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Tema -3

Instrumentation

Continuous-Wave (CW) Instrument

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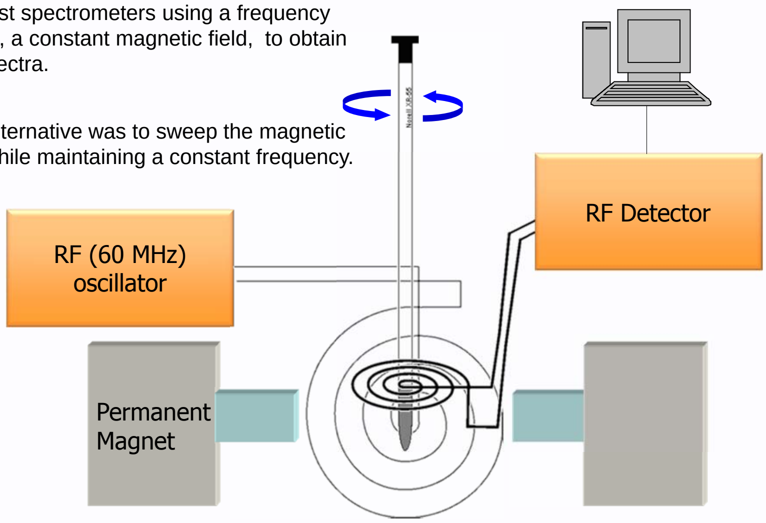
The first spectrometers using a frequency sweep, a constant magnetic field, to obtain the spectra.

One alternative was to sweep the magnetic field while maintaining a constant frequency.



Variable magnetic field – 1.41 T ± few millionths of T

Superconducting Magnet

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liquid helium temperature **(4K ≈ -269 °C),**
liquid N_2 (75K≈ -198 °C)

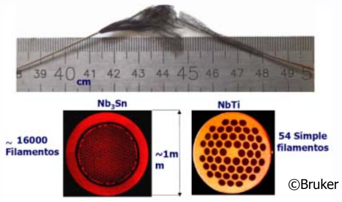
Solenoid coil from superconducting

Nb-Ti Wires

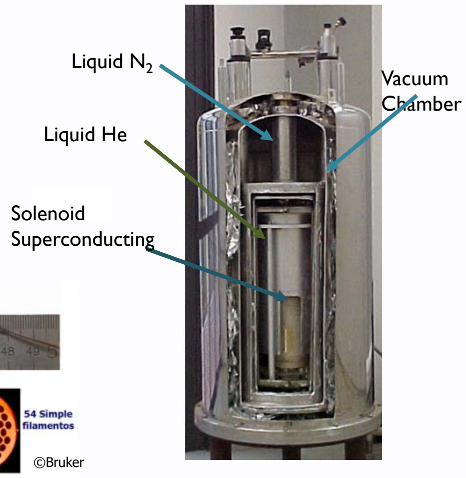
Nb_3Sn y Nb-Ti Wires

$(NbTa)_3Sn$ Wires (magnets at 2ºK)

Use up to 300 Km of wire!



Magnet



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Subcooled Magnet (800 MHz)

