- Atomic nuclei have spin $I$ and magnetic dipole moment $\mu = \gamma I$

- $^1H$, $^{13}C$, $^{15}N$ have spin ½ - good for NMR!

- Nuclei (with spin ½) have two states (up and down) in a magnetic field

- The energy difference between the states is $\Delta E = \gamma \hbar B$, which corresponds to frequency of electromagnetic radiation $\omega = \gamma B$ ($\nu = \frac{\gamma B}{2\pi}$) (Larmor frequency)

- The populations of the two states differs slightly $\therefore$ the bulk sample has a magnetic dipolar moment along z (aligned with $B$)
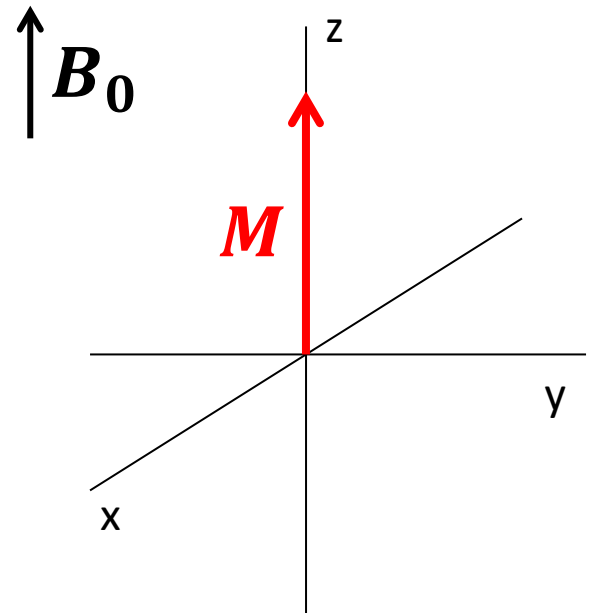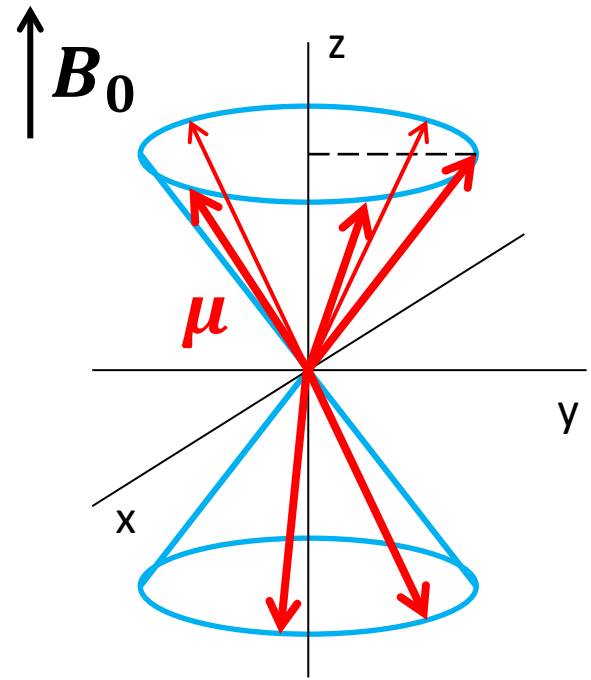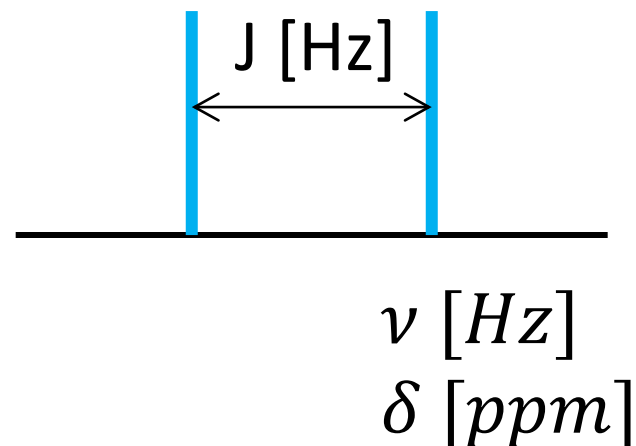
- The magnetic field at the nucleus differs from the external magnetic field due to the movement of electrons around the nucleus ∴ different nuclei in a molecule have slightly different Larmor frequencies

500.1306885 MHz

Reference:
500.1300000 MHz

688.5 Hz $\nu\ [Hz]$

1.3766 ppm $\delta\ [ppm]$

0 Hz

0 ppm
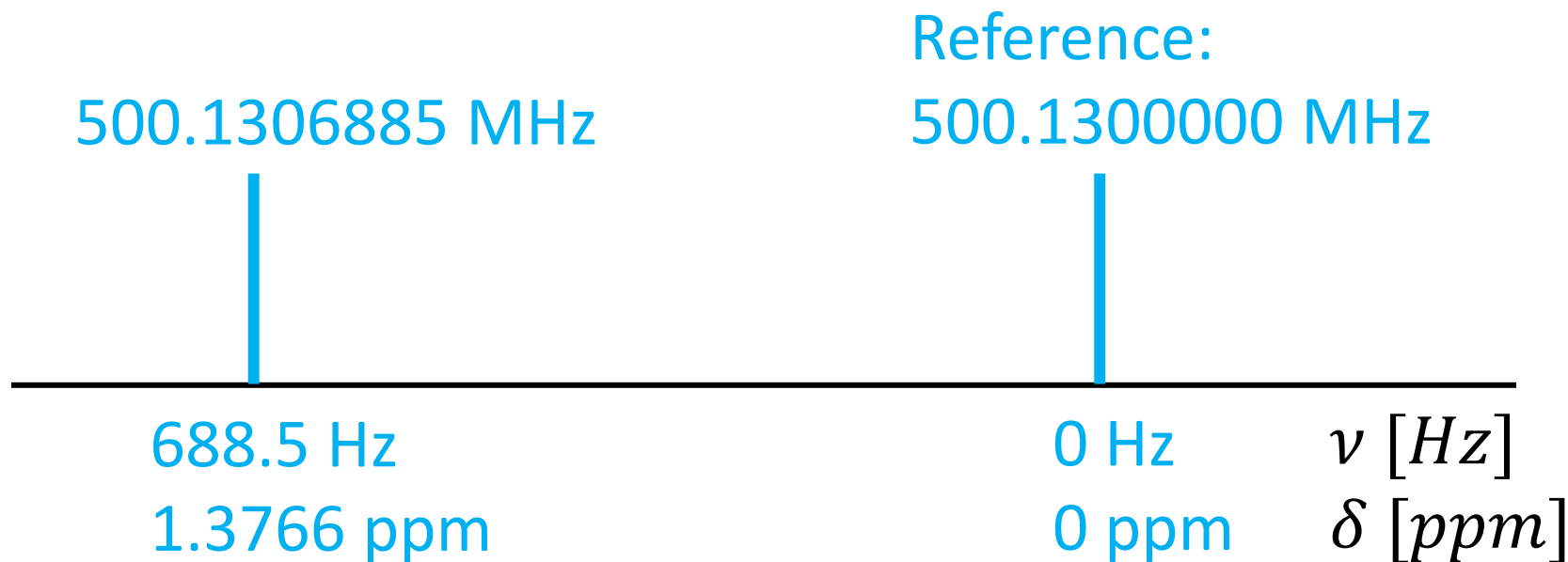
- Chemical shift (does not depend on external magnetic field!):

