The Importance of Understanding the Microscopic Distribution of Trityl Radicals for EPR Oxygen Images


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Why EPR?

Bad News: native diffusible unpaired electrons: Rare

- Good News: rare native electrons mean no background signal
- Bad News: Need to infuse subjects with materials with stable unpaired electrons
- Good News: Can infuse materials which target pharmacologic compartments
Why EPR?

- Spectroscopic Imaging: Specific quantitative sensitivity to Oxygen, Temperature, Viscosity, pH, Thiol

Free Radicals can be detected as well

Each property of interest is measured with a specifically designed probe.
Why EPR?

- Spectroscopic Imaging: Specific quantitative sensitivity to Oxygen, Temperature, Viscosity, pH, Thiol
- No water background for signal imaging (vs MRI)
Why EPR?

- **Spectroscopic Imaging:** Specific quantitative sensitivity to Oxygen, Temperature, Viscosity, pH, Thiol
- **No water background for signal imaging (vs MRI)**
- **Deep sensitivity at lower frequency (vs optical)**
  - 250 MHz vs 600 THz, 6 orders of magnitude lower
  - 7 cm skin depth vs < 1 mm
- **Vs typical EPR (0.009T vs 0.33T electromagnet)**
  - 250 MHz vs 10 GHz, factor of 40 lower
  - 7 cm skin depth
Why Electron Paramagnetic (Spin) Resonance Imaging?

- More sensitive and specific reporter of characteristics of the solvent environment of a paramagnetic reporter
- The reporter can be designed to report (mostly) only one characteristic of the environment
- E.G.: oxygen concentration, pH, thiol concentrations
- We have chosen to image molecular oxygen.
Imaging: Basic Strategy

Constraint: Electrons relax $10^6$ times faster than water protons

Image Acquisition: Projection

Reconstruction: Backprojection
Projection acquisition and image reconstruction

Image reconstruction: backprojections in spectral-spatial space

Resolution: \( \delta x = \frac{\delta B}{G_{\text{max}}} \)

THUS THE RESOLUTION OF THE IMAGE PROPORTIONAL TO \( \delta B \)

each projection filtered and subsampled;

Interpolation of Projections: number of projections x 4 with sinc(?) interpolation, Enabling for fitting

Spectral Spatial Object
Acquisition: Magnetic field sweep w stepped gradients (G)
Projections: Angle \( \alpha \):
\[ \tan(\alpha) = G \frac{\Delta L}{\Delta H} \]
The Ultimate Object: Spectral spatial imaging
Response of Symmetric Trityl (deuterated) to Oxygen

- So measuring the spectrum measures the oxygen. Imaging the spectrum: Oxygen Image
Why is oxygen important in cancer?
Known Since 1909 but in 1955 Thomlinson and Gray showed

necrosis

viable tissue
Dramatic differences in survival for patients with cervical cancer treated with radiation depending on mean tumor oxygenation: > or < 10 torr

This was thought to be the source of radiation resistance

• Hyperbaric oxygen trials √ (in human trials)

• Oxygen mimetic radiosensitizers √ (in animals only due to toxicity)

• Eppendorf electrode measurements (humans)
Intensity Modulated Radiation Therapy

- Sculpts radiation dose over distances of 5mm
- Able to spare normal tissues

But tumor volumes are homogeneous; no accounting for different regions with different sensitivity
Biological imaging to enhance targeting of radiation therapy: oxygen imaging

- Intensity modulated radiation therapy allows sophisticated control over spatial distribution of radiation dose.
Biological imaging to enhance targeting of radiation therapy: oxygen imaging

- Intensity modulated radiation therapy allows sophisticated control over spatial distribution of radiation dose.
- But: some regions may contain hypoxic cells.
Biological imaging to enhance targeting of radiation therapy: oxygen imaging

- Hypoxic cells are known to be radioresistant
Biological imaging to enhance targeting of radiation therapy: oxygen imaging

- Intensity modulated radiation therapy allows sophisticated control over spatial distribution of radiation dose.

So...

- Areas of hypoxia could be given extra dose if only we could identify them!
Why use radio-frequency for EPR?

