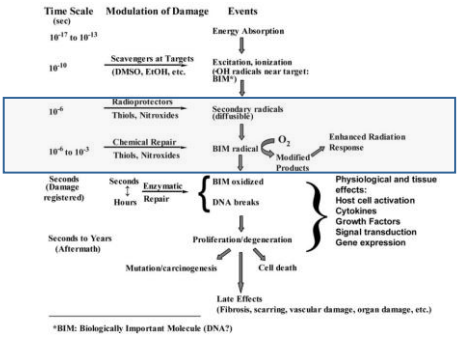
Overhauser Oxygenation Imaging: Physics, Instrumentation and Pre-Clinical Applications

D. Hristov, Kang-Hyun Ahn, Greig Scott
Stanford University, Stanford, CA

STANFORD ELECTRICAL ENGINEERING

STANFORD SCHOOL OF MEDICINE
Stanford University Medical Center

Motivation: What needs to be measured?



Mapping of all radiation damage modifying species ideal.

Motivation: On what magnitude scale?

Table 3. Critical O_2 tensions below which typical cellular functions in solid tumors progressively cease or anticancer treatments are impaired as a result of an inadequate O_2 availability

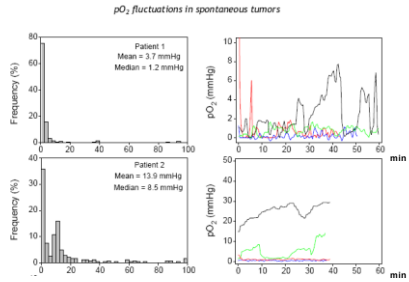
Critical O_2 tension, mmHg*	Function or parameter observed	Selective reference(s)
30–35	Effectiveness of certain (passive) immunotherapies	(*128*)
15–35	Cell death with photodynamic therapy	(*129–132*)
25–30	Cell death on exposure to x- and γ-radiation	(*2,3*)
10–20	Binding of hypoxia markers	(*87,95*)
1–15†	Proteome changes	(*22,114,121, 125,127*)
0.2–1	Genome changes	(*19,22,23,29, 97,133,134*)

*mmHg = millimeters of mercury.

Continuous oxygenation mapping preferable.

Michael Höckel, Peter Vaupel Journal of the National Cancer Institute, Vol. 93, No. 4, February 21, 2001

Motivation: On what timescale?

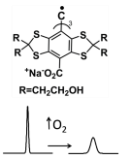


Fluctuations in pO_2 in poorly and well-oxygenated spontaneous canine tumors before and during fractionated radiation therapy
K.G. Brurberg et al. / Radiotherapy and Oncology 77 (2005) 220-226

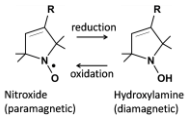
Significant fluctuations on a timescale of minutes exist.

Electron Paramagnetic Resonance Imaging (EPRI)

- Inject a free radical (unpaired electron) spin probe
- Use magnetic resonance to map spin probe properties and temporal dynamics



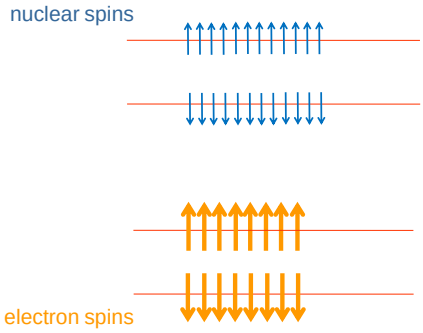
Trityl, oxygen-sensitive line broadening. Image broadening.



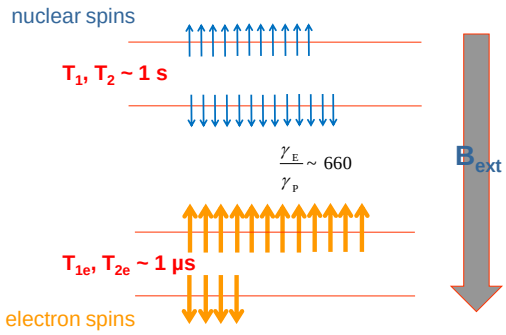
Nitroxide, redox potential similar to tissue. Image temporal decay.