$$\delta = \frac{500.1306885 - 500.1300000}{500.130000} * 10^6 = 1.3766\ ppm$$

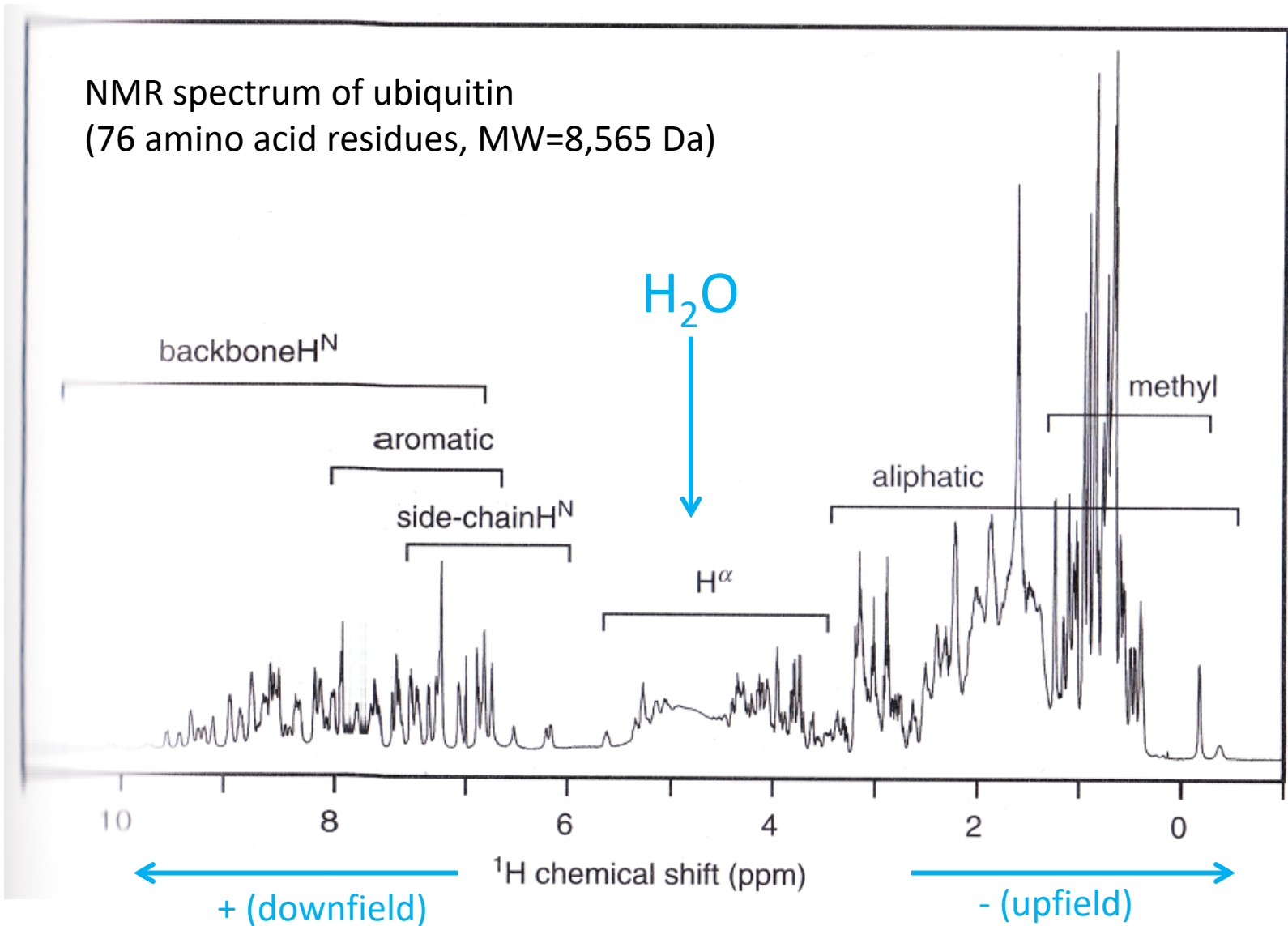- J-coupling (scalar coupling) = interaction between nuclear spins mediated by electrons that form the bond(s) between the nuclei
- Energy of spin I in the presence of J-coupling to spin S:

$m_I = -\frac{1}{2}$

$m_S = -\frac{1}{2}$

$m_S = +\frac{1}{2}$

J [Hz]

$\nu\ [Hz]$

$\delta\ [ppm]$

$m_I = +\frac{1}{2}$

$m_S = +\frac{1}{2}$

$m_S = -\frac{1}{2}$
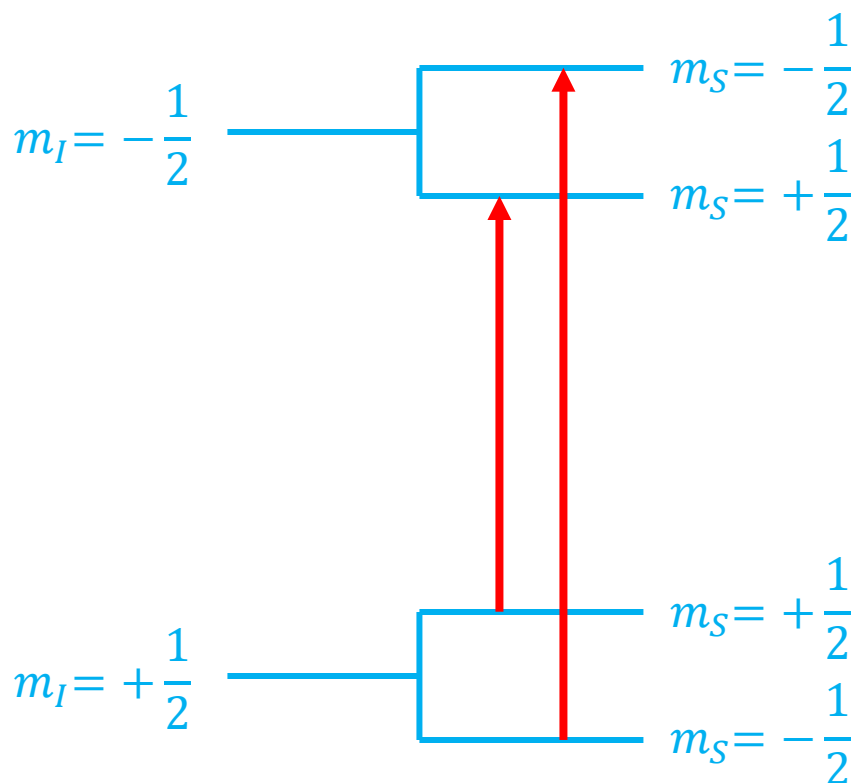
- J-coupling constant [Hz] does not depend on external magnetic field (but the splitting in ppm units will!)

NMR spectrum of ubiquitin
(76 amino acid residues, MW=8,565 Da)

H$_2$O

backboneH$^N$

aromatic

side-chainH$^N$

H$^\alpha$

aliphatic

methyl

$^1$H chemical shift (ppm)

+ (downfield)

- (upfield)

Cavanagh, J., Fairbrother, W.J., Palmer, A.G.III, Rance, M., Skelton, N.J. Protein NMR Spectroscopy: Principles and Practice, 2nd edition, 2007, Academic Press

## TABLE 1
### RANDOM COIL ¹H CHEMICAL SHIFTS FOR THE 20 COMMON AMINO ACIDS WHEN FOLLOWED BY ALANINE

| Residue | NH | H$^\alpha$ | H$^\beta$ | Others |
|---|---|---|---|---|
| Ala | 8.24 | 4.32 | 1.39 | |
| Cys (reduced) | 8.32 | 4.55 | 2.93, 2.93 | |
| Cys (oxidized) | 8.43 | 4.71 | 3.25, 2.99 | |
| Asp | 8.34 | 4.64 | 2.72, 2.65 | |
| Glu | 8.42 | 4.35 | 2.06, 1.96 | $\gamma$CH$_2$ 2.31, 2.31 |
| Phe | 8.30 | 4.62 | 3.14, 3.04 | 2,6H 7.28; 3,5H 7.38; 4H 7.32 |
| Gly | 8.33 | 3.96 | | |
| His | 8.42 | 4.73 | 3.29, 3.16 | 2H 8.58; 4H 7.29 |
| Ile | 8.00 | 4.17 | 1.87 | $\gamma$CH$_2$ 1.45, 1.16; $\gamma$CH$_3$ 0.91; $\delta$CH$_3$ 0.86 |
| Lys | 8.29 | 4.32 | 1.84, 1.75 | $\gamma$CH$_2$ 1.44, 1.44; $\delta$CH$_2$ 1.68, 1.68; $\epsilon$CH$_2$ 2.99, 2.99; $\epsilon$NH$_3^+$ 7.81 |
| Leu | 8.16 | 4.34 | 1.62, 1.62 | $\gamma$CH 1.59; $\delta$CH$_3$ 0.92, 0.87 |
| Met | 8.28 | 4.48 | 2.11, 2.01 | $\gamma$CH$_2$ 2.60, 2.54; $\epsilon$CH$_3$ 2.10 |
| Asn | 8.40 | 4.74 | 2.83, 2.75 | $\gamma$NH$_2$ 7.59, 6.91 |
| Pro | – | 4.42 | 2.29, 1.94 | $\gamma$CH$_2$ 2.02, 2.02; $\delta$CH$_2$ 3.63, 3.63 |
| Gln | 8.32 | 4.34 | 2.12, 1.99 | $\gamma$CH$_2$ 2.36, 2.36; $\delta$NH$_2$ 7.52, 6.85 |
| Arg | 8.23 | 4.34 | 1.86, 1.76 | $\gamma$CH$_2$ 1.63, 1.63; $\delta$CH$_2$ 3.20, 3.20; $\epsilon$NH 8.07 |
| Ser | 8.31 | 4.47 | 3.89, 3.87 | |
| Thr | 8.15 | 4.35 | 4.24 | $\gamma$CH$_3$ 1.21 |
| Val | 8.03 | 4.12 | 2.08 | $\gamma$CH$_3$ 0.94, 0.93 |
| Trp[a] | 8.25 | 4.66 | 3.29, 3.27 | 2H 7.27; 4H 7.65; 5H 7.18; 6H 7.25; 7H 7.50 |
| Tyr | 8.12 | 4.55 | 3.03, 2.98 | 2,6H 7.14; 3,5H 6.84 |

Chemical shifts are referenced to internal DSS at 25 °C, pH ~5.0.
[a] Measured using a peptide with free N- and C-termini.