250 MHz ~ 6 T MRI

S/N ~ $\omega^{0.8}$

$N \sim \omega^{1.2}$

IN LOSSY, CONDUCTIVE TISSUE
Aim:

**Image** crucial physiologic information: oxygen

Information:

Communicated by reporter molecules which are injected into animals/humans.

Communication through EPR spectrum.

Simple spectrum in a complex biological system
Response of Symmetric Trityl (deuterated) to Oxygen

- No temp. dependence: < 0.05 mG/K
- Low viscosity dependence
  \( \sim 1 \text{mG/cP} \)
- Minimal self quenching
Information about fluid in which reporter molecule dissolved obtained from *Spectral Parameters*

Standard penalty for deviation:
\[ \chi^2 = (y_i - \text{fit}(x_i, a_j))^2 \]

minimize this penalty by adjusting the *Spectral Parameters* \( a_j \) e.g., spectral width

Spectral parameters extracted from spectral data using *Spectral Fitting*

Bonus: Fitting gives parameter uncertainties
Spectral spatial imaging:
Major improvement: Synthesis of selectively deuterated OX31

Improves

1. Oxygen sensitivity: \( \delta \Delta B / \Delta B \)
2. Spatial resolution for a given gradient: resolvable \( \Delta x = \Delta B / G \)
3. Sensitivity
Line widths pO₂ calibration

Oxygen dependence of spin packet width obtained in a series of homogenous solutions of OX31: Since minimal viscosity dependence, aqueous=tissue
LINEWIDTH RESOLUTION

A measure of the line width resolution is the distribution of linewidhts from a homogeneous phantom. Here, the \( \sigma \) of the distribution is 0.17 \( \mu T \), or \( \sim 3 \) torr pO\(_2\) (8x8x8, projections in 20 minutes)
EPR spectral-spatial 4D imaging

- CW EPR imager at 250 MHz, with a loop-gap resonator
Mouse Image (OX063)

- PC3 tumor with 3D intensity image (Note no artifacts surrounding surface)
- XY and YZ pO2 slices corresponding to the slices in the volume
• Validation with Oxylite fiberoptic probe (measuring fluorescence quenching by oxygen)

Oxylite probe
Into tumor

Stereotactic Platform
For Needle Location
Good agreement!
Tumor Voxels Determined by Registered MRI
Induction of TNFα expression enhances IR-mediated cell killing
Effect of Gene Therapy + IR

Graph showing the effect of Gene Therapy + IR over time. The graph compares different conditions such as Control, Adeno, 50 Gy, and Adeno + 50 Gy. The y-axis represents a metric (not specified in the image) and the x-axis represents the day range from 0 to 30 days.
Probe the physiology with DCE and EPRI oxygen images

- 9mm PC3 human prostate tumors in nude mice
- Pre-treatment $T_2$ and DCE MRI plus EPRI Oxygen images
- 10 Gy Radiation plus AdEGRTNF$\alpha$ or sham
- 3 days post Rx $T_2$ and DCE MRI plus EPRI Oxygen images
Mouse 69: control, day 0 (top), day 3 (bottom)
Mouse 73, treated: day 0 (top), day 3 (bottom)
Image pO$_2$ Statistics

- **Control animals:** Day 0  Day 3
  - Mean pO2 55.1+/- 4  52.0+/-4.4
  - 10 torr hypoxic Fx 5.8+/-2.0  5.0+/-3.8
- **Treated animals**
  - Mean pO2 53.0+/-1.3  72.7+/-3.5
  - 10 torr hypoxic Fx 1.3 +/-0.3  0.2+/-0.2
- **Significance:**
  - Day 0 pO2  p=0.8
  - Day 3 pO2  p=0.01  Students 2 tailed t test
How can this be?

