Principles of EPR and Image Acquisition

Boris Epel
Quantum particle may have an intrinsic (not associated with a charge motion) magnetic moment.

**Stern–Gerlach experiment (1922)**
The beam of silver atoms was split in two by inhomogeneous magnetic field.

**Rabi experiment (1938)**
Varied magnetic field, can force the transition between the states.
First observation of EPR (1944)
Eugene Zavoyski

Kazan State University, Russia

133 MHz (4.75 mT)

Zavoyski laboratory journal ->
e\textsuperscript{-} has magnetic moment \( \mu \)

in the absence of \( B_0 \) \( \mu \)'s are oriented arbitrarily

\( \mu \) precesses around \( B_0 \)

A torque from external magnetic field applied to the e\textsuperscript{-} is required to change it’s rotation axis orientation
An unpaired electron can move between the two energy levels defined by $e^-$ interaction with magnetic field, $B$. It will absorb or emit photon of energy $h\nu$, where $\nu$ is frequency.

- **Fundamental equation of EPR spectroscopy:**
  
  $$h\nu = g\beta B_0$$

- For the given EPR frequency resonance position is at $B_0 = \text{const}$

- The uncertainty principle and magnetic interactions of $e^-$ with other $e^-$ or nearby magnetic nuclei result in resonance line broadening (signal is observed at $B$ different from $B_0$)
EPR Signal Anatomy

- Amplitude
- Integral of the spectrum
- Center field
- Line width
- Number of lines
- Distance between lines
- Line shape
- Relaxation

\[ S(T) = 1 - A \cdot \exp(-T/T_{1e}) \]
Imaging Principles

- Optics: Rayleigh criterion for resolution
  \[ r = \frac{0.61\lambda}{n\sin\theta} \]

- EPR wavelength is on the order of centimeters, too big to resolve microscopic objects
Paul Lauterbur method (1973)

Frequency encoding by application of the magnetic field gradients

The inverse Radon transform was used for image reconstruction

We use a similar method expanded to 3D and 4D
Magnetic Field Gradient

- The **direction** of B is always the same
- The direction of the magnetic field **gradient** can be changed
- Main equation:

\[ \Delta B = \vec{G} \vec{r} \]

Homogeneous field, \( B_0 \)

Linear gradient, \( 0^\circ \)

Linear gradient, \( 45^\circ \)

‘projection’
Gradient isocenter (B is always equal to B₀)

Rotation of the gradient direction is mathematically equivalent to rotation of the projection direction in Radon transformation → original object can be restored

Image Dimensionality

One dimensional

Two dimensional

Three-dimensional
Projection: Gradient Vector

\[ \rho(\vec{r}) \leftrightarrow P(\vec{G}, x) \quad \text{Radon(Inverse Radon) transformation} \]

• **1D** – **one** gradient is sufficient

• **2D** – a **plane** of gradients is required

• **3D** – a **sphere** of gradients is necessary
For the exact image reconstruction a complete (infinite) set of gradient has to be acquired.

Discrete image → discrete set of gradients
An approximate image can be reconstructed from any set of gradients
Uniform distribution of the gradient vectors over the unit sphere
  - Improves image quality & reduces artifacts
  - Equal Linear Angle (ELA) approximation
  - Equal Solid Angle (ESA) approximation
  - More complicated methods give slight improvements (Quasi Monte Carlo, Fekete)

Gradient Order

• Different gradient schemes fill the space in different order: MSPS vs PAR

<table>
<thead>
<tr>
<th>26 proj.</th>
<th>60 proj.</th>
<th>104 proj.</th>
<th>148 proj.</th>
<th>208 proj.</th>
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<td>MSPS:</td>
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Effect of the gradient order on reconstruction of dynamic phantom

(noiseless simulations)

Dynamic Phantom:
sub-volume pO₂ sinusoidally fluctuating between 20 and 40 torr
(period = imaging time)
Most sets of gradients used for imaging are not (totally) uniform.

Reconstruction methodology can compensate for the non-uniformity.

For the inverse Radon transformation this compensation can be expressed in terms of ‘projection coefficients’ calculated using Voronoi diagram.

Given set of points on unit sphere: $P = [P_1, P_2, ..., P_n]$; corresponding Voronoi areas $(V_k)$: area on surface with $d_k \leq d_j$, for all $j \neq k$.

Perfectly uniform points:
- Occupy equal portions of surface
- All $V_k$ are equal
**Parametric Imaging**

- EPR **spectral shape** in every point of the sample should be imaged

- **Amplitude**
  - Integral of the spectrum
  - Center field
  - Line width

- **Number of lines**
- **Distance between lines**
- **Line shape**
- **Relaxation**

- **High O₂**
  - Fast relax.