## TABLE 2
RANDOM COIL $^{13}$C CHEMICAL SHIFTS FOR THE 20 COMMON AMINO ACIDS WHEN FOLLOWED BY ALANINE

| Residue | C=O | $C^{\alpha}$ | $C^{\beta}$ | Others |
|---|---|---|---|---|
| Ala | 177.8 | 52.5 | 19.1 | |
| Cys (reduced) | 174.6 | 58.2 | 28.0 | |
| Cys (oxidized) | 174.6 | 55.4 | 41.1 | |
| Asp | 176.3 | 54.2 | 41.1 | $\gamma$CO 180.0 |
| Glu | 176.6 | 56.6 | 29.9 | $\gamma$CH$_2$ 35.6; $\delta$CO 183.4 |
| Phe | 175.8 | 57.7 | 39.6 | 1C 138.9; 2,6CH 131.9; 3,5CH 131.5; 4CH 129.9 |
| Gly | 174.9 | 45.1 | | |
| His | 174.1 | 55.0 | 29.0 | 2CH 136.2; 4CH 120.1; 5C 131.1 |
| Ile | 176.4 | 61.1 | 38.8 | $\gamma$CH$_2$ 27.2; $\gamma$CH$_3$ 17.4; $\delta$CH$_3$ 12.9 |
| Lys | 176.6 | 56.2 | 33.1 | $\gamma$CH$_2$ 24.7; $\delta$CH$_2$ 29.0; $\epsilon$CH$_2$ 41.9 |
| Leu | 177.6 | 55.1 | 42.4 | $\gamma$CH 26.9; $\delta$CH$_3$ 24.9, 23.3 |
| Met | 176.3 | 55.4 | 32.9 | $\gamma$CH$_2$ 32.0; $\epsilon$CH$_3$ 16.9 |
| Asn | 175.2 | 53.1 | 38.9 | $\gamma$CO 177.2 |
| Pro | 177.3 | 63.3 | 32.1 | $\gamma$CH$_2$ 27.2; $\delta$CH$_2$ 49.8 |
| Gln | 176.0 | 55.7 | 29.4 | $\gamma$CH$_2$ 33.7; $\delta$CO 180.5 |
| Arg | 176.3 | 56.0 | 30.9 | $\gamma$CH$_2$ 27.1; $\delta$CH$_2$ 43.3; $\epsilon$C 159.5 |
| Ser | 174.6 | 58.3 | 63.8 | |
| Thr | 174.7 | 61.8 | 69.8 | $\gamma$CH$_3$ 21.5 |
| Val | 176.3 | 62.2 | 32.9 | $\gamma$CH$_3$ 21.1, 20.3 |
| Trp[a] | 176.1 | 57.5 | 29.6 | 2CH 127.4; 3C 111.2; 4CH 122.2; 5CH 124.8; 6CH 121.0; 7CH 114.7; 8C 138.7; 9C 129.5 |
| Tyr | 175.9 | 57.9 | 38.8 | 1C 130.6; 2,6CH 133.3; 3,5CH 118.2; 4C 157.3 |

Chemical shifts are referenced to internal DSS at 25 °C, pH ~5.0.
[a] Measured using a peptide with free N- and C-termini.

Wishart DS, Bigam CG, Holm A, Hodges RS, Sykes BD. 1H, 13C and 15N random coil NMR chemical shifts of the common amino acids. I. Investigations of nearest-neighbor effects. J Biomol NMR. 1995 Jan;5(1):67-81.

Description and analysis of rotations (oscillations)
Rotation with angular velocity $\boldsymbol{\omega}$:

$$\frac{d\boldsymbol{\mu}(t)}{dt} = \boldsymbol{\omega} \times \boldsymbol{\mu}(t) = -\boldsymbol{\mu}(t) \times \boldsymbol{\omega}$$
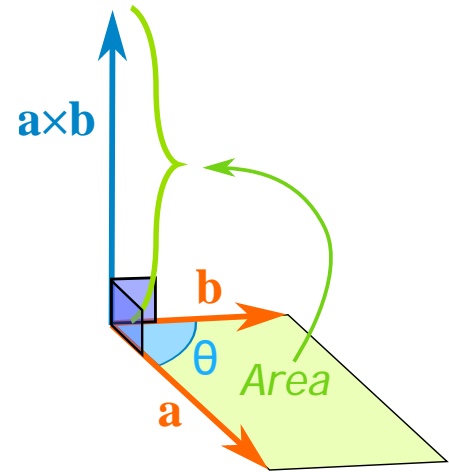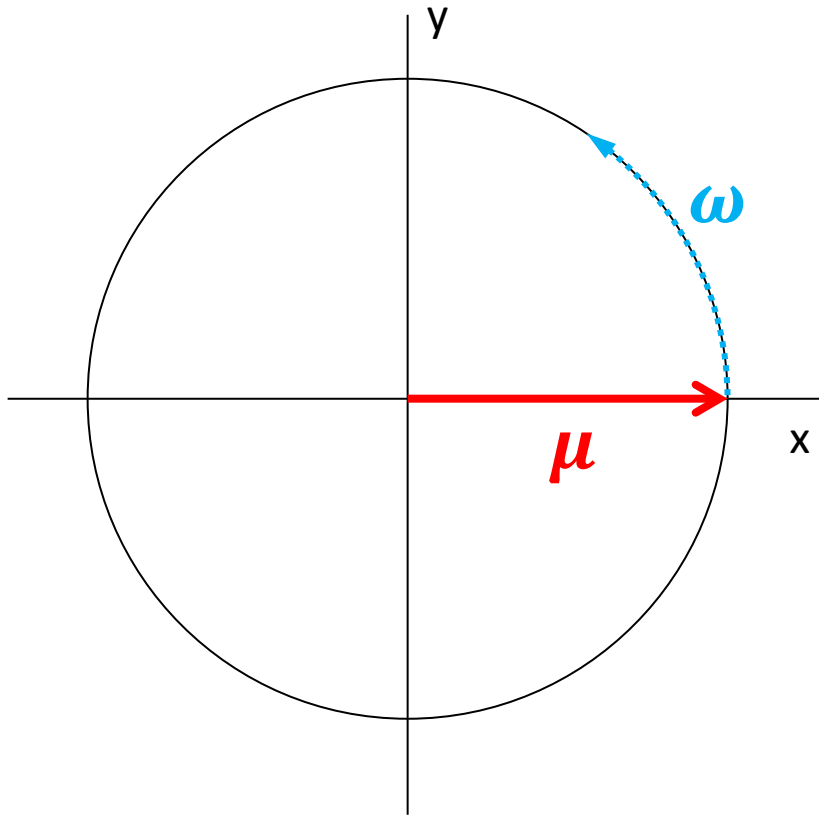
$\nu$: frequency in s$^{-1}$=Hz

$\omega = 2\pi\nu$
angular velocity
(angular frequency) in
radians/s

https://www.mathsisfun.com/algebra/vectors-cross-product.html

# Semi-classical model (Bloch equations)

Magnetic dipole $\boldsymbol{\mu}$ experiences torque in magnetic field:

$$\boldsymbol{\mu} = \gamma \boldsymbol{I}$$

$$\boldsymbol{\mu}(t) \times \mathbf{B} = \boxed{\frac{d\boldsymbol{I}(t)}{dt}} = \frac{1}{\gamma}\frac{d\boldsymbol{\mu}(t)}{dt}$$

torque

$$\frac{d\boldsymbol{\mu}(t)}{dt} = \boldsymbol{\mu}(t) \times \gamma\mathbf{B} = \boxed{-\gamma\boldsymbol{B}} \times \boldsymbol{\mu}(t)$$

$$\boldsymbol{\omega}$$

$\boldsymbol{\mu}(t)$ rotates (<u>precesses</u>) with angular velocity $\boxed{\boldsymbol{\omega} = -\gamma\boldsymbol{B}}$

Larmor frequency
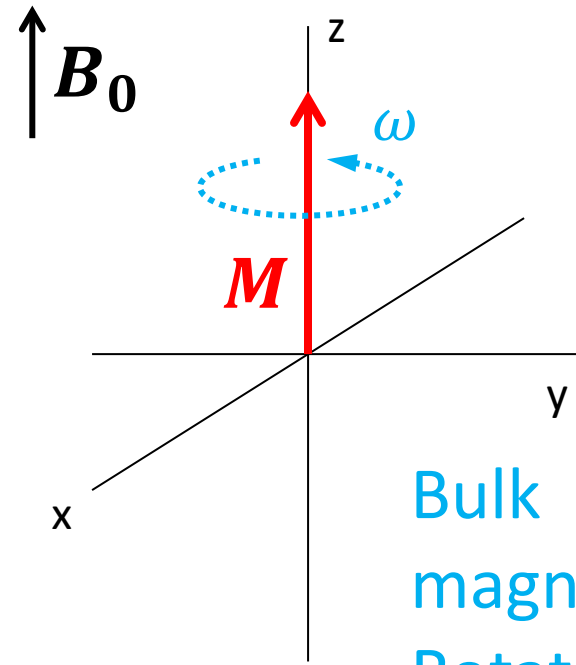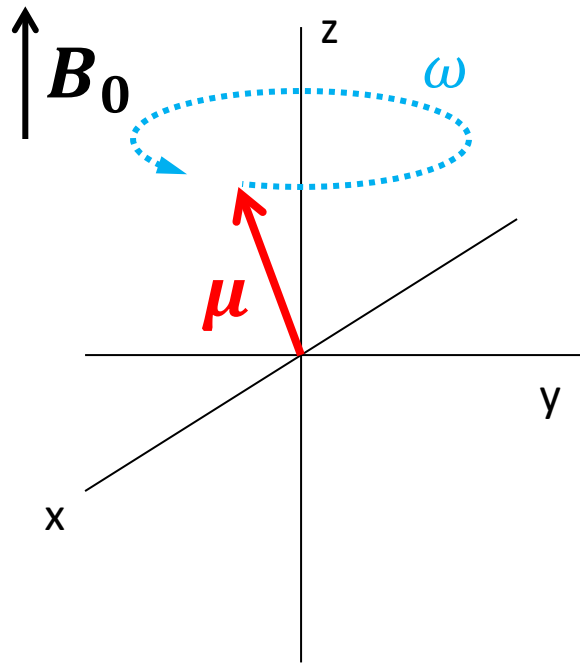
Bulk magnetization: Rotation not observable

Note: $\boldsymbol{\omega} = -\gamma\boldsymbol{B}$ ∴ $\boldsymbol{\omega}$ and $\boldsymbol{B}$ have opposite direction for positive $\gamma$ and the same direction for negative $\gamma$.
In all the diagrams, I'll draw them in the same direction.

