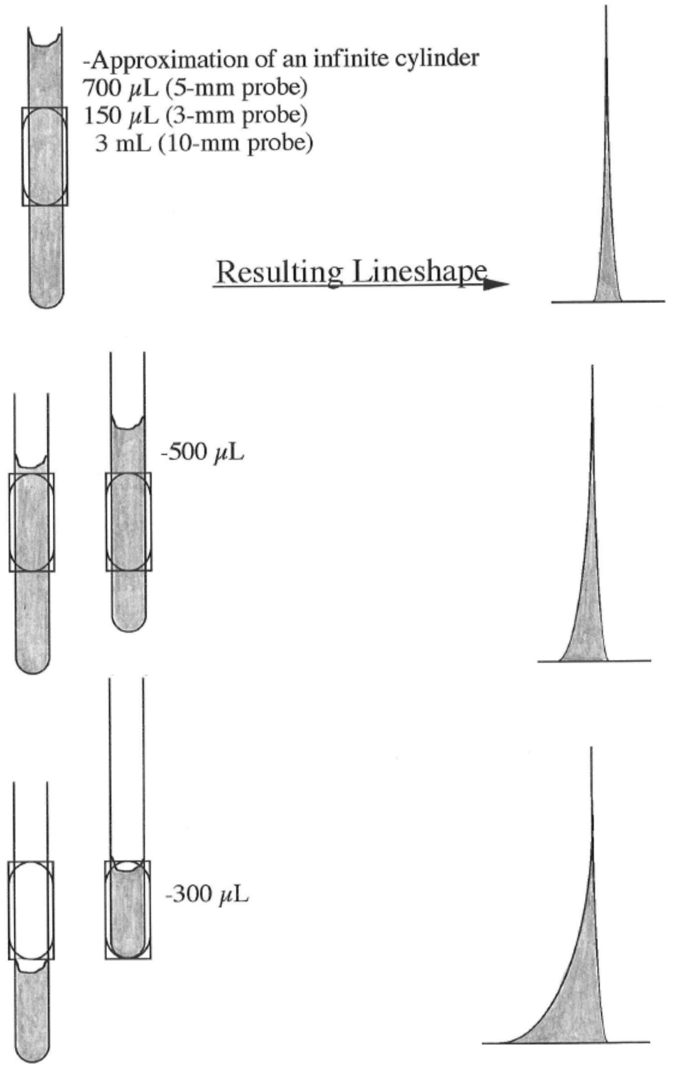


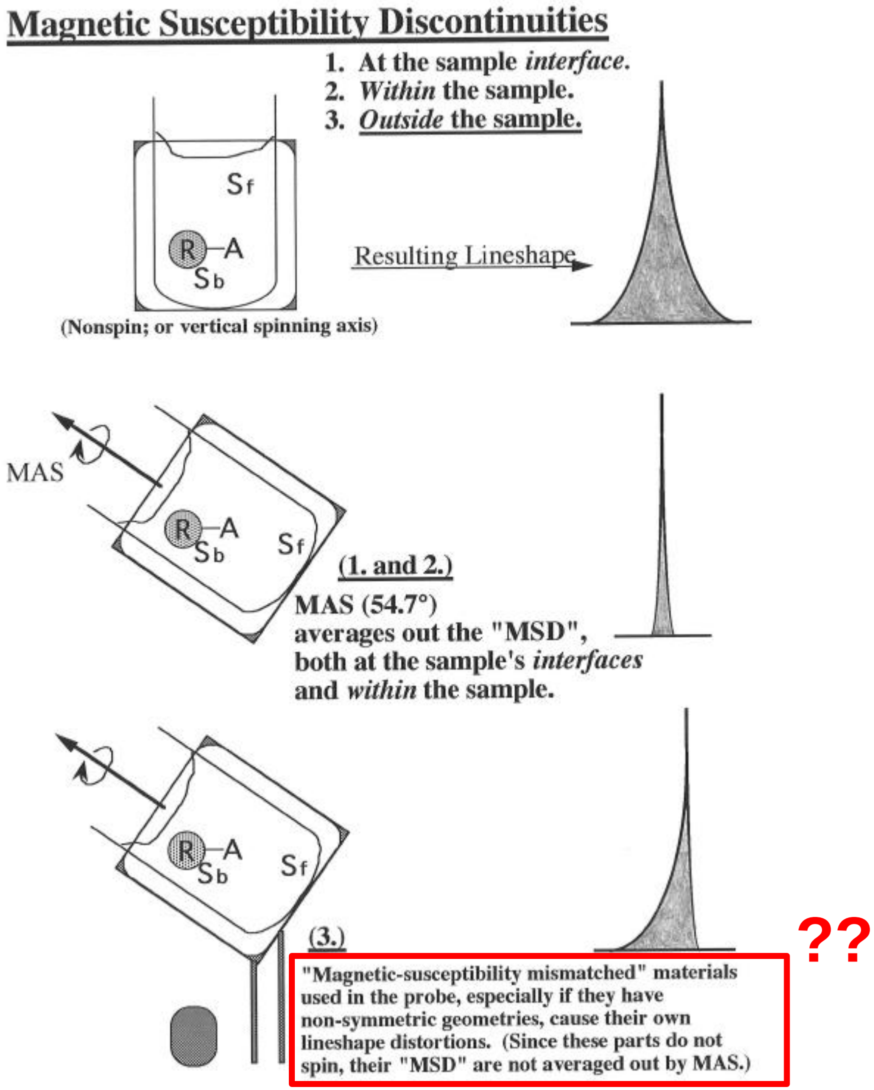
Each region has a different composition and...

...a different susceptibility
(distribution of resonance frequencies = line broadening)

Magnetic susceptibility: $\mathbf{M} = \chi\ \mathbf{H}$
M: sample magnetization; **H**: magnetic field strength

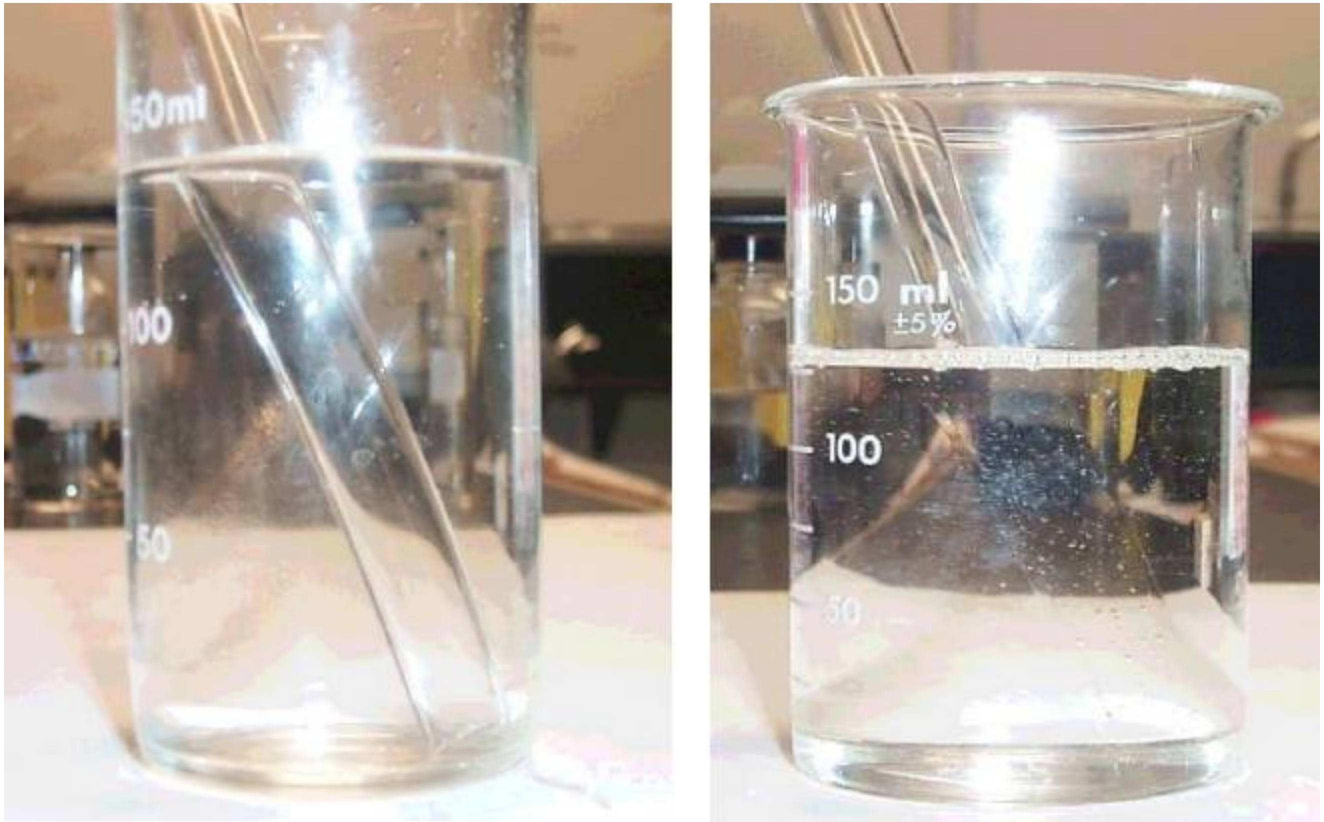
Basics





P. Keifer, "NMR spectroscopy for molecular diversity"

Optical equivalent of magnetic susceptibility match



From Thomas M. Barbara, Oregon University, http://www.enc-conference.org/Portals/0/Susceptibility%20in%20NMR-final.pdf

Previous Art

Susceptibility, lineshape, and shimming in high-resolution NMR

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[b] GE Corporate Research and Development, Schenectady, New York 12309, USA

Abstract

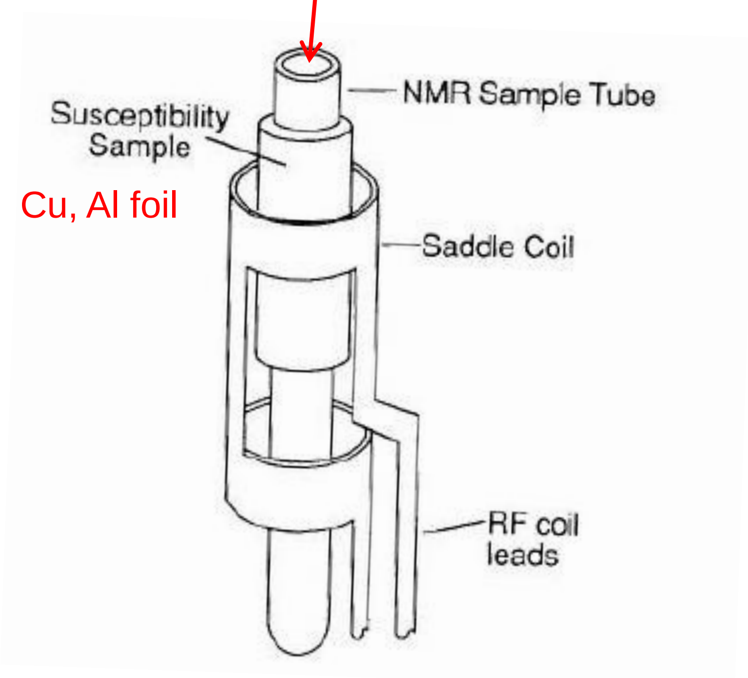
The field inhomogeneity caused by an object of known susceptibility and shape is calculated. The field perturbation is derived using the method of equivalent magnetic charges. Lineshapes are calculated by summing Lorentzians over the sample volume and are verified experimentally by observing changes caused by introducing material into the vicinity of a previously shimmed NMR sample. Such lineshapes can be used for the determination of susceptibility of materials.

To whom correspondence should be addressed at present address: Varian Associates, Palo Alto, California 94304.

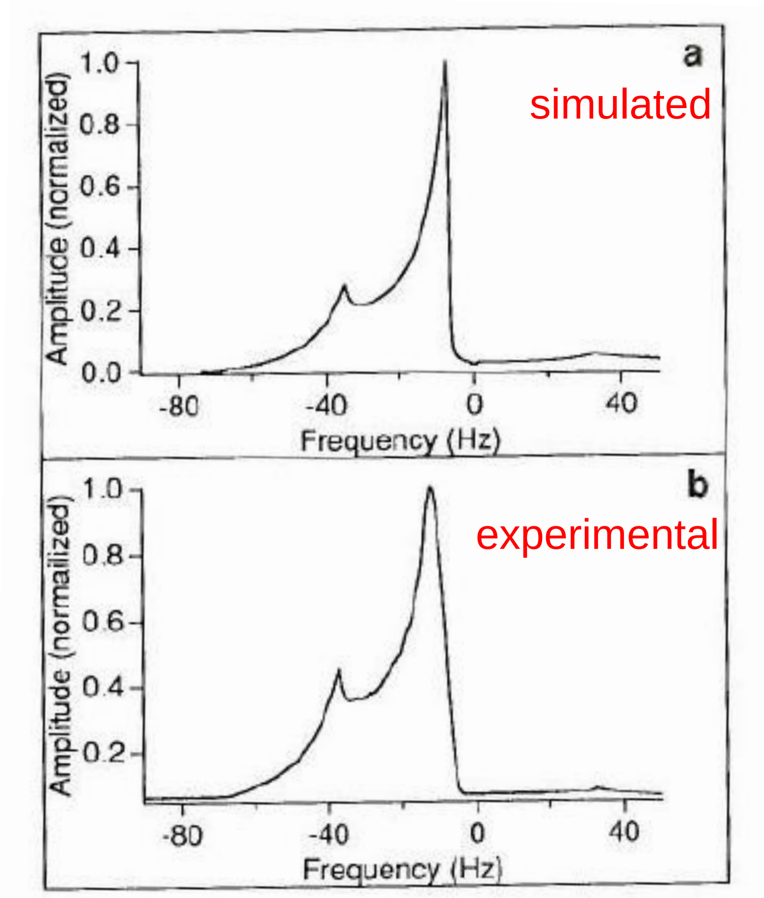
JMR, **1992**, *92*, 229-242

Previous Art

$CHCl_3$



Cu, Al foil



simulated

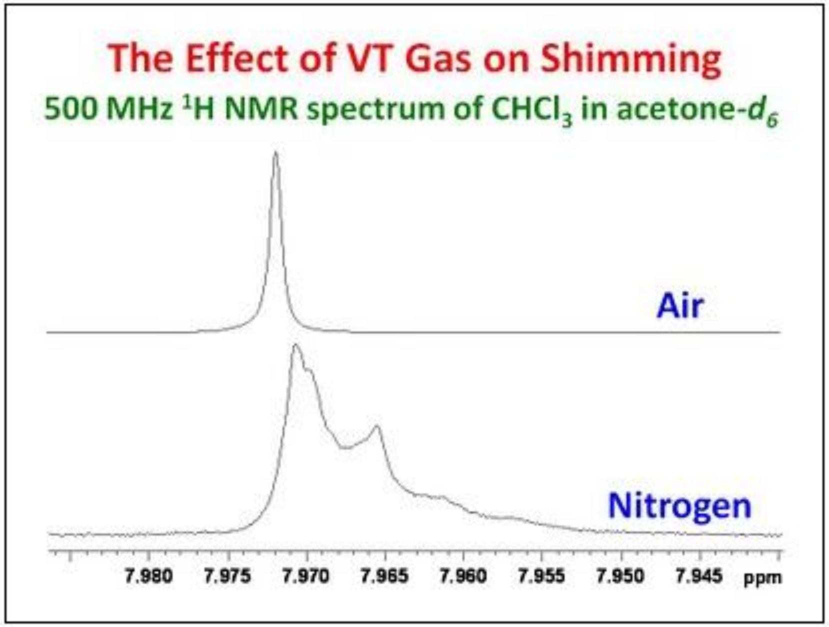
experimental

In both cases shimming by 20 spherical harmonics shims was either used or calculated

Are there everyday effects of all this?