- **Low O₂**
  - Slow relax.
Spectral-Spatial Imaging

\[ \rho(\vec{r}, B) \leftrightarrow P(\vec{G}, x) \]  
Radon(Inverse Radon) transformation

- Sampling gradient space by use of different gradients orientations and amplitudes
- Gradient vectors fill the volume of the unit sphere.
Spectral-spatial imaging

\[ \tan(\alpha) = G \frac{dL}{dB} \]

Change of the gradient amplitude in spectral-spatial image is mathematically equivalent to the rotation of the projection direction in spectral-spatial image.

Combination of 3D spatial and spectral acquisition gives a 4D spectral-spatial image in which EPR line shape is measured in every spatial location.
Filtered Back projection
experimental setup

\[ \alpha_n, \ n=1 \text{ to } N \]

\[ G_n = \tan(\alpha_n) \ \text{dB/dL} \]

\[ B_{swn} = \text{dB} / \cos(\alpha_n) \]

\[ A_n = I_n / \cos(\alpha_n) \]

\( \alpha \) – spectral angles between -90 and 90 degrees

\( \text{dB} \) – image support in spectral dimension [G]

\( \text{dL} \) – image support in spatial domain [cm]

\( B_{sw} \) – field sweep required for projection

\( N \) EPR spectra with different \( G \) and \( B_{sw} \) are acquired in spectral domain. For single line spectrum this protocol describes an optimal choice of the gradients and the field sweeps.
What EPR can measure

Oxygen, $pO_2$
Redox status
Acidosis, pH
Cell viability
Viscosity etc ...

Low $O_2$  $\leftrightarrow$  High $O_2$

Amplitude
Integral of the spectrum
Center field
Line width
Number of lines
Distance between lines
Line shape
Relaxation
What EPR can measure

The redox state of the glutathione (GSH)/glutathione disulfide (GSSG) couple is considered to be the major intracellular redox buffer

Oxygen, $pO_2$
Redox status
Acidosis, pH
Cell viability
Viscosity etc ...

What EPR can measure

Oxygen, $pO_2$
Redox status
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Oxygen

• $O_2$ is a di-radical – two unpaired electrons

• At room temperature exhibit very high relaxation rate – undetectable by EPR

• Interacts with other paramagnetic centers through Heisenberg spin exchange mechanism
  ▫ the R• and •O$_2$• electrons become indistinguishable
  ▫ Spin exchange dramatically accelerate the center relaxation

• Can be detected indirectly by observing relaxation of other paramagnetic species
  • Smoluchowski equation for collision frequency → relaxation
    $\omega = 4\pi R p[O_2](D(A) + D(O_2))$, R – distance, D - diffusivity
    (Subczynski WK, Hyde JS., Biophys J. 1984 Apr;45(4):743-748)
Oxymetry deep in tissues with low-frequency electron paramagnetic resonance

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EPR oximetry

Both spin-spin and spin-lattice relation rates exhibit linear relations with $p[O_2]$
Oxygen spin probes

**Soluble spin probes**

A Nitroxides
B Trityl radicals

**Particulate (insoluble) spin probes**

C Lithium phthalocyanine and its derivatives

- Concentration of oxygen dissolved in a fluid
- Concentration of oxygen in material pores
Trityls: bio-stable radicals for EPR oxygen imaging

Synthesized ~1996 by Nicomed Innovations, Sweden, currently GE Healthcare

Stable: The carbon based radical is sterically protected from environment.

Longer relaxation: Symmetric shape, fast motion.

Narrow EPR line: No g-anisotropy, low e-density on protons.

Polar (3+): Does not enter cells. Locates in the extracellular volume.

Bowman et al. *J Mag Res* 2004
Trityls

- At physiologic conditions and no O\textsubscript{2} $T_1 \approx T_2 \approx 6 - 7 \mu$s
- At 21% O\textsubscript{2} (blood saturated with O\textsubscript{2}) $T_1 \approx T_2 \approx 0.6 \mu$s
- High sensitivity to O\textsubscript{2} and still measureable using pulse EPR
- Clearance from a mouse: 20 - 30 minutes
- Non toxic, well tolerated by animals
- In development
  - $T_1/T_2$ up to 12 us, $T_2^*$ up to 3 us
  - Less polar molecules to probe inside cell
$pO_2$ images: Phase vs Spin-Lattice Relaxation
\( T_1 \) shows only weak dependence on spin probe concentration.

\( T_1 \) – based EPR imaging is the perfect method for precise oxygen imaging.
Verification: Oxylite™ probe


Track #2 Sep 21, 2004, Corr. Coef. R = 0.81
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http://epri.uchicago.edu

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