- Antivascular therapy is increasing the oxygenation: How could this be
- What if the antivascular therapy specifically targeted chaotic tumor vessels and had less effect on the well functioning ones
- Ad EGR TNFα is locally produced. Tumor vessels can’t get rid of it but fully functional vessels can.
- It’s a large molecule: on form 51KD, monomer 17KD
EPRI Oxygen Images Predict Radiation Curability of Tumors

- MDAnderson FSa Fibrosarcomas in C3H mice
- Radiation given to tumors of air breathing animals + loosely and tightly clamped tumors
- Doses given near the TCD_{50}
- Images obtained prior to therapy in same state
- Tumor voxels determined with either co-registered MRI or stereotactic surface “touch”
EPRI Hypoxic Fraction Predicts Radiation Curability of Tumors

XRT: $p=0.01$  EPRI oxygen: $p=0.023$
EPRI Hypoxic Fraction Predicts Radiation Curability of Tumors

- Bivariate logit model of response
- Dose correlates with cure $p=0.01$
- Fraction of voxels correlates with cure $p=0.06$
- Slope of the boundary curve is approximately $1\ \text{Gy/\%voxels less than 10 torr}$
- Minimum cure dose (abscissa intercept): 25 Gy
Spin Echo Imaging

- Diminishes the dependence of image noise on dead time
- Mode: Straight $\pi/2 - \tau - \pi - \tau$ spin echo imaging
$T_2$ distribution (mG equivalent)

\[ \sigma: 0.28 \text{ mG equivalent to } \sim 0.5 \text{ torr} \]
Real Test of EPR Oxygen Imaging

• Treat with radiation to a single dose – TCD50
• Evaluate tumor oxygenation status with EPROI
• Question:
  – Do EPROI voxels with low pO_2 predict radiation failure or success?
• Use Electron Spin Echo Oxygen Imaging
Typical ESEOI slices from failed and controlled tumors
EPROI predicts tumor cure after a single TCD50 dose radiation.

Single dose 33.8 Gy

HF10<9% 1/15 failed
HF10>9% 12/18 failed
P = 0.012 (Stud. 2 tail)

HF10 separates cures from recurrences

EPROI HF10 predicts tumor cure
New Radiation Paradigm: Few Large Radiation Dose Fractions

- Radiation dose sculpting enables safe delivery of high tumor radiation doses/fraction (3Fx 18-24Gy)
- Single dose (≥24Gy) gives ~90% local control of oligometastatic (M1) cancer regardless of the histologic type or the target organ
- Single large doses (>24 Gy) gives 80% disease free survival at 18 months for Stages I and II medically inoperable lung cancer, comparable with surgery
The earliest visible response of tumors to high-dose radiation is an acute wave of endothelial apoptosis.

- Initiated by a plasma membrane, not DNA damage, response
- Involves ASMase activation and ceramide generation
- Inhibited in apoptosis-deficient cells (asmase\(^{-/-}\), Bax\(^{-/-}\))
- Does not effect DNA repair-deficient phenotypes (SCID, atm\(^{-/-}\))
The Apoptosis Inhibits Repair of Radiation Damage

- Oxygen necessary for radiation damage repair
- The vascular apoptosis creates rapid hypoxia
- This sensitizes tumors to large doses of radiation
- THE EFFECT HAS ONLY BEEN SEEN IN HISTOLOGIC SECTIONS, NOT IN VIVO
IS THERE A RAPID HYPOXIC RESPONSE IN VIVO?

- Electron Spin Echo allows oxygen images in 10 minutes
- Oxygen images can be obtained before a 20 Gy radiation dose
- Compared with the oxygenation after the dose
- Voxels from the tumor provide a large statistical sample.
- Large variability of pO2 voxels in the tumor
pO$_2$ slice before/after 20 Gy in ASMase $+/+$ C57 mouse

Tumor T2 MRI before experiment.
Results: $\Delta s$ in 50 min (~30 min) post 20 Gy

$+/+$: Significantly ↓ $pO_2$ ⇒ ↓ repair ⇒ ↑ sensitivity
These T2 based pO2 images are sensitive to the spin probe concentration

- Concentration broadening sensitive to the salt concentration of the solvent.
- Trityl molecules are triply charged and in water repel, giving an artificially low concentration dependence, 2 mG/mM
Saline Concentration Dependence of OX063H Relaxation Rate R2e