Application of perpendicular (horizontal) magnetic field $\boldsymbol{B_1}(t)$ that oscillates with angular velocity $\omega = -\gamma B$:
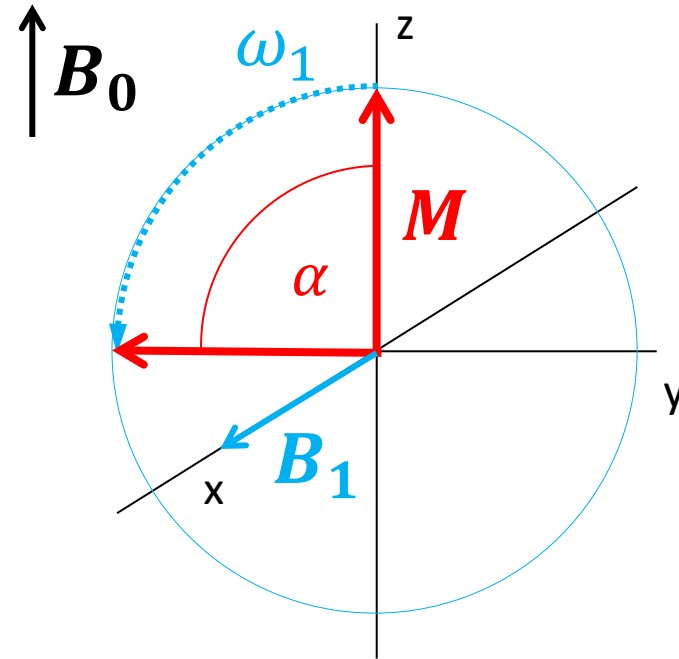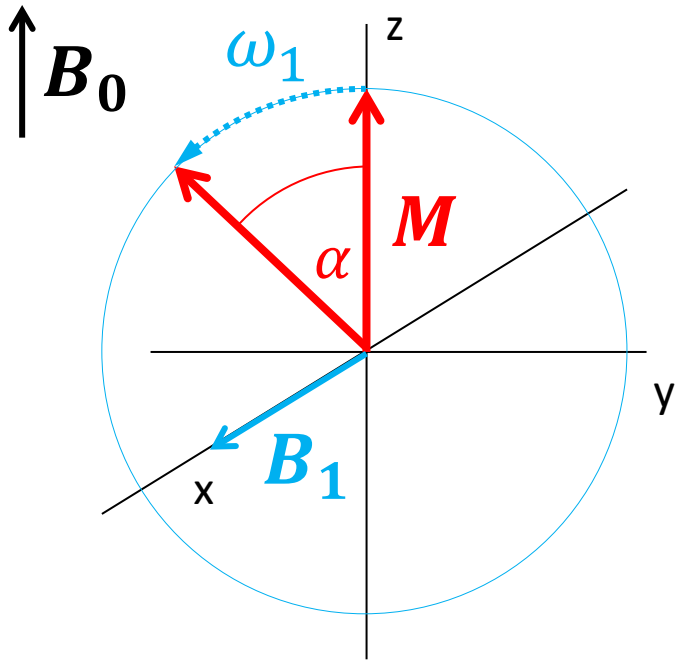
$$\frac{d\boldsymbol{\mu}(t)}{dt} = -\gamma(\boldsymbol{B} + \boldsymbol{B_1}(t)) \times \boldsymbol{\mu}(t)$$

Switch to a coordinate frame that rotates with angular velocity $\omega = -\gamma B$:

$$\frac{d\boldsymbol{\mu}(t)}{dt} = -\gamma\boldsymbol{B_1} \times \boldsymbol{\mu}(t)$$

$\boldsymbol{\mu}(t)$ rotates with angular velocity $\boldsymbol{\omega_1} = -\gamma\boldsymbol{B}_1$ around $\boldsymbol{B}_1$ in the rotating frame!
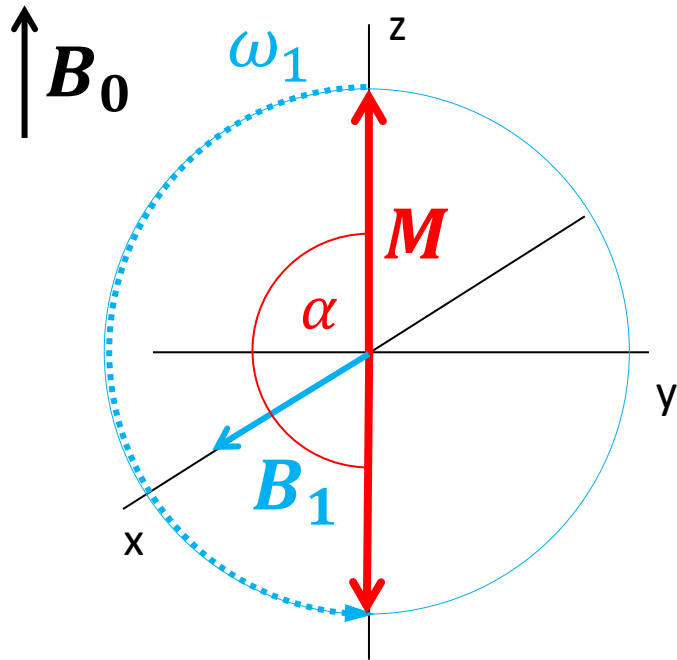
Rotating frame (angular velocity $\boldsymbol{\omega} = -\gamma\boldsymbol{B}$):



$\boldsymbol{B_1}$ along x:
Rotation in the y-z plane
with angular velocity $\boldsymbol{\omega_1}$
$\alpha = \omega_1 t$

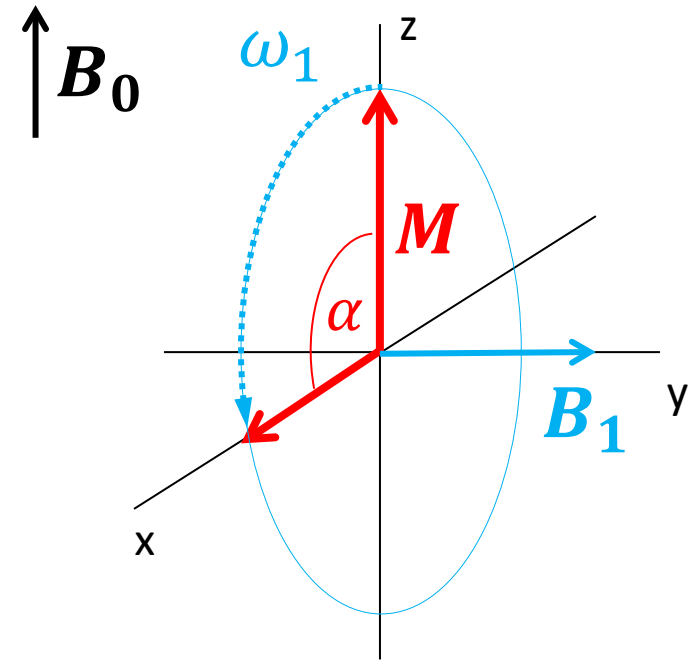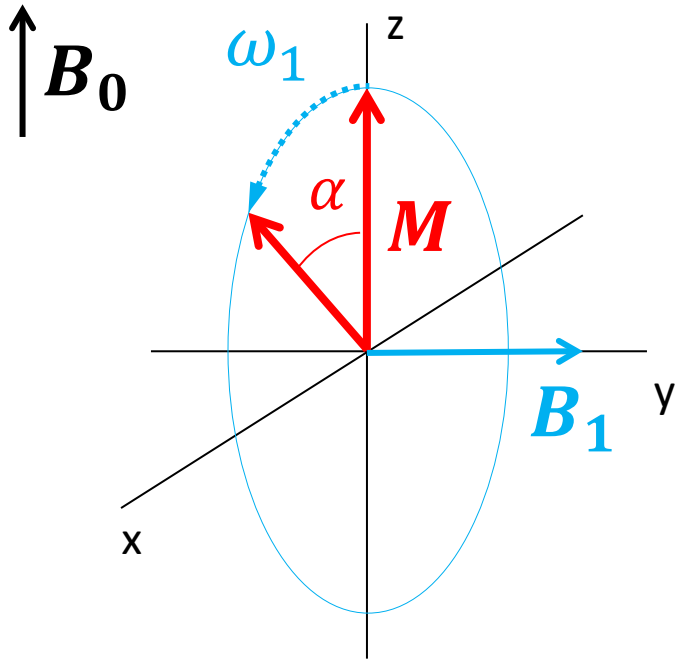90° pulse: $\alpha = \omega_1 t = \dfrac{\pi}{2}$

$M_z = M_0 \rightarrow -M_y = M_0$

Rotating frame (angular velocity $\boldsymbol{\omega} = -\gamma\boldsymbol{B}$):



180° pulse: $\alpha = \omega_1 t = \pi$

$M_z = M_0 \rightarrow -M_z = M_0$

Rotating frame (angular velocity $\boldsymbol{\omega} = -\gamma\boldsymbol{B}$):



$\boldsymbol{B_1}$ along y:
Rotation in the x-z plane
with angular velocity $\boldsymbol{\omega_1}$
$\alpha = \omega_1\, t$