With proper probes EPR sensitive to redox and oxygenation

NMR vs. EPR



NMR vs. EPR



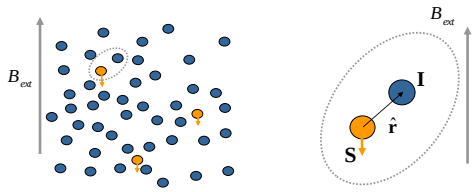
For the same B0, electrons have more polarization, higher resonance frequency, shorter T1, T2.

"Overhauser proposed ideas of startling originality, so unusual that they initially took portions of the scientific community back, but of such depth and significance that they opened vast new areas of science."

- Dynamic nuclear polarization, DNP
- Proton Electron Double Resonance, PEDRI

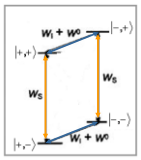
Nuclear-electron spin interaction

$$H = -\hbar\gamma_I B_{ext} I_z - \hbar\gamma_S B_{ext} S_z + \hbar H'$$



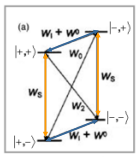
$$H' = -\left(\hbar\gamma_I\gamma_S / r^3\right)\left[\frac{3(\mathbf{I}\cdot\hat{\mathbf{r}})(\mathbf{S}\cdot\hat{\mathbf{r}})}{r^2} - \mathbf{I}\cdot\mathbf{S}\right]$$

Nuclear-electron spin interaction



$$\left|m_{i=1,4}\right\rangle = \left|I_Z, S_Z\right\rangle$$

$$\underbrace{\left|+,-\right\rangle, \left|-,-\right\rangle, \left|-,+\right\rangle, \left|+,+\right\rangle}_{\text{non - interactin g spin states}}$$



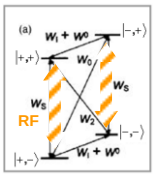
net nuclear magnetization

$$< I_Z >= (N_{|++\rangle} + N_{|+-\rangle}) - (N_{|-+\rangle} + N_{|--\rangle})$$

net electron magnetization

$$< S_Z >= (N_{|+-\rangle} + N_{|--\rangle}) - (N_{|-+\rangle} + N_{|++\rangle})$$

J. Chem. Phys. 128, 052211 (2008)© 2008 American Institute of Physics



$$< I_Z > / I_0 = 1 - kf\,\frac{\gamma_S}{\gamma_I}\left(\frac{< S_Z > - S_0}{S_0}\right)$$

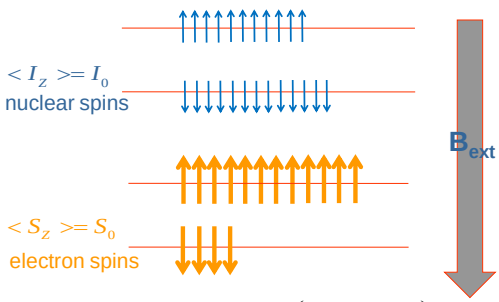
$k \sim 0.5$ (coupling factor)

Leakage factor $\quad f = \dfrac{w_0 + 2w_1 + w_2}{(w_0 + 2w_1 + w_2 + w^0)} = \dfrac{rcT_{10}}{1 + rcT_{10}} = rcT_1$

Saturation factor $\quad s = \dfrac{< S_Z > - S_0}{S_0} = \dfrac{\gamma^2 B_{RF}^2 T_{1e} T_{2e}^*}{1 + \gamma^2 B_{RF}^2 T_{1e} T_{2e}^*}$

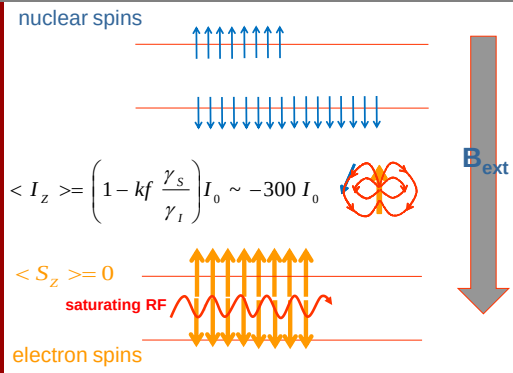
J. Chem. Phys. 128, 052211 (2008)© 2008 American Institute of Physics

