# Pulsed vs. Continuous Wave (CW)

## Electron Paramagnetic Resonance (EPR)

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- CW-EPR
- Pulsed EPR (FID and Echo)
- Relaxation Times T1 and T2
- ENDOR-EPR

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# What is EPR ?
# (Electron Paramagnetic Resonance)

- EPR is a form of magnetic resonance spectroscopy that is used to detect unpaired (or “free”) electrons.

- The physical principle of EPR is similar to NMR, but EPR measures unpaired electrons instead of protons.

- EPR is the only technique that provides direct detection of free radicals and other samples that contain unpaired electrons.

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# EPR

- Interaction of **ELECTRONS** with external **MAGNETIC FIELDS** and with its **SURROUNDING**

**Reguirements:**

**(1)At least ONE unpaired electron**
**(2)Resonance condition matched**
**(3)Laws of the conservation of both energy and momentum obeyed**

# What is EPR ?
# (electron paramagnetic resonance)

EPR samples have one absolute requirement…they must contain unpaired electrons.

Common examples:

**Transition metal ions** - Fe, Cu, Mn, Co, Mo, Ni

**Free Radicals** – Typically carbon, nitrogen or oxygen containing compounds

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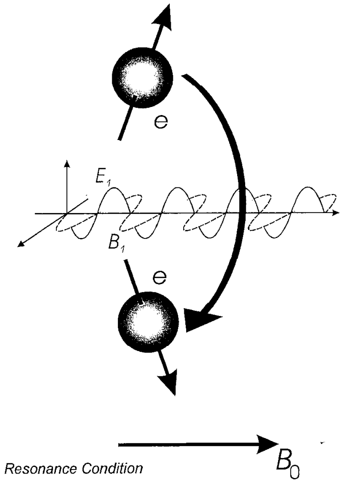
# Electron Spin Resonance in the Presence of the Magnetic Field



Resonance Condition

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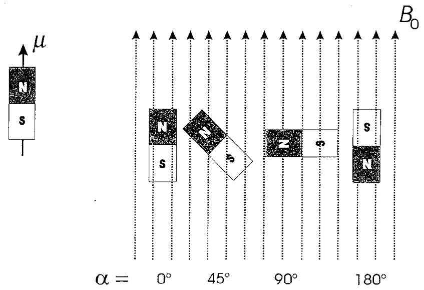
*Energy of a Magnetic Moment $\mu$ in a Magnetic Field $B_0$*

**Classical:**



*Every Orientation is allowed*

*The Energy depends on the orientation:*

$$E = -\mu \cdot \mathbf{B}_0$$
$$= -|\mu \cdot \mathbf{B}_0| \cdot \cos\alpha$$

*Energy at maximum:* $E_{max}$ at $\alpha = 180°$

*Energy at minimum:* $E_{min}$ at $\alpha = 0°$

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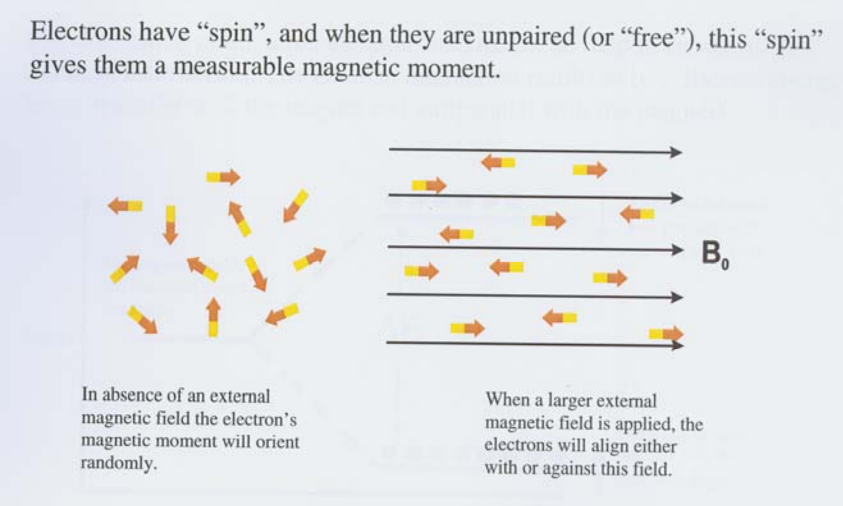


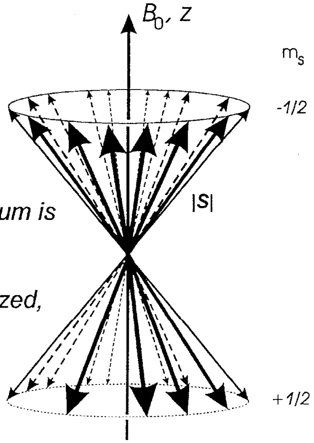
Electrons have "spin", and when they are unpaired (or "free"), this "spin" gives them a measurable magnetic moment.

In absence of an external magnetic field the electron's magnetic moment will orient randomly.

When a larger external magnetic field is applied, the electrons will align either with or against this field.

*Energy of a Magnetic Moment $\mu$ in a Magnetic Field $B_0$*

**Quantum Mechanics:**



*Only distinct orientations are allowed: The angular momentum is QUANTISIZED*

*Thus, the energy E is quantisized, too.*

$\hat{S} = (S_x, S_y, S_z)$    $m_S = +S, S-1, \ldots, -S$

$|\hat{S}| = S(S+1))^{1/2}$

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## What information does the EPR spectrum provide?

### Information commonly gained from the EPR spectrum

- Unambiguously determines that “free” or unpaired electrons (e.g.,free radicals) are present in the sample.
- Reveals the molecular structure and the environment near the electron.
- Indicates the degree of molecular motion in a sample with unpaired electrons.

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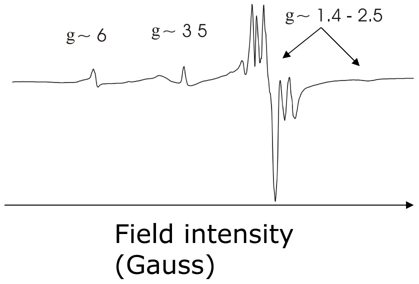
## What information does the EPR spectrum provide?

The **g-factor** helps us characterize the type of EPR sample we are measuring. For example, it can identify a specific metal ion, its oxidation state, spin state and coordination environment.

Cytochrome oxidase is a metalloprotein with more than one metal center. The g-values are used to identify and characterize the different centers.