90° pulse: $\alpha = \omega_1 t = \dfrac{\pi}{2}$

$M_z = M_0 \rightarrow M_x = M_0$

When the angular velocity $\boldsymbol{\omega_{rf}}$ of the perpendicular magnetic field $\boldsymbol{B_1}(t)$ differs (somewhat) from Larmor frequency $\boldsymbol{\omega} = -\gamma \boldsymbol{B}$:

Pulses still work as long as $|\gamma \boldsymbol{B_1}| \gg |\boldsymbol{\omega} - \boldsymbol{\omega_{rf}}|$

Rotating frame (angular velocity $\boldsymbol{\omega_{rf}}$):

$$\frac{d\boldsymbol{\mu}(t)}{dt} = -\gamma(\boldsymbol{B} + \boldsymbol{B_1}) \times \boldsymbol{\mu}(t) - \boldsymbol{\omega_{rf}} \times \boldsymbol{\mu}(t)$$

$$= (\boxed{-\gamma\boldsymbol{B}} - \boldsymbol{\omega_{rf}} \boxed{-\gamma\boldsymbol{B_1}}) \times \boldsymbol{\mu}(t)$$

$\boldsymbol{\omega}$
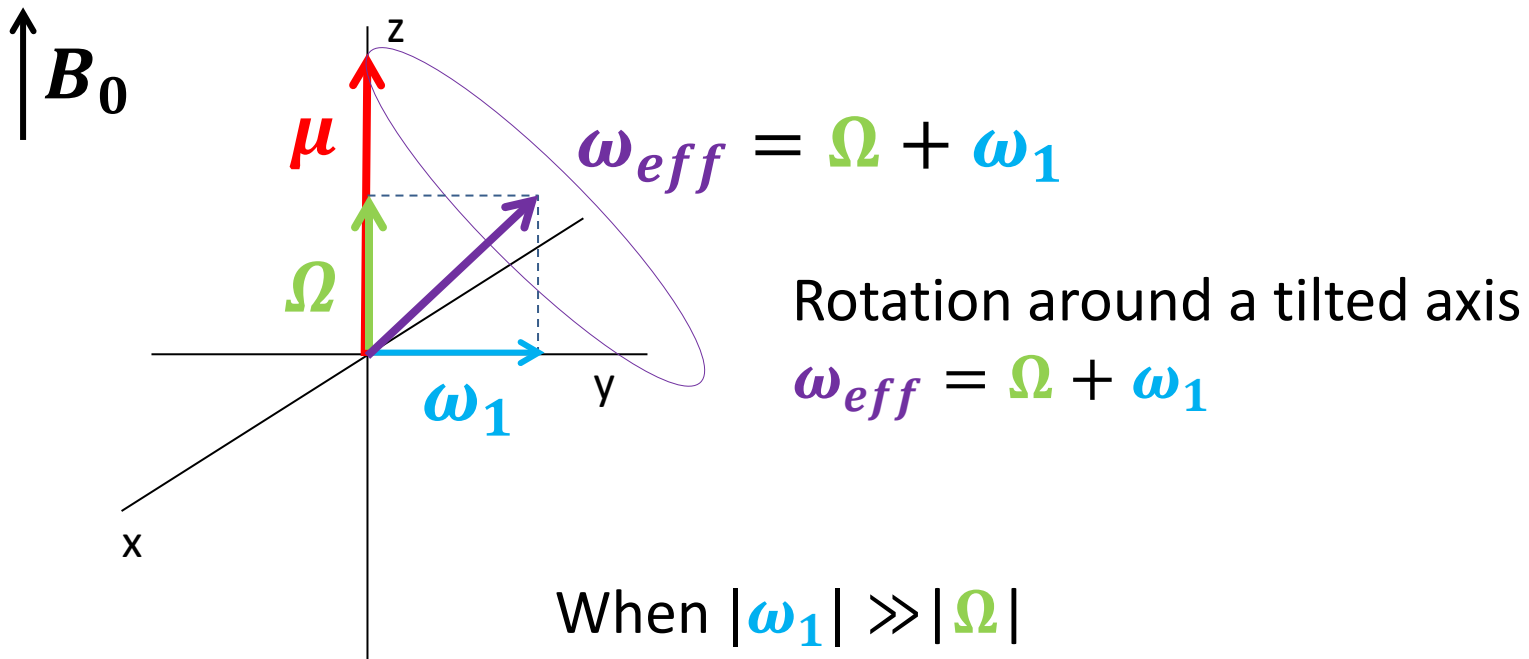
$\boldsymbol{\omega_1}$

Rotation around $B_1$ (stationary in the rotating frame)

Correction for rotation of the rotating frame

Rotation around $\boldsymbol{B}$ with Larmor frequency

Rotating frame (angular velocity $\boldsymbol{\omega_{rf}}$):

$$\frac{d\boldsymbol{\mu}(t)}{dt} = \left(\boxed{\boldsymbol{\omega} - \boldsymbol{\omega_{rf}}} + \boldsymbol{\omega_1}\right) \times \boldsymbol{\mu}(t)$$

$\boldsymbol{\Omega}$ (offset)



$\boldsymbol{\omega_{eff}} = \boldsymbol{\Omega} + \boldsymbol{\omega_1}$

Rotation around a tilted axis
$\boldsymbol{\omega_{eff}} = \boldsymbol{\Omega} + \boldsymbol{\omega_1}$

When $|\boldsymbol{\omega_1}| \gg |\boldsymbol{\Omega}|$
$\boldsymbol{\omega_{eff}} \approx \boldsymbol{\omega_1}$
Rotation around axis in a horizontal plane

Another way to look at it:

Rotating frame (angular velocity $\boldsymbol{\omega_{rf}}$):

$$\frac{d\boldsymbol{\mu}(t)}{dt} = -\gamma(\boldsymbol{B} + \boldsymbol{B_1}) \times \boldsymbol{\mu}(t) - \boldsymbol{\omega_{rf}} \times \boldsymbol{\mu}(t)$$

$$= -\gamma\left(\boxed{\boldsymbol{B} + \frac{\boldsymbol{\omega_{rf}}}{\gamma}} + \boldsymbol{B_1}\right) \times \boldsymbol{\mu}(t)$$

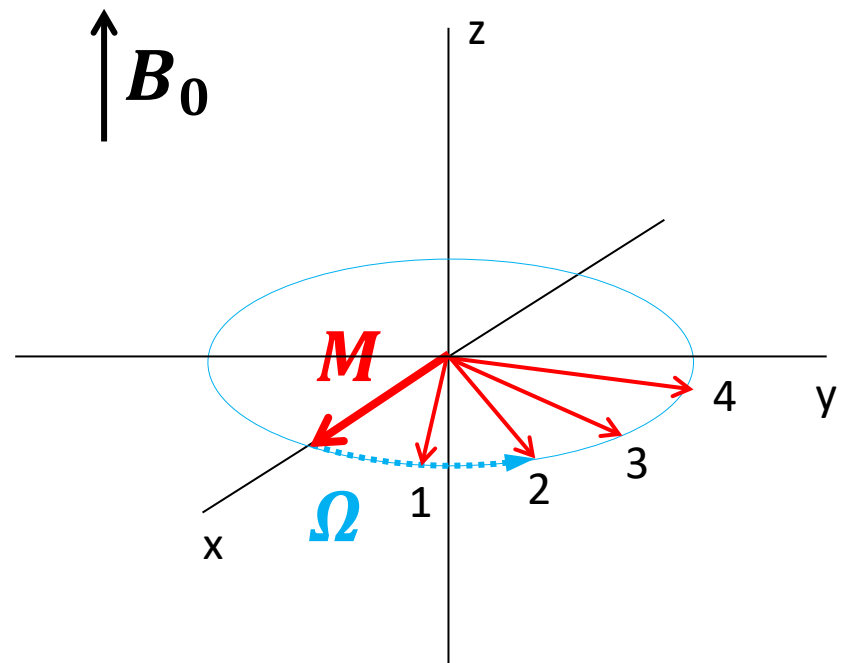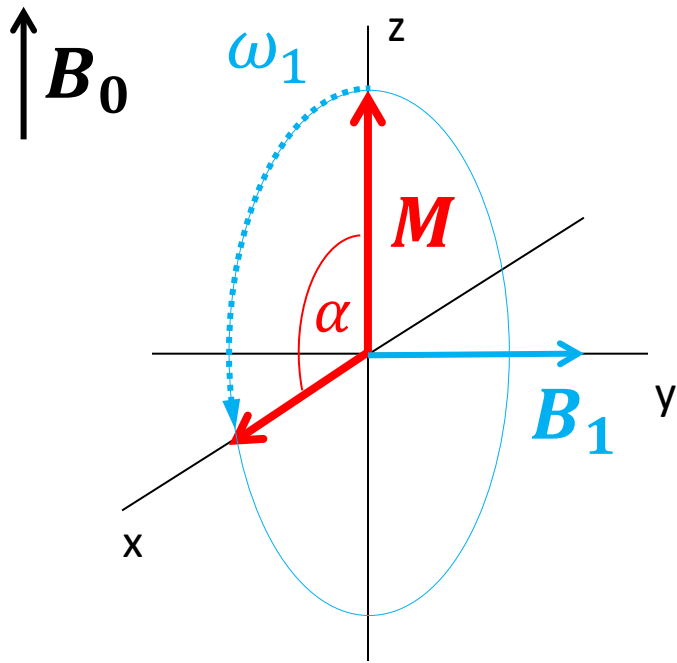$\Delta\boldsymbol{B}$ (residual vertical magnetic field in the rotating frame)