No saturation case

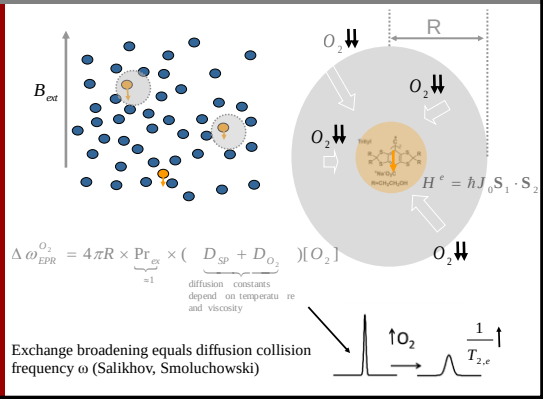


$$< I_Z > / I_0 = 1 - kf\,\frac{\gamma_S}{\gamma_I}\left(\frac{< S_Z > - S_0}{S_0}\right)$$

Complete saturation



Heisenberg Spin Exchange Interaction



Exchange broadening equals diffusion collision frequency ω (Salikhov, Smoluchowski)

Oxygen effect

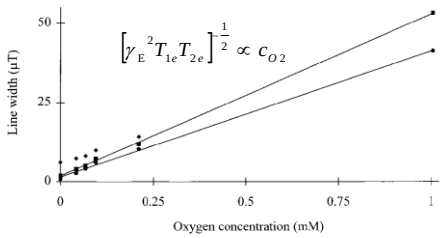
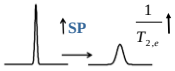


FIG. 10. The oxygen effect on (◆) the peak–peak linewidth, (■) the Lorentzian linewidth, $2(\sqrt{3}\gamma_e T_{2e})^{-1}$, and (●) $2(\sqrt{3}\gamma_e T_{1e})^{-1}$, for the deuterated hydroxy trityl in water at 37°C. (—) Linear regression fits.

Ardenkjaer-Larsen et al., EPR and DNP properties of certain novel single electron contrast agents intended for oximetric imaging. J Magn Reson. 1998 Jul;133(1):1-12.

Heisenberg Spin Exchange

$$\Delta\omega_{EPR}^{SP} = 2\pi R \times D_{SP} \times [SP]$$



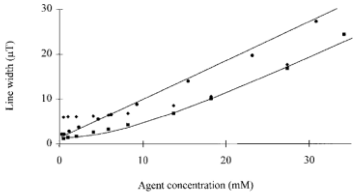


FIG. 7. (◆) The peak–peak linewidth and (■) the Lorentzian linewidth, $2(\sqrt{3}\gamma_e T_{2e})^{-2}$, from Voigt lineshape analysis in water, and (●) the Lorentzian linewidth in isotonic saline for the deuterated hydroxy trityl at 37°C. (—) Linear regression fit in isotonic saline and fit to Eq. [23] in water.

Overhauser enhancement summary

$$\text{O.E.} = \frac{I_Z - I_0}{I_0} = -(\gamma_S/\gamma_I)\, fks$$

$$f = rcT_1 \text{ (leakage factor)}$$

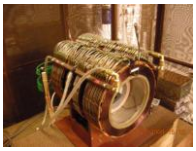
$$k = 0.5 \text{ (coupling factor, purely dipolar interaction)}$$

$$s \equiv \frac{\gamma_E^2 B_{RF}^2 T_{1e} T_{2e}}{1+\gamma_E^2 B_{RF}^2 T_{1e} T_{2e}} = \frac{\alpha\gamma_E^2 P_{RF} T_{1e} T_{2e}}{1+\alpha\gamma_E^2 P_{RF} T_{1e} T_{2e}} \text{ (saturation factor)},$$

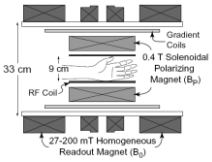
$$\left[\gamma_E^2 T_{1e} T_{2e}\right]^{-\frac{1}{2}} = a_1 c_{O2} + a_2 c + a_3$$

Overhauser Enhancement reports spin probe parameters via its dependence on spin probe concentration and relaxation times. It also depends on EPR rf power (B_1), EPR pulse duration.