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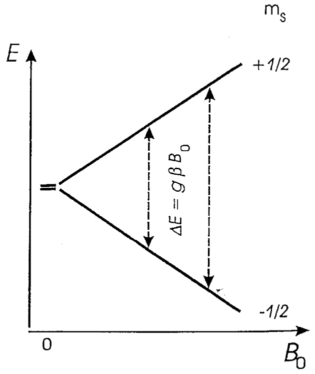
## Electron Zeeman Effect

***Zeeman Interaction***

$\hat{H} = -\hat{\mu}\cdot \boldsymbol{B}$    $\hat{\mu} = -g\,\beta\,\hat{\boldsymbol{S}}$

$E = \pm\frac{1}{2}\,g\,\beta\,\boldsymbol{B}_0$



# *g*-Factor

*The g-Factor*

*A dimensionless parameter*

*Classical Approach:*

*„$g_e$ would be the correction factor for the anomalous magnetic moment of the electron“*

*Free Electron:*

$g_e = 2.0023193043$

*In matter:*

$h\nu = g\beta B_0$ ; $g \neq g_e$

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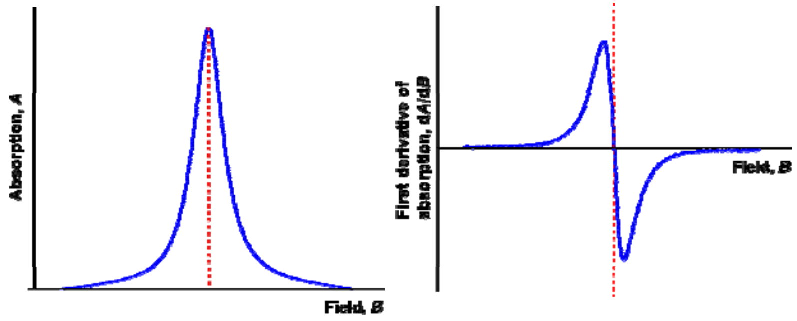
# Spectra



Since in EPR spectroscopy, one typically modulates the applied field H or $B_0$ (in order to obtain an a.c. signal for amplification), the derivative of the absorption is recorded.

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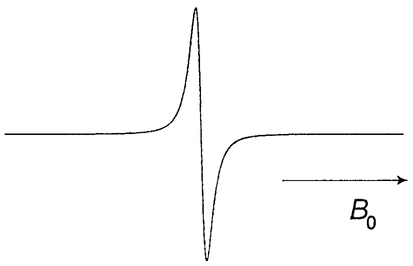
*Introduction*:

What parameters characterize CW-EPR spectra?



*g*-value

Linewidth

Line shape

Line Intensity

Saturation bahaviour

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# Transition, Saturation and Relaxation

**Problem:**

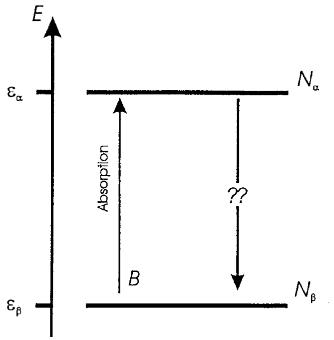
*(i) Absorption of energy $\Delta E$*

$N_\alpha/N_\beta \longrightarrow 1$

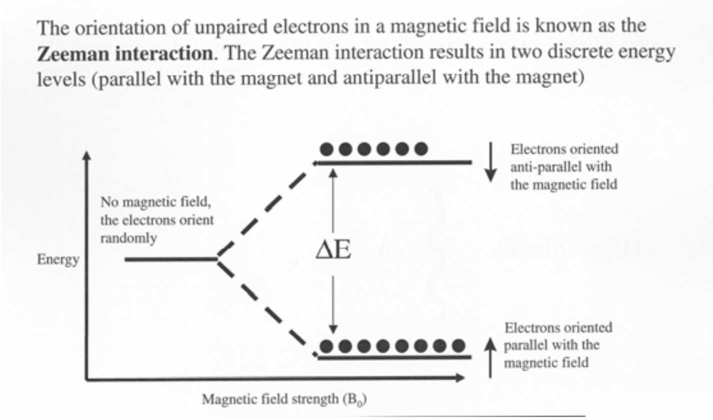
$\Delta N \longrightarrow 0$

*(ii) Thermal Equilibrium*

$N_\alpha/N_\beta = \exp\{-\Delta E/k_B T\}$

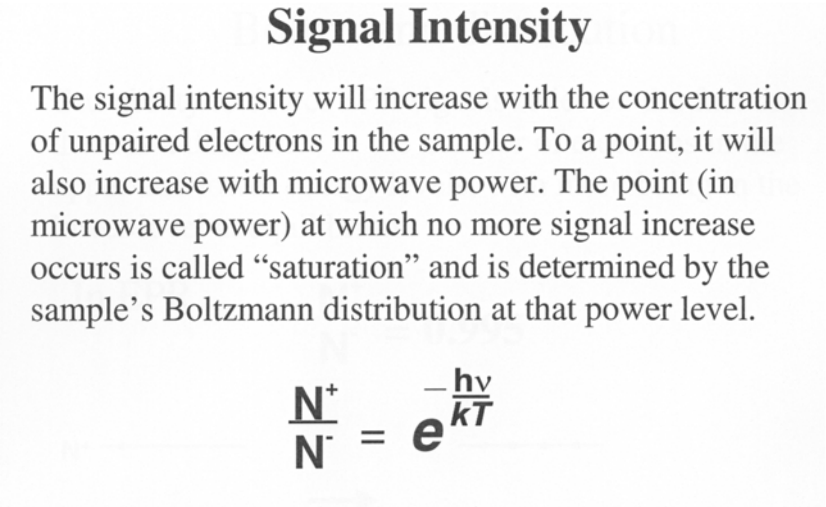


# Electron Zeeman Effect



The orientation of unpaired electrons in a magnetic field is known as the **Zeeman interaction**. The Zeeman interaction results in two discrete energy levels (parallel with the magnet and antiparallel with the magnet)

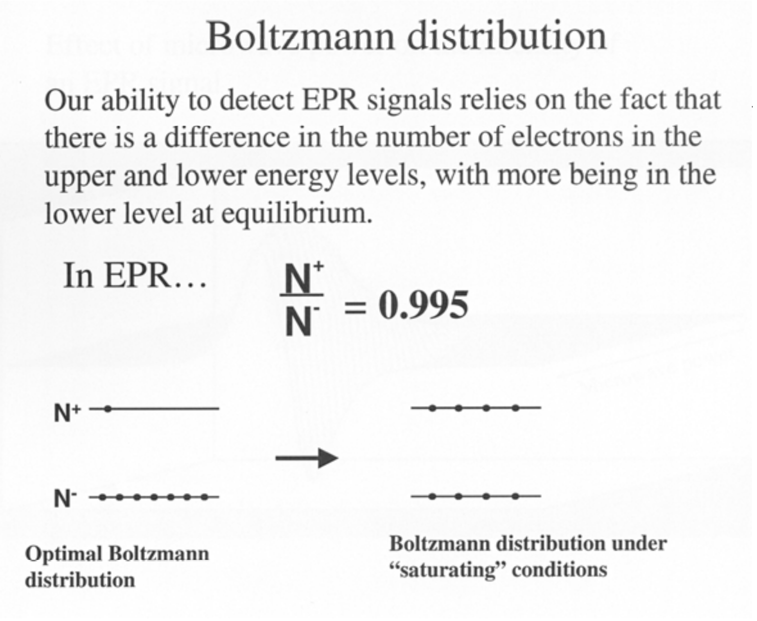
# Signal Intensity



The signal intensity will increase with the concentration of unpaired electrons in the sample. To a point, it will also increase with microwave power. The point (in microwave power) at which no more signal increase occurs is called “saturation” and is determined by the sample’s Boltzmann distribution at that power level.

$$\frac{N^+}{N^-} = e^{-\frac{h\nu}{kT}}$$

# Boltzmann distribution



Our ability to detect EPR signals relies on the fact that there is a difference in the number of electrons in the upper and lower energy levels, with more being in the lower level at equilibrium.