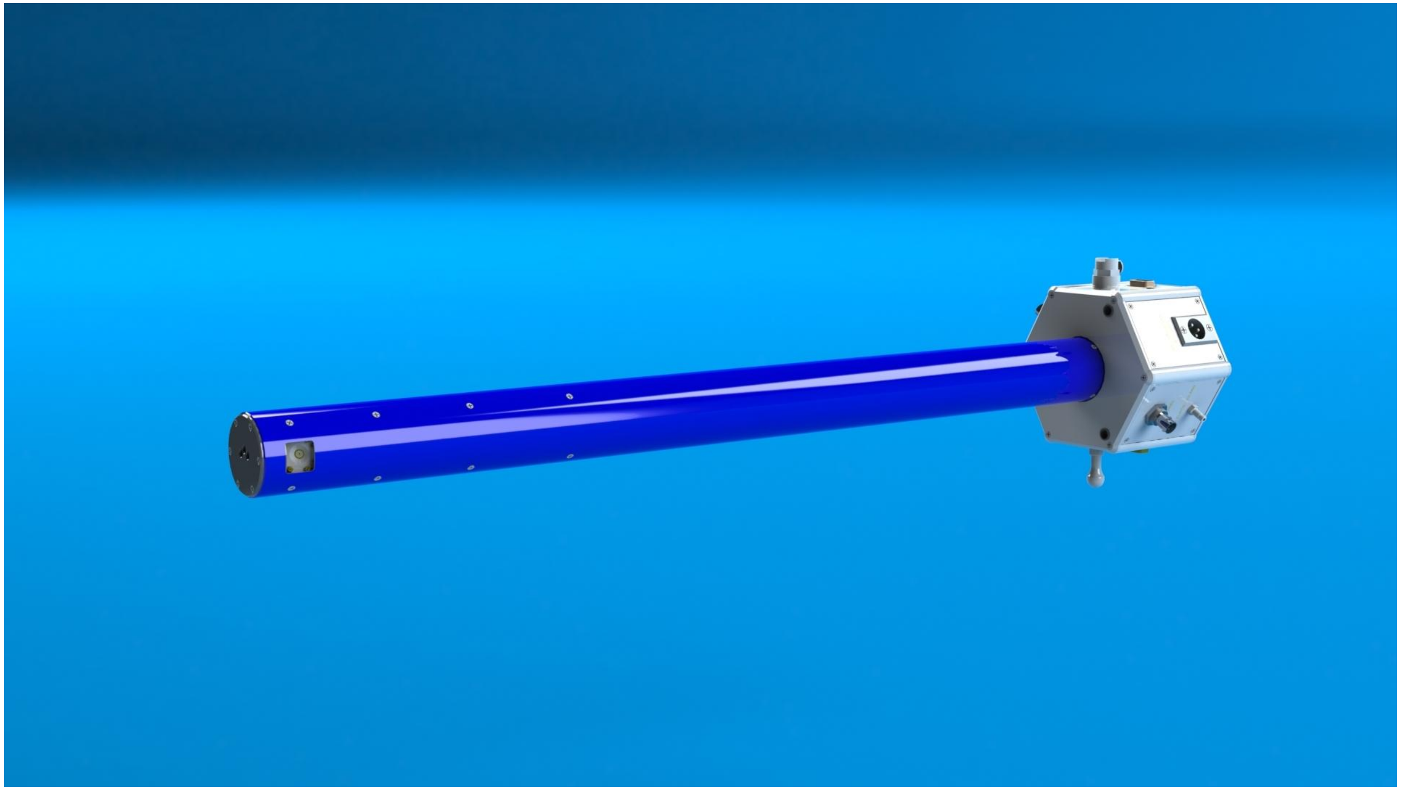
Fuks et al. JMR, **1992**, *92*, 229-242

An easy experiment…



From Glenn Facey NMR Blog, http://u-of-o-nmr-facility.blogspot.it/2010_01_01_archive.html

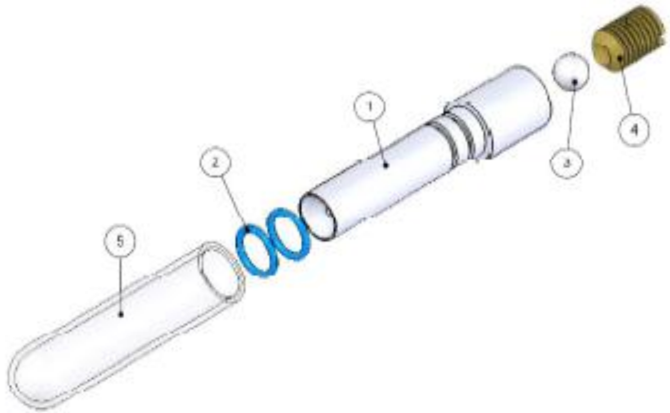
Fast Nanoprobe

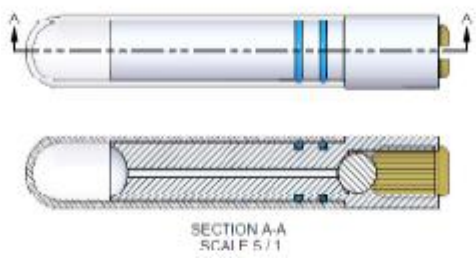


Not solid, nor liquid...

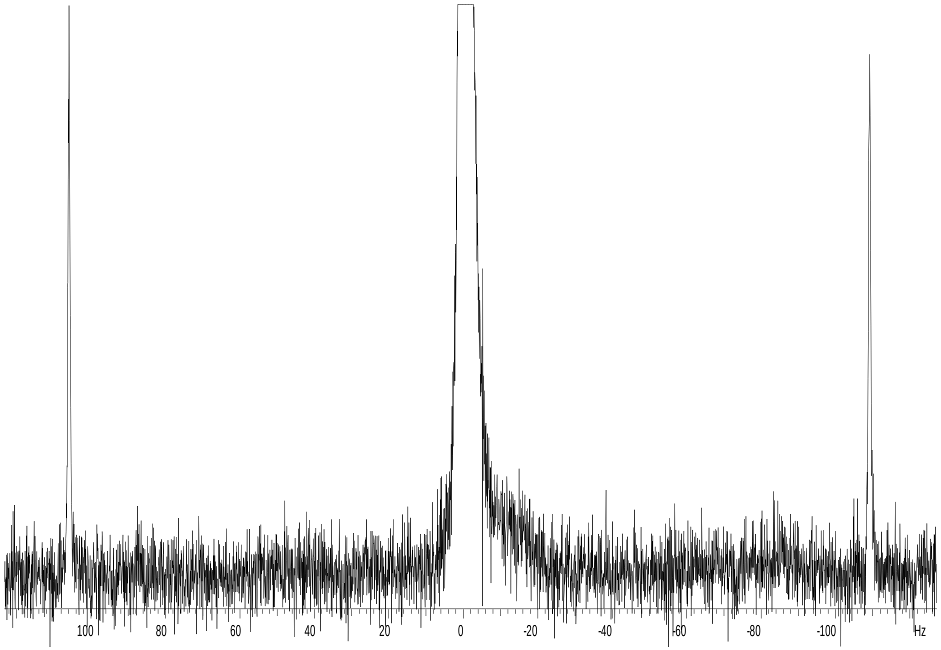
Fast Nanoprobe

- it is capable of higher spin rates than a Nanoprobe (up to ~15 KHz; as opposed to up to ~2.5 KHz), which is useful for more "solid-like" samples
- it uses the solids spin-rate controller (both bearing and drive)
- it is an ID-style Probe (^{15}N – ^{31}P on the heteronuclear channel)
- gradient up to 50 G/cm
- 16, 25, 43 μL sample volume
- available from 600 to 800 MHz
- the original-design Nanoprobes (gH{X} and X{H}) are offered at 400-600 MHz (gradient up to 130 G/cm)





Fast Nanoprobe



Linewidth of 0.7/6.5/19 Hz

S/N 142@600 MHz

43 µl ETB sample

History of the Nanoprobe (1)

High-Resolution [1]H NMR in Solid-Phase Organic Synthesis

William L. Fitch,* George Detre, and Christopher P. Holmes

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James N. Shoolery

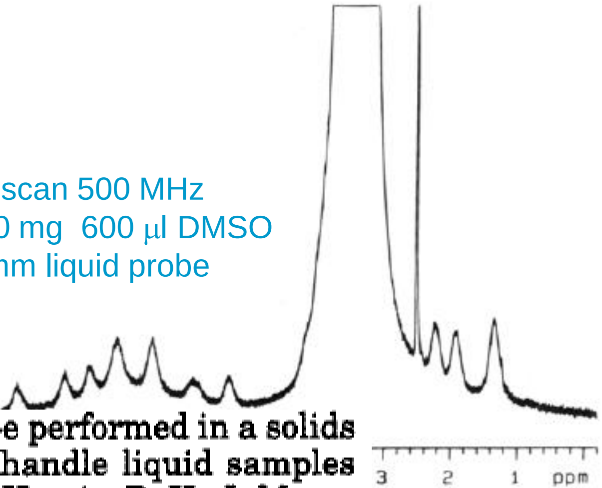
NMR Consultant, 2301 Bowdoin Street, Palo Alto, California 94306

Paul A. Keifer

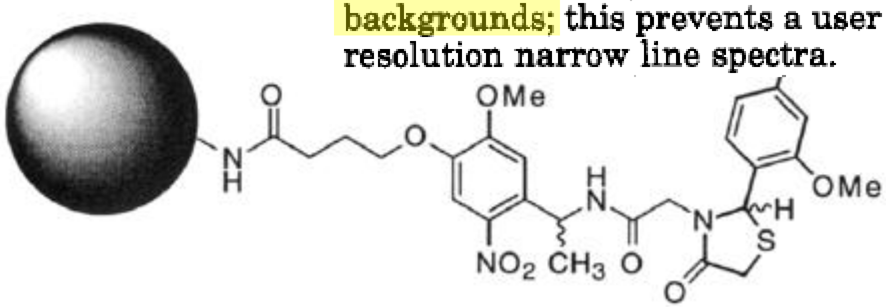
Varian NMR Instruments, 3120 Hansen Way, Palo Alto, California 94304-1030

Received October 19, 1994®

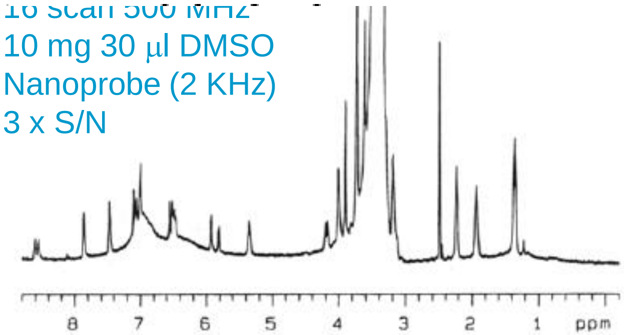
16 scan 500 MHz
100 mg 600 µl DMSO
5 mm liquid probe



(13) In principle this experiment could also be performed in a solids NMR MAS probe which had been adapted to handle liquid samples (see: Merwin, L. H.; Sabald, A.; Espidel, J. E.; Harris, R. K. *J. Magn. Reson.* **1989**, *84*, 367 and references cited therein.) However, it should be noted that solids probes are never susceptibility matched in their coil design. As such, they produce spectra in which the resonances have wider half-widths, much wider line shapes, and significantly larger backgrounds; this prevents a user from generating good quality high-resolution narrow line spectra.



16 scan 500 MHz
10 mg 30 µl DMSO
Nanoprobe (2 KHz)
3 x S/N



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History of the Nanoprobe (2)

A Comparison of NMR Spectra Obtained for Solid-Phase-Synthesis Resins Using Conventional High-Resolution, Magic-Angle-Spinning, and High-Resolution Magic-Angle-Spinning Probes*

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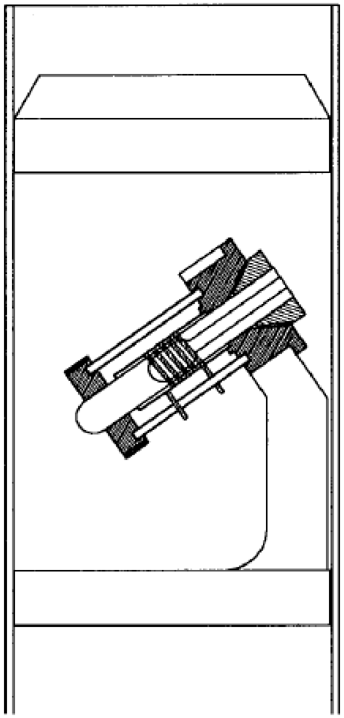
†*Varian NMR Instruments, 3120 Hansen Way, Palo Alto, California 94304-1030;* §*Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey 08543-4000; and* ¶*NMR Consultant, 2301 Bowdoin Street, Palo Alto, California 94306*

JMR A, **1996**, *119*, 65-75

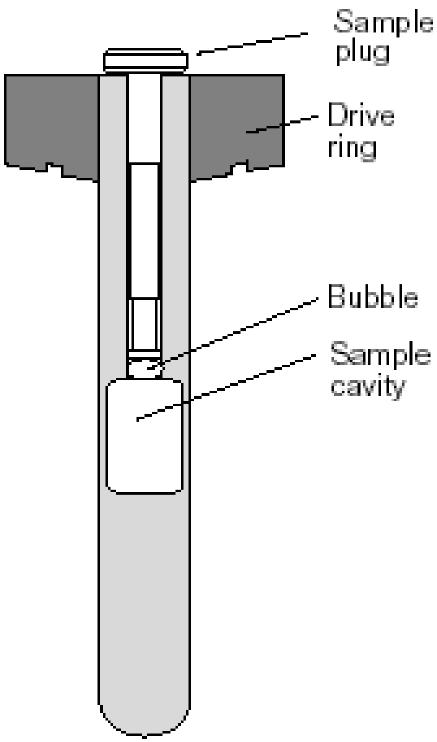


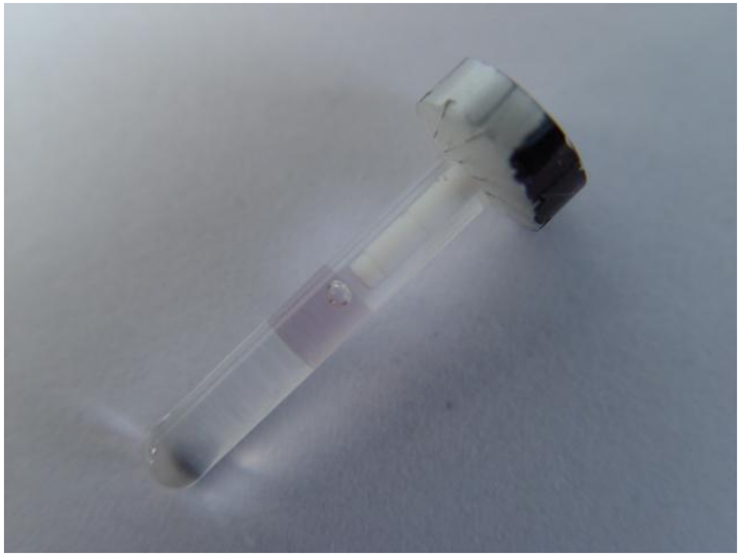
Resolution depends on

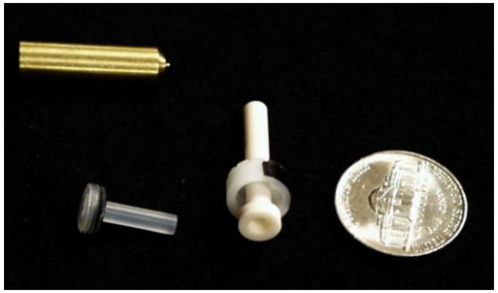
1) molecular mobility
2) magnetic-susceptibility interface within the sample
3) magnetic-susceptibility interface related to probe construction
4) frequency (or gyromagnetic ratio)

History of the Nanoprobe (2)

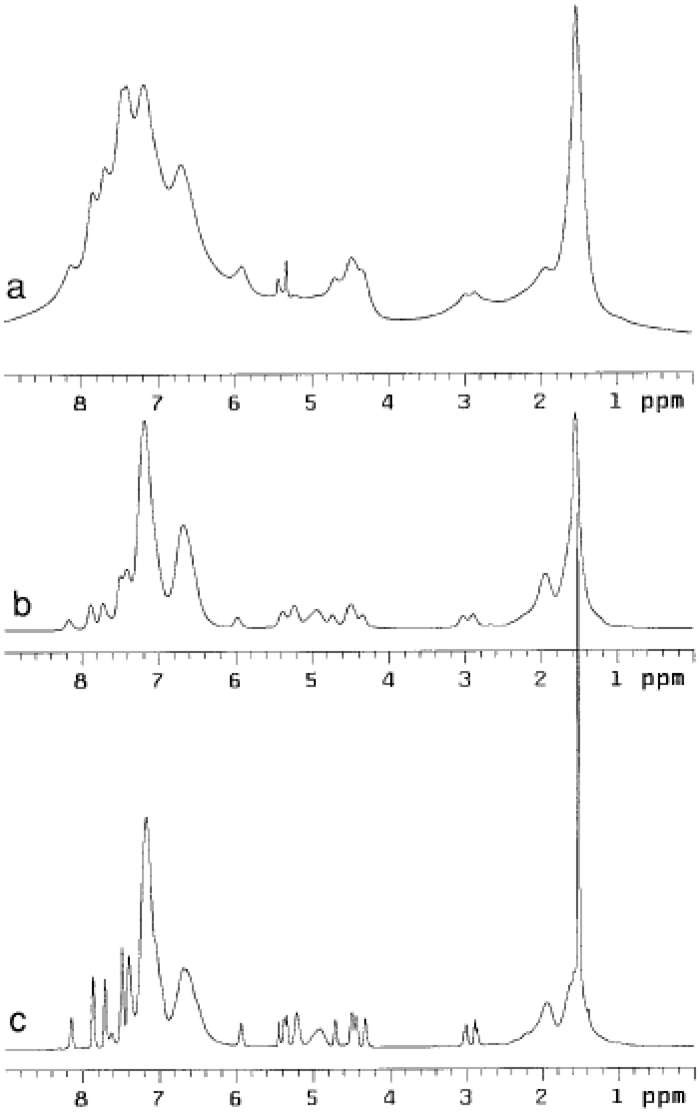
Magic angle spinning
(abt 2 KHz)







History of the Nanoprobe (probes comparison – ^{1}H spectra)



Liquid probe
100-300 Hz lw
Solvent (CD_2Cl_2) 15 Hz

Spec: 0.4 Hz lsh

Normal CP-MAS probe (3.8 KHz)
Narrower resonances
Solvent (CD_2Cl_2) 65 Hz

Spec: abt 30 Hz ^{1}H

Nanoprobe (2 KHz)
11 Hz lw
Solvent (CD_2Cl_2) 3.5 Hz

Spec: 0.4 Hz lsh

BEST!