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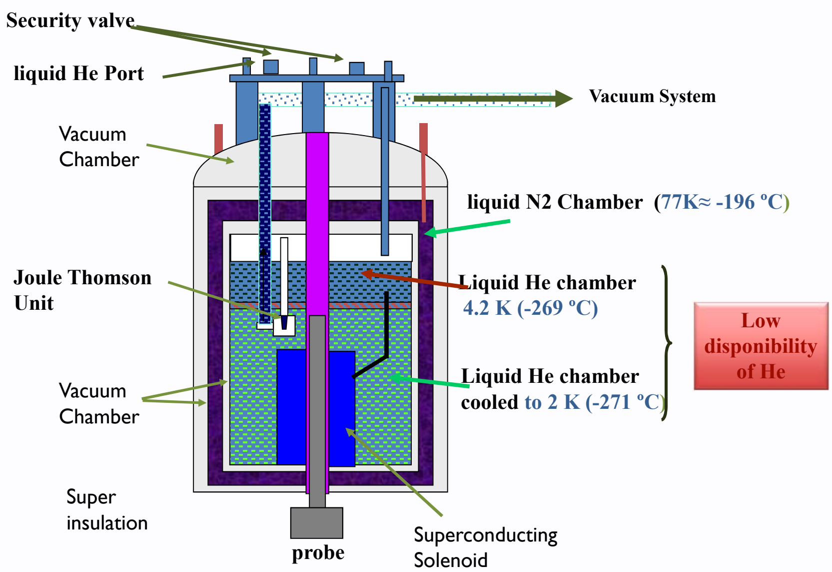

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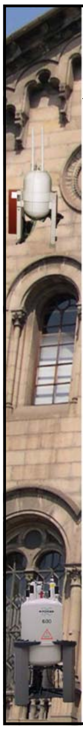

Shielded Magnets

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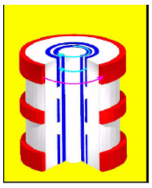
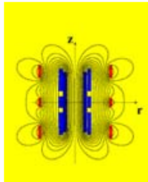


Blue Main Coil
Red Coils to Shielded

coil outside of the main coil which cancel out much of the fringe field



©Bruker

New Coils to minimize the stray field

- Excellent homogeneity and stability of Magnetic field
- External field perturbations are efficiently attenuated
- Low external residual magnetic field
- Minimize laboratory space requirements

Shielded Magnets in NMR Facility

3 Mercury-400 MHz NMR
1 VNMRS-500 MHz
1 Bruker Avance III 600 MHz

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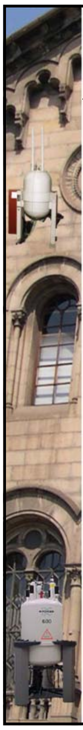

The Stray field in the NMR spectrometers

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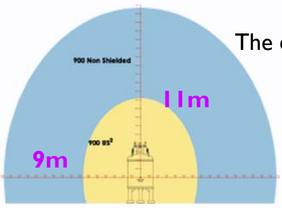
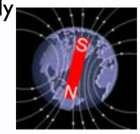
NMR magnetic field: Tesla or specifying the ^{1}H Larmor frequency for the magnet.



The earth's magnetic field is approximately

10^{-4} T (about 0.5-0.1 gauss)



1 T=10.000 Gauss

The 5 gauss Level

Magnets in NMR Facility

Field (Teslas)	^{1}H frecuency (MHz)	Radial Distance	Axial Distance
18.78	800	6.3 m	8.0 m
14.08	600	3.6 m	4.0 m
14.08	600	0.7m	1.4 m
11.74	500	2.8 m	3.6 m
11.74	500	0.8m	1.25 m
9.39	400	0,9 m	1,5 m
7,05	300	1.8 m	2.0 m

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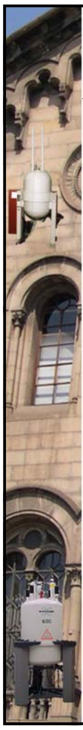