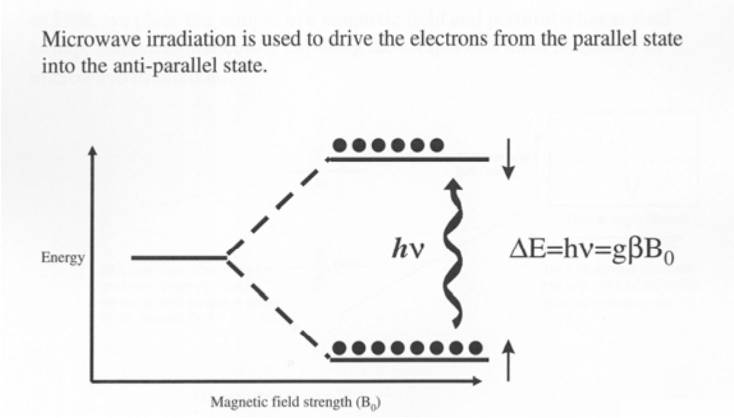
In EPR… $\frac{N^+}{N^-} = 0.995$

**Optimal Boltzmann distribution**

**Boltzmann distribution under “saturating” conditions**

| | NMR | EPR | UV/VIS |
|---|---|---|---|
| **Frequency ν** | (10-1000) MHz | (1-600) GHz | $10^{15}$ Hz |
| **Wavelength λ** | meters | cm - mm | (200 - 730) nm |
| **spon. Em.** | *neglectable* | *neglectable* | *important* |
| ***ΔE vs. therm. E*** ($T > 77$ K) | $k_BT >> \Delta E$ | $k_BT >> \Delta E$ | $k_BT << \Delta E$ |
| ***ΔN/N*** (3.4 kG, 300 K) | ca. $10^{-6}$ | ca. $10^{-3}$ | ca. 1 |

# The resonance absorption is undertaken by microwave irradiation



Microwave irradiation is used to drive the electrons from the parallel state into the anti-parallel state.

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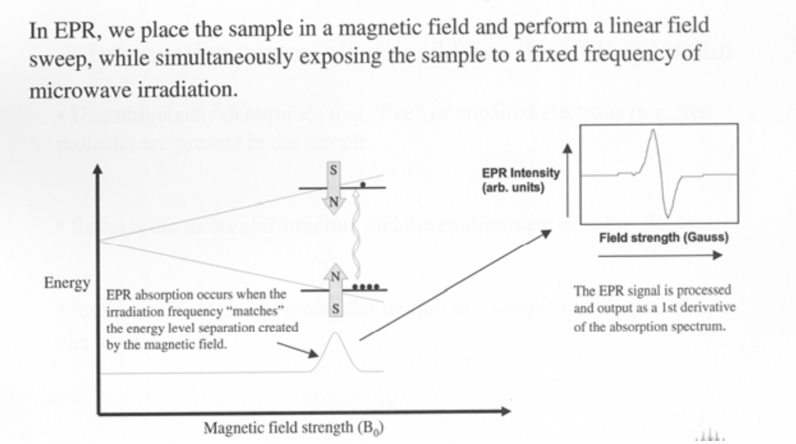
# To use the field sweep to obtain a fixed frequency. $\Delta E \approx 0.3\ \mathrm{cm}^{-1}$ ($h$*9GHz)/3*$10^3$



In EPR, we place the sample in a magnetic field and perform a linear field sweep, while simultaneously exposing the sample to a fixed frequency of microwave irradiation.

EPR absorption occurs when the irradiation frequency "matches" the energy level separation created by the magnetic field.

The EPR signal is processed and output as a 1st derivative of the absorption spectrum.

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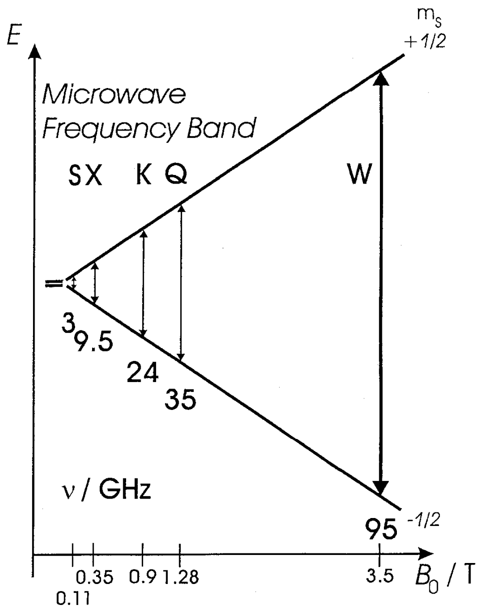
*The g-Factor: Microwave Frequency Bands*

*From S- to W-band*



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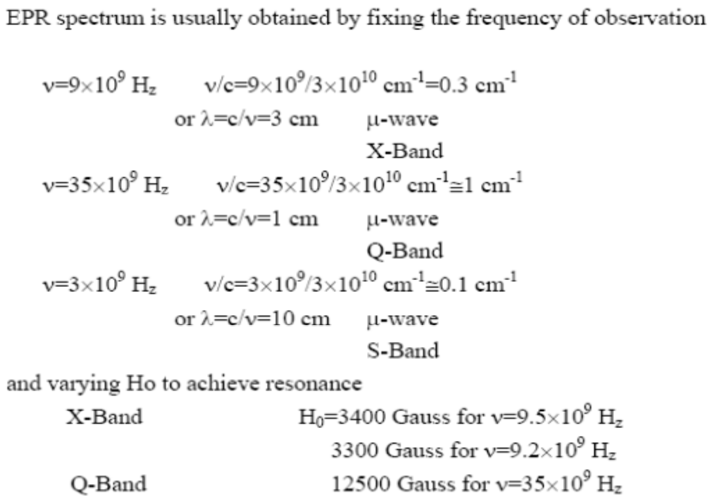


EPR spectrum is usually obtained by fixing the frequency of observation

$\nu=9\times10^9$ Hz $\quad \nu/c=9\times10^9/3\times10^{10}\ \mathrm{cm}^{-1}=0.3\ \mathrm{cm}^{-1}$
or $\lambda=c/\nu=3$ cm $\quad$ μ-wave
X-Band

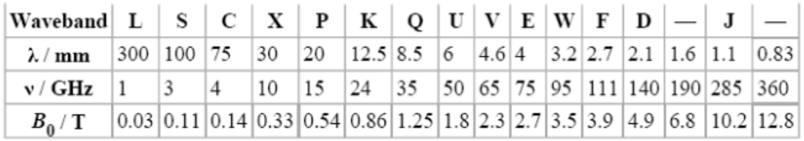
$\nu=35\times10^9$ Hz $\quad \nu/c=35\times10^9/3\times10^{10}\ \mathrm{cm}^{-1}\cong1\ \mathrm{cm}^{-1}$
or $\lambda=c/\nu=1$ cm $\quad$ μ-wave
Q-Band

$\nu=3\times10^9$ Hz $\quad \nu/c=3\times10^9/3\times10^{10}\ \mathrm{cm}^{-1}\cong0.1\ \mathrm{cm}^{-1}$
or $\lambda=c/\nu=10$ cm $\quad$ μ-wave
S-Band

and varying Ho to achieve resonance

X-Band $\quad H_0=3400$ Gauss for $\nu=9.5\times10^9$ Hz
3300 Gauss for $\nu=9.2\times10^9$ Hz
Q-Band $\quad$ 12500 Gauss for $\nu=35\times10^9$ Hz

# ESR of a Nitroxide Radical at different frequency of observation



| Waveband | L | S | C | X | P | K | Q | U | V | E | W | F | D | — | J | — |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| λ / mm | 300 | 100 | 75 | 30 | 20 | 12.5 | 8.5 | 6 | 4.6 | 4 | 3.2 | 2.7 | 2.1 | 1.6 | 1.1 | 0.83 |
| ν / GHz | 1 | 3 | 4 | 10 | 15 | 24 | 35 | 50 | 65 | 75 | 95 | 111 | 140 | 190 | 285 | 360 |
| $B_0$ / T | 0.03 | 0.11 | 0.14 | 0.33 | 0.54 | 0.86 | 1.25 | 1.8 | 2.3 | 2.7 | 3.5 | 3.9 | 4.9 | 6.8 | 10.2 | 12.8 |

EPR spectra of a nitroxide radical as a function of frequency. Note the improvement in resolution from left to right. See this page (http://hf-epr.sitesled.com/) for an animation of the above figure.