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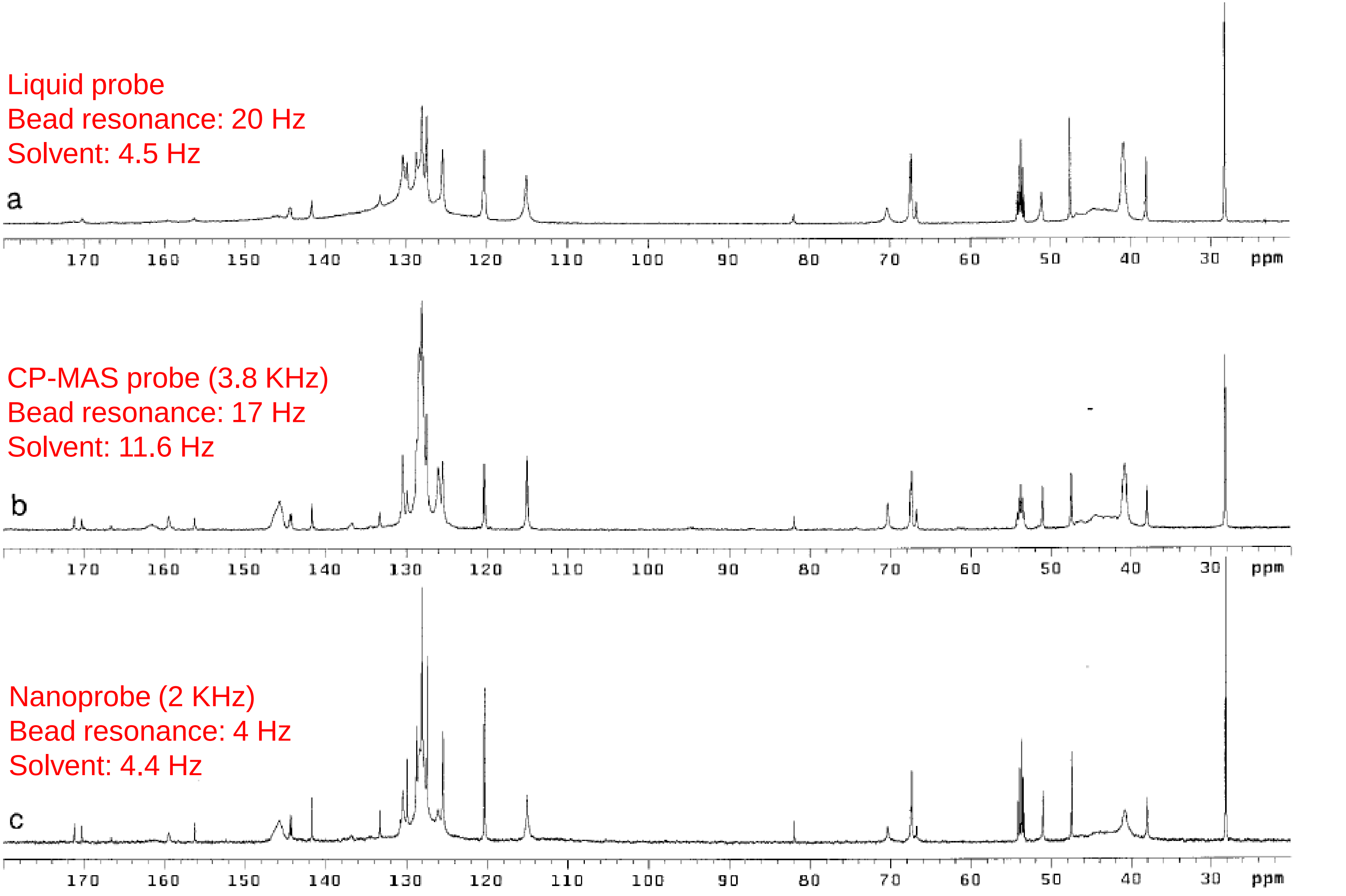
History of the Nanoprobe (probes comparison – ^{13}C spectra)



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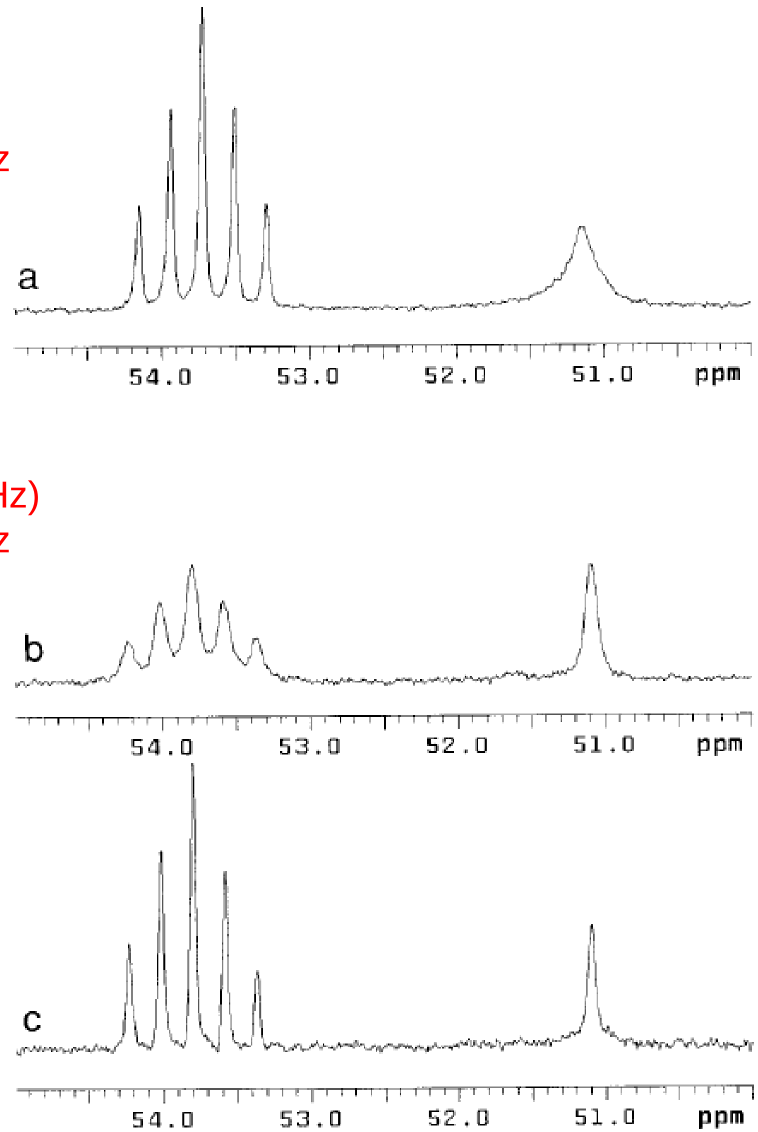
History of the Nanoprobe (probes comparison – ^{13}C spectra)

SENSITIVITY?



Liquid probe
Bead resonance: 20 Hz
Solvent: 4.5 Hz

More material (15 x the amount of the Nanoprobe) but broader: S/N only 20% more than Nanoprobe

CP-MAS probe (3.8 KHz)
Bead resonance: 17 Hz
Solvent: 11.6 Hz

Good trade-off: larger volume and narrower than liquid probe

Nanoprobe (2 KHz)
Bead resonance: 4 Hz
Solvent: 4.4 Hz

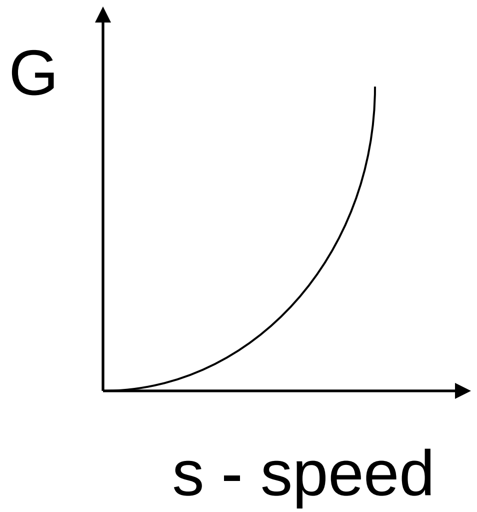
Best for sample-limited materials. S/N abt 50% of CP-MAS probe with 32% as much sample

Some **Nanoprobe** applications

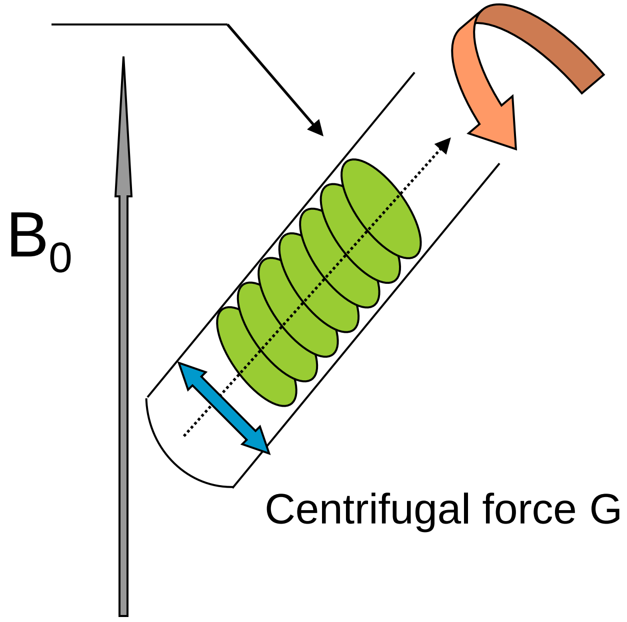
- DOSY
- Reaction Control
- Food Science
- Agricultural Science
- Cultural Heritage
- Medicine

Magic angle gradient and spinning

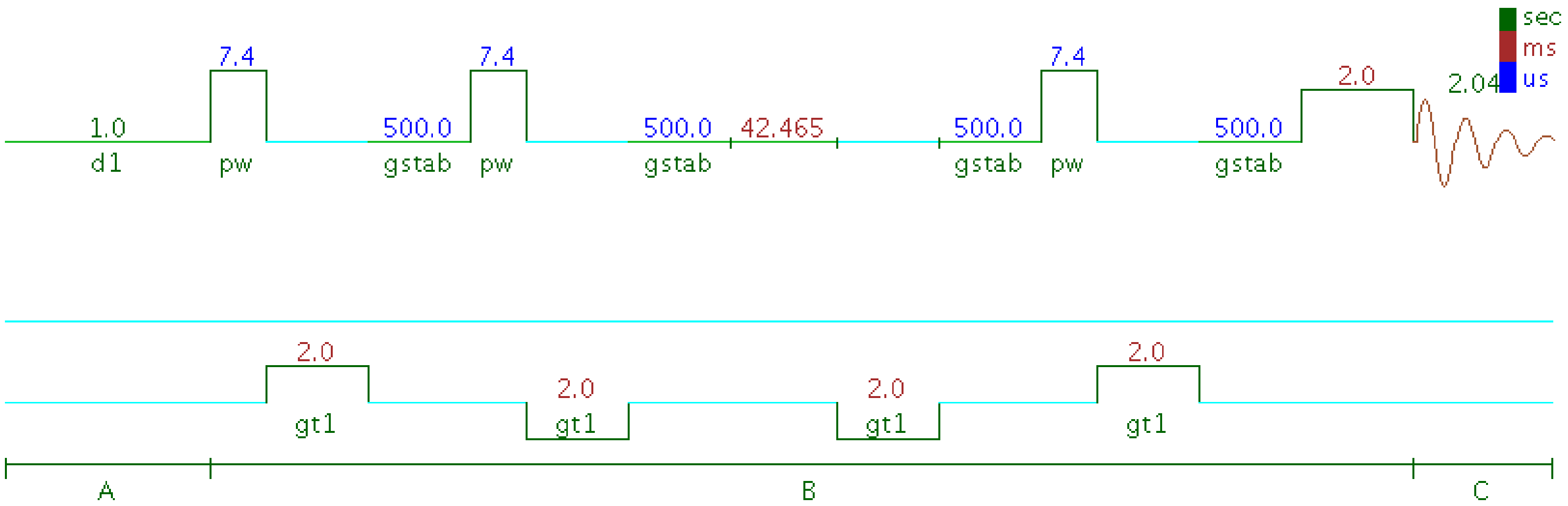
Centrifugal force $G \sim s^2$

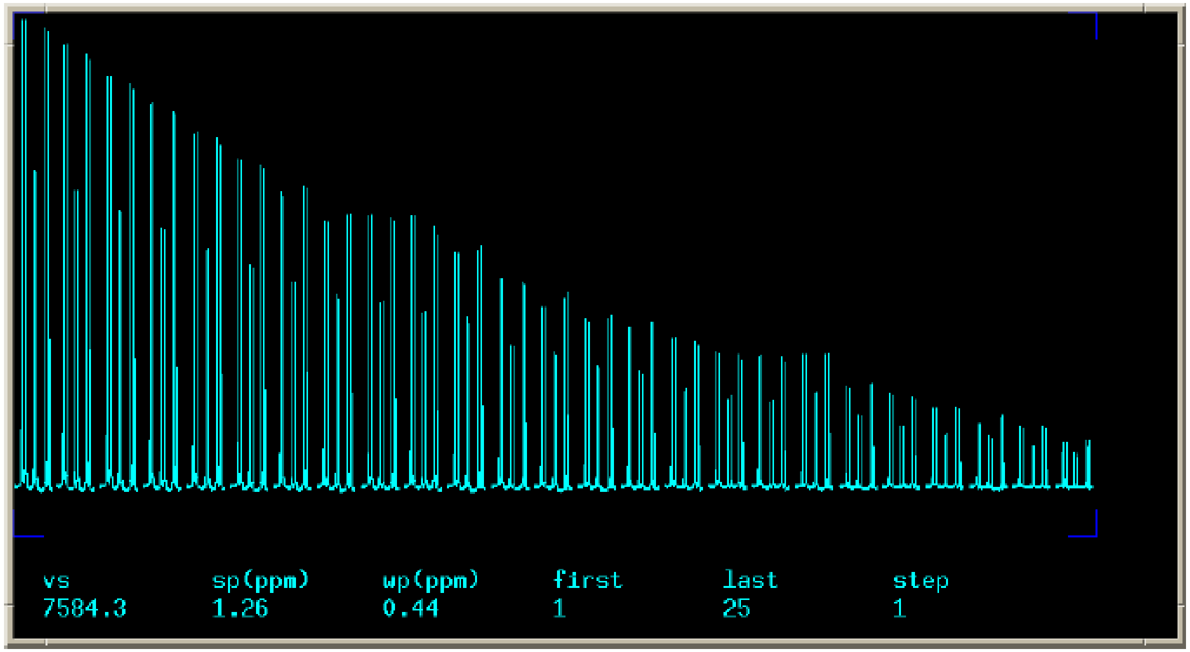


Gradient isoplanes



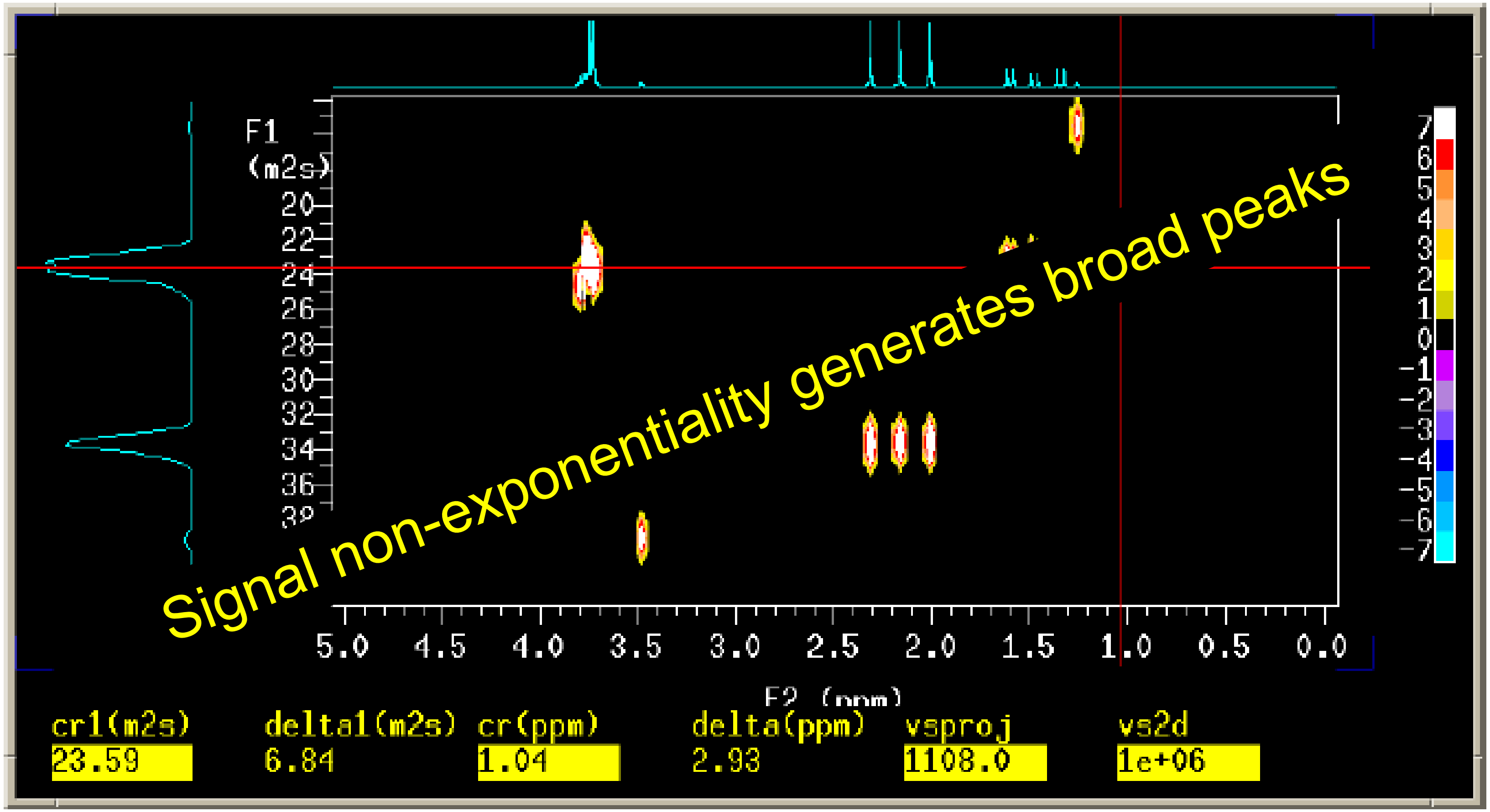
DOSY (beware of rotation..)





