









**Unitat de RMN**

## Introduction












## NMR: Nuclear Magnetic Resonance


- **Definition**

NMR is a physical phenomenon that occurs when applying a radio frequency to atomic nuclei in a magnetic field.

γ-rays	x-rays	UV	VIS	IR	μ-wave	radio
						
$10^{-10}$	$10^{-8}$	$10^{-6}$	$10^{-4}$	$10^{-2}$	$10^0$	$10^2$
wavelength (cm)						

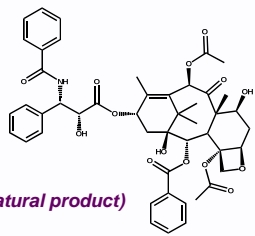
<ul style="list-style-type: none"> <li>• Gamma rays</li> <li>• X-rays</li> <li>• UV-Vis</li> <li>• IR</li> <li>• radiofrequencies resonance)</li> </ul>	<ul style="list-style-type: none"> <li>Nuclear transitions</li> <li>Electronic transitions (inner electrons)</li> <li>Electronic transitions (outer electrons)</li> <li>Vibration transitions</li> <li>NMR and EPR (electronic paramagnetic resonance)</li> </ul>
---	---

Unitat de RMN Centres Científics i Tecnològics

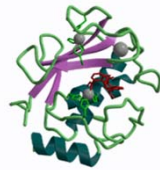

  
 U
   
 B Universitat de Barcelona

## Typical Applications of NMR


- 1) **Structural (chemical) elucidation**
  - Natural product chemistry
  - Synthetic organic chemistry
  - analytical tool of choice of synthetic chemists
  - used in conjunction with MS and IR
- 2) **Study of dynamic processes**
  - reaction kinetics
  - study of equilibrium (chemical or structural)
- 3) **Structural (three-dimensional) studies**
  - Proteins, Protein-ligand complexes
  - DNA, RNA, Protein/DNA complexes
  - Polysaccharides
- 4) **Drug Design**
  - Structure Activity Relationships by NMR
- 5) **Medicine-MRI**



*Taxol (natural product)*




*NMR Structure of MMP-13 complexed to a ligand*



*MRI image of the Human Brain*

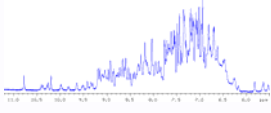
Unitat de RMN Centres Científics i Tecnològics

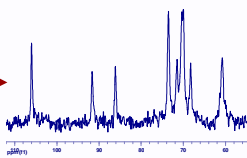

  
 U
   
 B Universitat de Barcelona

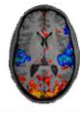
## NMR

NMR → Flexibility / adaptability

- NMR liquid state
  - Structural determination
    - ❖ Low and medium molecular weight compounds
    - ❖ High molecular weight compounds 10kDa
  - .....
- NMR solid state
  - Organic and inorganic compounds
  - Biopolimers ( proteins, DNA RNA)
  - .....
- Magnetic Resonance Imaging Spectroscopy (MRI)
  - MRI functional
  - Micro-imagen
  - MRI Locating










Unitat de RMN Centres Científics i Tecnològics

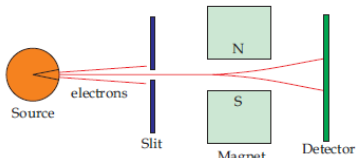
## The first observable evidence of nuclear magnetic moment



Universitat de Barcelona

**1921** Otto Stern & Walther Gerlach: Beam of electrons split into two paths when passed through an inhomogeneous magnet due to the electron's magnetic moment.



**1943 Stern wins Nobel Prize in Physics**

Angular momentum ( $p$ )

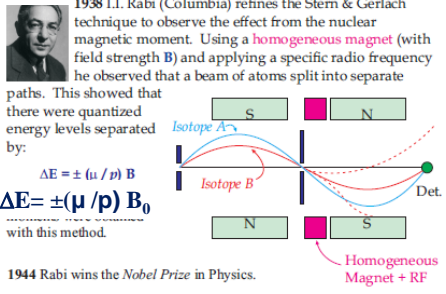
Magnetic moment ( $\mu$ )

**1938** I.I. Rabi (Columbia) refines the Stern & Gerlach technique to observe the effect from the nuclear magnetic moment. Using a homogeneous magnet (with field strength  $B$ ) and applying a specific radio frequency he observed that a beam of atoms split into separate paths. This showed that there were quantized energy levels separated by:

$$\Delta E = \pm (\mu / p) B$$

$$\Delta E = \pm (\mu / p) B_0$$


with this method.



