Published in final edited form as:

Adv Exp Med Biol. 2014 ; 812: 113–119. doi:10.1007/978-1-4939-0620-8_15.

How *in vivo* EPR Measures and Images Oxygen

Boris Epel^a, Gage Redler^a, and Howard J. Halpern^a

^aDepartment of Radiation Oncology, University of Chicago, Chicago, IL, USA, Center for EPR Imaging In Vivo Physiology, Chicago, IL, USA

Abstract

The partial pressure of oxygen (pO₂) in tissues plays an important role in the pathophysiology of many diseases and influences outcome of cancer therapy, ischemic heart and cerebrovascular disease treatments and wound healing. Over the years a suite of techniques for reliable oxygen measurements has been developed. This is a mini-review of EPR *in vivo* oxygen imaging methods that utilize soluble spin probes. Recent developments in pulse EPR imaging technology have brought an order of magnitude increase in image acquisition speed, enhancement of sensitivity and considerable improvement in the precision and accuracy of oxygen measurements.

1 Spin probe

The oxygen molecule is a diradical with two unpaired electrons in a triplet state that exhibits very fast relaxation. Upon interaction with a spin probe, oxygen enhances the relaxation rate of the probe via Heisenberg exchange [1]. The Smoluchowski diffusion equation predicts a linear relationship between pO₂ and relaxation rates that is validated for multiple radicals. This relationship allows a direct EPR measure of pO₂ with high precision [2].

The success of non-invasive oximetry in the last decade is strongly linked to triarylmethyl radicals or trityls (Figure 1) developed by Nycomed Innovations (later acquired by GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) possessing a narrow single EPR line [3]. The trityls that are commonly used for *in vivo* imaging are methyl-tris[8-carboxy-2,2,6,6-tetrakis[2-hydroxyethyl]benzo[1,2-d:4,5-d']bis[1,3]dithiol-4-yl]-trisodium salt, OX063 (16 μT p-p) and its partially deuterated form Ox63H24D (8 μT p-p). These spin probes are distributed in the extracellular fluid compartment [2,4]. In the blood stream of a mouse, the clearance halftime of these probes is approximately 9 – 10 min, whereas in tumors they remain and provide strong signals for about 40 – 50 min [5]. The lethal dose (LD₅₀) of analogs of OX063 is very high, 8 mmol/kg, which allows high dose injections [6].

2 Imaging methods

For imaging, the spatial position of a paramagnetic species is encoded by use of linear magnetic field gradients, $\mathbf{G}$. The additional magnetic field experienced by a species at position $\mathbf{x}$ in the sample is then $\mathbf{B}=\mathbf{G}\cdot\mathbf{x}$. The time evolution of signal from a sample after an RF pulse is

$$s(t) = \int_V f(x) \exp\left(-\frac{t}{T_x^*(x)}\right) \exp[-i2\pi k(t)x] dx \quad (1)$$

The time evolution of echoes can be treated in a similar way. Here, V describes an integral over the sample volume, and $f(\mathbf{x})$ is the spatial distribution of the magnetization. The

relaxation term $\exp\left(-\frac{t}{T_2^*(x)}\right)$ describes the attenuation of signal. The switching speed of the gradients achieved by EPR *in vivo* imaging hardware is slow in comparison to the relaxation rates of electrons (units of microseconds). Thus gradients constant on the time scale of spin probe relaxation are used in the definition of k -space trajectories:

$$K(k) = \frac{\gamma_e}{2\pi} \int_0^t G(t') dt' = \frac{\gamma_e}{2\pi} Gt \quad (2)$$

For correct reconstruction of an image, sufficient k -space data must be acquired. Two general ways of filling k -space by using static gradients have been elaborated: projection-based and Fourier imaging.

2.1 Projection-based reconstruction

The gradient in Eq. 2 is a scaling factor between time and k -values. By increasing of the gradient, the time, t , necessary to cover the desired k -space can be made small enough that the relaxation term in the Eq. 1 become negligible. In this case Equation 1 describes the Radon transformation of an object in Fourier space along the direction defined by the gradient [7]. The k -space trajectory of EPR signal detected under static gradient is a radial line passing through the origin of the k -space coordinates (Fig. 2). For spatial imaging, EPR projections are obtained while static gradients, G , with constant amplitude and different directions are applied. Different reconstruction algorithms have been developed for radially sampled data. Most of them fall into one of two categories: filtered back projection (FBP) [8,9] or iterative reconstruction [10]. All reconstruction procedures on sparsely sampled data give inexact results: they represent a compromise between accuracy and the computation time required. FBP demands fewer computational resources, whereas iterative reconstruction generally produces fewer artifacts at a higher computing cost.

2.2 Single point imaging

The SPI method is based on phase encoding of spatial information. The method, was originally used to overcome the influence of the EPR line width (or R_2^*) on the imaging resolution [11]. Use of a single data point at delay $t=\tau$ (Eq. 1) makes relaxation term constant and allows for k -space sampling at will by choice of an appropriate static gradient. Typically, gradients are sampled on a rectangular grid (Fig. 2) and object [12,13]. The object is then reconstructed by use of multidimensional Fourier transformation of k -space data.