$\boldsymbol{B_{eff}} = \Delta\boldsymbol{B} + \boldsymbol{B_1}$
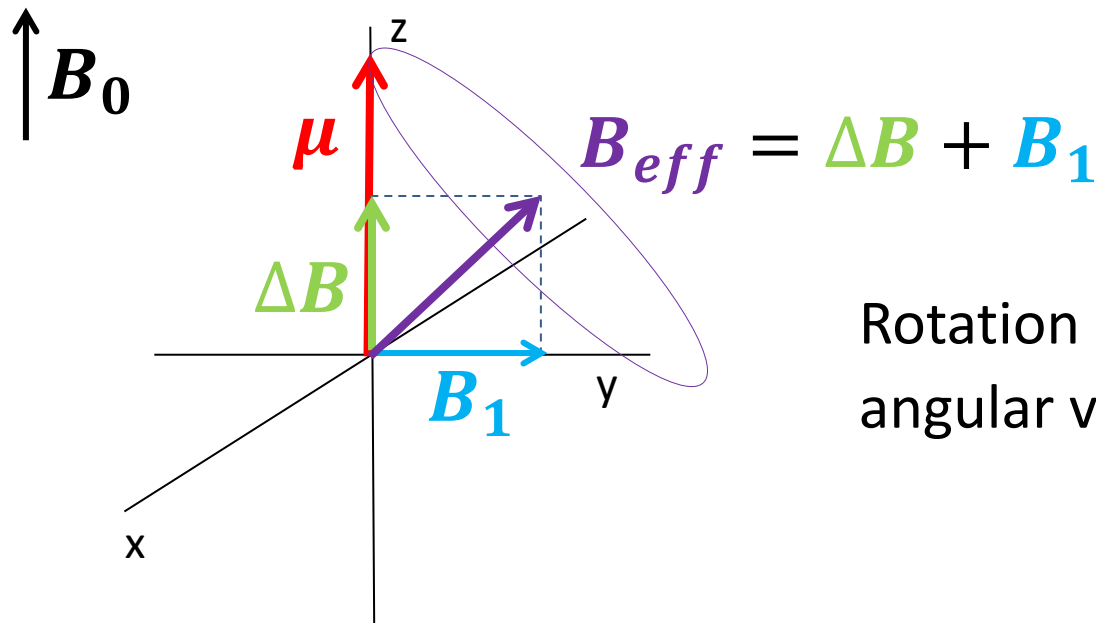
Rotation around $\boldsymbol{B_{eff}}$ with angular velocity $\boldsymbol{\omega_{eff}} = -\gamma\boldsymbol{B_{eff}}$

Rotating frame (angular velocity $\boldsymbol{\omega_{rf}}$):

$$\frac{d\boldsymbol{\mu}(t)}{dt} = \left(\boxed{\boldsymbol{\omega} - \boldsymbol{\omega_{rf}}} + \boldsymbol{\omega_1}\right) \times \boldsymbol{\mu}(t) = -\gamma\left(\boxed{\boldsymbol{B} + \frac{\boldsymbol{\omega_{rf}}}{\gamma}} + \boldsymbol{B_1}\right) \times \boldsymbol{\mu}(t)$$

$\boldsymbol{\Omega}$ (offset)

$\Delta\boldsymbol{B}$ (residual vertical magnetic field in the rotating frame)

# Basic NMR experiment

90° pulse

Free induction decay (FID)
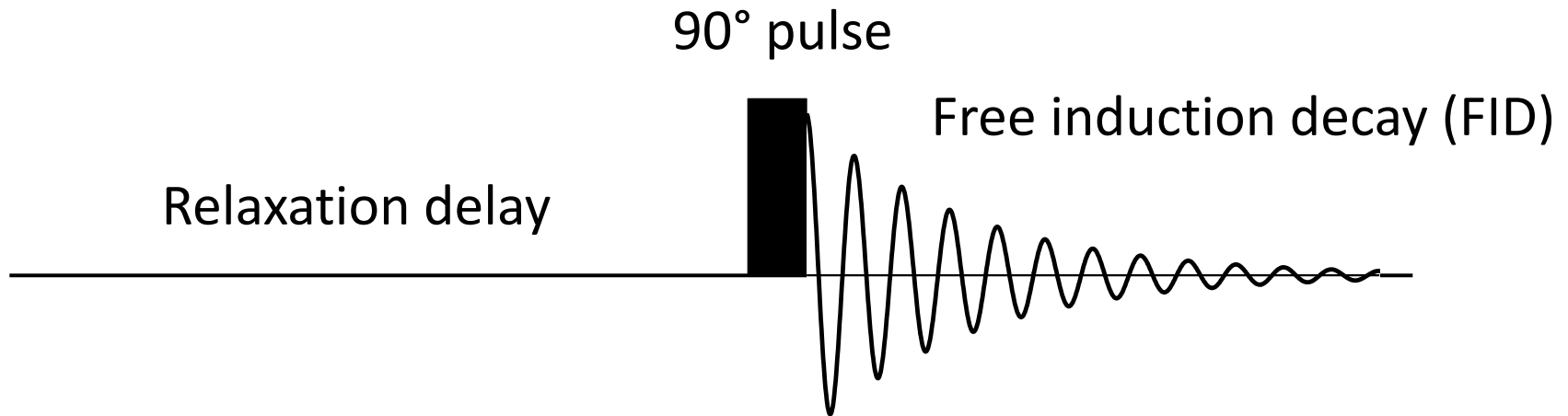
Relaxation delay

Relaxation delay - Allows magnetization to return to equilibrium along z
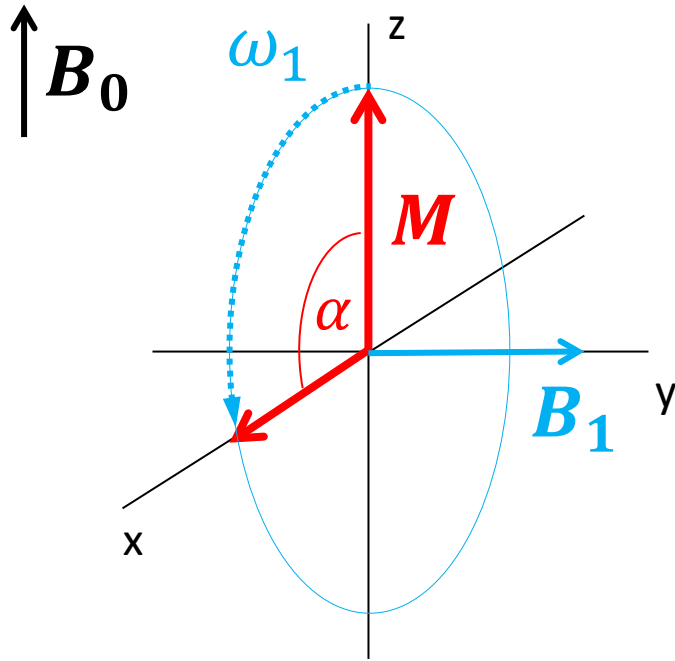
90° pulse - Tips magnetization into the x-y plane

Free induction decay (FID) - Magnetization precesses in the x-y plane, this induces electrical current in a coil and is detected

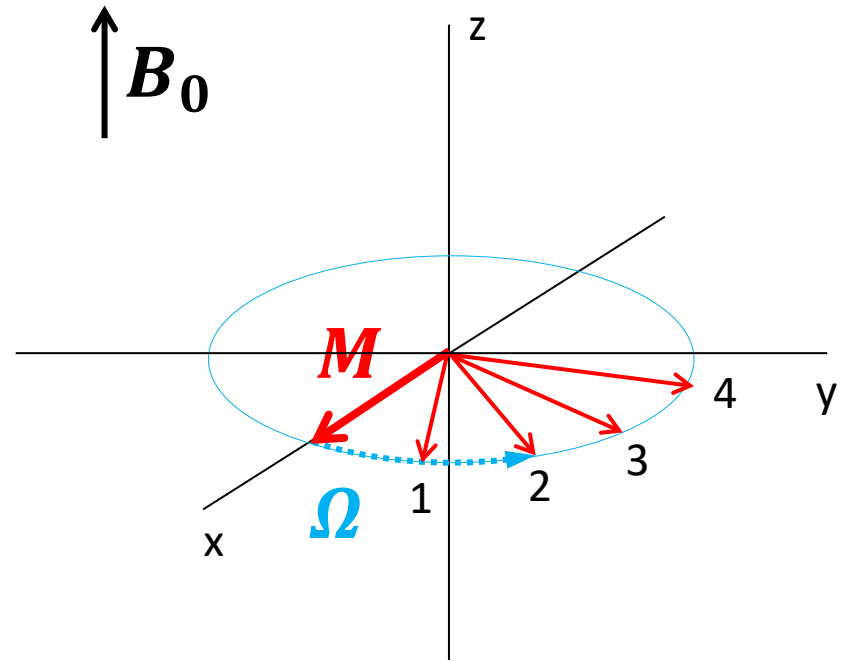Repeat $n$ times (to improve signal to noise ratio)