Magnetic forces

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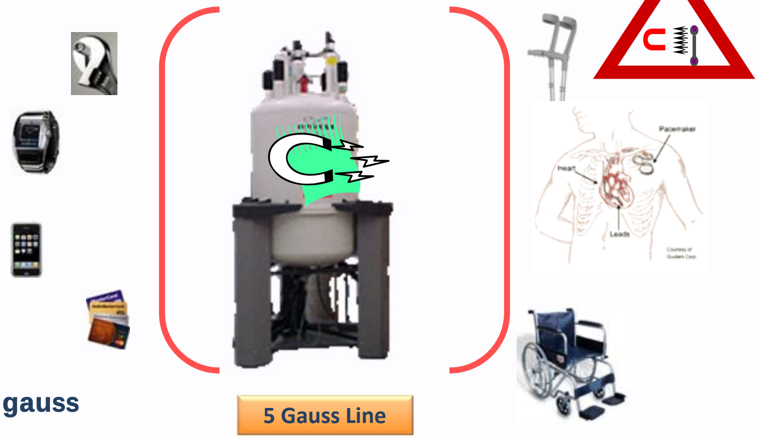
Metal objects must remain outside the 5-gauss perimeter.
The greater the mass of the object, the more strongly it is attracted by the magnet.
The shorter the distance to the magnet, the stronger the force.



10 gauss

5 Gauss Line

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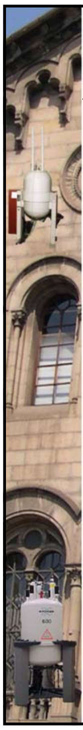

QUENCH

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Quench: is the sudden loss of supercondutivity in the magnet's main coil that produces a rapid evaporation helium liquid to gas

Quench Video

Don´t

•Sock or moving the magnet
•Manipulating security ports
•Incorrect transferring cryogenic liquids.

In the event of a "magnet quench:

•Leave the room immediately
•Do not re-enter the room until the oxygen level has returned to normal
•Activate the ventilation system

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NMR-Sensitivity

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NMR signal (**s**) depends on:

1) Number of Nuclei (**N**) (limited to field homogeneity and filling factor)
2) Gyromagnetic ratio (in practice γ^3)
3) Inversely to temperature (**T**)
4) External magnetic field ($B_o^{2/3}$, in practice, homogeneity)
5) B_1^2 exciting field strength (RF pulse)

$$N_\alpha / N_\beta = e^{\Delta E / kT} \qquad \Delta E = \gamma h B_o / 2\pi$$

Increase energy gap → Increase population difference → Increase NMR signal

$$\uparrow \Delta E \equiv \uparrow B_o \equiv \uparrow \gamma$$

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NMR-Sensitivity in different nuclei

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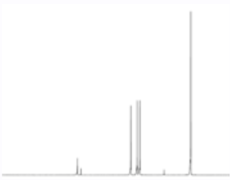
Relative sensitivity of ^1H, ^{13}C, ^{15}N and other nuclei NMR spectra depend on

- Gyromagnetic ratio (γ)
- Natural abundance of the isotope

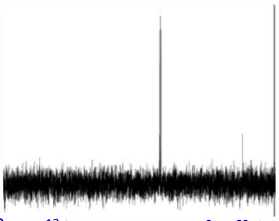
γ - Intrinsic property of nucleus can not be changed.

^1H is ~ 64x as sensitive as ^{13}C and 1000x as sensitive as ^{15}N !

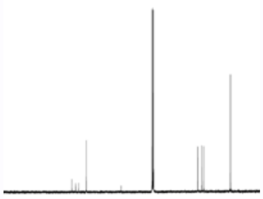
Considering that the natural abundance of ^{13}C is **1.1%** and ^{15}N is **0.37%** the relative sensitivity increases to **~6,400x** and **~2.7x10^5x** !!



^1H NMR spectra of caffeine
8 scans ~12 secs



^{13}C NMR spectra of caffeine
8 scans ~12 secs



^{13}C NMR spectra of caffeine
10,000 scans ~4.2 hours

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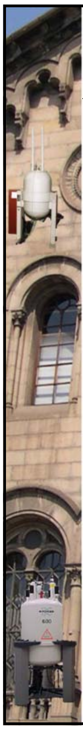