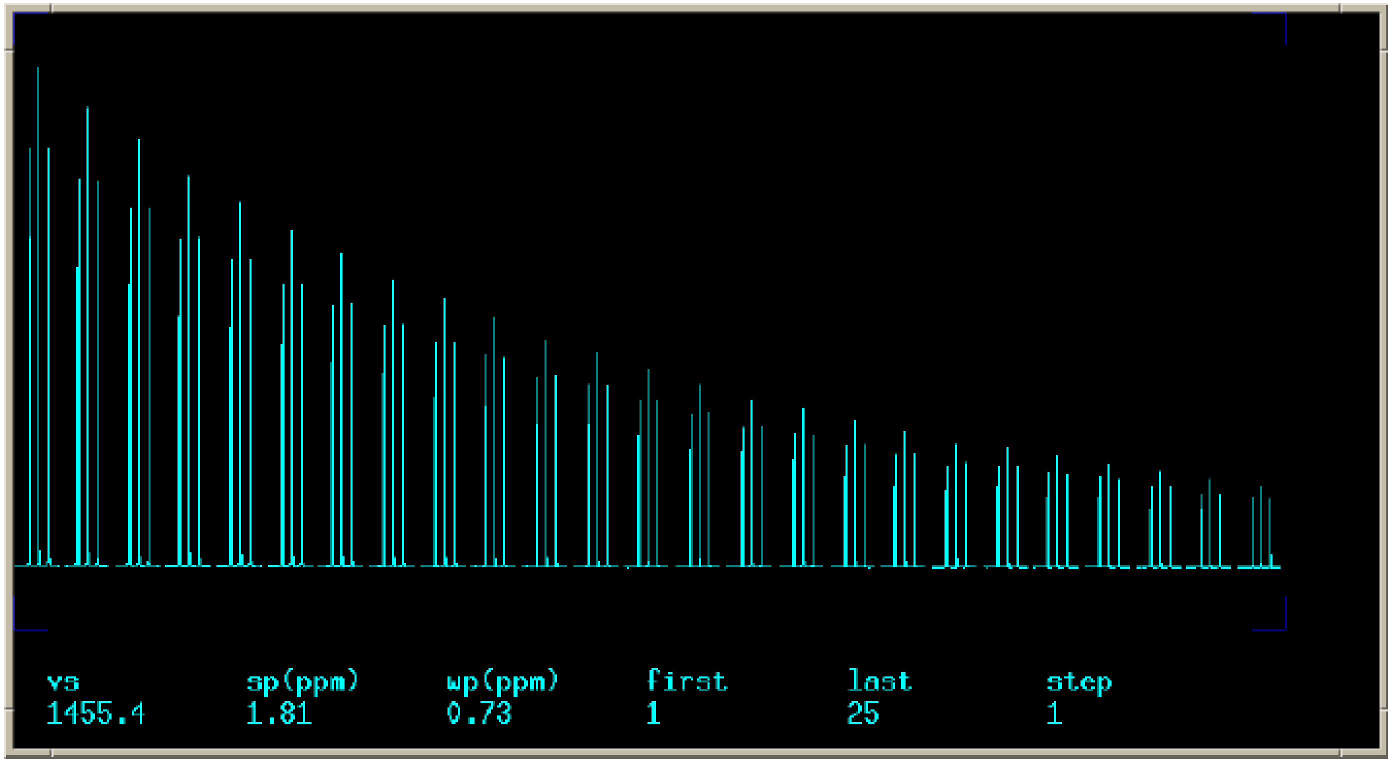
DgcsteSL sequence, 1-22 G/cm, spinning speed 2300 Hz.

DOSY (beware of rotation...)



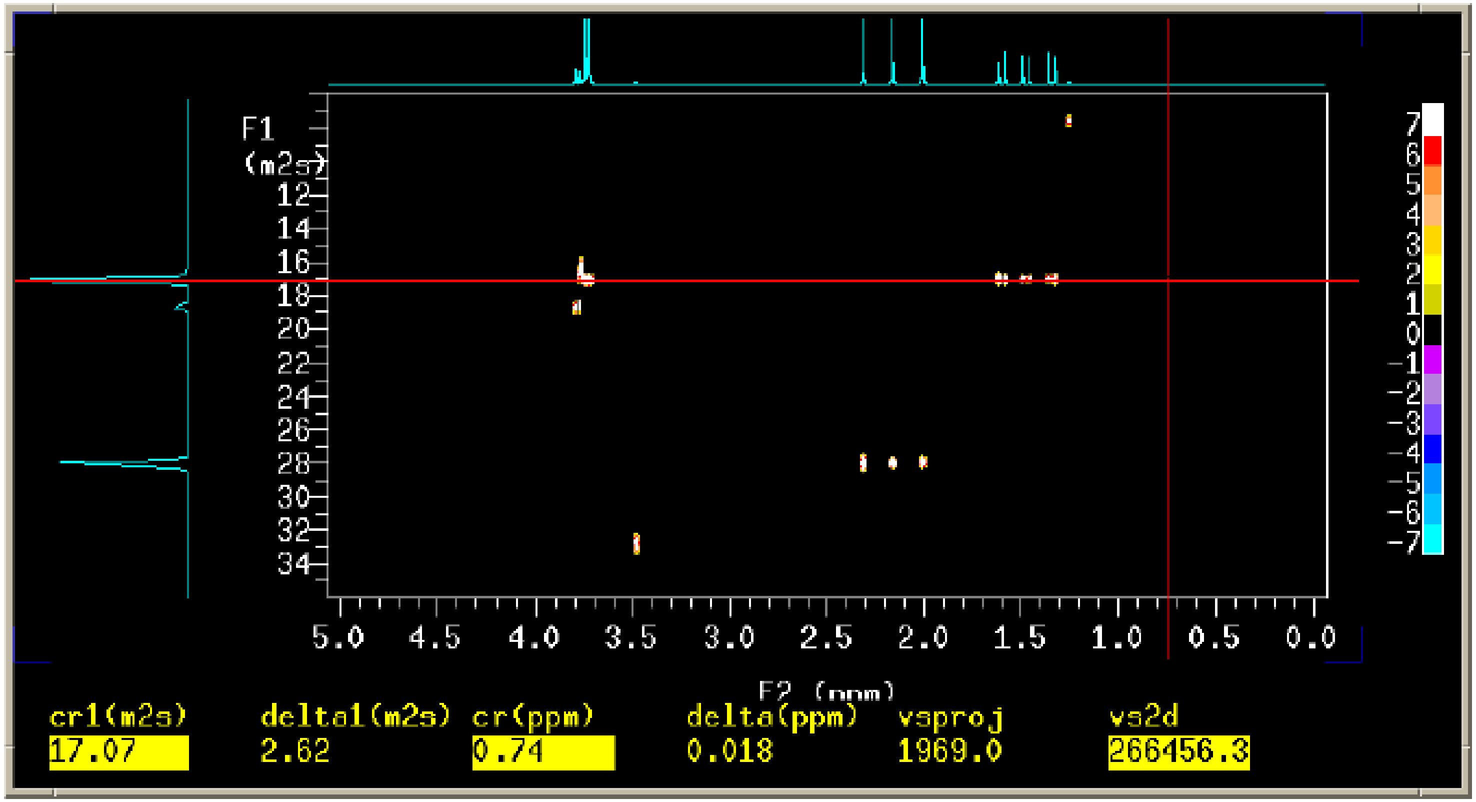
DgcsteSL sequence, 1-22 G/cm, spinning speed 2300 Hz.

DOSY (synchronized)



DgcsteSL sequence, 1-22 G/cm, spinning speed 2300 Hz with **rotor synchronization**

DOSY (synchronized)



DgcsteSL sequence, 1-22 G/cm, spinning speed 2300 Hz with **rotor synchronization**

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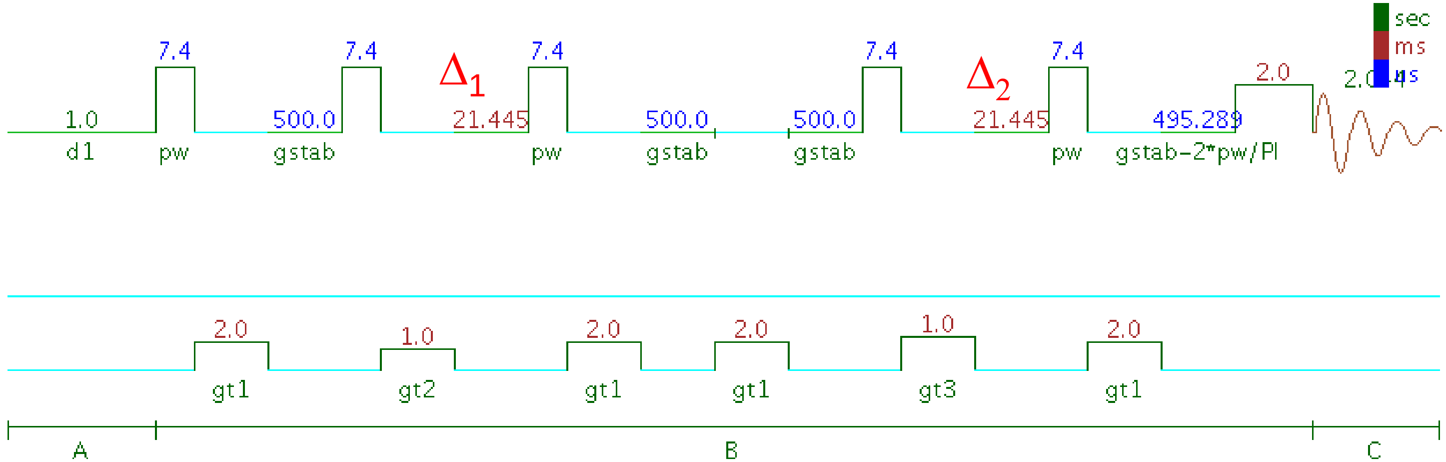
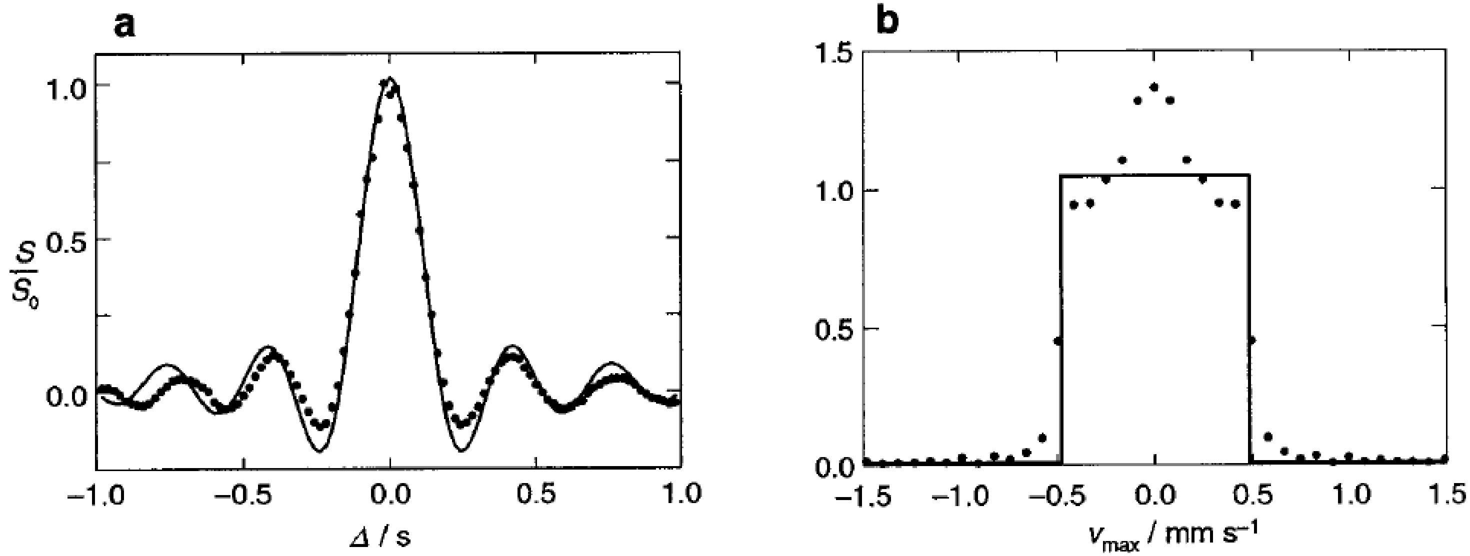
DOSY (does convection take place?)

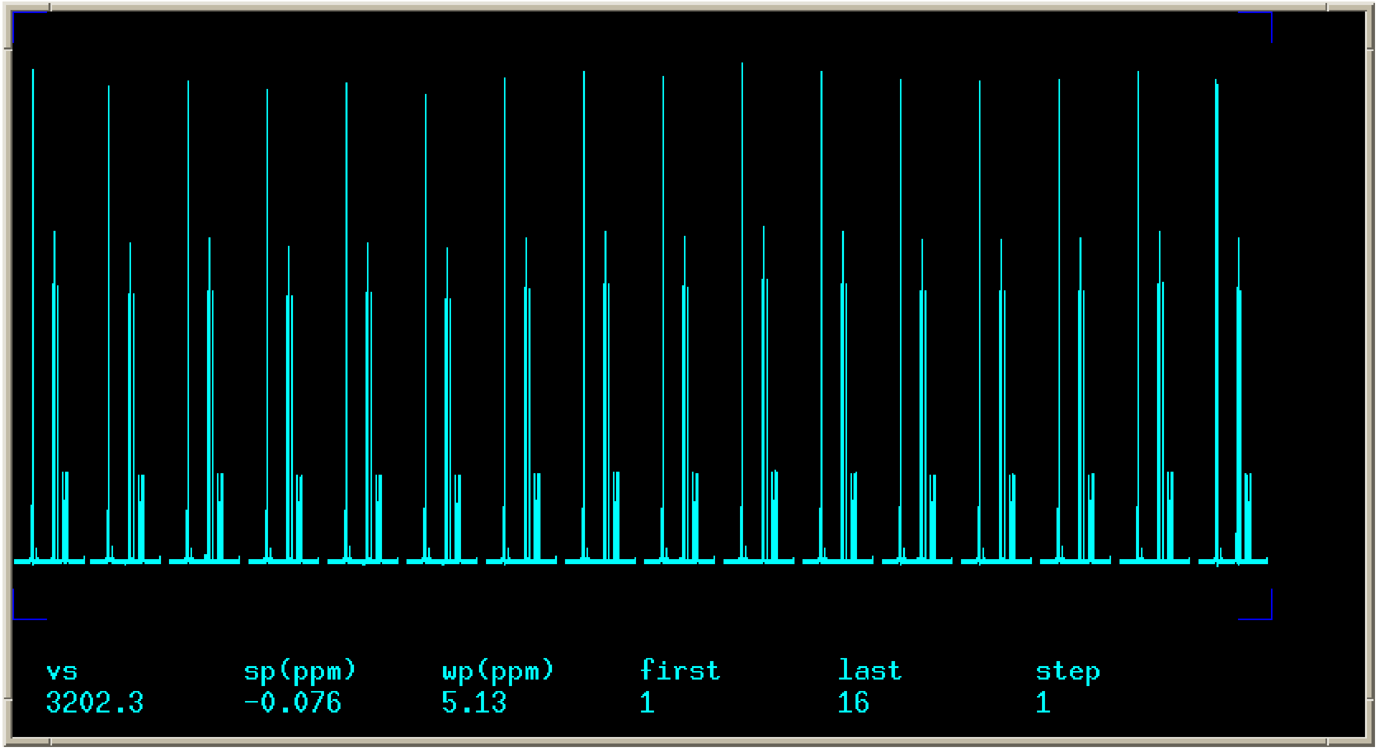




$\Delta = \Delta_1 - \Delta_2$

from Loening and Keeler, JMR, **1999**, *139*, 334-341

DOSY (does convection take place?)



DgcsteSL_cc sequence, 15 G/cm, spinning speed 2300 Hz with rotor synchronization and Δ = 0 – 13 ms

T = 45 deg $CDCl_3$!

DOSY (packing issue?)

MAGNETIC RESONANCE IN CHEMISTRY
Magn. Reson. Chem. 2005; **43**: 31–35
Published online 26 October 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/mrc.1504

MRC

DOSY of sample-limited mixtures: comparison of cold, nano and conventional probes

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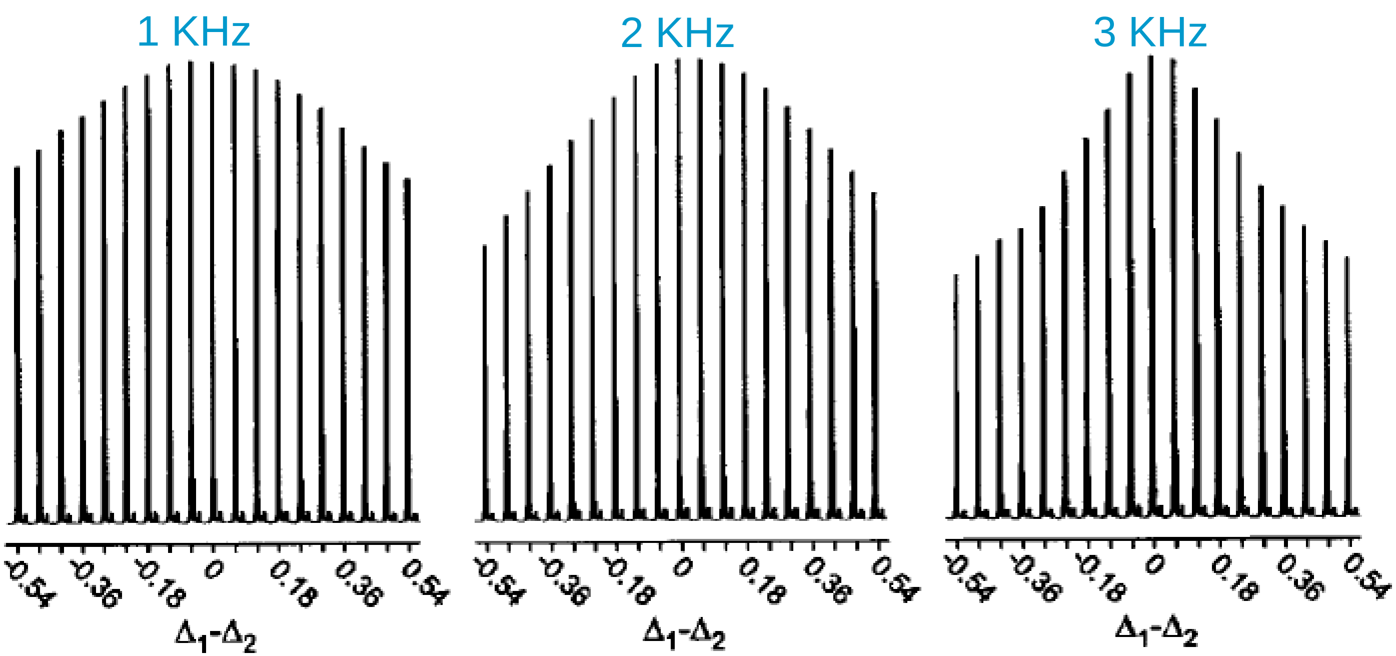
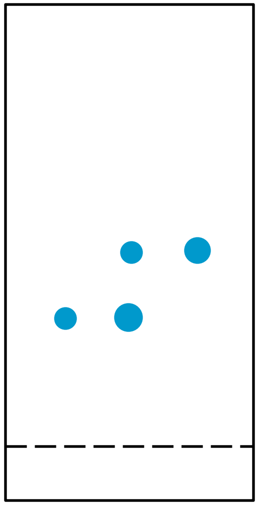
[1] Discovery Drug Disposition, Lilly Research Laboratories, Indianapolis, IN 46285, USA
[2] Discovery Chemistry Research, Lilly Research Laboratories, Indianapolis, IN 46285, USA