Rotating frame (angular velocity $\boldsymbol{\omega_{rf}}$)
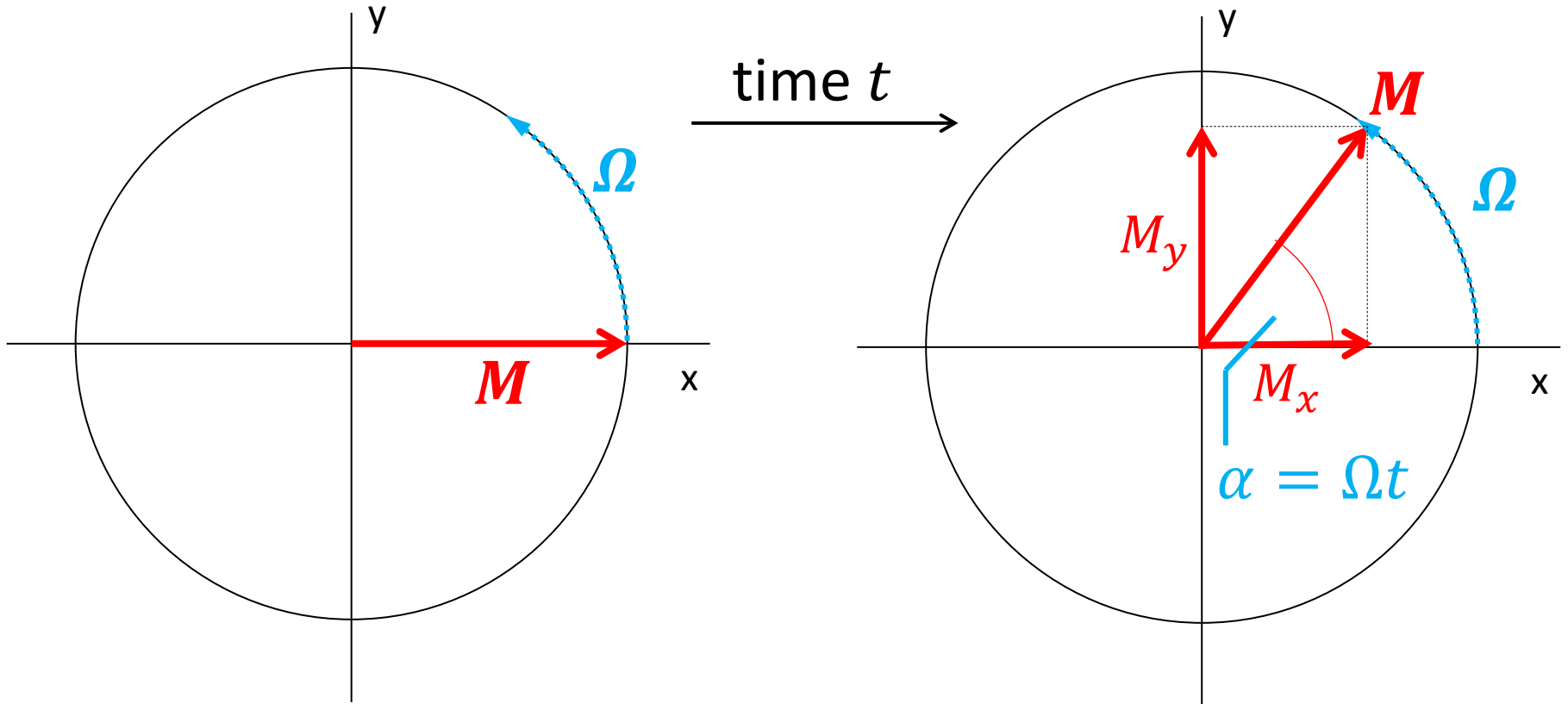
90° pulse

Free induction decay (FID)

$\boldsymbol{B_0}$ — $\omega_1$ — z — $\boldsymbol{M}$ — $\alpha$ — y — $\boldsymbol{B_1}$ — x

$\boldsymbol{B_0}$ — z — $\boldsymbol{M}$ — $\boldsymbol{\Omega}$ — 1 — 2 — 3 — 4 — y — x

Turn on $\boldsymbol{B_1}$ for time $t$ such that
$$\alpha = \omega_1 t = |-\gamma B_1| t = \frac{\pi}{2}$$

Magnetization precesses in the x-y plane with frequency $\boldsymbol{\Omega}$ (and relaxes back to equilibrium)

# Description and analysis of rotations (oscillations): Rotation with angular velocity $\boldsymbol{\Omega}$



$M_x = M_0$
$M_y = 0$

$M_x = M_0 \cos \Omega t$
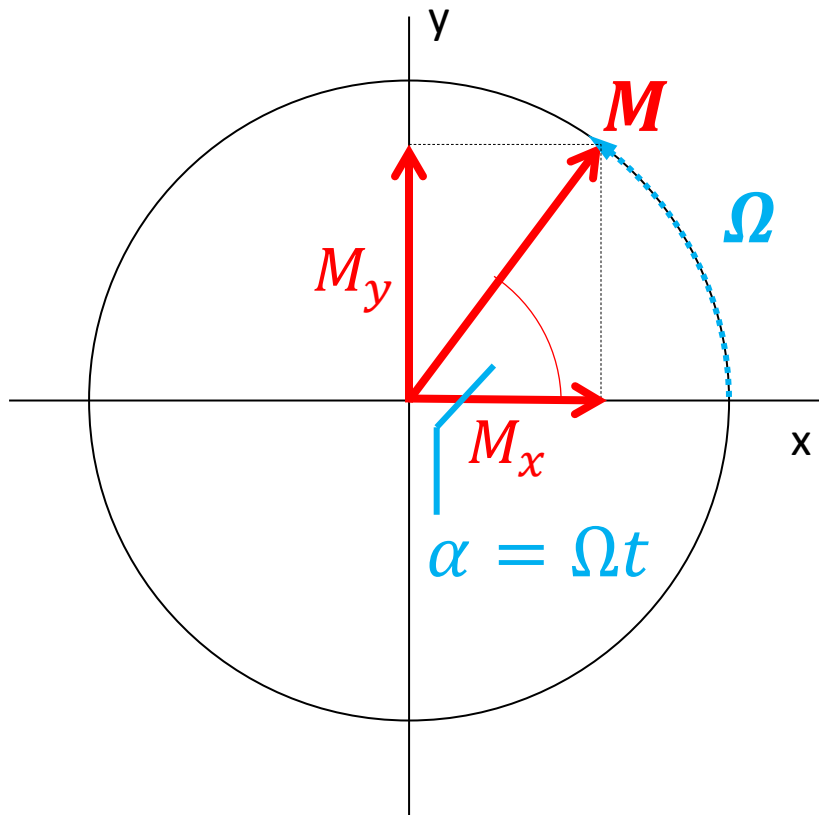$M_y = M_0 \sin \Omega t$
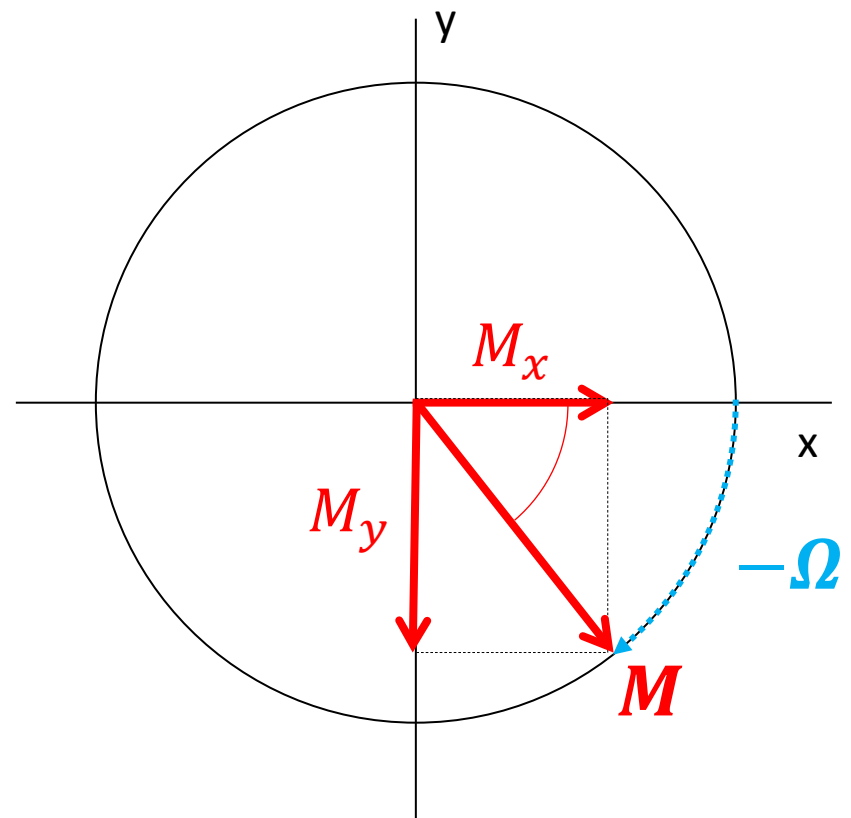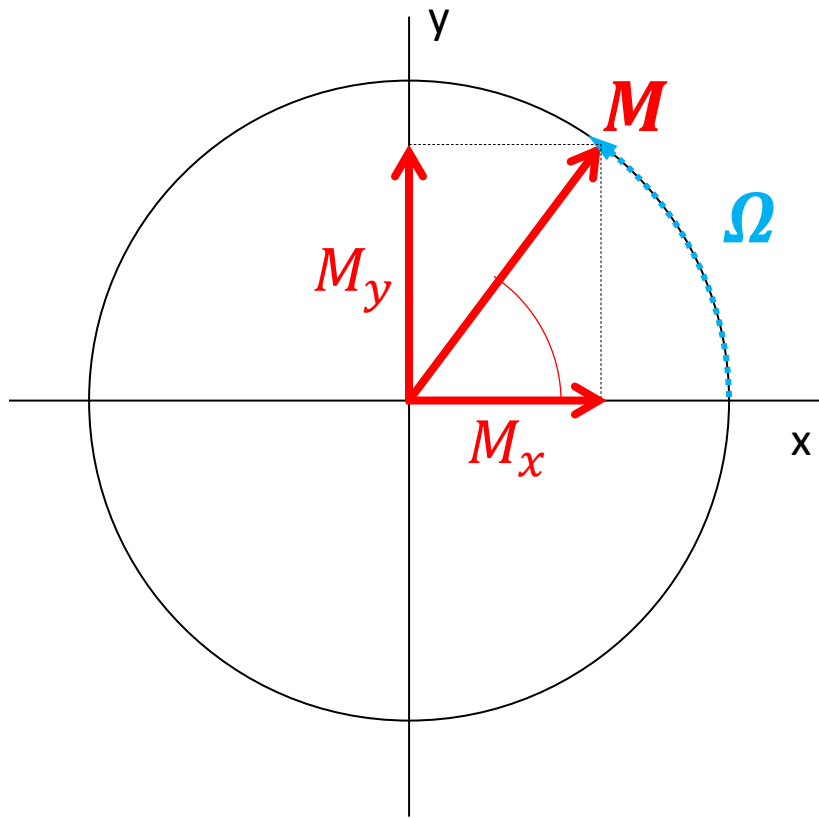
$$M_x = M_0 \cos \Omega t$$
$$M_y = M_0 \sin \Omega t$$