Magnet evolution

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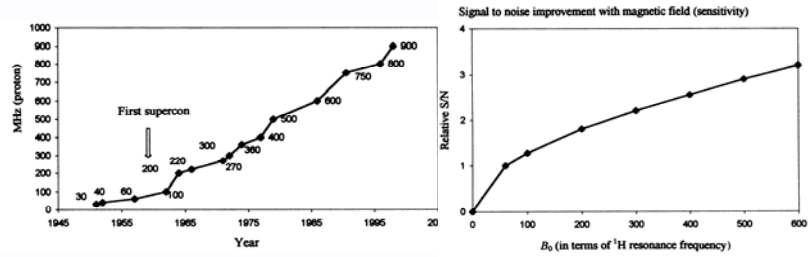

Increase in Magnet Strength is a way to Increase Sensitivity

The sensitivity is dependent on γ^3 and B_0^2

~90 MHz
(1967)

(18.78 Tesles)
~2,000,000 $

2009-2010
1GHz (23.5 Tesla)
13.000.000 €

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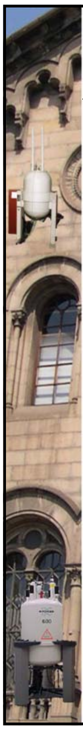

Higher frequency → more dispersion

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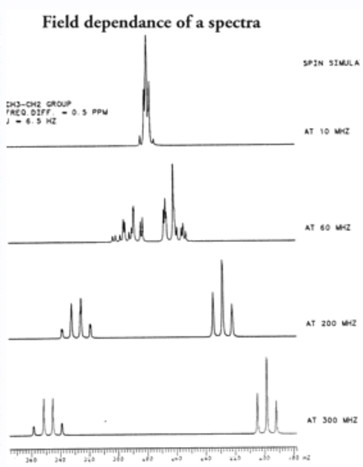

500 MHz
PPM
10 0

600 MHz
PPM
10 0

800 MHz
PPM
10 0

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Probe

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The coils have inductors and capacitors that serve as antennae to transmit and receive RF to/from sample.

Contain coils for

- Transmitting the RF pulse
- Detecting the NMR signal
- Create RF Gradient field
- Observing the lock signal

Neumatic System for:

- Insert and eject the sample
- spin the sample
- temperature regulation

Thermocouples and heaters to temperature regulation

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Sensitivity of an NMR Probe

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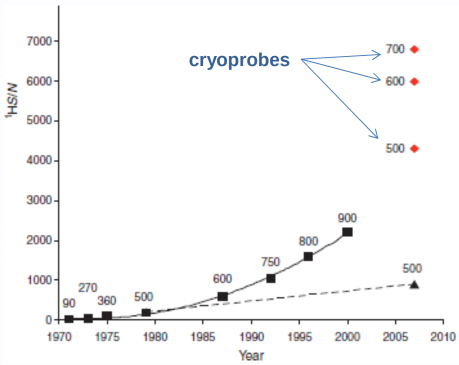

$$\frac{S}{R} = K \frac{N^2\, \nu^{3/2}}{T_S} \sqrt{\frac{C_f\, Q_p\, V_s}{T_p^2\, T_R}}$$

N = concentration Nuclear spins

V_0 = resonance frequency

V_s = volume sample

Ts = temperature sample

C_f = Coil filling factor

Q_p = Quality factor probe

T_p = noise temperature probe

T_R = noise temperature receiver

Núcleo	B800	B600-II	B600-I	V500S	V500	M400	I300
1H	>7000:1	>7000:1	708:1	730:1	726:1	220:1	84:1
13C	> 700:1	> 1100:1	335:1	240:1	83:1	158:1	83:1
31P	---	---	200:1	135:1	24:1	183:1	50:1
19F	---	---	450:1	450:1	---	175:1	66:1

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Cryoprobes.