0% O₂ saline 37°C

0.5 mM in saline 37°C

Average concentration observed in mice is ~0.3 mM

8.3 mG/mM
Concentration Dependence in vivo

\[ pO_2 = \kappa (R - R(0 \text{ torr}, 0 \text{ mM}) - \varepsilon C) \]

The concentration of the spin probe in a mouse was increased step-wise.

\[ \varepsilon \text{ in water } = 2 \text{ mG/mM} \]
\[ \varepsilon \text{ in saline } = 8 \text{ mG/mM} \]
\[ \varepsilon \text{ in vivo } = 15-40 \text{ mG/mM, varies with animals} \]
Partial Volume Effect

\[ C = \frac{N}{V} \]

\[ C = 4, \quad C^{\text{REAL}} = 4 \]

\[ C = 4, \quad C^{\text{REAL}} = 16 \]
Excluded Volume

Trityl spin probe cannot penetrate cell membrane.

Blood: hematocrit volume is 35-49%

Muscle: excluded volume is up to 80%
Concentration Dependence of OX063H Relaxation Rates

0% O₂ saline 37°C

0.5 mM in saline 37°C

Average concentration observed in mice is ~0.3 mM
Need to use EPR microscopy to determine the tissue distribution
And microscopic maps of $R_{2e}^*$
Conclusions

• CW 4D Images of phantoms can be obtained with ~1 mm spatial resolution and ~1.5mG spectral resolution or ~ 3 torr in ~ 45 minutes

• Quantitative oxygen images spatially correspond to Oxylite oxymeter measurements.

• Images of increased oxygenation levels due to adeno-EGR-TNFα radiation anti-vascular therapy a

• EPR oxygen images show that anti-vascular agents attack ineffective tumor vessels -> improve oxygen delivery

• EPR Oxygen images + dose predict tumor cure

• Electron Spin Echo images give more precise oxygenation images in less time for small animals

• It is crucial to understand the distribution of trityl in living animal tissues to understand the self broadening effects of the trityl. These are within a few torr but could be troublesome
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<tr>
<td>Howard Halpern</td>
<td>Greg Karczmar</td>
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<td>Martyna Elas</td>
<td>Jonathan River</td>
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Stimulated Echo

Sequence bandwidth in the absence of relaxation:

- FID: $\sim 1/t_p$
- ESE: $\sim 0.25/t_p$
- SE: $\sim 0.5/t_p$

Two pulse sequence has a twice higher signal than stimulated echo


*Stimulated echo requires 16 times less power for equivalent bandwidth coverage as compare to IRESE / ESE*

This might be crucial for imaging of large objects, since RF power requirements are growing proportional to the resonator volume.
Technical Enhancements

- CW oxygen imaging of mouse tumors:
  - **Overmodulation**
  - Rapid projection acquisition
  - Interpolation to recover spectral fidelity
- Implementation of an intermediate size imaging system to
  - Explore rapid acquisition while obtaining data from the older small imager
  - Test proof of principle for the design of the larger magnet
  - Explore scaling to larger objects
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Increased sampling sharpens the image
Technical Enhancements

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AIR BREATHING

CARBOGEN (95% OXYGEN) BREATHING
HETEROGENEOUS TUMOR SLICE: SIGNIFICANT DIFFERENCES IN $PO_2$

**CARBOGEN (95% OXYGEN)**

- 215 voxels selected = 63.4221 percent of non-zero total

**AIR**

- 213 voxels selected = 61.561 percent of non-zero total

**Graphs:**
- Mean = 36.2 SEM = 1.5
- Mean = 31.3 SEM = 1.5
BOLD MRI of Fsa fibrosarcoma, April 12th

Air Control

Carbogen-Air \[n-(n-1)]\]
Quantitative Oxygen Map

BOLD MRI

dd010412
BOLD MRI of Fsa fibrosarcoma, June 1st

Air Control

Carbogen-Air
Quantitative oxygen map

BOLD MRI
Physiology of Oxygenation Response to Ad.EGR-TNFα + 10 Gy X-ray

Day 0

Day 3

Day 16
Carr-Purcell-Meiboom-Gill

Measure here
Spin Echoes from CPMG pulse sequence
CPMG Image

3 ECHO XZ IMAGE 2 TUBES 3 MM DIA SEPARATED BY 3 MM 16 PROJECTIONS

ECHO 1 T = 5 USEC

ECHO 2 T = 10 USEC

ECHO 3 T = 15 USEC

FOV CM
Slices of 3D concentration and pO2 ESEOI image of a live mouse leg bearing a tumor
Basic Interaction