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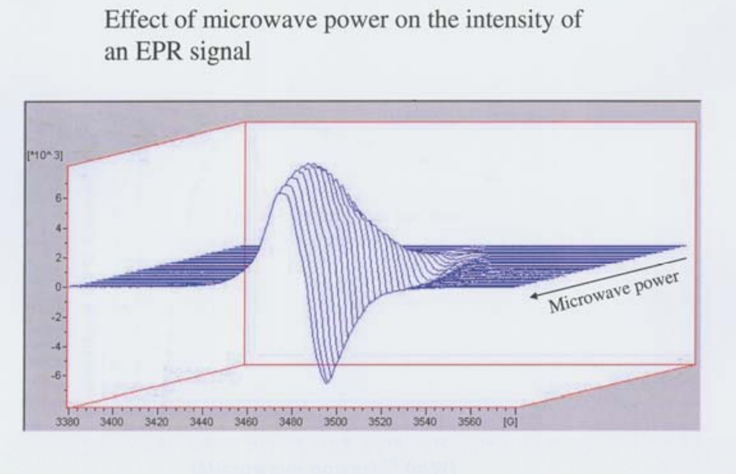

Effect of microwave power on the intensity of an EPR signal

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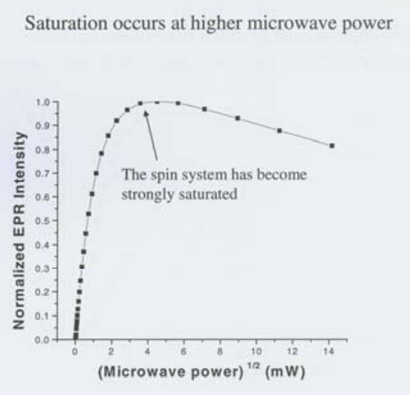
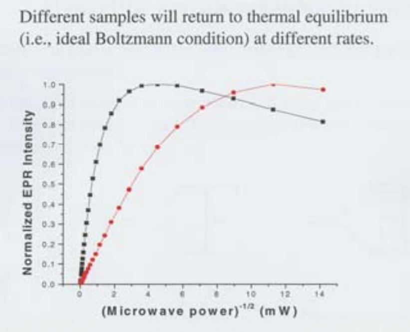


Saturation occurs at higher microwave power



Different samples will return to thermal equilibrium (i.e., ideal Boltzmann condition) at different rates.

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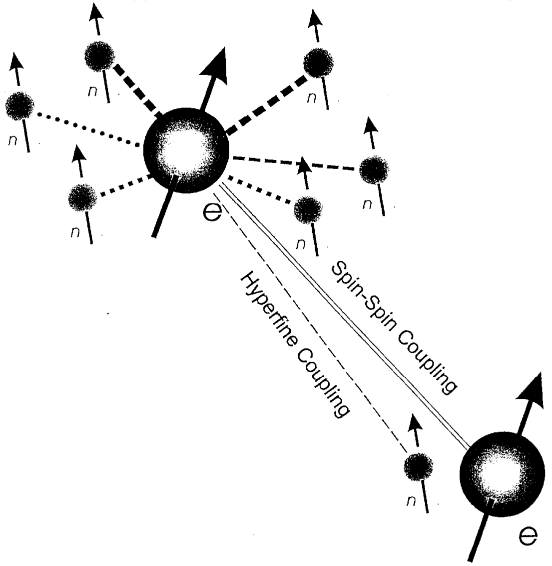


*Hyperfine Coupling and Spin-Spin Coupling*

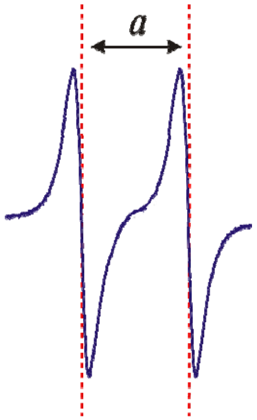
# Hyperfine Interactions

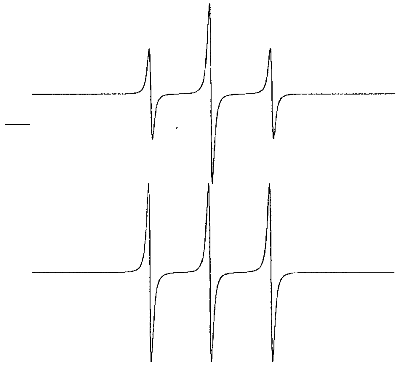
- EPR signal is ‘split’ by neighboring nuclei
  - Called hyperfine interactions
- Can be used to provide information
  - Number and identity of nuclei
  - Distance from unpaired electron
- Interactions with neighboring nuclei

$$E = g\mu_B B_0 M_S + aM_s m_I$$

  $a$ = hyperfine coupling constant

  $m_I$ = nuclear spin quantum number
- Measured as the distance between the centers of two signals





Number and amplitude ratio of lines ($I=1/2, 1, \ldots; N_I$)

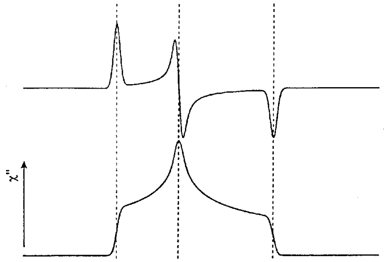
splitting of the lines ($a$)

$g$-value of the center of gravity

Linewidths of the individual lines

Line shapes (differences)

Saturation bahaviour (differences)



Anisotropy; Symmetry

Principal $g$-values

Linewidth

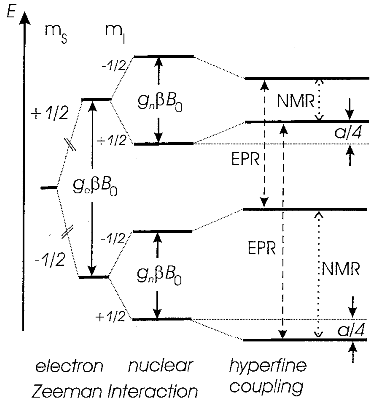
Line shape

Line Intensity

Temperature dependence

*Hyperfine Coupling: EPR and NMR Transitions*

*Electron and Nuclear Zeeman Terms + Hyperfine*



$$H = g_e\beta B_0 S_z + g_n\beta B_0 I_z + aS_z I_z$$

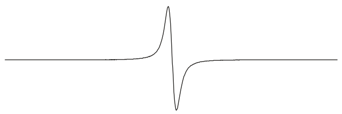
*Resonance Line Positions*

|  | EPR | NMR |
|---|---|---|
| $\Delta E =$ | $h\nu_S \pm a/2$ | $h\nu_I \pm a/2$ |

## What information does the EPR spectrum provide?

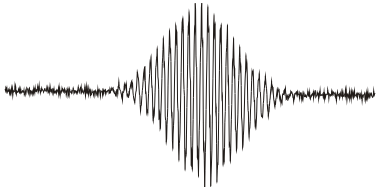
**Hyperfine helps us determine molecular structure**

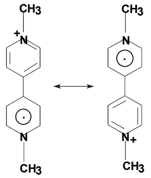
No hyperfine interaction



Simple hyperfine interaction

Multiple hyperfine interactions





## What information does the EPR spectrum provide?

### Lineshape of EPR spectra

- Samples with rapidly relaxing electrons have broader lines, while those with slower relaxation times have sharper lines.