$$M_x = M_0 \cos(-\Omega t) = M_0 \cos \Omega t$$
$$M_y = M_0 \sin(-\Omega t) = -M_0 \sin \Omega t$$

Need both $M_x$ and $M_y$ components to distinguish the direction (sign) of rotation

$M_x$ and $M_y$ components formally treated like real and imaginary components of a complex number:
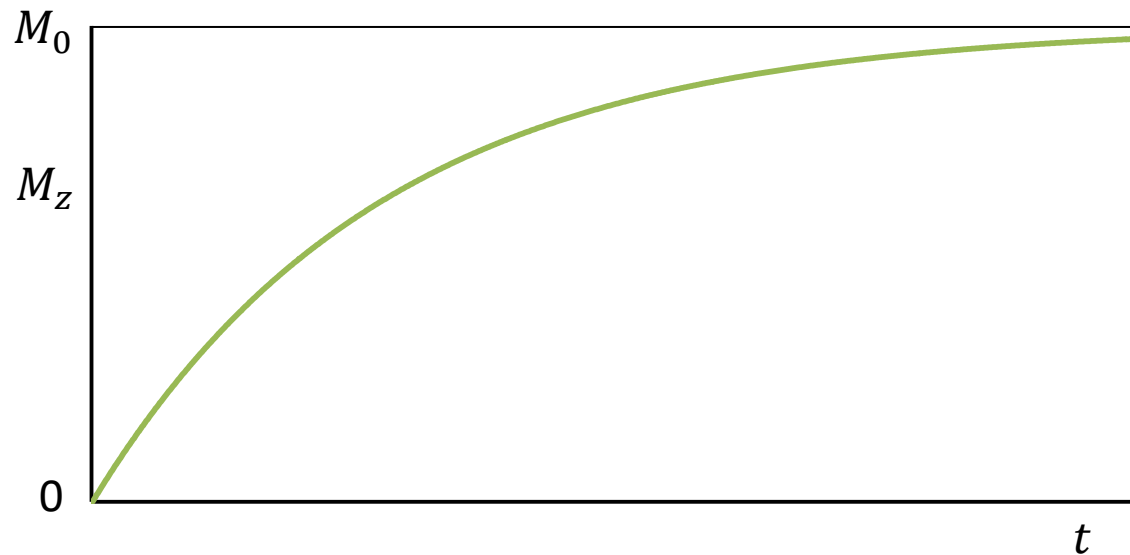
$$M = M_x + iM_y = M_0 \cos \Omega t + iM_0 \sin \Omega t$$

$$M = M_0 \left( \cos \Omega t + i \sin \Omega t \right) = M_0 e^{i\Omega t}$$

# Return to equilibrium: relaxation

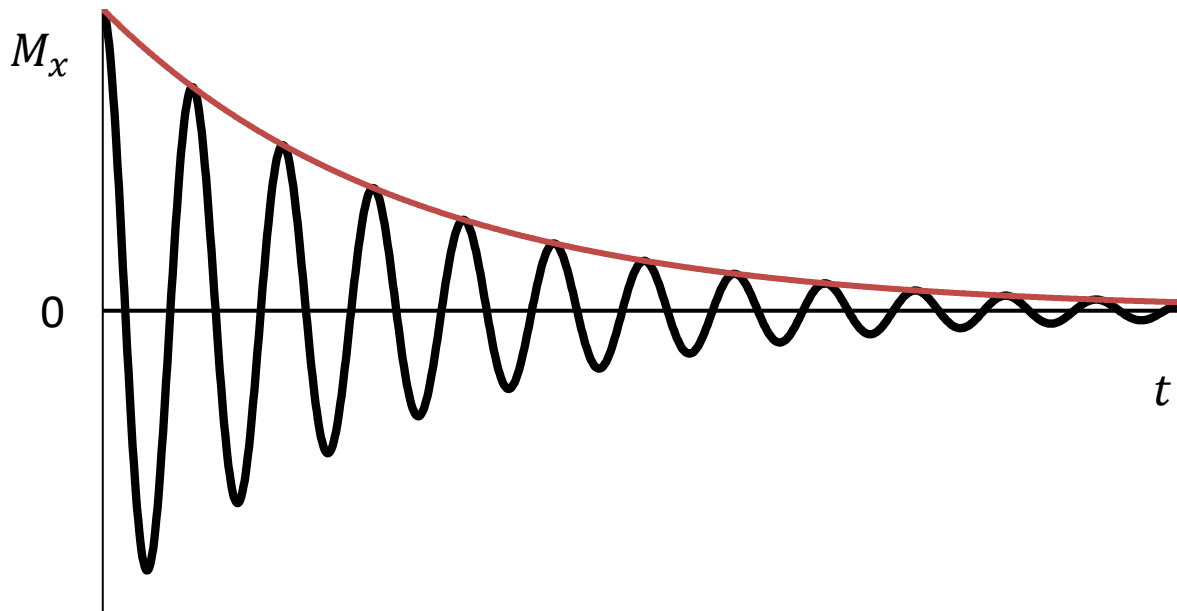- Longitudinal (spin-lattice) relaxation

$$M_z = M_0(1 - e^{-R_1 t}) = M_0(1 - e^{-\frac{t}{T_1}})$$

# Return to equilibrium: relaxation

- Transverse (spin-spin) relaxation:

$$M_{xy} = M_0 e^{-R_2 t} e^{i\Omega t} = M_0 e^{-\frac{t}{T_2}} e^{i\Omega t}$$

# Converting oscillating signal to a frequency spectrum

Fourier transform $S(\omega) = \int_{-\infty}^{\infty} s(t) e^{-i\omega t} \, dt$

Oscillation $s(t) = M_0 e^{-R_2 t} e^{i\Omega_0 t}$

$$S(\omega) = \int_0^\infty M_0 e^{-R_2 t} e^{i\Omega_0 t} \, e^{-i\omega t} \, dt$$

$$\boxed{\begin{aligned} [i(\Omega_0 - \omega) - R_2]t &= y \\ [i(\Omega_0 - \omega) - R_2]dt &= dy \end{aligned}}$$

$$= \int_0^\infty M_0 e^{[i(\Omega_0 - \omega) - R_2]t} \, dt = \int M_0 e^y \frac{dy}{i(\Omega_0 - \omega) - R_2}$$

$$\boxed{\int e^y dy = e^y + C}$$

$$= \frac{M_0}{i(\Omega_0 - \omega) - R_2} \int e^y \, dy = \frac{M_0}{i(\Omega_0 - \omega) - R_2} [e^{i(\Omega_0 - \omega)t} e^{-R_2 t}]_{t=0}^{\infty}$$

$$\underbrace{\phantom{[e^{i(\Omega_0 - \omega)t} e^{-R_2 t}]_{t=0}^{\infty}}}_{(0-1)}$$

$$(B + A)(-B + A) = -B^2 + A^2$$

$$S(\omega) = \frac{-M_0}{i(\Omega_0 - \omega) - R_2} = \frac{-M_0}{i(\Omega_0 - \omega) - R_2} \frac{-i(\Omega_0 - \omega) - R_2}{-i(\Omega_0 - \omega) - R_2}$$

$$= M_0 \frac{i(\Omega_0 - \omega) + R_2}{(\Omega_0 - \omega)^2 + R_2{}^2} = M_0 \left[ \underbrace{\frac{R_2}{(\Omega_0 - \omega)^2 + R_2{}^2}}_{A(\omega)} + i \underbrace{\frac{\Omega_0 - \omega}{(\Omega_0 - \omega)^2 + R_2{}^2}}_{D(\omega)} \right]$$

$A(\omega)$
Absorption

$D(\omega)$
Dispersion

Lorentzian line shape

Full width at half height

$$\Delta\omega = 2R_2$$
$$\left(\Delta\nu = \frac{R_2}{\pi}\right)$$

$\Omega_0$

$\omega$