$CDCl_3$
RT
77μm/s

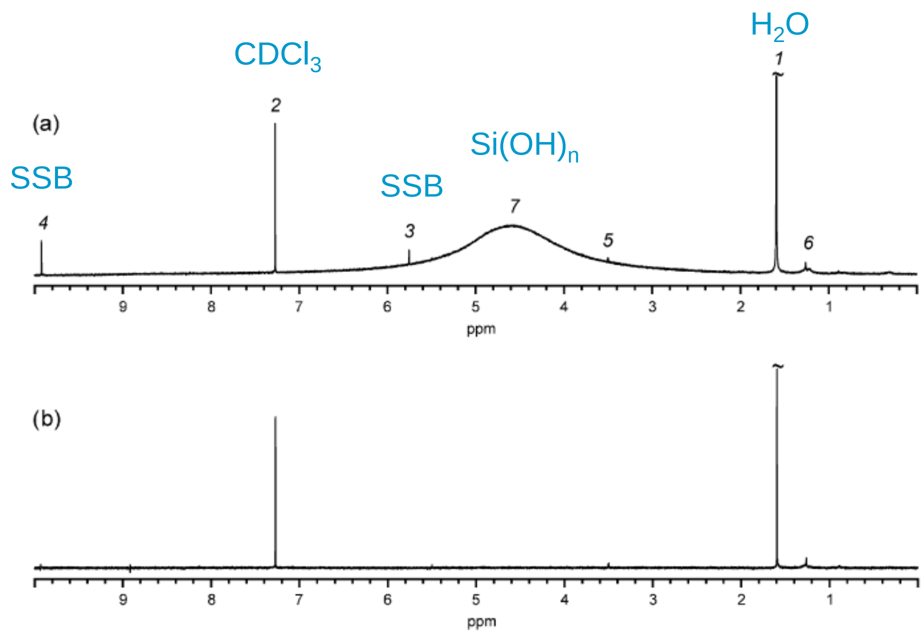


Nanoprobe and Reaction Control



1) scrap, ~~extract, dry, solve in deuterated solvent~~, record NMR spectrum

2) scrap, suspend in deuterated solvent, record NMR spectrum

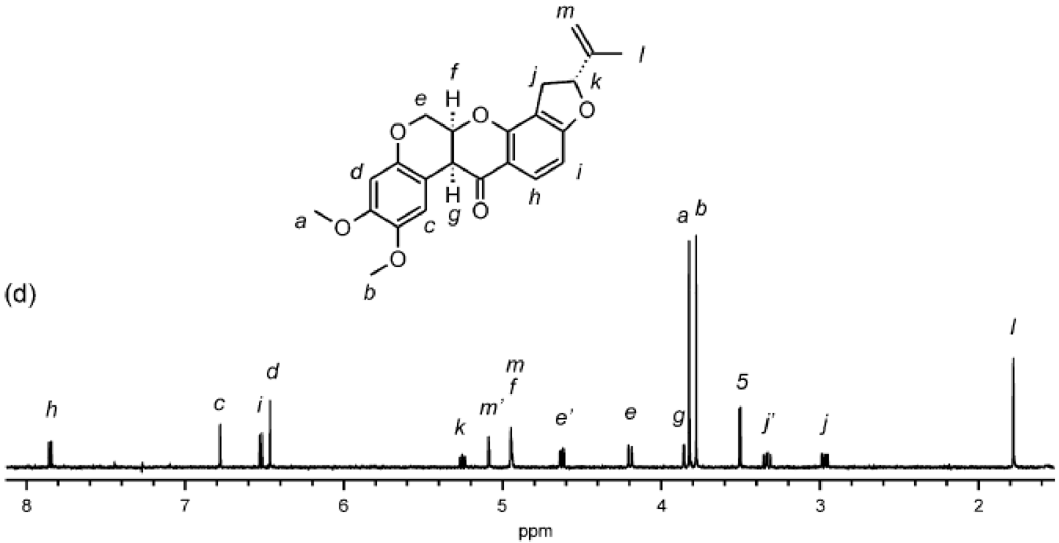


Stationary phase in $CDCl_3$

Stationary phase in CDCl3
(+ abt 10 ms CPMG step)

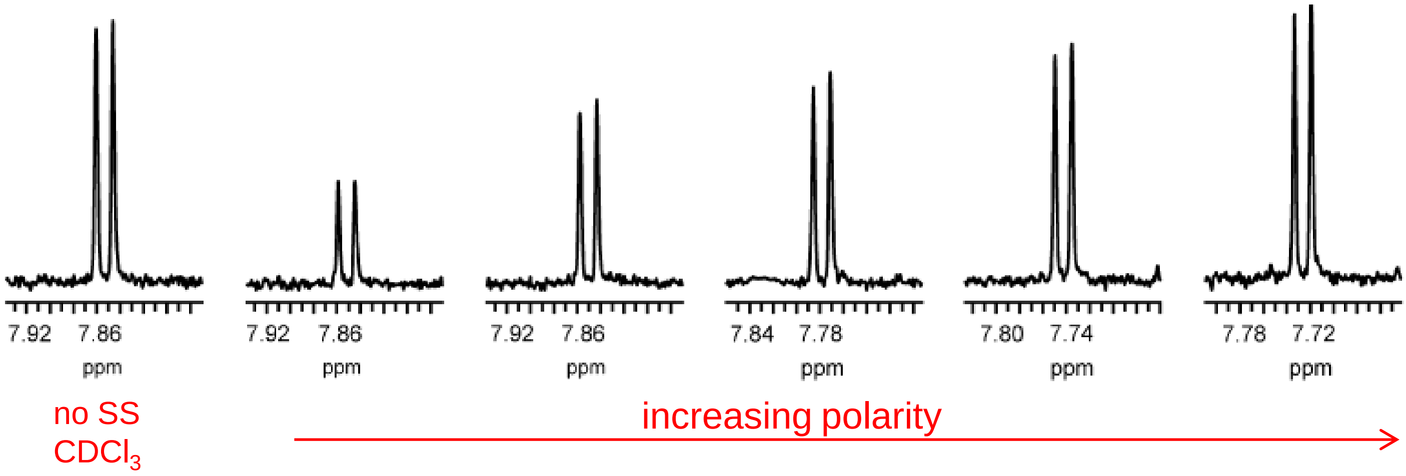
Bradley, McLaughlin MRC 45, 814-818 (2007)

Nanoprobe and Reaction Control



Rotenone 10 nmol
stationary phase 1.05 mg
CPMG, nt=256

What are we actually looking at?



Bradley, McLaughlin MRC 45, 814-818 (2007)

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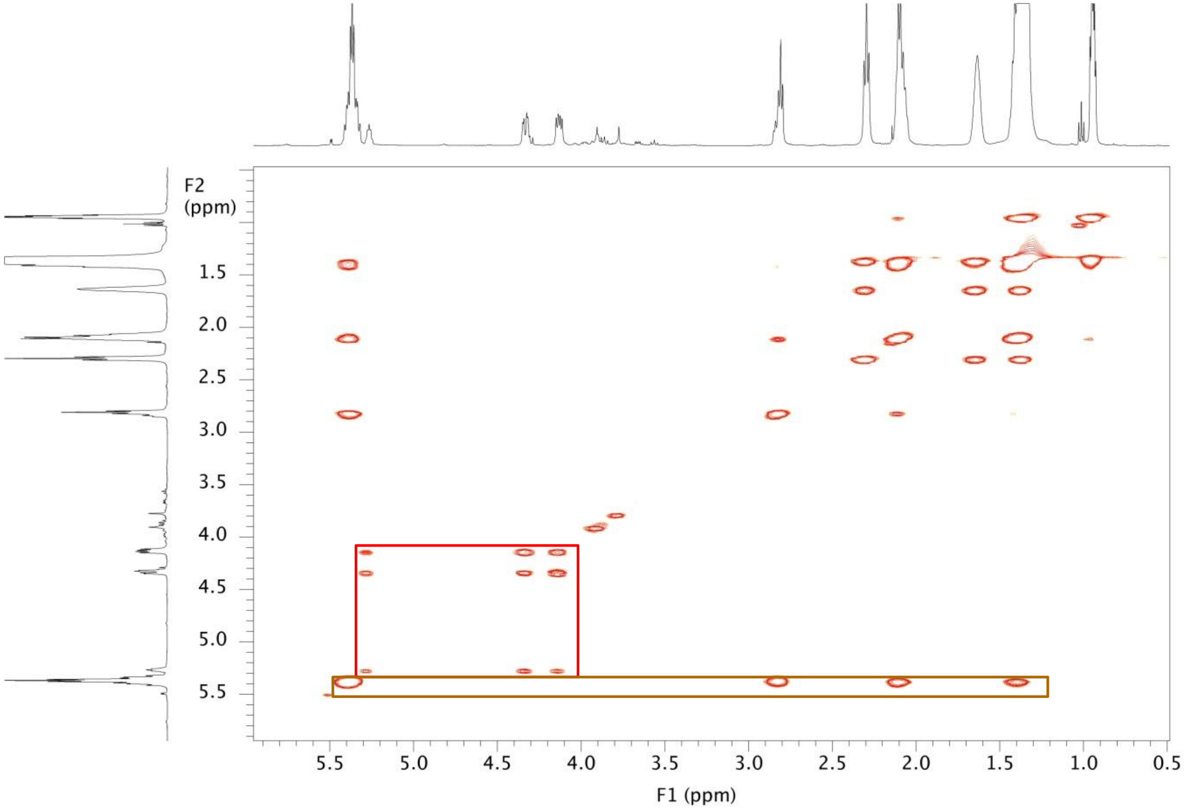
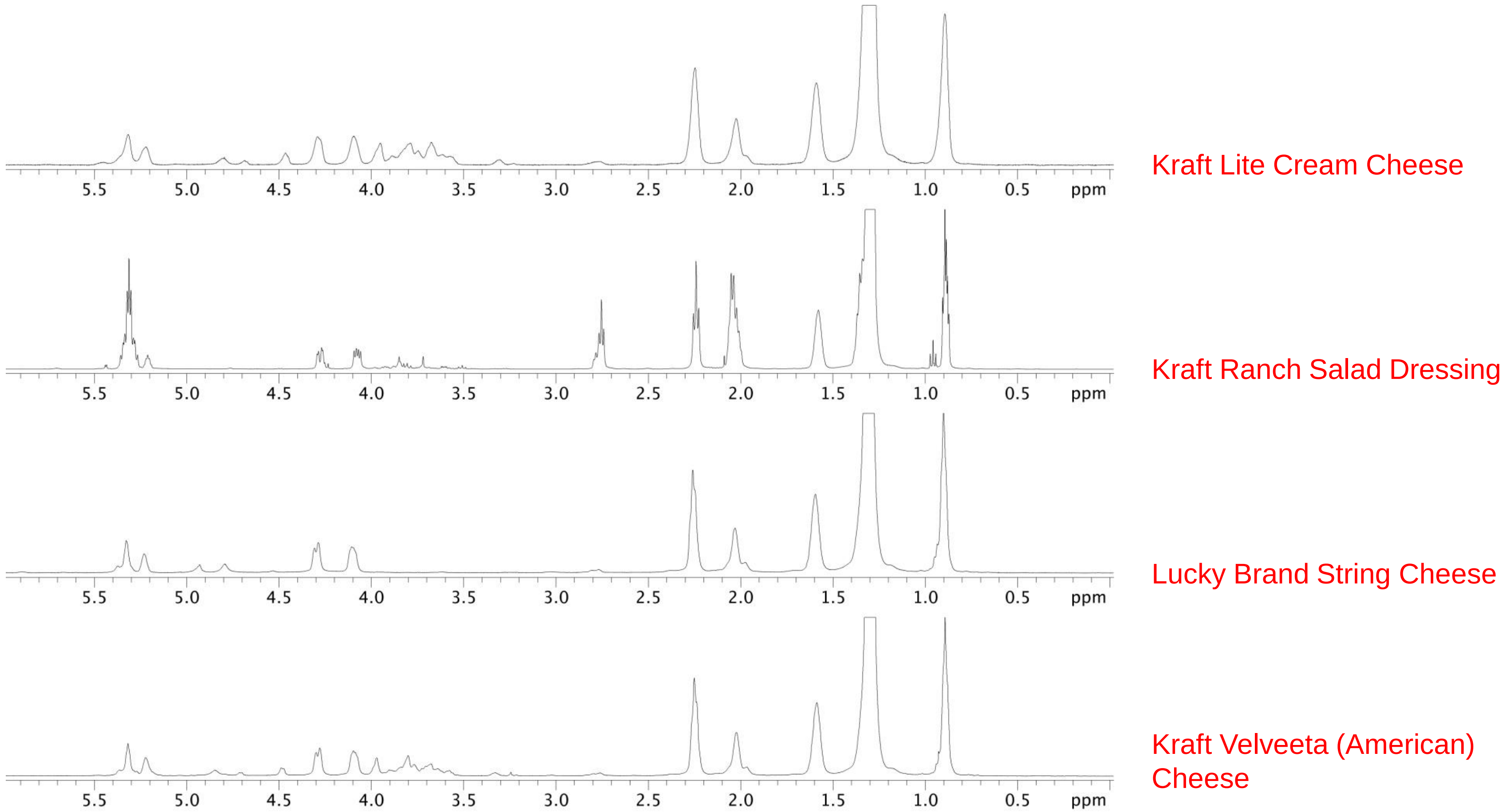
Nanoprobe and Emulsions



Nanoprobe

Comparison between emulsions and cheese (^1H spectra)



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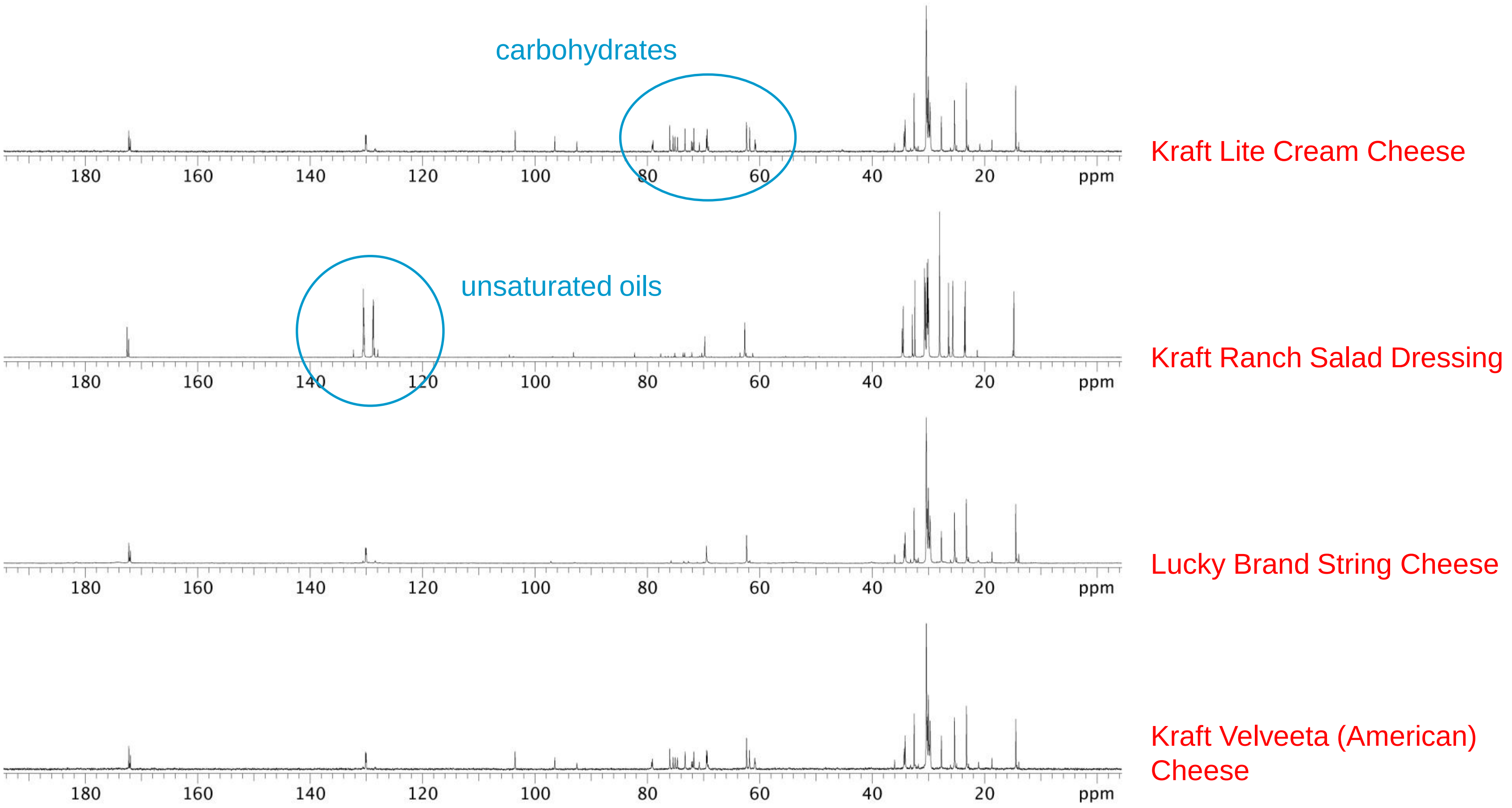
Nanoprobe