- Magnetic Moment $\mu$ (let bold indicate vectors)
- Magnetic Field $\mathbf{B}$
- Energy of interaction:
  - $E = -\mu \cdot \mathbf{B} = -\mu B \cos(\theta)$
Magnetic Moment

• ~ Classical Orbital Dipole
  – Orbit with diameter $r$, area $a = \pi r^2$
  – Charge $q$ moves with velocity $v$; Current is $qv/2\pi r$
  – Moment $\mu = a \cdot I = \pi r^2 \cdot qv/2\pi r = qvr/2 = qmvr/2m$

• $mv \times r \sim$ angular momentum: let $mvr = "S$

• $\beta = q/2m$; for electron, $\beta_e = -e/2m_e$ (negative for e)

• $\mu_B = \beta_e S$; $S$ is in units of $\hbar$
  – Key 1/m relationship between $\mu$ and $m$

• $\mu_B$ (the electron Bohr Magneton) = $-9.27 \times 10^{-24}$ J/T
Magnitude of the Dipole Moments

• Key relationship: $|\mu| \sim 1/m$

• Source of principle difference between
  – EPR experiment
  – NMR experiment

• This is why EPR can be done with cheap electromagnets and magnetic fields $\sim 10\text{mT}$

*not requiring superconducting magnets*
Torque on the Dipole in the Magnetic Field $B_0$

- Torque, $\tau$, angular force
  \[ \tau = -\frac{\partial E}{\partial \theta} = \mu B_0 \sin \theta = -|\mu \times B| \]
- For moment of inertia $I$
  \[ I \frac{\partial^2 \theta}{\partial t^2} = -|\mu|B_0 \sin \theta \sim -|\mu|B_0 \theta \text{ for small } \theta \]
- This is an harmonic oscillator equation
  \[ (m\frac{\partial^2 x}{\partial t^2} + kx = 0) \]
- Classical resonant frequency \( (\omega_0 = \sqrt{k/m}) \)
  \[ \omega_0 = \sqrt{|\mu|B_0/I} \]
Damped Oscillating Moment: Pulsed Experiment

- Solution to above is $\psi = A \exp(i\omega t) + B \exp(-i\omega t)$: Undamped, Infinite in time, Unphysical
- To $I \frac{\partial^2 \theta}{\partial t^2} + \mu B_0 \theta = 0$ add a friction term $\Gamma \frac{\partial \theta}{\partial t}$ to get:

  $$I \frac{\partial^2 \theta}{\partial t^2} + \Gamma \frac{\partial \theta}{\partial t} + \mu B_0 \theta = 0$$

  $$\omega = i\Gamma/2I \pm (4I\mu B_0 - \Gamma^2)^{1/2}/2I$$ so that

  $$\theta(t) = A \exp(-t \Gamma/2I - i ((4I\mu B_0 - \Gamma^2)^{1/2}/2I)t) + B \exp(-t \Gamma/2I + i ((4I\mu B_0 - \Gamma^2)^{1/2}/2I)t)$$

  Lifetime: $T \sim 2I/\Gamma$

  Redfield Theory: $\Gamma \sim \mu^2$ so $T_1s \& T_2s \propto 1/\mu^2$

Interaction with environment

“Reality Term”
Damped Driven Oscillating Moment: Continuous Wave Measurement

- If we add a driving term to the equation so that \( I \frac{\partial^2 \theta}{\partial t^2} + \Gamma \frac{\partial \theta}{\partial t} + \mu B_0 \theta = B_1 \exp(i \omega_1 t) \),
- In the steady state \( \theta(t) = \frac{B_1 \exp(i \omega_{1hh} t)}{[\omega_0^2 - \omega_1^2 + 2i \omega_1 \Gamma / 2I]} \)
a resonant like profile with