**1944 Rabi wins Nobel Prize in Physics**


Unitat de RMN Centres Científics i Tecnològics

## First NMR signals 1946




Universitat de Barcelona

**1945-6** Edward Purcell with Henry C. Torrey and Robert V. Pound (MIT) observe NMR signal from paraffin wax (December 15, 1945).

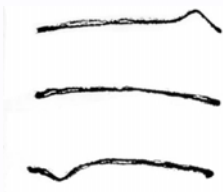


**1945-6** Felix Bloch, with W.W. Hansen and Martin Packard (Stanford) use a different method they call nuclear induction to observe proton signal from  $H_2O$  in early January 1946.



Both groups agreed that they were observing the same phenomena and called it NMR. (Bloch's method is closer to techniques used today).


**1944 Bloch and Purcell win the Nobel Prize in Physics**




First NMR signals 1946

Phys. Rev. 70 474 (1946)

Unitat de RMN Centres Científics i Tecnològics






## Chemical Shifts 1950-1

1950 Proctor and Yu accidentally discover that two different nitrogens of  $\text{NH}_4\text{NO}_3$  resonate at different frequencies.

1950 Other reports of similar differences in resonance frequencies of different compounds for proton (water vs. mineral oil) and fluorine ( $\text{CF}_3$  vs. aromatic F).

1951 Arnold, Dharmatti and Packard (Stanford) are the first to report different resonance frequency for protons in the same molecule.

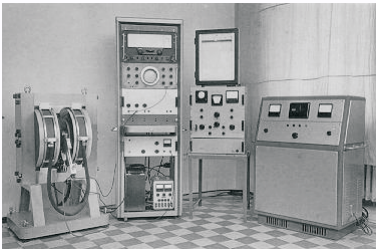
**The Chemical Shift was discovered**




First published 'high-resolution'  $^1\text{H}$  NMR spectrum (1951)


The three peaks of methanol was observed by Packard group

The operating field of magnet was 7.600 gauss.  $^1\text{H}$  frequency 32.4 MHz

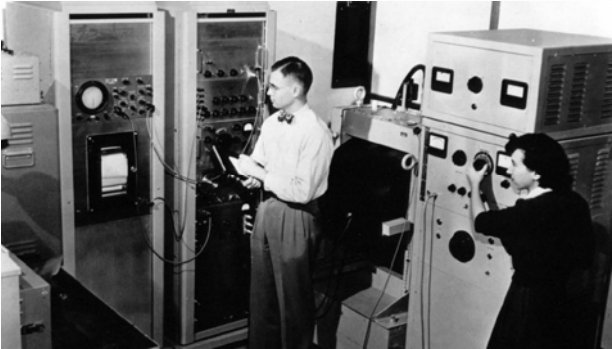


Unitat de RMN Centres Científics i Tecnològics





## First NMR Applications Laboratory, 1952



**The first commercial NMR spectrometer, the Varian HR-30, was installed at Humble Oil company in Baytown, Texas in September 1952.**

Unitat de RMN Centres Científics i Tecnològics

## 1951 Chemical Shift, and reference

U  
B Universitat de Barcelona

The scale was the higher field to right

At the first the Chemical Shifts was expressed in gauss

Swept field spectrum CW

**Down -field**  
Higher Frequency  
De-shielded

**Up -field**  
Lower Frequency  
Shielded

The position of resonance are related whit the environment of the nucleus

The chemical shifts was reported as the ratio difference from the resonance Reference (TMS)

$$\text{ppm} = \frac{V_{\text{obs}} - V_{\text{ref}}}{V_{\text{ref}}} \times 10^6$$

The area of the signals is directly proportional to the protons generating each signal

*Unitat de RMN Centres Científics i Tecnològics*

## Spin-Spin Coupling

U  
B Universitat de Barcelona

The position of resonance are related with the environment of the nucleus

The area of the signals is directly proportional to the protons generating each signal

**1951** Herbert Gutowski et. al. find that resonances split due to interactions between nuclei called Spin-spin coupling .