Comparison between emulsions and cheese (^{13}C spectra)



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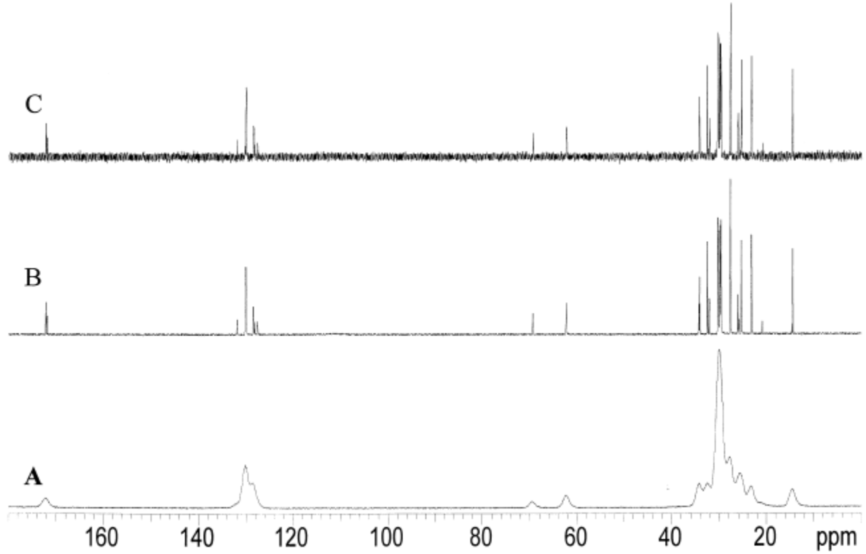
Nanoprobe

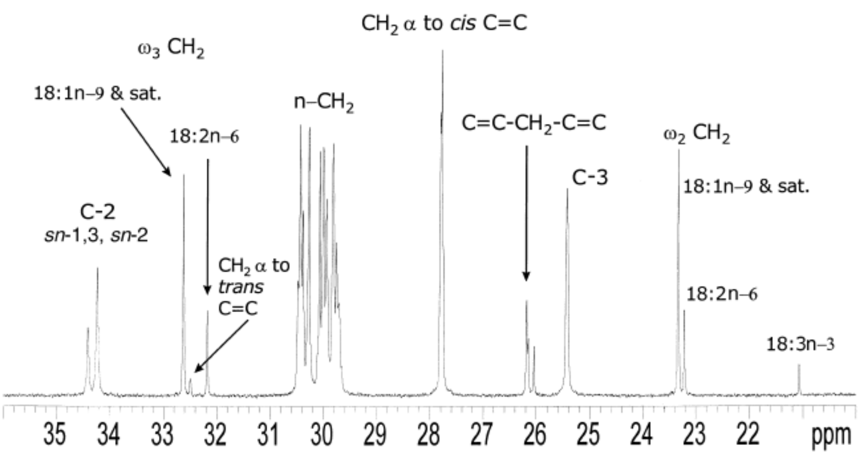
NMR of intact Canola seeds (CANadian Oil Low Acid – low erucic acid)



One seed in a tube for nanoprobe (10 mins)

One seed in a tube for nanoprobe (4 h)

Abt 80 seeds in normal 5mm tube



Seed line[b]	Acyl chain ^{13}C NMR (mol%)[b] and GC–FID (wt%) 18:3n-3	18:3n-6	18:1n-9[c]	Saturates
LEAR	9.3 (11.7)	24.0 (19.1)	60.6 (62.3)	6.1 (6.6)
8:0, 10:0	8.1 (9.3)	8.7 (8.4)	49.7 (54.2)	33.5 (27.6)
12:0	6.2 (7.1)	7.6 (9.8)	29.3 (30.8)	56.9 (52.3)
14:0	<1.0 (1.7)	21.8 (22.3)	20.7 (21.4)	57.5 (54.3)
16:0	3.8 (5.0)	21.3 (15.8)	35.0 (37.4)	39.8 (41.6)
18:0	6.8 (8.1)	19.6 (17.5)	46.0 (45.1)	27.6 (28.2)
18:1	<1.0 (1.9)	13.1 (7.8)	82.7 (84.0)	4.2 (6.0)
HEAR	10.8 (10.7)	17.6 (14.7)	69.3 (69.0)	2.3 (4.8)
24:1	9.3 (8.0)	18.2 (14.4)	69.0 (71.2)	3.4 (5.3)

Hutton et al. Lipids 34, 1339-1346 (1999)

Nanoprobe and Cultural Heritage

Recent advances in swollen-state NMR spectroscopy for the study of drying oils

Giacomo Cipriani[a], Antonella Salvini[a], Luigi Dei[b], Azzurra Macherelli[b], Francesco Saverio Cecchi[a], Carlo Giannelli[a]

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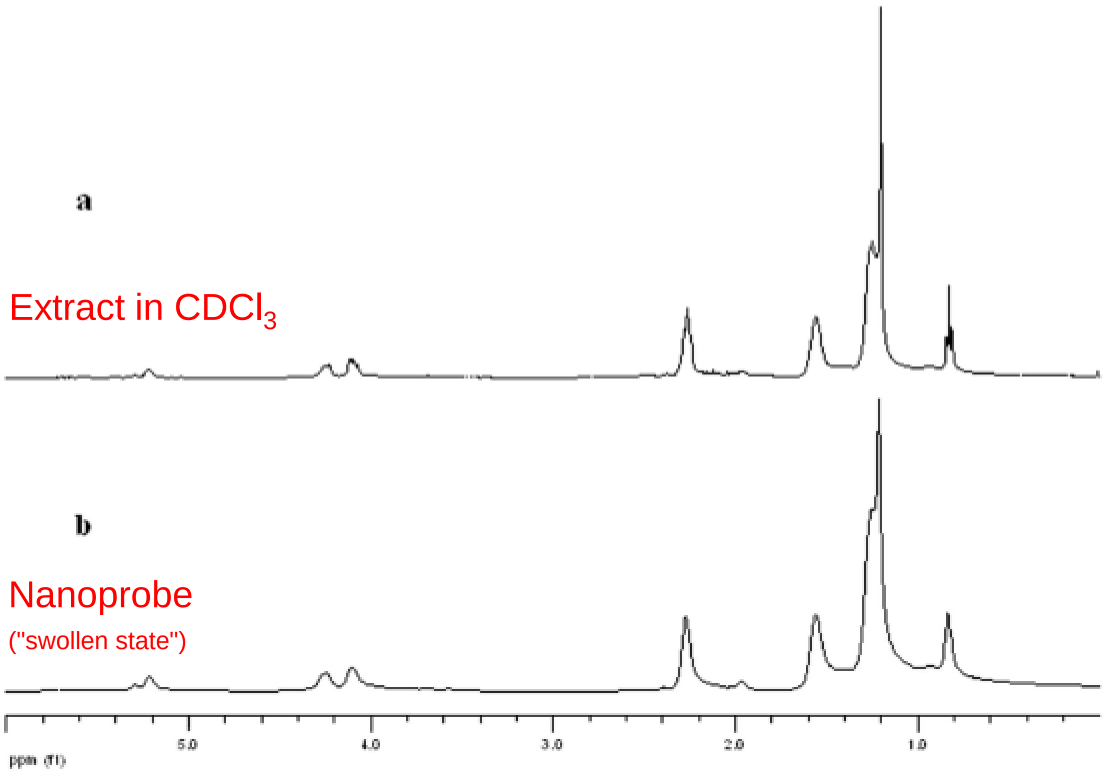
Abstract

The cross-linking processes of three drying oils (poppy, linseed, walnut), a class of organic compounds very important in the field of artworks, were studied by means of ^{1}H-NMR spectroscopy, following the change of the protons signals when oils are heated at 60 °C in the presence of air or nitrogen gas. This preliminary information is particularly important to identify the presence of drying oils within a solid painting film. We demonstrated that it was possible to study these films by swollen-state NMR, an innovative spectroscopy method that can directly analyze very small semisolid samples instead of solutions. The main advantages of this method are the short time of analysis, the possibility to analyze samples without any preliminary treatment, and the small quantity of the sample required. Therefore, swollen-state NMR technique was used to characterize three real painting films: in this way, we succeeded in recognizing in real paintings specimens the presence of drying oils employed as binders, avoiding any interferences due to other organic compounds acting as binding agents, like waxes or egg-yolk media.

J. Cult. Herit., **2009**, *10*, 388-395

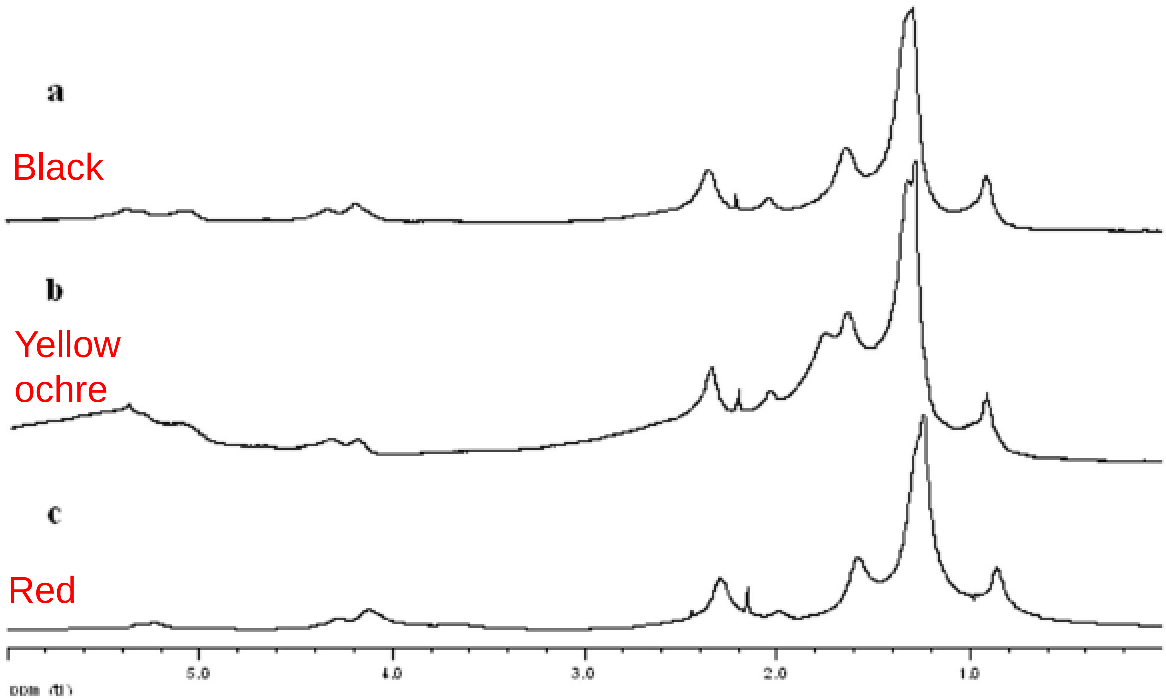
Nanoprobe and Cultural Heritage



Lineseed oil (aged 240 h) – 400 MHz

J. Cult. Herit., **2009**, *10*, 388-395

Nanoprobe and Cultural Heritage

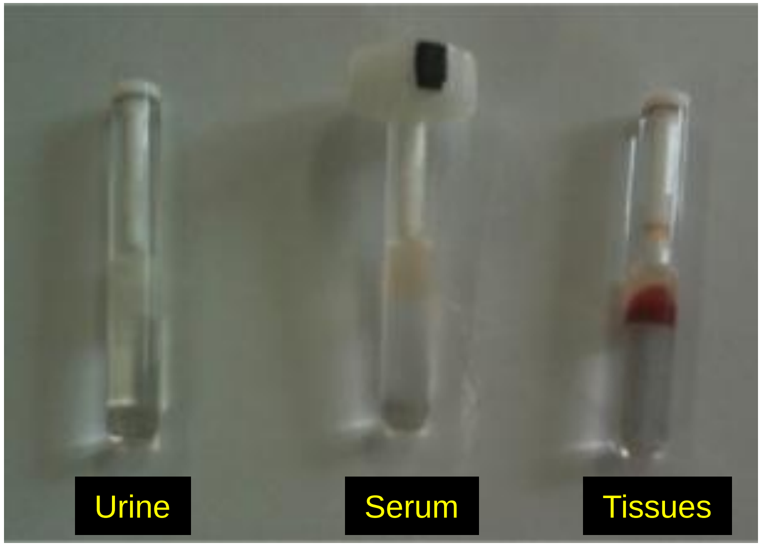


Painting Film (aged 3y)

J. Cult. Herit., **2009**, *10*, 388-395

Nanoprobe and Medicine

Nanoprobe and Medicine

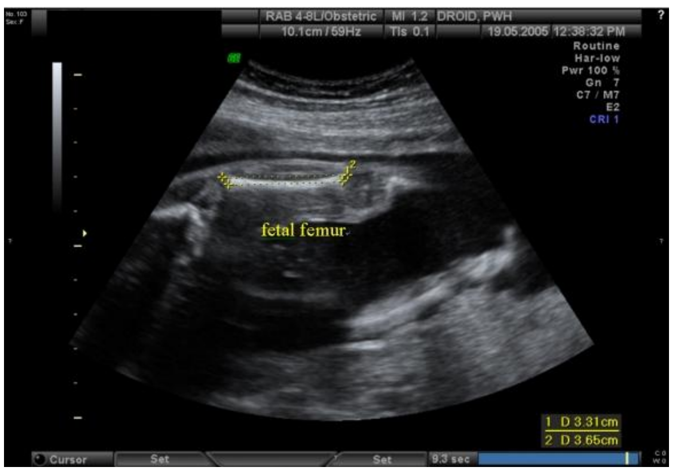


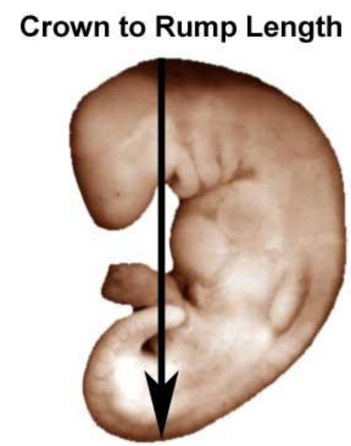
Nanoprobe and Medicine

? IMAGING

Calculation of gestational age in late second and third trimesters by ex vivo magnetic resonance spectroscopy of amniotic fluid

Brad R. Cohn, MD; Emily Y. Fukuchi, MD, MPH; Bonnie N. Joe, MD, PhD; Mark G. Swanson, PhD; John Kurhanewicz, PhD; Jingwei Yu, PhD, MD; Aaron B. Caughey, MD, PhD, MPH



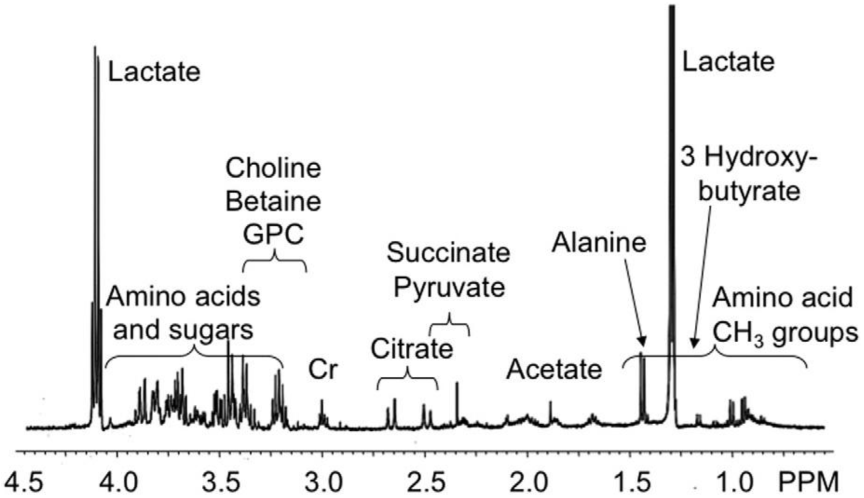


± 3.6 weeks

± 1 weeks

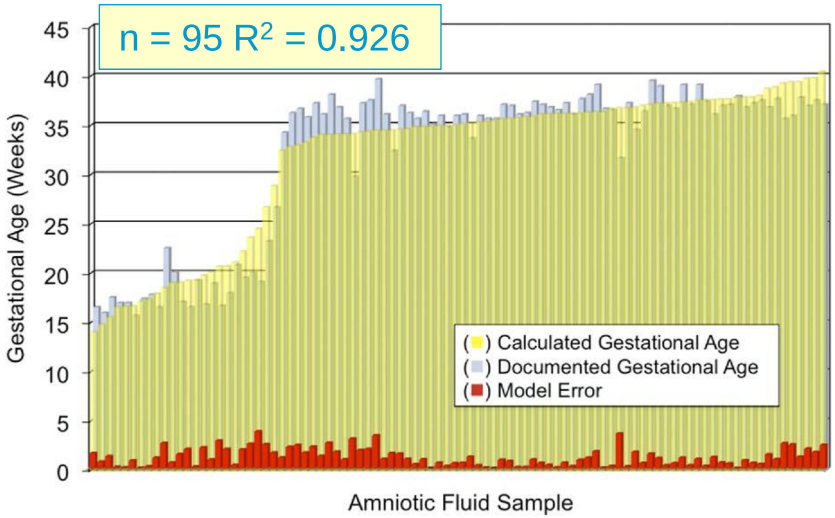
Am. J. Obstet. Gynec. 203, 76e1-76e10 (2010)

Nanoprobe and Medicine



- quantification by ERETIC (old INOVA system)
- today in DD2 systems it can be replaced by qEstimate + CRAFT

GA = 64.922 –
(14.456 × alanine) +
(4.965 × ln [creatinine]) –
(0.931 × glucose) –
(5.202 × valine)



± 1.75 weeks

Am. J. Obstet. Gynec. 203, 76e1-76e10 (2010)

Tissues

Magnetic Resonance in Medicine 55:1257–1264 (2006)