\( \omega_0^2 = \mu B / I \), proportional to the equilibrium energy.

\[ \text{Re} [\theta(t)] \sim B_1 / \omega_1^2 [(\Delta \omega)^2 + (\Gamma / 2I)^2] \text{ for } \omega_0 \sim \omega_1 \]
Lorentzian shape, Linewidth \( \Gamma / 2 \)
Q.M. Time Evolution of the Magnetization

- $S$ is the spin, an operator
- $H$ is the Hamiltonian or Energy Operator
- In Heisenberg Representation:
  \[
  \frac{\partial S}{\partial t} = \frac{1}{i\hbar} [H, S], \text{ As we recall, } H = -\mu_B \cdot B = -\beta_e S \cdot B.
  \]
  \[
  [H, S_i] = HS_i - S_i H = -\beta_e (S_j S_i - S_i S_j) B_j = \beta_e \hbar S \times B
  \]
  \[
  \frac{\partial S}{\partial t} = \beta_e S \times B; \text{ follows also classically from the torque on a magnetic moment } \sim S \times B \text{ as seen above}
  \]
  Bloch equations follow with (let $M = S$):
  \[
  \frac{\partial S}{\partial t} = \beta_e S \times B - 1/T_2(S_x \hat{1} + S_y \hat{j}) - 1/T_1(S_z - S_0) \hat{k}
  \]
Density Matrix

- Define an Operator $\rho$ associating another operator $S$ with its average value:
- $<S> = \text{Tr}(\rho S) ; \rho = \Sigma |a_i><a_i| ;$ basically is an average over quantum states or wavefunctions of the system
- $\rho :$ Density Matrix: Characterizes the System
- $\partial \rho / \partial t = 1/i\hbar [H, \rho], 1/i\hbar [H_0+H_1, \rho],$
  Here we break the Hamiltonian into two terms, the basic energy term, $H_0 = -\mu \cdot B_0,$ and a random driving term $H_1 = \mu \cdot \dot{B}_r(t),$ characterizing the friction of the damping
- $\rho^* = \exp(iH_0t/\hbar) \rho \exp(-iH_0t/\hbar) = e^+ \rho e^- ; H_1^* = e^+H_1e^-$
- $\partial \rho^* / \partial t = 1/i\hbar [H^*_1(t), \rho^*(t)],$
- $\rho^* \sim \rho^*(0) + 1/i\hbar \int dt' [H^*(t'), \rho^*(0)] + 0$
  $(1/i\hbar)^2 \int dt' dt'' [H^*_1(t'), [H^*_1(t''), \rho^*(0)]]$
The Damping Term (Redfield)

\[
\begin{align*}
\frac{\partial \rho^*}{\partial t} &= \frac{1}{\hbar} [H^*(t'), \rho^*(0)] \rightarrow 0 \\
(1/\hbar)^2 \int dt' [H^*_{1}(t), [H^*_{1}(t-t'), \rho^*(t')] \\
Each of the H^*_{1} has a term in it with H= -\mu \cdot B_r and \mu \sim q/m \\
\frac{\partial \rho^*}{\partial t} \sim (-) \mu^2 \rho^* \text{ This is the damping term: } \\
\rho^* \sim \exp(- \mu^2 t \text{ with other terms}) \\
Thus, state lifetimes, e.g. T_2, are inversely proportional to the square of the coupling constant, \mu^2 \\
The state lifetimes, e.g. T_2 are proportional to the square of the mass, m^2
\end{align*}
\]
Consequences of the Damping Term

- The coupling of the electron to the magnetic field is $10^3$ times larger than that of a water proton so that the states relax $10^6$ times faster.
- No time for Fourier Imaging techniques.
- For CW we must use:
  1. Fixed stepped gradients
  1. Vary both gradient direction & magnitude (3 angles)
  2. Back projection reconstruction in 4-D
Projection Acquisition in EPR