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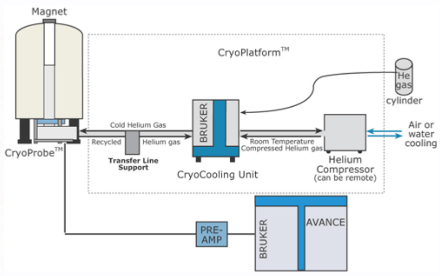
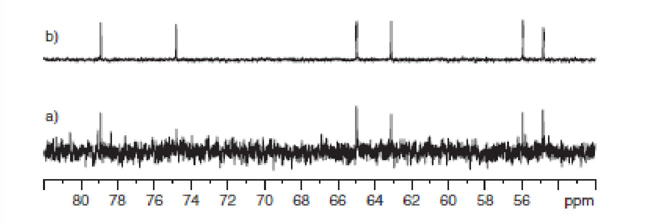


Increased sensitivity at a "**reasonable**" cost
~300.000 €

The same sample, the same conditions **but** Different results



Cryoprobe

Standard probe

Científics i Tecnològics

Cryoprobes description

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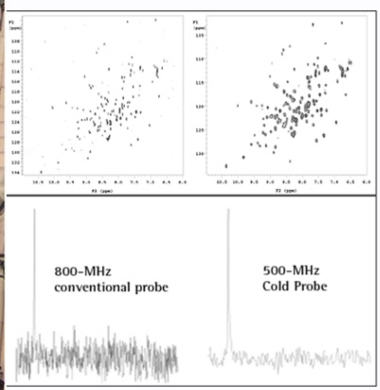
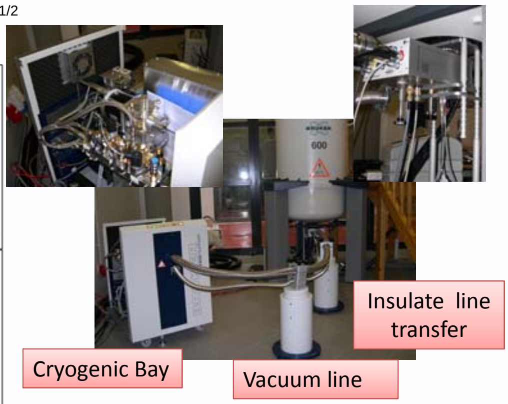
Cryoprobes use helium gas to cool the coils to 20-25K while the sample remains at room temperature. The sensitivity of the probe can be increased by a factor of four.
1H in B600 SN > 7000

Reduce the Noise in the probe (25 K) and in the Preamp (70 K)

$S/N \sim 1/(T_cR_c + T_a[R_c + R_s] + T_sR_s)^{1/2}$





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Tunning the probe

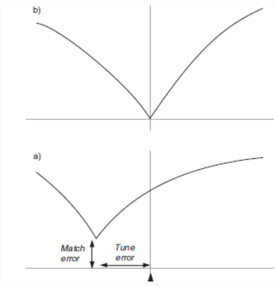
Placing the sample into the probe affects the probe tuning

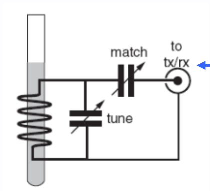
Solvent, buffers, salt concentration, sample concentration and temperature all have significant impact on the probe tuning

The coils should be optimized for each sample: **"tunning the probe"**

Adjust two capacitors: match and tune

Goal is to minimize the reflected power at the desired frequency





Power submitted to transmitter and receiver is maximized

Tuning capacitor changes resonance frequency of probe

A poorly tuned probe causes a degradation of sensitivity

Matching capacitor matches the impedance to a 50 Ohm cable

Optimize the absorption of RF to the sample (less heat dissipation)

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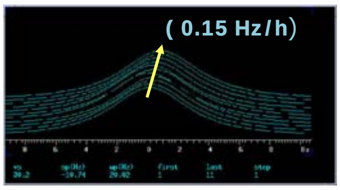
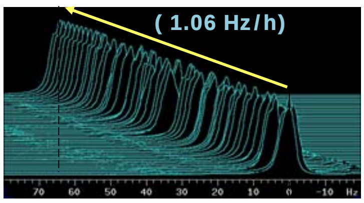
Lock System, *Field Drift*