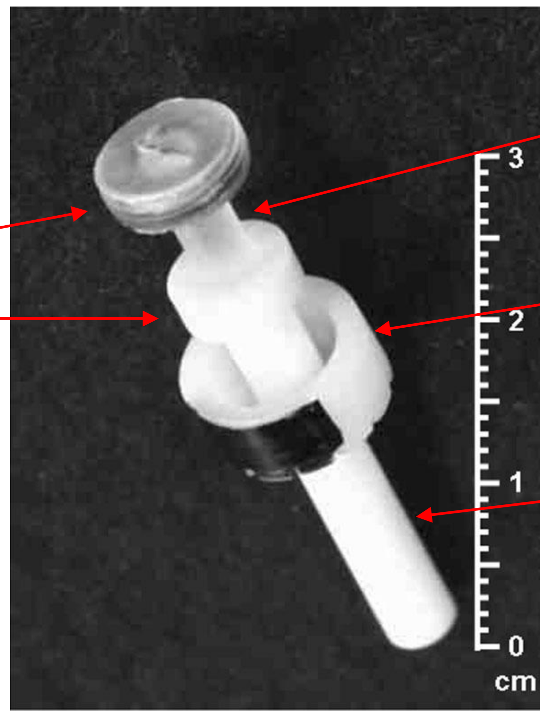
Quantitative Analysis of Prostate Metabolites Using ^{1}H HR-MAS Spectroscopy

Mark G. Swanson,[1*] Andrew S. Zektzer,[1] Z. Laura Tabatabai,[2] Jeffry Simko,[2]
Samson Jarso,[1] Kayvan R. Keshari,[1] Lars Schmitt,[3] Peter R. Carroll,[3]
Katsuto Shinohara,[3] Daniel B. Vigneron,[1] and John Kurhanewicz[1]

Note that:

plug threads into drive ring for tight seal,

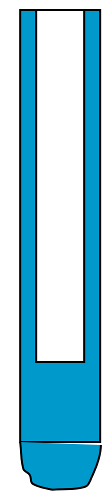
tube is widemouth



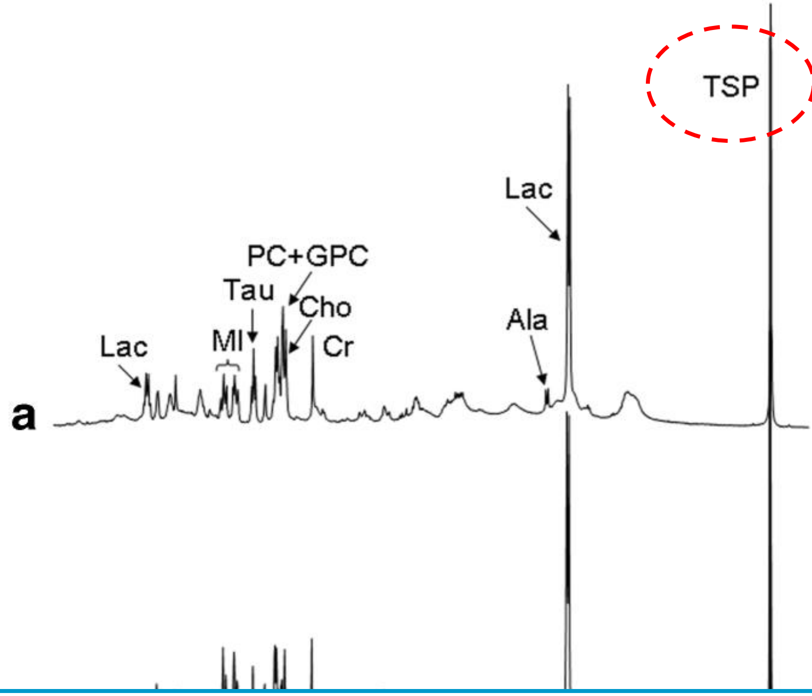
Plug

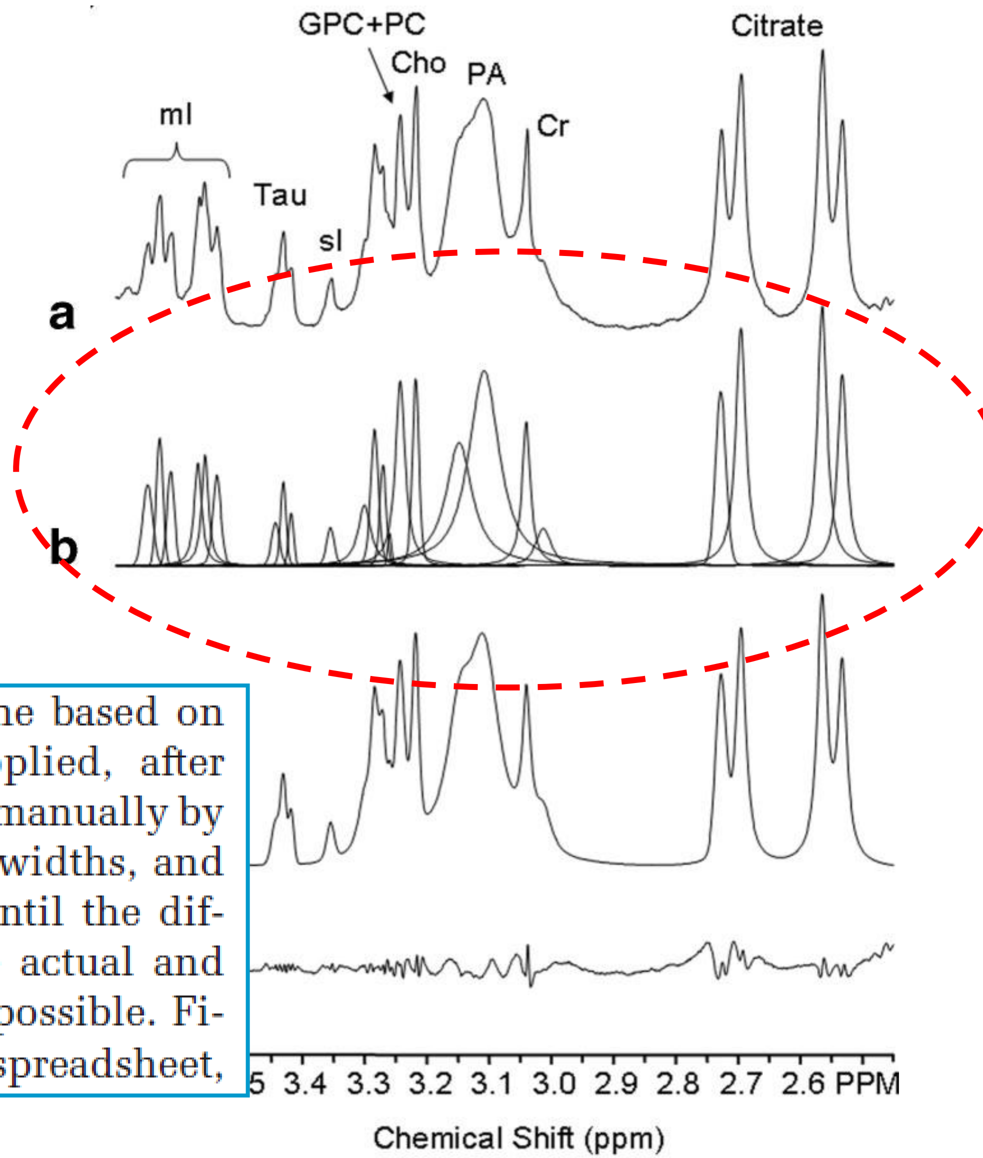
Drive Ring

Sample Tube



Tissues

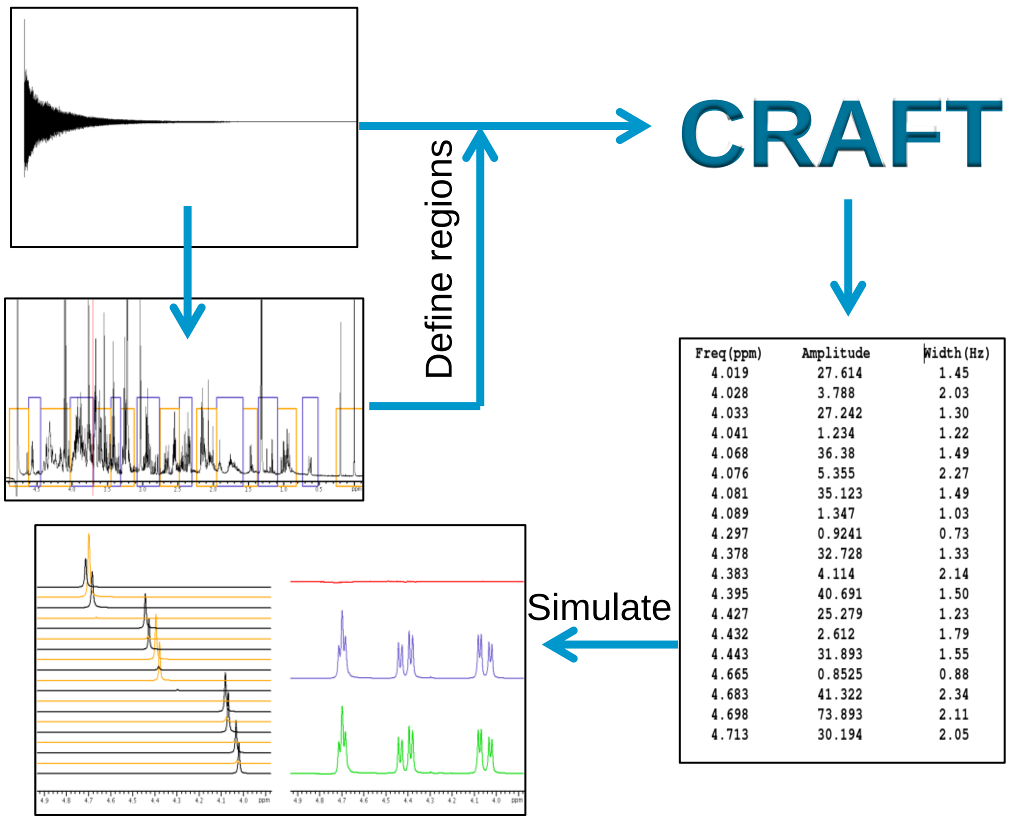




using four peaks. An automated fitting routine based on the Levenberg-Marquardt algorithm was applied, after which the resulting peak fits were touched up manually by adjusting the peak positions, intensities, linewidths, and percent Lorentzian and Gaussian character until the difference (i.e., residual spectrum) between the actual and summed spectra was minimized as much as possible. Final peak areas were imported into an Excel spreadsheet,

Swanson et al. MRM, **2006**, *55*, 1257-1264

CRAFT – Deconstructing a Spectrum

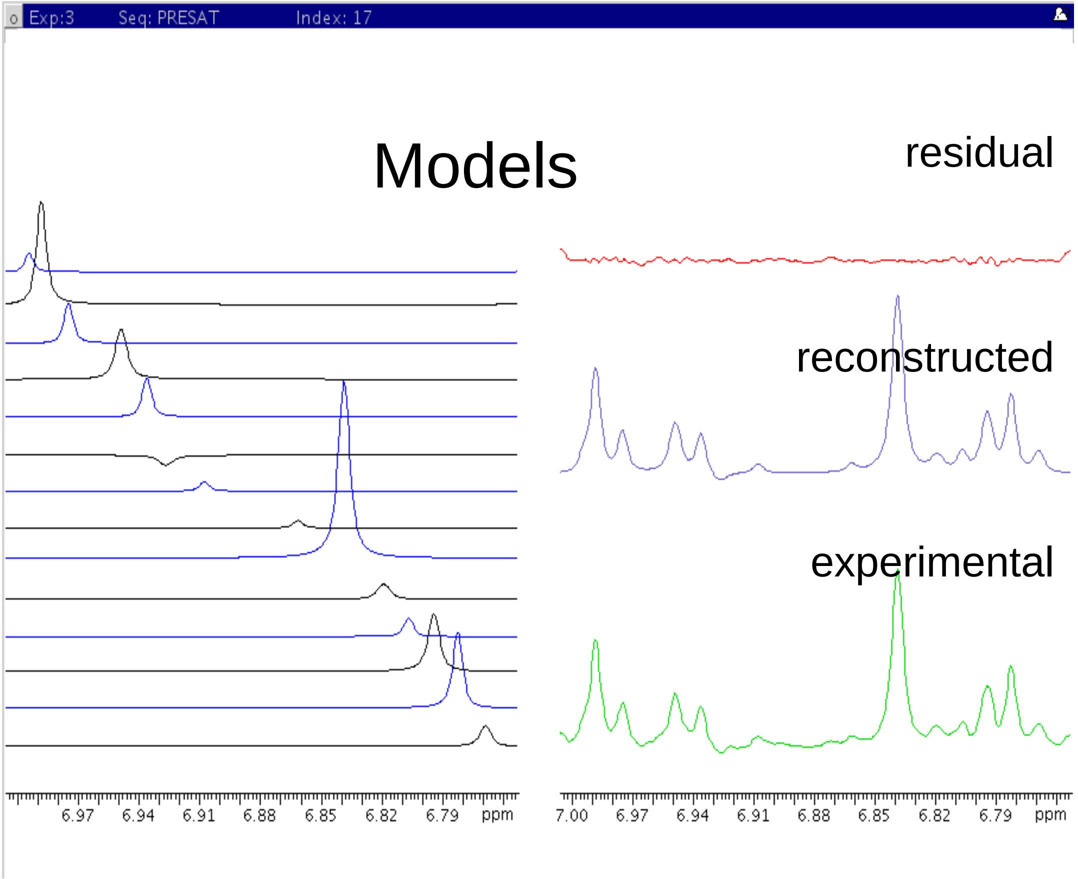


Freq(ppm)	Amplitude	Width(Hz)
4.019	27.614	1.45
4.028	3.788	2.03
4.033	27.242	1.30
4.041	1.234	1.22
4.068	36.38	1.49
4.076	5.355	2.27
4.081	35.123	1.49
4.089	1.347	1.03
4.297	0.9241	0.73
4.378	32.728	1.33
4.383	4.114	2.14
4.395	40.691	1.50
4.427	25.279	1.23
4.432	2.612	1.79
4.443	31.893	1.55
4.665	0.8525	0.88
4.683	41.322	2.34
4.698	73.893	2.11
4.713	30.194	2.05

Once data have been captured in the spreadsheet, analysis is essentially complete.

Most users want to see how well the data reduction step worked. CRAFT includes a complete set of tools to allow visual inspection of the results.

CRAFT – Reconstructing Spectra for Comfort



The common method for displaying CRAFT data is to show the experimental NMR spectrum after Fourier Transform, the CRAFT spectrum reconstructed from the final spreadsheet, and the residual signal, or the “difference between the two” spectrum.

This step is for user convenience; it is not required for analysis!

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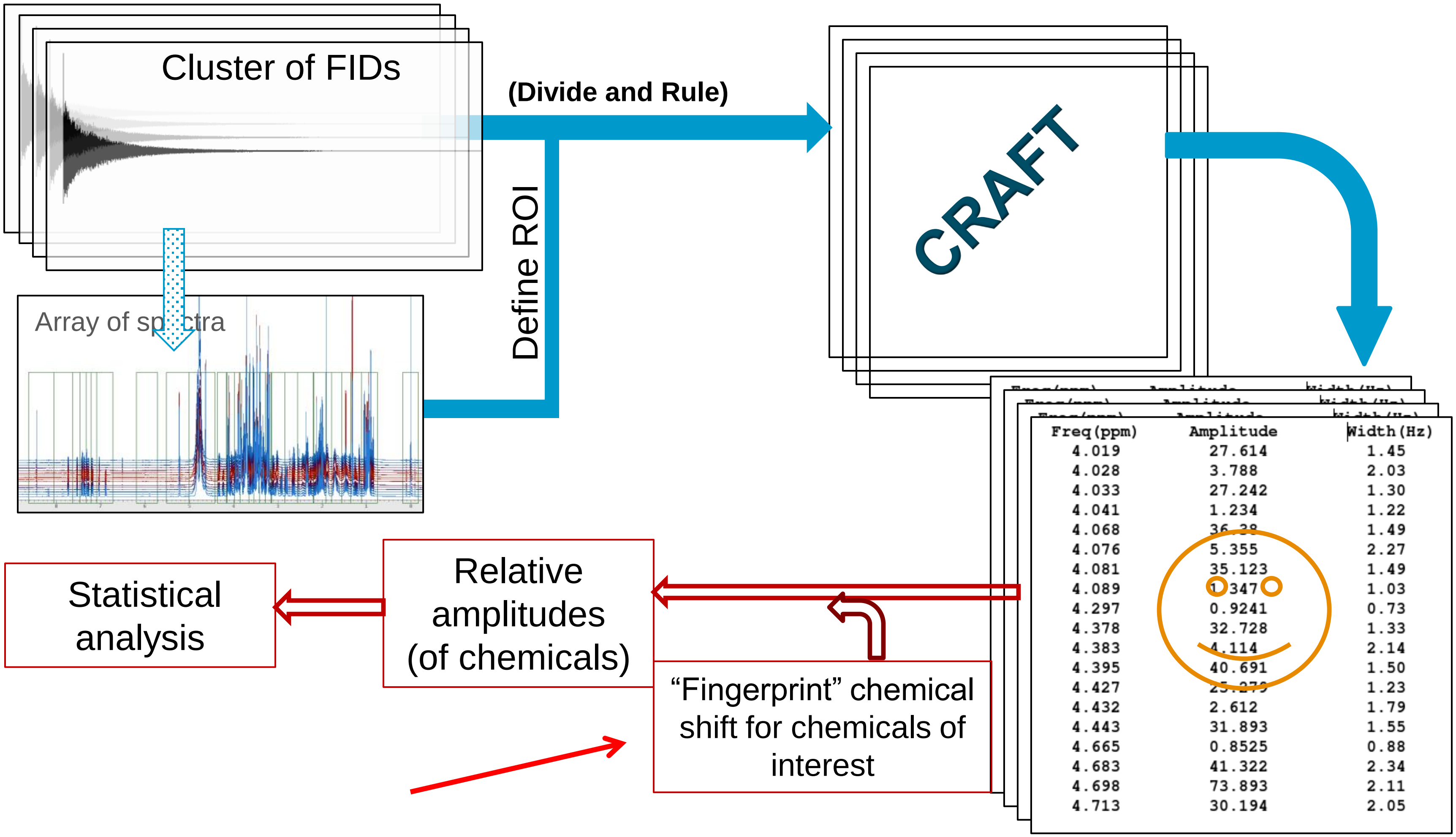
CRAFT workflow