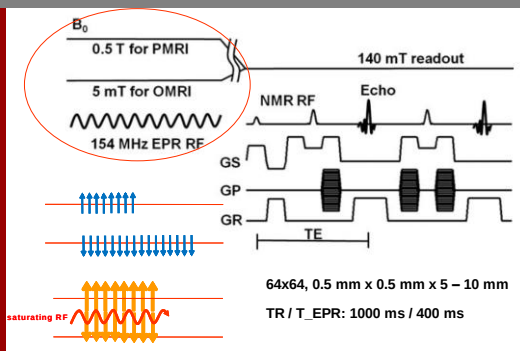
Instrumentation



Field-cycled PEDRI imaging of free radicals with detection at 450 mT, Lurie, D.J.; Davies, G.R.; Foster, M.A.; Hutchison, J.M.S. (2005) Magnetic Resonance Imaging, 23, pp.175–181



Pulse sequences

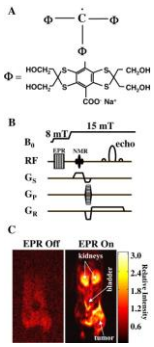


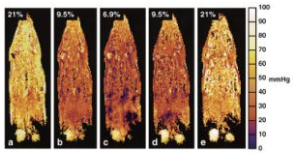
64x64, 0.5 mm x 0.5 mm x 5 – 10 mm

TR / T_EPR: 1000 ms / 400 ms

Ahn, K.-H., Scott, G., Stang, P., Conolly, S. and Hristov, D. (2011), Multiparametric imaging of tumor oxygenation, redox status, and anatomical structure using overhauser-enhanced MRI–prepolarized MRI system. Magnetic Resonance in Medicine, 65: 1416–1422.

Overhauser enhanced magnetic resonance imaging for tumor oximetry

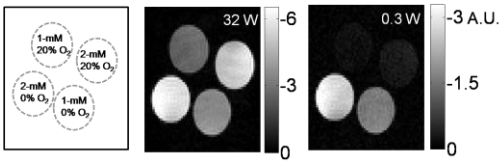




Golman, K., Petersson, J. S., Ardenkjær-Larsen, J.-H., Leunbach, I., Wistrand, L.-G., Ehnholm, G. and Liu, K. (2000), Dynamic in vivo oxymetry using overhauser enhanced MR imaging. Journal of Magnetic Resonance Imaging, 12: 929–938.

Krishna M. C. et.al. PNAS 2002;99:2216-2221

Oxygen Imaging

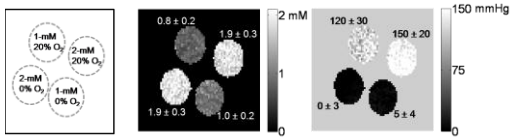


Spin-echo OMRI acquired using two RF power levels for phantoms with various trityl concentrations and oxygenations.

Table 1
OE Factors Obtained from Trityl Phantoms

	High power (32 W)		Low power (0.3 W)	
Trityl concentration	Deoxygenated	Oxygenated	Deoxygenated	Oxygenated
1 mM	123 ± 20	89 ± 14	59 ± 11	12 ± 4
2 mM	190 ± 25	148 ± 18	92 ± 14	15 ± 4

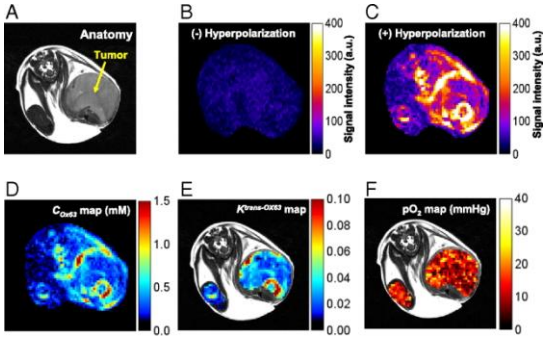
Oxygen Imaging



Spin probe concentration (a) and pO2 (b) computed from two-power level oximetry. Trityl produces stronger EPR signal with less oxygen.