NMR magnetic field slowly drifts with time

The field drift can be affect to the NMR Signals





- NMR probes contains an additional transmitter coil tuned to deuterium frequency
- Need to constantly correct for the field drift during data collection
- Deuterium NMR resonance of the solvent is continuously irradiated and monitored to maintain an on-resonance condition

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The feedback of lock System

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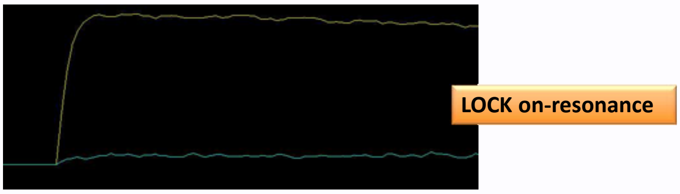
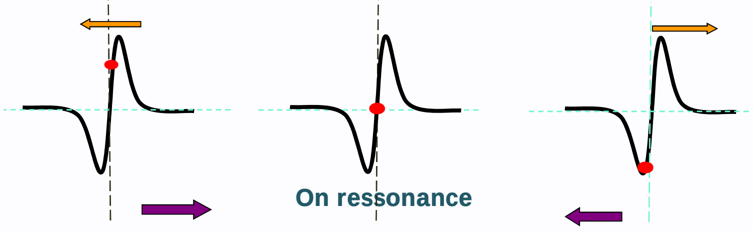


Changes in the intensity of the reference signal, controls a feedback circuit



With shim coil (Z0) creates a small magnetic field to moved the main field to place and the lock-signal back into resonance

Homogeneity in the magnetic field

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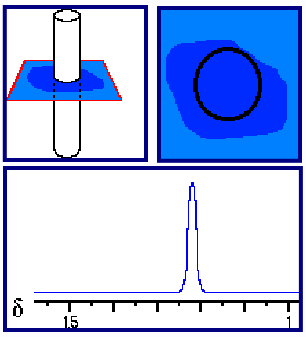
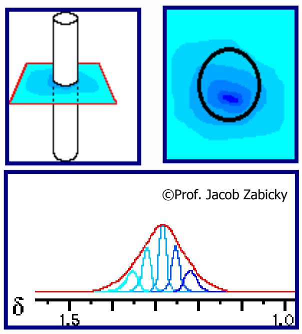


If the magnetic field is the same throughout the volume of active probe

narrow NMR signals



If the magnetic field is heterogeneous across the sample

broader NMR signals

The same proton experience different B_0 magnetic field

Adjust the homogenity : Shim Coils

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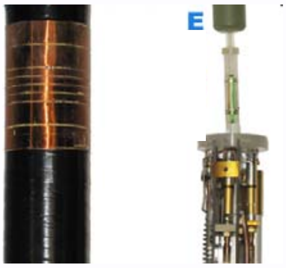
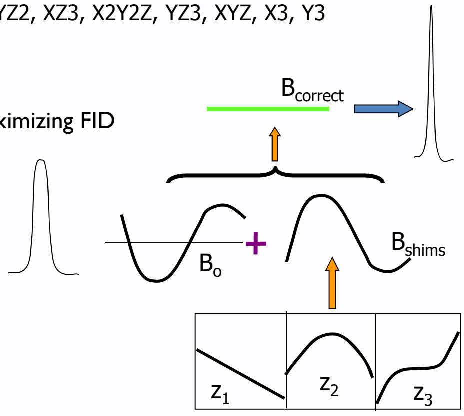
The electric currents in the shim coils create small magnetic fields which compensate the inhomogenieties in the magnet
The shim coils vary in the geometric orientation and function

- **Z1, Z2, Z3, Z4**, Z5, Z6, Z7
- X, XZ, XZ2, X2Y2, XY, Y, YZ, YZ2, XZ3, X2Y2Z, YZ3, XYZ, X3, Y3

Optimize shims by:

- minimizing line-width
- maximizing lock signal or maximizing FID





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Shimming

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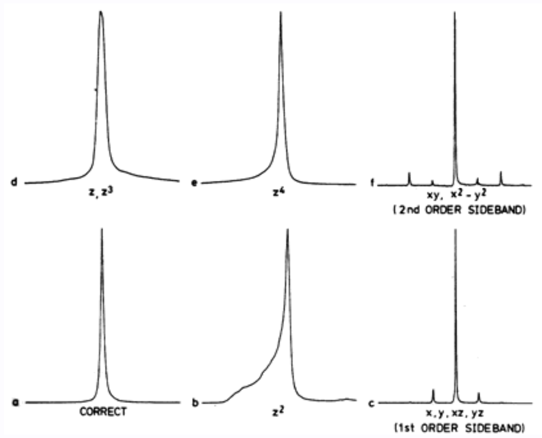
Optimize shims by

- minimizing line-width,
- maximizing lock signal
- maximizing FID

Examples of poor line-shapes due to shimming errors



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Gradient Shimming

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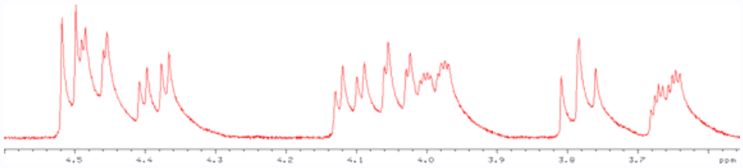
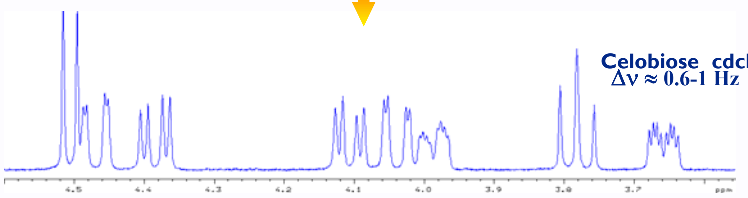
Mercury and VNMRS Gradient map (^{2}H or ^{1}H)



***N° iterations* (10 max)**

gradient shim (Z 1-4)
3 iterations (aprox 1 minute)



Celobiose cdcl3
Δν ≈ 0.6-1 Hz

Line widths near of 1Hz

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Spinning the Sample

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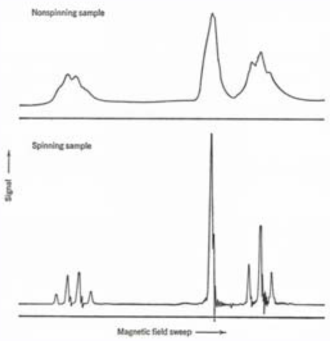
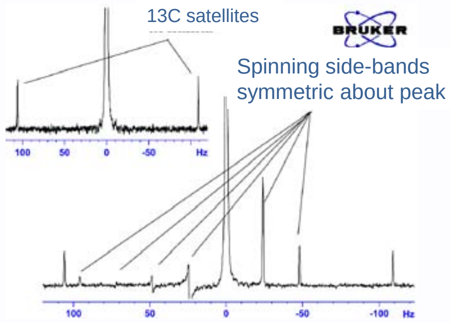
Improves effective magnetic field homogeneity by averaging inhomogeneities in the magnet
- Z – shims are also known as spinning shims

Spinning the sample causes symmetric side-bands at intervals related to spinning rate
- Non-spinning shims (X,Y) problems
- Creates artifacts → streaks or T_1 ridges from spinning side-bands and spinning instability

Samples are never spun for multi-dimensional NMR experiments





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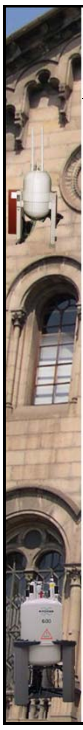