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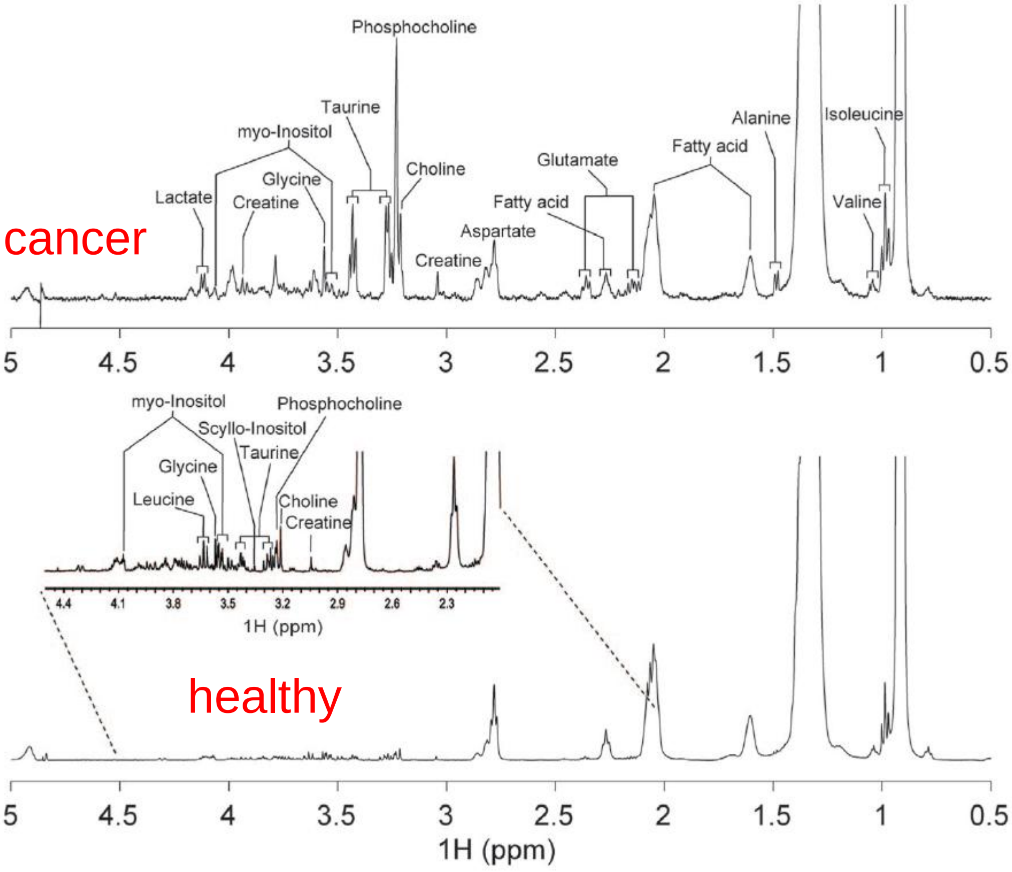
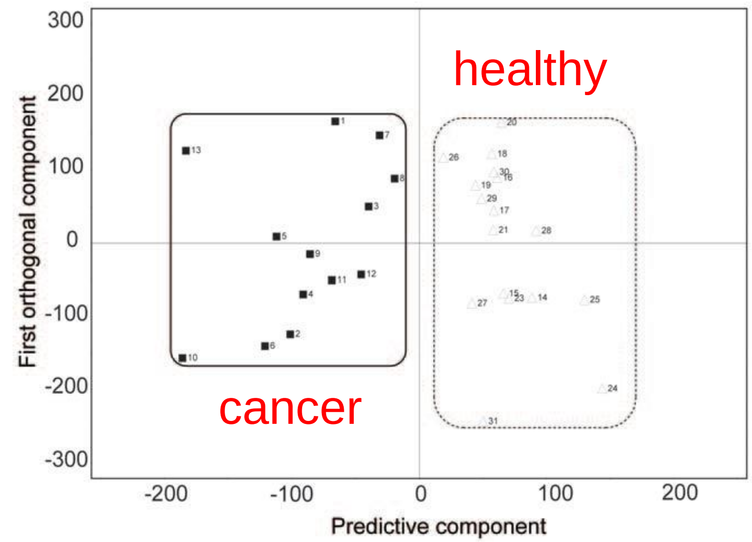
4.713	30.194	2.05

Tissues

An HR-MAS MR Metabolomics Study on Breast Tissues Obtained with Core Needle Biopsy

MuLan Li[1], Yonghyun Song[2], Nariya Cho[1], Jung Min Chang[1], Hye Ryoung Koo[1], Ann Yi[1], Hyeonjin Kim[1], Sunghyouk Park[2*], Woo Kyung Moon[1*]





PLoS One., **2011**, *6*, e25563

The faster the better? (not always)

Since the centrifugal force increases by speed^2 tissues and living matter may not survive

- Findings of Dr. Hongjun Pan University of Tennessee

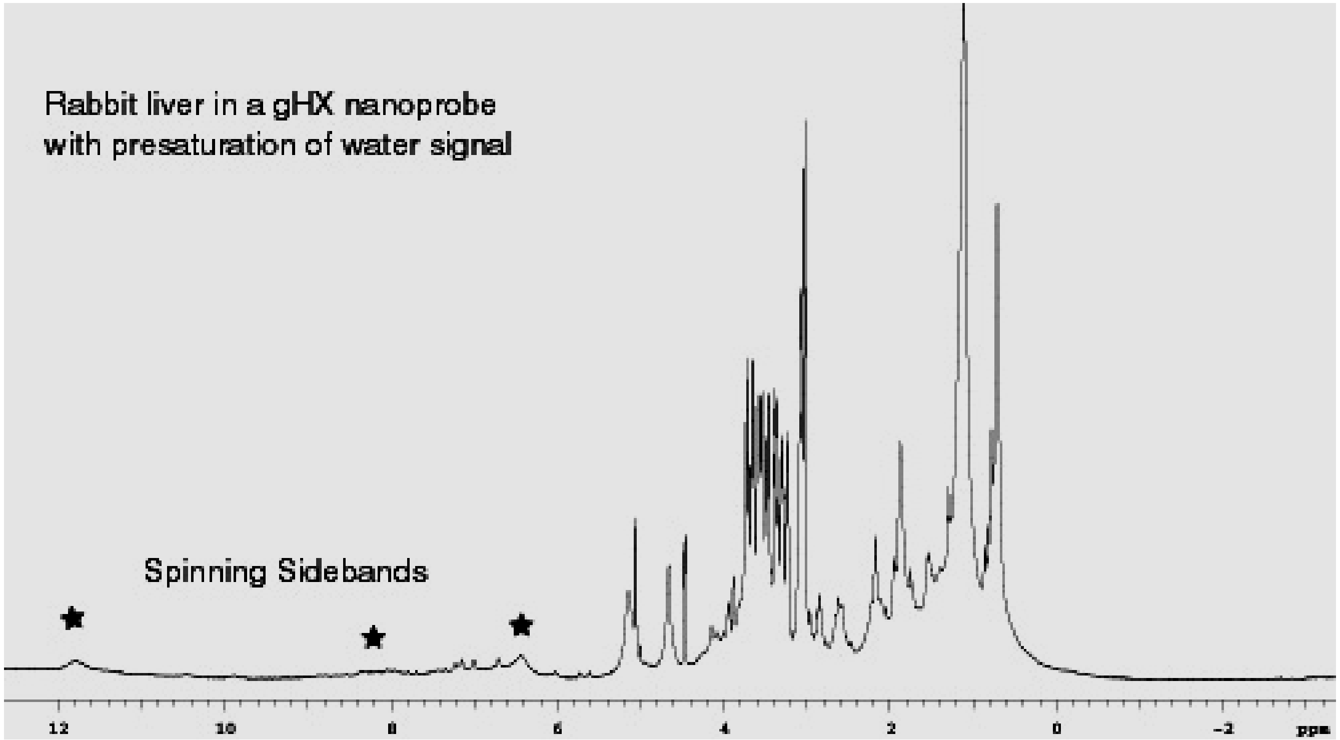
The effect of mechanical spinning on mosquito egg hatching

Spinning rate (Hz)	0	0	600	500	1100	1200
Time (min)	0	0	15	5	15	5
Total eggs	688	759	239	158	260	273
Hatched eggs	426	359	52	41	2	21
Hatching rates	62%	47%	22%	26%	0.8%	7%

For living objects, spinning of about 500 Hz has significant effect on the life. The effect is proportional to the spinning rate and spinning time. **For a spinning rate higher than 1200 Hz, no hatching occurred.**

The faster the better? (not always)

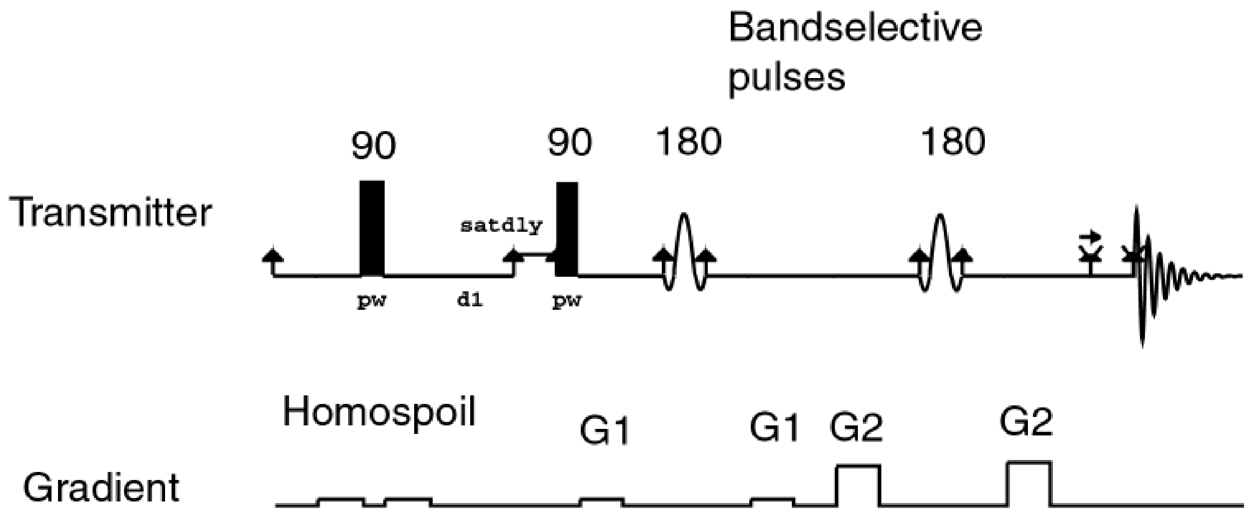
Removing spinning sidebands



The faster the better? (not always)

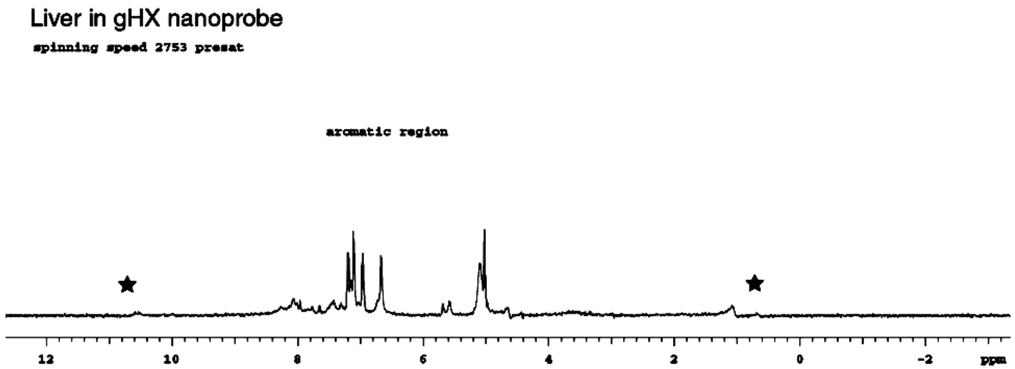
Removing spinning sidebands

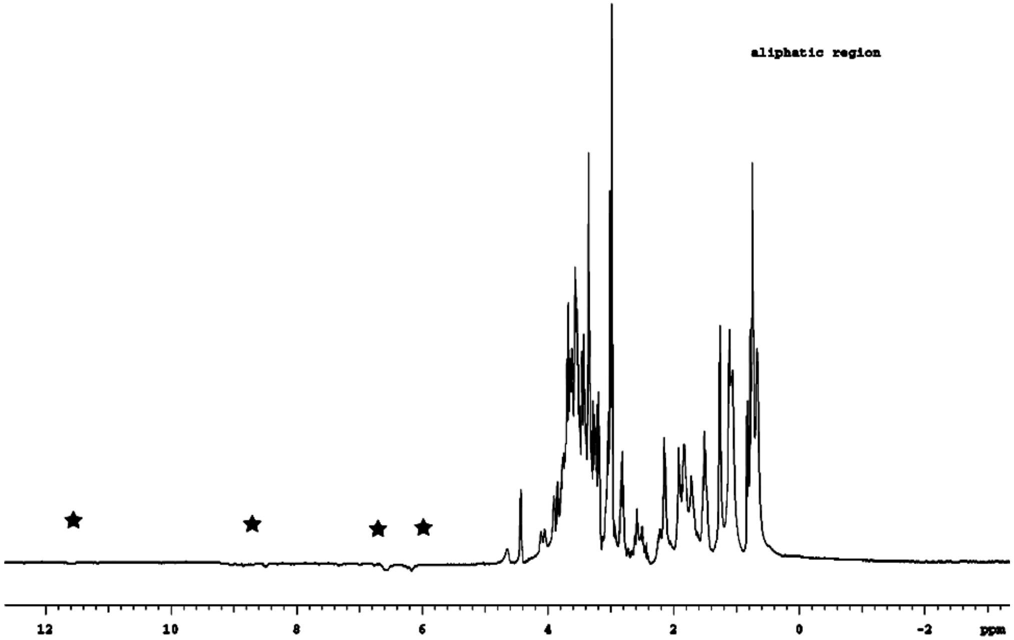
- record the spectrum in parts using selective excitation.
- split the spectrum in regions from 2 Hz to the spinning speed.
- preferred method: DPFGSE with shapes created with Pbox.
- spinning sidebands are outside the excited region.
- suppression of broad signals – no need for CPMGT2
- helps suppressing the H_2O signal
- combine the filtered spectra together as FID and re-create the full spectrum



The faster the better? (not always)

Removing spinning sidebands





Starting spectra

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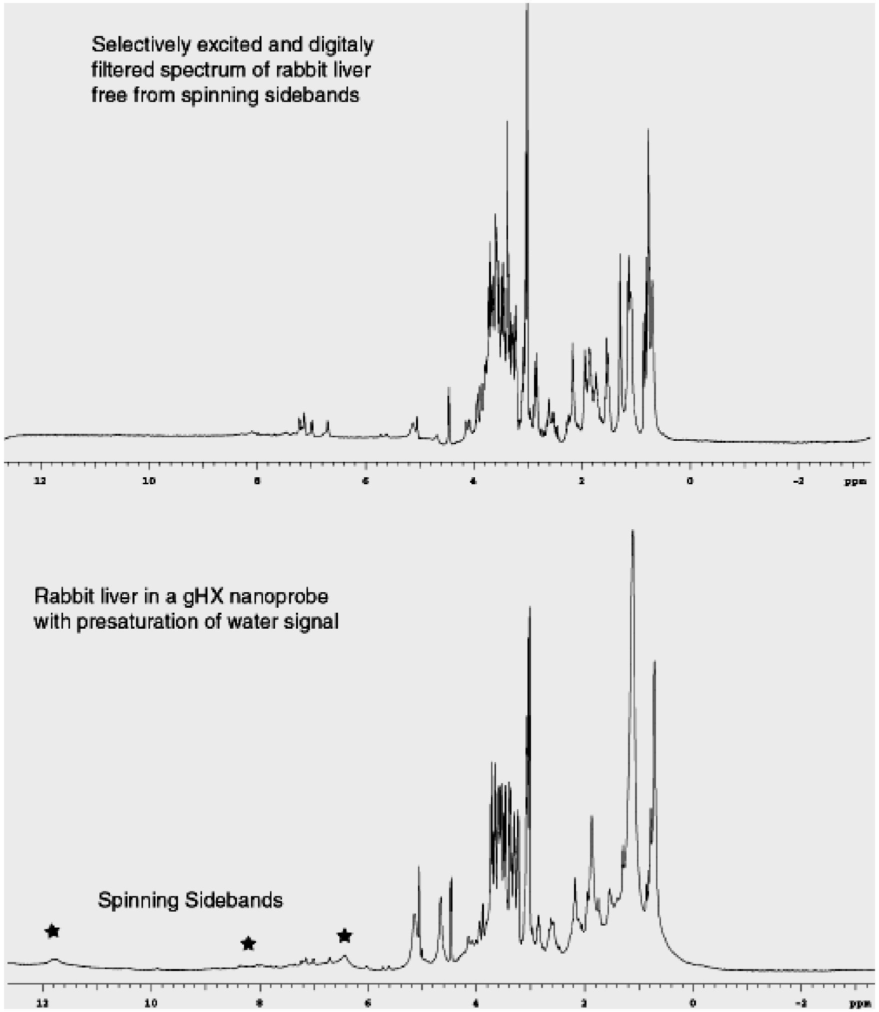
The faster the better? (not always)

Removing spinning sidebands



See also for recent work on this subject: Renault et al. Sci. Reports 3, 3349 (2013)

Agilent Technologies

Grazie!

Agilent Technologies

mauro.cremonini@agilent.com