- Weak hyperfine interactions and/or anisotropic interactions contribute to the linewidth of an EPR signal (inhomogeneous broadening)

- A high concentration of unpaired electrons in a sample can lead to line broadening due to increased “spin-spin” relaxation.

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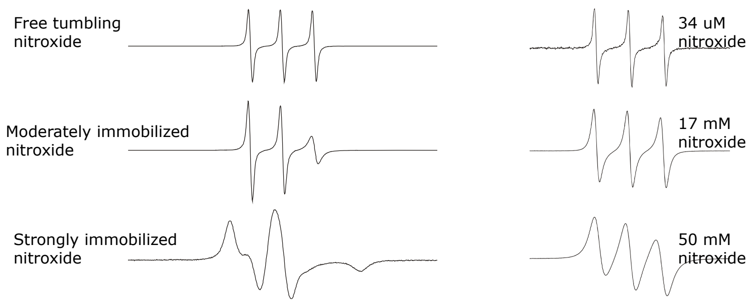
## What information does the EPR spectrum provide?

### Lineshapes help us determine molecular dynamics

Effects from molecular motion

Effects from relaxation



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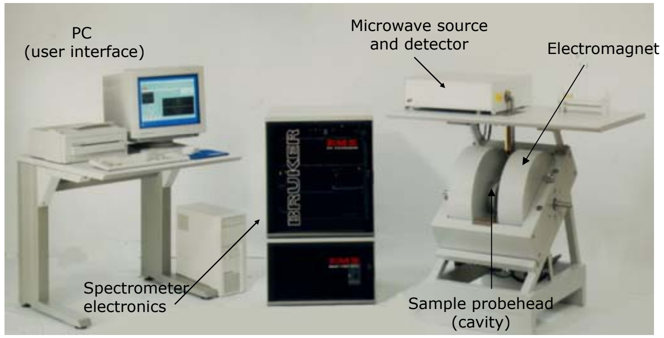
## What is required of an EPR Spectrometer?

### The modern EPR spectrometer



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## What is required in an EPR Spectrometer?

### General components

- A Gunn diode in the bridge provides stable attenuated microwave source.

- Transmission lines (usually wave guide) transmit microwaves to the cavity where they are concentrated at the sample.

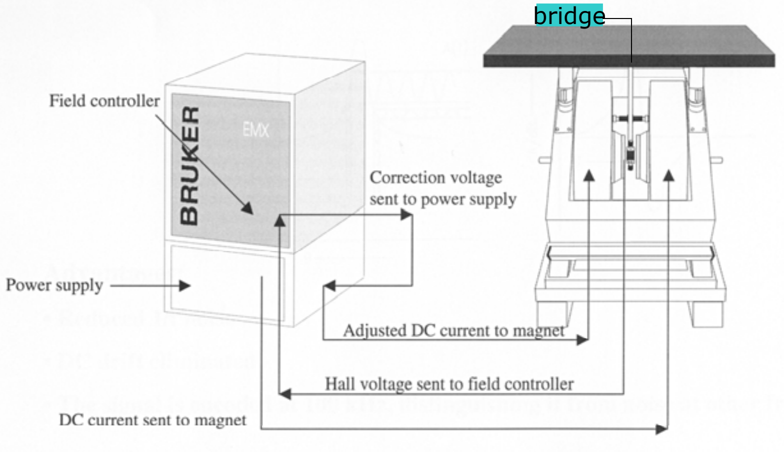
- An AFC locks the source at the cavity’s resonant frequency.

- A field controller and hall probe provide a linear, homogeneous field sweep of the electromagnet.

- A diode in the bridge detects the EPR absorption.

- The EPR signal is processed using field modulated, phase sensitive detection and is then digitized.

- Magnet/power supply generates magnetic field
- Field controller provides linear sweep of the field
- Hall probe detects field in magnet gap and provides feedback voltage to the field controller



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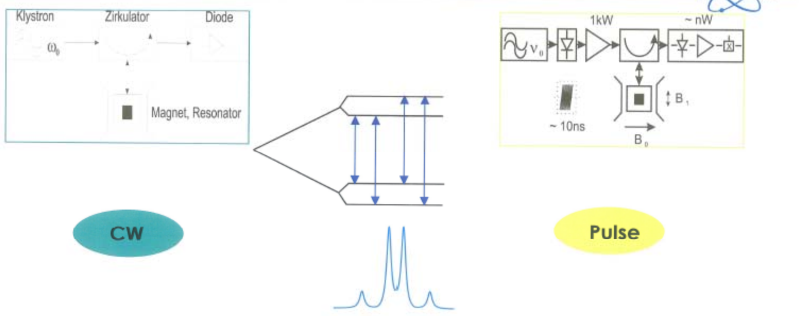
## CW- and Pulse-EPR



$H_{obs} = H_{int} + H_{ext}$

$H_{ext} << H_{int}$

$B_{mod}, v_{mod}, B_1 << A, \Delta g, 1/T_1, 1/T_2$

$H_{ext} >> H_{int}$

$\gamma B_1 >> A, \Delta g, 1/T_1, 1/T_2$

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### Basics of Pulsed Magnetic Resonance

*CW vs. FT Magnetic Resonance.* In order to understand pulsed EPR, first the difference between CW (continuous wave) and FT (Fourier Transform) techniques must be realized. We can compare these techniques by using an analogy which relates them to musical instruments, where our sample is a guitar which is continuously playing a unique chord, and our magnet is a piano. For CW techniques, we would play each key on the piano in succession, detecting resonances between the frequencies in the guitar chord and frequencies of the piano notes along the way. We basically perform the same experiment in CW EPR, only the magnetic field is swept instead of its frequency and we detect any resonances in our sample. An alternative approach is to strike each key on the piano at the same time and Fourier transform the resulting sound to obtain the frequency spectrum of the guitar; this fact is called the multiplex advantage and is fundamental to pulsed magnetic resonance experiments. In pulsed EPR, we apply a short and intense microwave pulse consisting of a finite bandwidth of frequencies, digitize the signals coming from our sample, and perform a Fourier transformation to obtain the EPR spectrum in the frequency domain.