Ahn, K.-H., Scott, G., Stang, P., Conolly, S. and Hristov, D. (2011), Multiparametric imaging of tumor oxygenation, redox status, and anatomical structure using overhauser-enhanced MRI–prepolarized MRI system. Magnetic Resonance in Medicine, 65: 1416–1422.

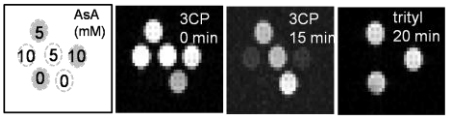
Simultaneous imaging of tissue pO2 and Ktrans of OX63 in vivo using Overhauser-enhanced MRI.



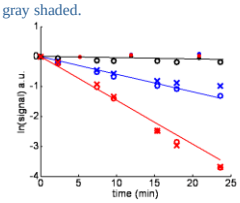
Matsumoto S et al. PNAS 2009;106:17898-17903

PNAS

Imaging of redox and oxygenation

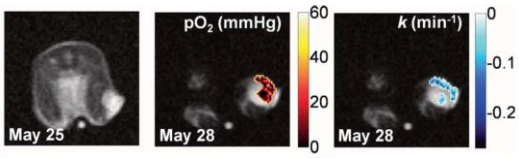


Five phantoms contained 3CP nitroxide. Various amounts of ascorbic acid were added as shown in the left diagram. Phantoms with additional 1-mM trityl are gray shaded.



Red, blue, and black denote 0, 5, 10 mM AsA addition. Solid circles, open circles, and cross marks represent trityl signal, 3CP signals with and without 1-mM trityl, respectively.

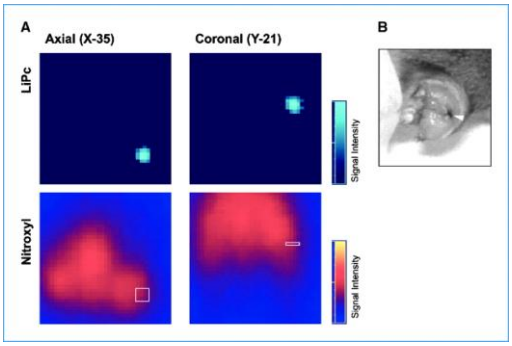
Applications



PMRI (May 25) demonstrates anatomic information with 5 mm tumor of bright T2 contrast. Fiducial tubing (1.6 mm i.d. filled with water) is visible between the two legs. The mouse underwent pO2 and redox OMRI 3 days later. Trityl and 3CP were consecutively infused by intratumoral injection.

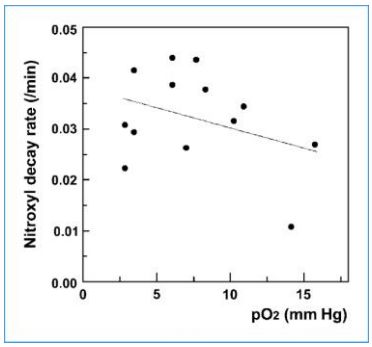
Ahn, K.-H., Scott, G., Stang, P., Conolly, S. and Hristov, D. (2011), Multiparametric imaging of tumor oxygenation, redox status, and anatomical structure using overhauser-enhanced MRI–prepolarized MRI system. Magnetic Resonance in Medicine, 65: 1416–1422.

Determination of position of LiPc implantation on an image of HM-PROXYL probe.



Takeshita K et al. Cancer Res 2010;70:4133-4140

Correlation between pO2 and rate of nitroxyl signal decay in a tumor under air-breathing.



Takeshita K et al. Cancer Res 2010;70:4133-4140

Summary

- Dynamic nuclear polarization (Overhauser) offers possibility for quantitative imaging of oxygenation and redox status in pre-clinical models
- RF power deposition main obstacle to clinical translation
- Offers potential for correlative studies with number of simultaneously monitored parameters and anatomical context

STANFORD ELECTRICAL ENGINEERING

STANFORD SCHOOL OF MEDICINE Stanford University Medical Center

Thank you