- Spectral Spatial Object
  
  \[ f(B, \bar{x}) \]

  \[ B_{\text{app}}(\bar{x}) = B_0 + B_{sw} + \bar{G} \cdot \bar{x} \]

  \[ h\nu = g \beta B_{\text{TOT}} \]

  \[ \left[ B_{v_0} - \frac{\Delta B}{2}, B_{v_0} + \frac{\Delta B}{2} \right] \times \Omega_{\bar{x}} \]
Projection Description

- With $s(B_{sw}, \hat{G})$ defined as the spectrum we get with gradients imposed

$$s\left(B_{sw}, \hat{G}\right) = \int \int f_{sw}(B, \bar{x}) \delta\left(B - B_{sw} - \hat{G} \cdot \bar{x}\right) d\bar{x} dB$$
More Projection

• The integration of $f_{sw}$ is carried out over the hyperplane in 4-space by

$$B = B_{sw} + \hat{G} \cdot \bar{x}$$

Defining with

$$\kappa = c \hat{G} \cdot \bar{x}$$

The hyperplane becomes

$$B = B_{sw} + G(\hat{G} \cdot \bar{x}) = B_{sw} + c^{-1}G\kappa$$

with

$$\tan \alpha = -c^{-1}G$$

$$c = \frac{\Delta B}{\Delta L}$$
And a Little More

So we can write

$$B = B_{sw} + c \tan \alpha \hat{G} \cdot x$$

$$s(B_{sw}, \bar{G}) = \cos \alpha \int \int f_{sw}(B, \bar{x}) \delta \left( \cos \alpha B - \cos \alpha B_{sw} + \sin \alpha c \hat{G} \cdot \bar{x} \right) d\bar{x} dB$$
So finally it’s a Projection

\[ s \left( B_{sw}, \tilde{G} \right) = \cos \alpha \int_{\Omega_{\bar{r}}} f_{\bar{r}} (\bar{r}) \delta \left( \xi - \hat{\alpha}_G \cdot \bar{r} \right) \, d\bar{r} \]

with

\[ \xi \equiv \cos \alpha B_{sw} \]
\[ \bar{r} \equiv (B, c \bar{x}) \]
\[ \hat{\alpha}_G \equiv \left( \cos \alpha, \sin \alpha \hat{G} \right) \]
\[ \Omega_{\bar{r}} \equiv \left[ \frac{\Delta B}{2}, \frac{\Delta B}{2} \right] \times c \Omega_{\bar{x}}. \]
Good agreement!
Adjacent tracks in areas of rapid oxygen variation agree with Oxylite
Line Width Fidelity: Agreement Between Image and Individually measured line-widths (1.6 mm) heterogeneous phantom. Side lines Approximately ±3 torr.
New insight into the oxygen status of tumors and tissues
Concurrence between position of tumor estimated from the stereotactic platform and high trityl amplitude tumor measurements.
Further validation of concurrence of tumor and high EPR OX063 peak height from Correlation with MRI Using:

- MRI setup in roughly the same position
- Interactive surface rendering and match
Dimensions of the resonator (1.5 cm thick)
Sensitive Region of the resonator (total of just over 2 -2.5 cm)
Electron Spin Echo Experiment

Measure near here
2-D Spin Echo Image of 3 Vials with Different Oxygen Concentrations
3-D Spin Echo Images of Vials with 0 and 3% Oxygen for Different $\tau$

- T = 1.5 $\mu$sec
- T = 3.5 $\mu$sec
- T = 5.5 $\mu$sec
- T = 7.5 $\mu$sec
Histogram of Decay Rates Across the Image for 0% and 2% (15torr) Oxygen
Mouse leg/tumor Sagittal

A

B

C

D

1 cm

Amp [mM]  Amp [mM]  pO₂ [torr]  pO₂ [torr]

CW  ESE  CW  ESE
Fraction of tumor voxels with pO2 < 2.5 torr and < 10 torr

*: failure
22 Animals/tumors: pO2 statistics of failures vs controlled tumors

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Median pO2</th>
<th>HF 2.5</th>
<th>HF5</th>
<th>HF10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Student 2 tail t-test p value</td>
<td>0.051</td>
<td>0.047</td>
<td>0.024</td>
<td>0.009</td>
</tr>
</tbody>
</table>
SBRT and IGRT enable safe delivery of high radiation doses/fraction (1-3Fx18-24Gy) to tumors residing in soft tissues, bone, lung and the liver.