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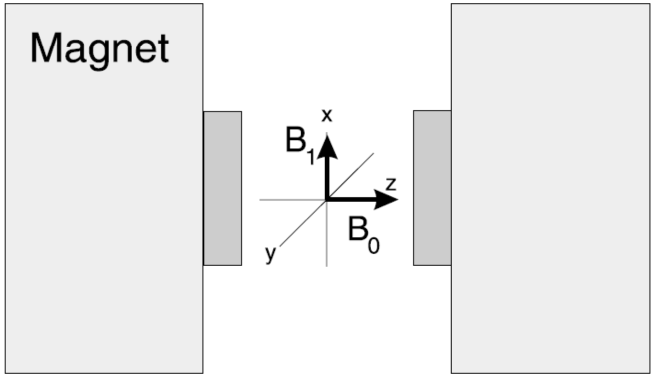


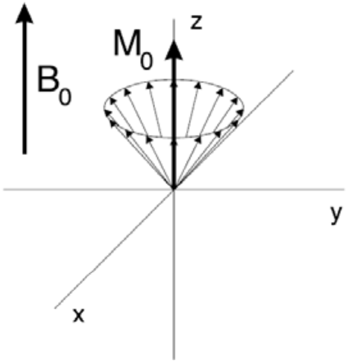
Elucidation of Pulsed EPR by Laboratory Frame

# Larmor Frequency

- When an electron spin is placed in a magnetic field, a torque is exerted on the electron spin, causing its magnetic moment to precess about the magnetic field just as a gyroscope precesses in a gravitational field. The angular frequency of the precession is commonly called the Larmor frequency and it is related to the magnetic field by

$$\omega_L = -\gamma B_0$$

# The Larmor precession and the resultant stationary magnetization.



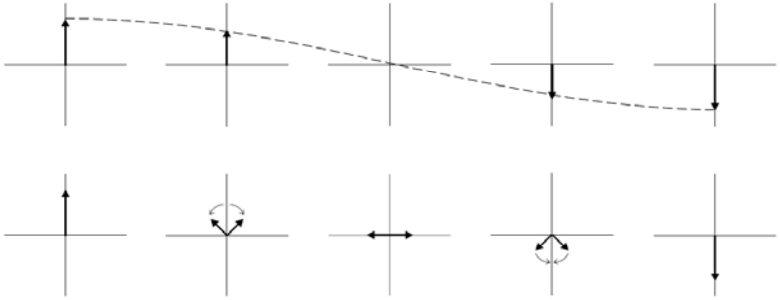
According to the Boltzmann distribution, therefore, there should be a net magnetization parallel to the z axis.

# Larmor Frequency

- where $\omega_L$ is the Larmor frequency, $\gamma$ is the constant of proportionality called the gyromagnetic ratio, and $B_0$ is the magnetic field. The sense of rotation and frequency depend on the value of $\gamma$ and $B_0$. A free electron has a $\gamma /2 \pi$ value of approximately -2.8 MHz/Gauss, resulting in a Larmor frequency of about 9.75 GHz at a field of 3480 Gauss. The Larmor frequency corresponds to the EPR frequency at that magnetic field.

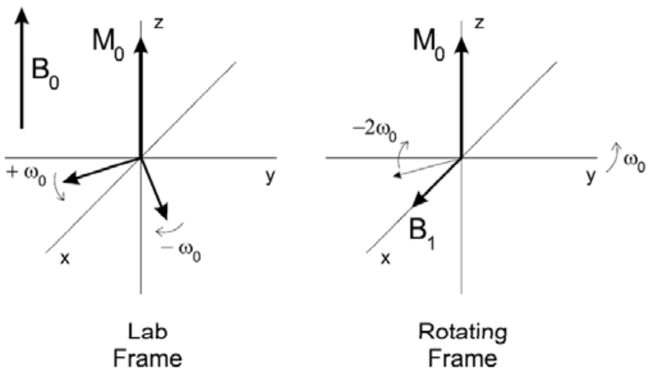
# **Linearly polarized microwaves represented as two circularly polarized components**

Linearly polarized microwaves vs. magnetic field oscillating at the microwave frequency



The sum of two magnetic fields rotating in opposite directions at the microwave frequency will produce a field equivalent to the linearly polarized microwaves

## The microwave magnetic field in both reference frames



$\omega_L = \omega_0$ **On Resonance**

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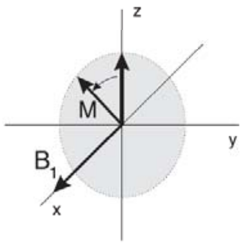
The interaction of a static magnetic field with the magnetization; the magnetization will precess about **B1** at a frequency



$\omega_1 = -\gamma B1$

Rabi Frequency

Rotating the magnetization.

As long as the microwaves are applied, the magnetic field will rotate the magnetization about the +x axis.

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The angle by which $\mathbf{M_0}$ is rotated, commonly called the tip angle, is equal to,

$$\alpha = -\gamma\,|\mathbf{B}_1|t_p$$

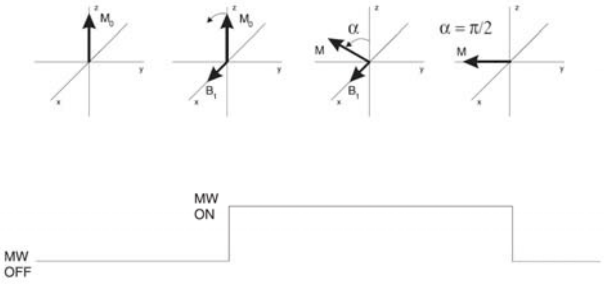

Figure 2-6 The effect of a $\pi/2$ pulse.

The most commonly used tip angles are $\pi/2$ and $\pi$ (90 and 180 degrees)

$\mathbf{B_1}$ of 10 Gauss $\pi/2$ pulse length of approximately 9 ns

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## Free Induction Decay (FID)

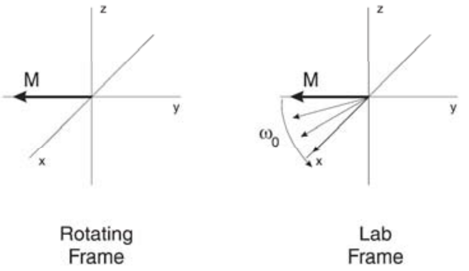
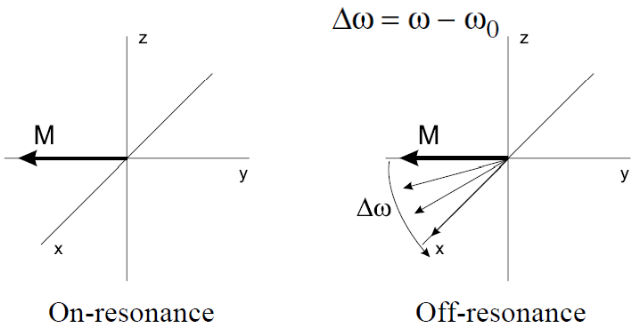


Figure 2-8 Generation of a FID.

The stationary magnetization along -y then becomes a magnetization rotating in the x-y plane at the Larmor frequency. This generates currents and voltages in the resonator just like a generator. The signal will be maximized for the magnetization exactly in the x-y plane. This microwave signal generated in the resonator is called a FID (Free Induction Decay).

# On Resonance vs. Off Resonance

## The magnetization in the rotating frame exactly on-resonance and Δω off-resonance



$$\Delta\omega = \omega - \omega_0$$

Not all parts of the EPR spectrum can be exactly on-resonance
Δω>0 counter-clockwise
Δω<0 clockwise

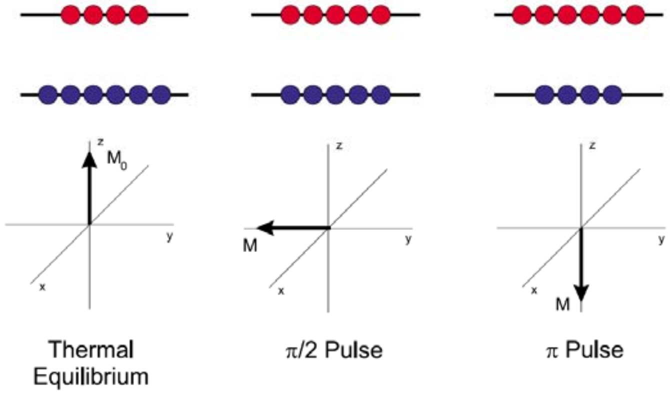
---

# FID (Free Induction Decay)

- This frequency behavior gives us a clue as how the EPR spectrum is encoded in the FID. The individual frequency components of the EPR spectrum will appear as magnetization components rotating in the x-y plane at the corresponding frequency, Δω. If we could measure the transverse magnetization in the rotating frame, we could extract all the frequency components and hence reconstruct the EPR spectrum.