For proton and other spin = 1/2 nuclei, the number of lines in a multiplet is equal to the number of neighboring spins plus one (n+1 ).

**Super High Resolution NMR in 1956**


spinning in NMR tube

*Unitat de RMN Centres Científics i Tecnològics*





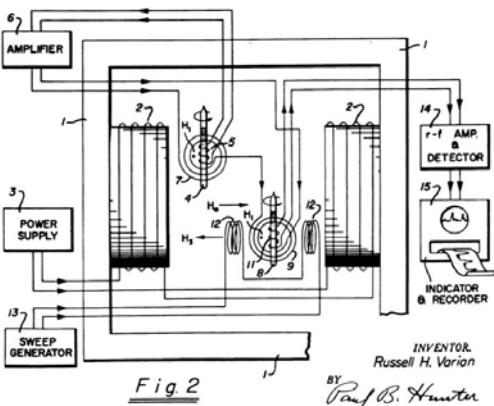
## Field/Frequency Lock, 1956



Universitat de Barcelona


Russell Varian developed the concept of the field/frequency lock utilizing a feedback mechanism.

Oct. 29, 1963      R. H. VARIAN      3,109,138  
GYROMAGNETIC RESONANCE METHODS AND APPARATUS  
Filed Aug. 29, 1956      2 Sheets-Sheet 1




correct for the field drift during data collection



Unitat de RMN Centres Científics i Tecnològics



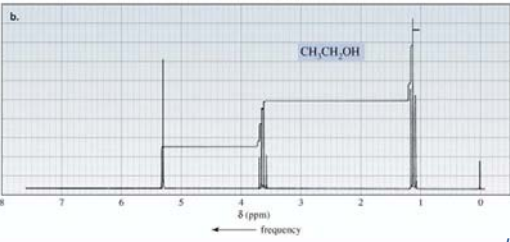
## 60 MHz NMR Spectrometer




Universitat de Barcelona

The Varian A-60 NMR spectrometer, introduced in 1961




Unitat de RMN Centres Científics i Tecnològics




## Starting point

“I put a sample in the instrument, adjusted the resolution, and ran a spectrum on the precalibrated chart. It was perfect. But could the A-60 reproduce a spectrum with the fingerprint quality of the IR instrument? I moved the pen back to the start and restarted the scan. I was momentarily distracted, and when I looked back I saw only one line on the chart. "Why didn't the second scan run properly?" I asked. The answer came back, "It did!" Amazed and almost incredulous, I returned the pen three more times. It laid down five identical spectra with a single trace showing on the paper!


***At that instant, I knew the field of organic chemistry would never be the same again.*** (from *Shoolery*, J.N. "NMR spectroscopy in the beginning." *Anal. Chem.* **1993**, *65*(17), 731A-741A)

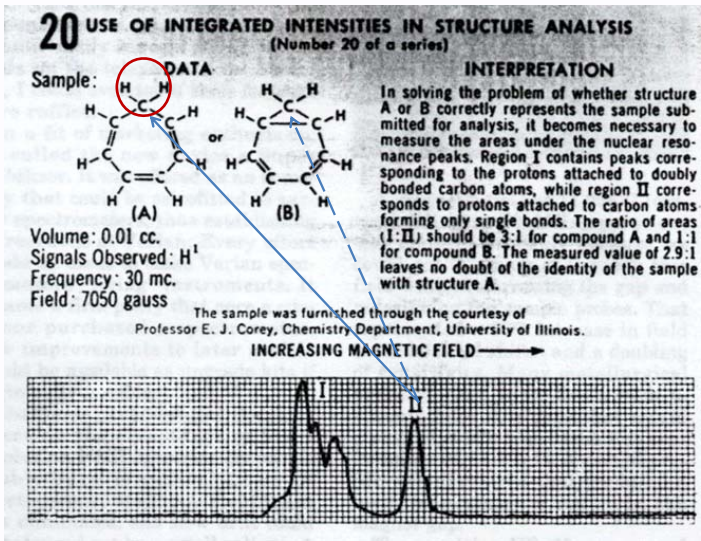


Unitat de RMN Centres Científics i Tecnològics



## The first problem solved by NMR





The sample was furnished through the courtesy of Professor E. J. Corey, Chemistry Department, University of Illinois.