- Single dose ($\geq 24$Gy) results in $\sim 90\%$ long-term local control of oligometastatic (M1) cancer regardless of the histological phenotype or the target organ of the metastatic lesion.

- Single large doses (>24 Gy) results in 80% disease free survival at 18 months for Stages I and II medically inoperable lung cancer, comparable with surgery.
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Bigger: Rat tumor
4 cm Rabbit Tumor

Key: local arterial trityl perfusion
CW vs ESE
ESE vs CW

![Bar chart comparing ESE and CW](chart.png)
Scaling up in volume

- CW oxygen imaging of mouse tumors:
  - Overmodulation
  - Rapid projection acquisition
  - Interpolation to recover spectral fidelity
- Implementation of an intermediate size imaging system to
  - Explore rapid acquisition while obtaining data from the older small imager
  - Test proof of principle for the design of the larger magnet
  - Explore scaling to larger objects
30 cm DHV Large Magnet

Achieves design homogeneity

Multidirectional access
OX063H $T_{1e}$ Imaging

• At animal body temperature ($\sim$37°C) $T_{1e}$ is governed by local mode relaxation and has weak spin rotation contributions due to highly isotropic g-factor and small nuclei hyperfine interactions.

• $T_{2e}$ is largely governed by $T_{1e}$ and dipolar spin-spin interactions responsible for linear concentration dependence in saline. At 37°C the hyperfine anisotropy is averaged completely ($T_{2e} = T_{1e}$).

• In saline $T_{2e}$ has a concentration dependence $\sim$8 mG/mM, considerably weaker than that for nitroxides. $T_{1e}$ concentration dependence in saline was not studied.

$T_{1e}$ Sequences for FBP imaging

Saturation by fast repetition (SRT)

Inversion recovery (IRESE)

Stimulated Echo (SE)

Fourier transformation of the echo is used to generate projections.

The same sequence of gradients is used for both IRESE and SE
Precision of Relaxation Time Imaging

<table>
<thead>
<tr>
<th>Pulse Sequence</th>
<th>$T_{2e}$ or $T_{1e}$</th>
<th>Error of $T_{2e}$ or $T_{1e}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESE ($T_{2e}$)</td>
<td>5.15 $\mu$s</td>
<td>0.19 $\mu$s</td>
</tr>
<tr>
<td>SRT ($T_{1e}$)</td>
<td>6.2 $\mu$s</td>
<td>1.4 $\mu$s</td>
</tr>
<tr>
<td><strong>IRESE ($T_{1e}$)</strong></td>
<td>5.9 $\mu$s</td>
<td>0.29 $\mu$s</td>
</tr>
<tr>
<td>SE ($T_{1e}$)</td>
<td>5.8 $\mu$s</td>
<td>0.38 $\mu$s</td>
</tr>
</tbody>
</table>

Error: S.D. of $T_{1e}, T_{2e}$ from all voxels of phantom

*Non-imaging conditions:*

$T_{2e} = 5.07$ ms (ESE)  \hspace{2em}  $T_{1e} = 5.8$ ms (IRESE)
Promise of EPRI Oxygen Images

Use the DOSE vs HYPOXIC FRACTION diagram to modify the dose to the hypoxic region only.
IMRT Dose “Painting” increasing dose as above based on EPRI oxygen map

Primary Tumor Dose

Hypoxic Boost
Concentration Dependence of OX063H Relaxation Rates

Average concentration observed in mice is \(~0.3\) mM
Concentration Dependence of OX063H Relaxation Rates

0% O₂ saline 37°C

Average concentration observed in mice is ~0.3 mM

0.5 mM in saline 37°C
Red contours outline the borders of tumor identified in the registered $T_2$-weighted MRI image. The $pO_2$ histogram of tumor voxels is shown in red.