---

# Populations before and after π/2 and π pulses

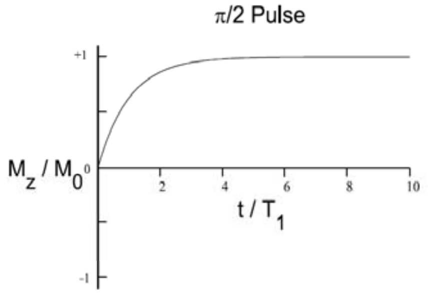


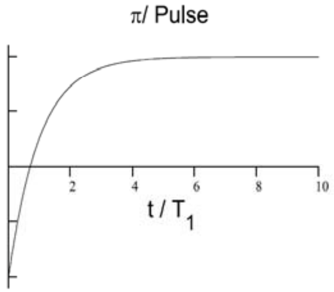
For 10,000 spins, 5004 spins are parallel and 4096 spins are antiparallel.
X band (9.8 GHz) at 300 K

---

# Spin Lattice Relaxation Time ($T_1$)

- The spin system is not in thermal equilibrium after a π/2 or π pulse and through its interactions with the surroundings, it will eventually return to thermal equilibrium. This process is called spin-lattice relaxation.





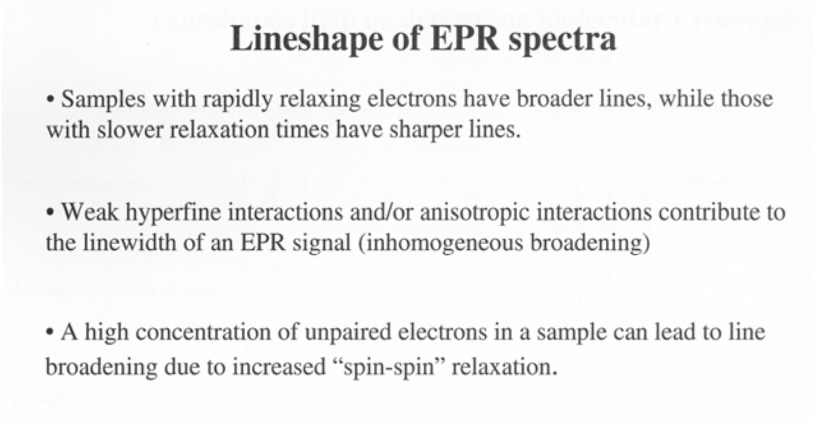
$$M_z(t) = M_0 \cdot \left[1 - e^{-\frac{t}{T_1}}\right]$$

$$M_z(t) = M_0 \cdot \left[1 - 2 \cdot e^{-\frac{t}{T_1}}\right]$$

---

**Transverse Relaxation Time ($T_2$)**

The transverse relaxation time corresponds to the time required magnetization to decay in the x-y plane



## Lineshape of EPR spectra

- Samples with rapidly relaxing electrons have broader lines, while those with slower relaxation times have sharper lines.
- Weak hyperfine interactions and/or anisotropic interactions contribute to the linewidth of an EPR signal (inhomogeneous broadening)
- A high concentration of unpaired electrons in a sample can lead to line broadening due to increased “spin-spin” relaxation.

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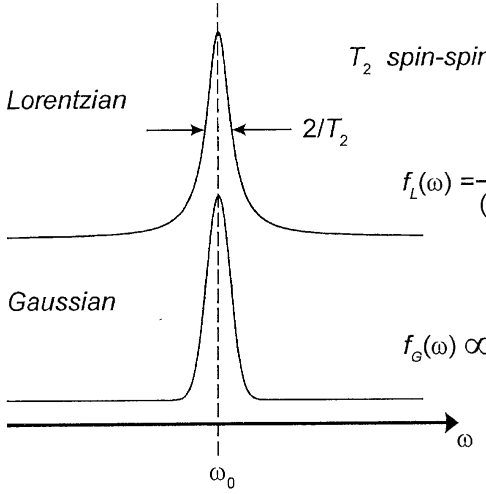
*Line Shapes:*

*Lorentzian vs. Gaussian; Homogeneous*
*Inhomogeneous Line Shape*

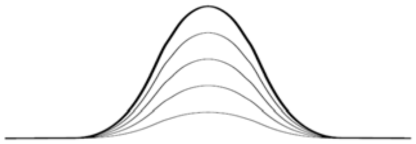


*$T_2$ spin-spin relaxation time*

$$f_L(\omega) = \frac{(1/\pi T_2)}{(1/T_2)^2 + (\omega_0 - \omega)^2}$$

$$f_G(\omega) \propto \exp(\omega_0 - \omega)^2$$

---



(a) Homogeneous broadening. The lineshape is determined by the relaxation times and therefore lorentzian lineshapes are a common result. (See Equation [2-13] and Figure 2-21.)
The EPR spectrum is the sum of a large number of lines each having the same Larmor frequency and linewidth.

The decay from this mechanism is general exponential .

b) Inhomogeneous broadening. The lineshape is determined by unresolved couplings because the EPR spectrum is the sum of a large number of narrower individual homogeneously broadened lines that are each shifted in frequency with respect to each other. Gaussian lineshapes are a common result.

# Spin Echo

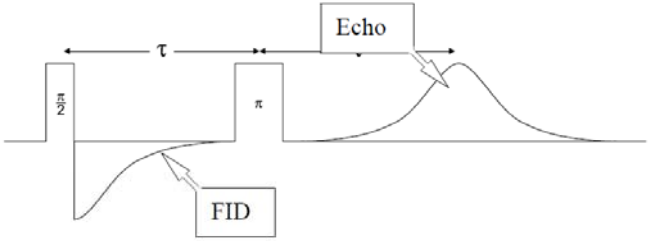


Figure 2-34 A Hahn echo.

**Echoes are essential in EPR**
**FID (fast decay)**
**Allow longer τ**

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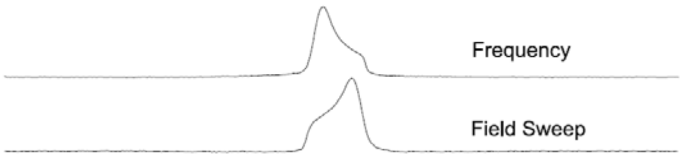
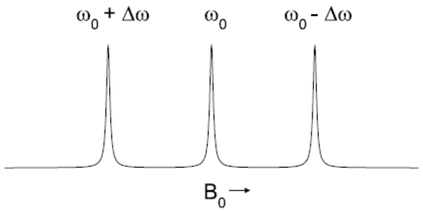
# Field Sweeps vs. Frequency Spectra