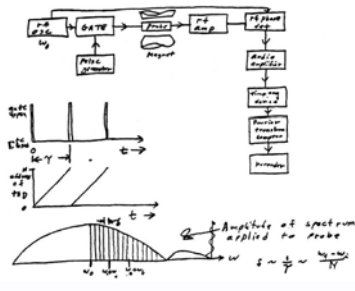
Unitat de RMN Centres Científics i Tecnològics



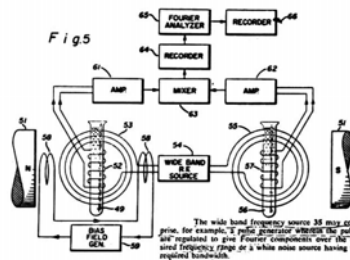
## RF Pulse. 1965

1965-6: Richard Ernst and Weston Anderson (Varian) use pulse excitation to produce the first spectrum.

From Weston Anderson's Engineering Notebook dated June 3, 1964, outlining the idea of pulse-excited high resolution NMR spectroscopy.



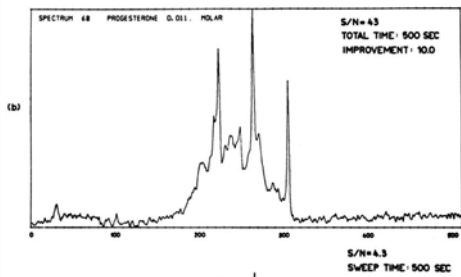
Nov. 22, 1966 R. H. VARIAN 3,287,629  
 STROMAGNETIC RESONANCE METHODS AND APPARATUS  
 Original Filed Aug. 29, 1966 3 Sheets-Sheet 3



Unitat de RMN Centres Científics i Tecnològics

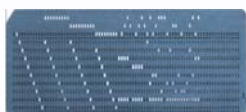


Richard Ernst & Wes Anderson, Rev. Sci. Instr. 37, 93 (1966)



Initially it took several hours for the processing of spectra

The computers used punch cards for data entry




Fourier transform (top) and conventional spectra of 0.011 M progesterone showing sensitivity enhancement by a factor ten

Unitat de RMN Centres Científics i Tecnològics





## Commercial FT-NMR 1967..... 2XXX



---

### Nicolet NT-150 NMR Spectrometer (1971)






### Advantages of FT over CW

- Faster data collection (Fellgett Advantage or Multiplex advantage).
- Signal averaging allowed better sensitivity (S/N) and the observation of less sensitive and less abundant nuclei.
- Allowed advancements in solid-state NMR.
- Allows the use of multipulse sequences.
- Allowed the development of NMR Imaging.

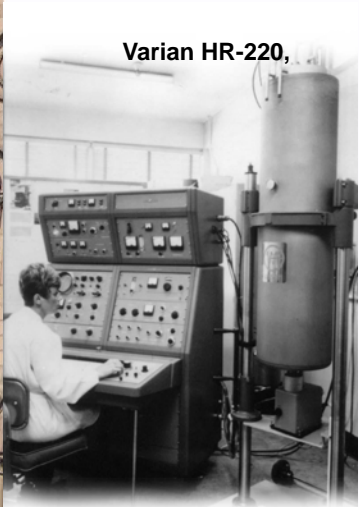
*Unitat de RMN Centres Científics i Tecnològics*

## Superconducting magnets




---

### Varian HR-220,



1966


### Bruker Avance 1000 MHz



Lyon, France in July 2009

*Unitat de RMN Centres Científics i Tecnològics*

## Solid State NMR

U    
 B Universitat de Barcelona

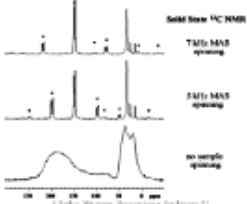
**Solid-State NMR:**

While advances in NMR through resolution improvements occurred with liquids, solid-state spectra remained broad and featureless.

1958 Andrew and Lowe show that rapid sample spinning narrows solid-state resonances.


1965-1975 Using methods incorporating sample spinning and *FT* pulse techniques, several labs make solid-state NMR as information-rich as liquids spectra (Waugh, Pines, Mansfield and Vaughn).

1990's- Applications for Bio-molecules become increasingly useful (membrane bound proteins, etc.).