Environment Stability

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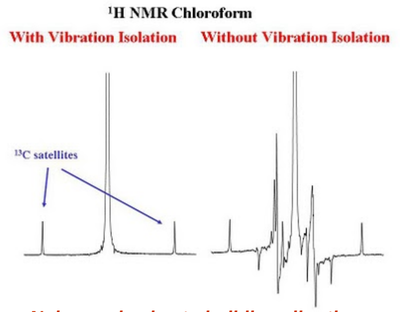
Changes in the environment during data acquisition may have strong negative impacts on the quality of the NMR data

Common causes of spectra artifacts are:

- Vibrations (building, HVAC, etc)
- Temperature changes

The longer the data acquisition, the more likely these issues will cause problems

The lower the sample concentration (lower S/N) the more most obvious are these artifacts.



Noise peaks due to building vibrations

Dampers

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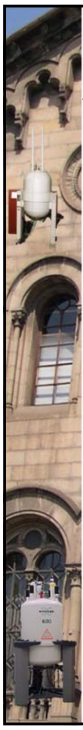

Variable temperature accessory

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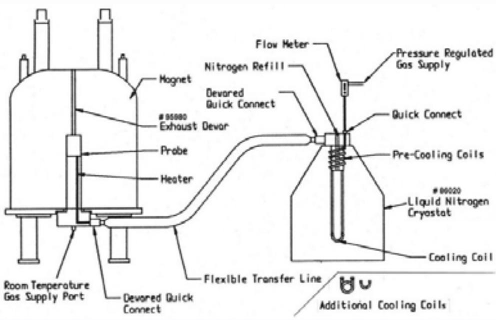
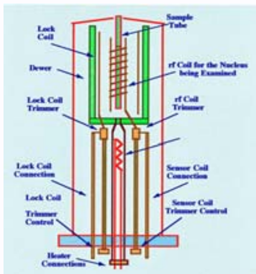
All NMR experiments must be performed with temperature conditions regulated.

You can change the temperature of the experiments.

- Raise the temperature to promote the exchange process.
- Lower the temperature in the case of instability of the compound or to slow a process.





The temperature range depend to the probe characteristics

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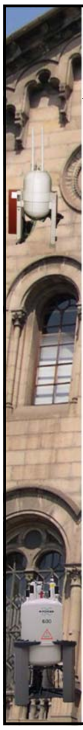

Efects the temperature changes

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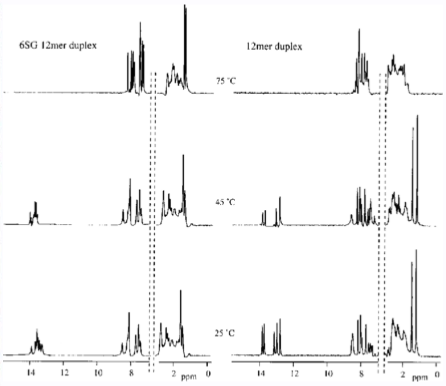
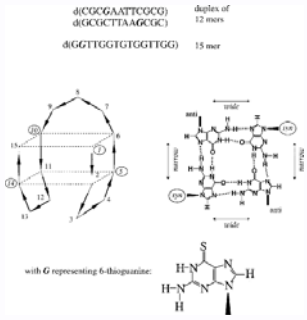
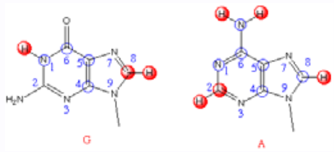
The chemical shift may depend of temperature:

Changes in the equilibrium position, proton exchange , others





The 400 MHz proton spectra of the unmodified and 6SG-containing 12mer DNAs are shown as a function of temperature with the samples in 95% H_2O/ 5% D_2O. The resonances of **the imino protons are in the 12–14 p.p.m. region.**



Nucl. Acids Res. (1999) 27 (14): 2860-2867.

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