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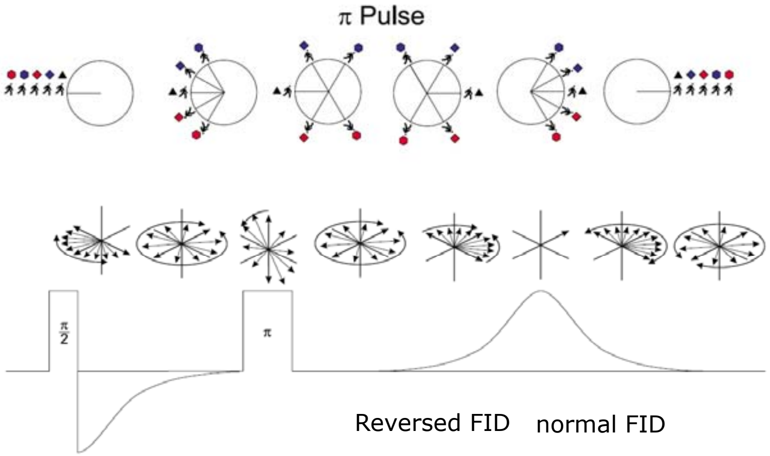
## Refocusing of the magnetization during an echo



Fourier transfer the second half of the FID ⟶ EPR spectrum

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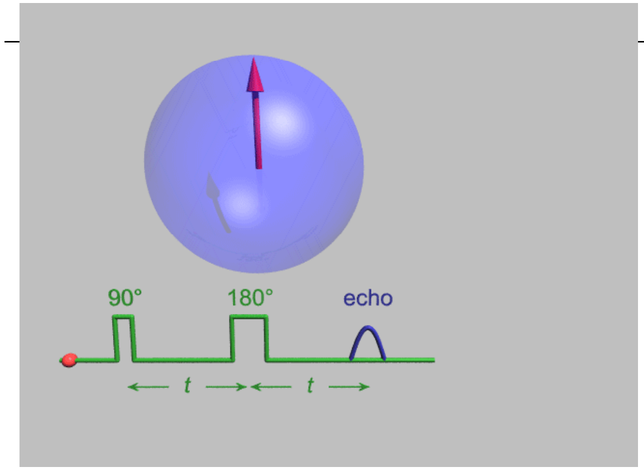
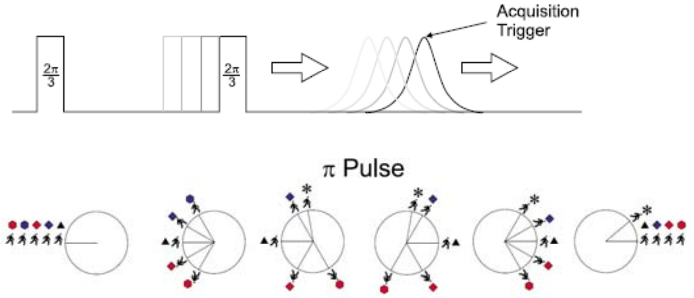


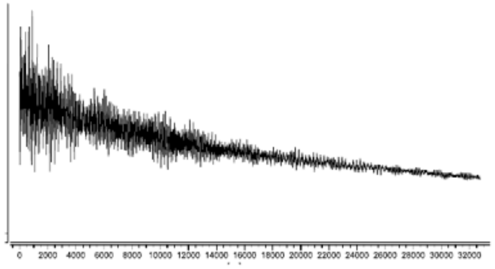
## Dephasing due to a sudden frequency shift. The asterisk marks the runner whose frequency suddenly become less.



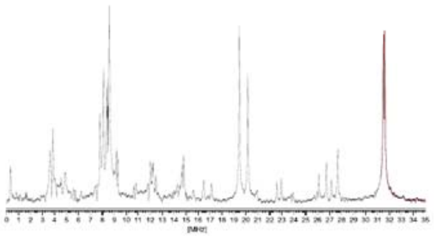
**Vary τ to obtain echo. Transverse relaxation would lead to an exponential decay in echo height due to phase memory time $T_M$ that was contributed by T2 (spin-spin relaxation, spectral, spin and instantaneous diffusion).**

## ESEEM (Electron Spin Echo Envelope Modulation)

- The electron spins interact with the nuclei in their vicinity and this interaction causes a periodic oscillation in the echo height superimposed on the normal echo decay. The modulation or oscillation is caused by periodic dephasing by the nuclei. Armed with this information, one can identify nearby nuclei and their distances from the electron spin and shed light on the local environment of the raidcal or metal ion.



Modulation of the echo height with τ due to ESEEM.



The Fourier transform of the ESEEM showing proton couplings.

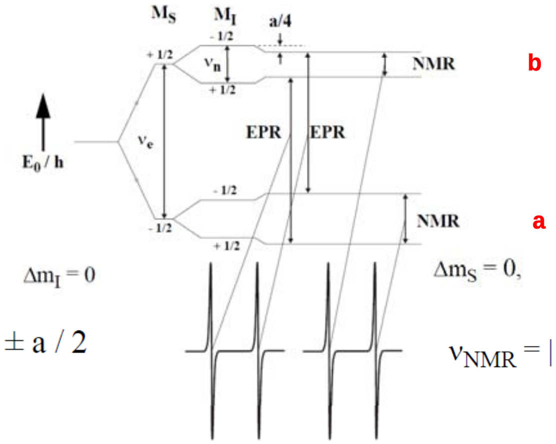
## Resolution Enhancement of ENDOR

$$\mathcal{H} = \mu_B \mathbf{B_0 \cdot g \cdot S} + \mathbf{S \cdot A \cdot I} + \mathbf{I \cdot P \cdot I} + \mu_N \mathbf{B_0 \cdot g_n \cdot I}$$

$$E_{m_S,\, m_I} = g\, \mu_B\, B_0\, m_S - g_n\, \mu_N\, B_0\, m_I + ha\, m_S\, m_I$$

$$\nu_e = g\,\mu_B B_0 / h \quad \text{and} \quad \nu_n = g_n\,\mu_N B_0 / h$$

$$E_{m_S,\,m_I} / h = \nu_e m_S - \nu_n m_I + a\, m_S m_I$$



$\Delta m_S = \pm 1, \quad \Delta m_I = 0$

$\nu_{EPR} = \nu_e \pm a / 2$

$\Delta m_S = 0, \quad \Delta m_I = \pm 1$

$\nu_{NMR} = | \nu_n \pm a / 2 |$

---

## The ENDOR experiment

(i) Saturate one of EPR transitions (EPR intensity = 0)

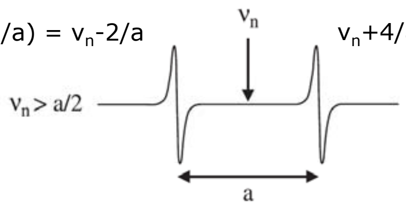
(ii) Slowly vary NMR frequency. At resonance, the population of level (b + 1/2) will decreases, and population of level (a-1/2) will increase. EPR will no longer be saturated and EPR intensity ≠ 0.

---

## ENDOR frequencies for $\nu_n > a/2$ and $\nu_n < a/2$.

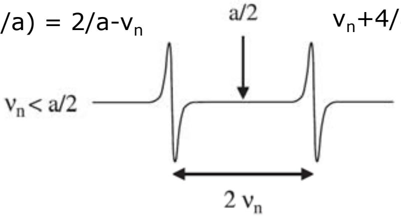
$\nu_n$-4/a-(4/a) = $\nu_n$-2/a

$\nu_n$+4/a-(-4/a) = $\nu_n$+2/a



4/a-($\nu_n$-4/a) = 2/a-$\nu_n$

$\nu_n$+4/a-(-4/a) = $\nu_n$+2/a



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## Energy level diagram for the interaction of an electron with four equivalent protons in the high field limit. a > 0, $g_n$ > 0, and $\nu_n$ > a / 2