Unitat de RMN Centres Científics i Tecnològics


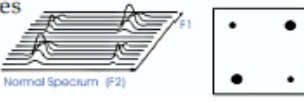
## 2D techniques

U    
 B Universitat de Barcelona

**Multi-Dimensional Techniques:**


1971 Jean Jeneer conceives the first two dimensional experiment.

1976 Richard Ernst executes and publishes the first 2D experiment (COSY).

1976- Explosions of new techniques and applications.

1985 Kurt Wüthrich publishes first solution-phase protein structure determined by NMR measurements.




1991 Ernst wins the Nobel Prize in Chemistry

2002 Wüthrich wins the Nobel Prize in Chemistry

14

Unitat de RMN Centres Científics i Tecnològics

## R.R. Ernst Nobel Prize 1991



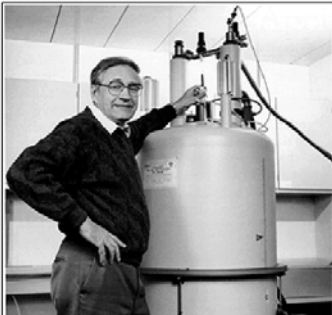
Universitat de Barcelona

### The Nobel Prize in Chemistry 1991

The Royal Swedish Academy of Sciences has awarded this year's Nobel Prize in Chemistry to

**Richard R. Ernst**  
ETH, Zürich, Switzerland


for his contributions to the development of the methodology of high resolution nuclear magnetic resonance (NMR) spectroscopy.



Richard R. Ernst's revolutionary development of the methodology of nuclear magnetic resonance spectroscopy has transformed NMR into maybe the most important instrumental technique within chemistry today. Ernst has contributed more than anybody else to this development by the discoveries of Fourier-transform NMR and two-dimensional (2D) NMR.


*Unitat de RMN Centres Científics i Tecnològics*

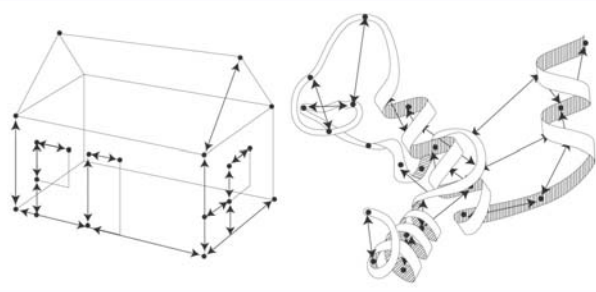
## 2002 Nobel Prize in Chemistry



Universitat de Barcelona

### Kurt Wüthrich






Showed that NMR was possible for proteins

*Unitat de RMN Centres Científics i Tecnològics*

**More**


U  
B Universitat de Barcelona

**NMR Imaging (MRI)**

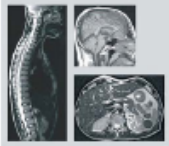
 **1973** Paul Lauterbur (SUNY, Stony Brook) uses magnetic field gradients in three dimensions and computer axial tomography (CAT)-scan (back-projection) to produce the first NMR images.

**1975** Kumar, Welte and Ernst develop a switched time domain gradient method for the basic reconstruction method (which is used in MR imaging today).

**1975** Peter Mansfield et. al., develops a phase encoding technique for image collection and publishes first human body part image (finger cross-section).



**1983** First commercial imager is introduced.



**2003** Lauterbur and Mansfield win Nobel Prize in "Medicine or Physiology"

19

Unitat de RMN Centres Científics i Tecnològics

U  
B Universitat de Barcelona

**NMR Overview:**

The technique has developed rapidly during its brief history. Advances in instrumentation, especially magnet technology has allowed many different applications. The applications fall into three fairly different groups:

**Liquid-State**  
(High Resolution)

- Small Molecule Characterization
- Biomolecular Structure & Function


**Imaging:**


- Micro-Imaging (non-clinical)
- In-vivo spectroscopy
- Diagnostic Applications
  - Clinical Imaging
  - Functional MRI

**Solid-State**

- Material Characterization
- Biomolecular Structure & Function

Unitat de RMN Centres Científics i Tecnològics



 Universitat de Barcelona

*Unitat de RMN Centres Científics i Tecnològics*