SPI generates images free from artifacts at the expense of the necessity to repeat the measurement in every point of k -space. Thus this method has higher spatial fidelity but somewhat lower SNR.

In the simplest form of SPI, FID detection is used, and phase relaxation times are extracted from multiple images with different τ [12]. The disadvantage of this method is that, for a given set of gradients, the images reconstructed from different τ have different k -space

samplings and, therefore, different spatial extents and resolutions. Resampling of these images to a common scale causes artifacts, especially around the edges of an object. More advanced sampling techniques that involve acquisition with use of multiple gradient grids have been suggested. This has led to the same k -space sampling for different τ and consequently the elimination of artifacts [13].

2.3 Pulse sequences and 4D images

Pulse sequences for measuring R_2^* , R_2 and R_1 relaxation are available. In all cases, the pulse sequence is chosen in a way that amplitude of the EPR signal time evolution become dependent on the relaxation time and sequence parameter (for example delay between pulses). Multiple 3D images with different values of this parameter are acquired and reconstructed independently. Then these images are stacked together to form 4D image, the additional dimension of which represents the evolution of signal amplitude in every voxel as a function of the sequence parameter. The final image of relaxation times is produced by fitting of this evolution to an appropriate dependence. This process is illustrated in Figure XX.

For SPI and single $\pi/2$ - pulse, the fit of individual voxels to an exponential decay gives the FID decay rate R_2^* , related to R_2 as $R_2^* = \Sigma(R_2^i) + R_2$, where $\Sigma(R_2^i)$ is the sum of the known oxygen-independent contributions to R_2 [14].

For projection based imaging, the FID detection was not successful. Pulse imagers do not allow signal detection immediately after the excitation. The delay between excitation and opening of the detector (t_{dead}), leads to a missing volume in k -space, $k < \gamma_e G t_{\text{dead}}$, and to image distortion.

To avoid incomplete coverage of k -space, projection-based pulse imaging techniques use dead time free spin echo sequences [15,16]. The electron spin echo is detected by use of the two-pulse sequence $(\pi/2)-\tau-(\pi)-\tau$ -echo; here, τ is the time delay between pulses. For acquiring of a phase relaxation image, separate images with different τ -delay values are obtained. These delays have to cover the range of times suitable for correct determination of the relaxation time. Logarithmically spaced delays yield a more precise determination of the relaxation time [16]. The echo sequence allow direct measurement of R_2 , which results in higher pO_2 precision in comparison to R_2^* methods. Recently to obtain direct measurement of R_2 spin echo acquisition was introduced to SPI [17].

Finally, for generation of a spin-lattice relaxation (SLR) image, IRESE, the inversion recovery sequence $(\pi)-T-(\pi/2)-\tau-(\pi)-\tau$ -echo can be used (Fig. 3) [18]. The first π -pulse inverts the populations of electron levels; the delay, T , allows this polarization to relax at the SLR rate. The detection sequence and imaging method are identical to that of the two pulse spin echo. The SLR images can be obtained using SPI as well.

3 Importance of Spin-Lattice Relaxation Imaging

Although oxygen induced relaxation is the largest factor under physiologic conditions, other relaxation mechanisms can affect the accuracy of EPR oximetry. Some factors, such as

temperature, viscosity, and salinity, are tightly controlled by a living body. Variations in these factors are relatively small and position-independent; and so, their effects on relaxation rates can be accounted for. In contrast, spin-probe self-broadening, the effect of local spin-probe concentration on the relaxation rate, may be substantial and non-uniform and thus require special treatment. Reducing trityl injection into the animal obviously reduces the trityl concentration *in vivo*, and, thereby the self-broadening. However, this strategy for reducing the self-broadening uncertainty in pO_2 also lowers the image SNR. These considerations stress the importance of a methodology that is less susceptible to concentration broadening. In trityls, the oxygen dependences of R_1 and R_2 are identical. However, the concentration dependence of R_2 , especially at physiologic solvent salinity can be up to 6 times higher than that of R_1 . Thus SLR measurement provides considerable improvement in the pO_2 image accuracy.

4 Conclusions

EPR oxygen imaging possesses the full suite of methods capable for accurate and precise imaging of small animals.

References

1. Eastman PE, Kooser RG, Pas MR, Freed JH. Studies of Heisenberg spin exchange in ESR spectra I. Linewidth and saturation effects. *J. Chem. Phys.* 1969; 54:2690.
2. Tatum JL, et al. Hypoxia: Importance in tumor biology, noninvasive measurement by imaging, and value of its measurement in the management of cancer therapy. *Int J Radiat Biol.* 2006; 82:699–757. [PubMed: 17118889]
3. Ardenkjaer-Larsen JH, et al. EPR and DNP properties of certain novel single electron contrast agents intended for oximetric imaging. *J. Magn. Reson.* 1998; 133:1–12. [PubMed: 9654463]
4. Williams BB, et al. Imaging spin probe distribution in the tumor of a living mouse with 250 MHz EPR: correlation with BOLD MRI. *Magn Reson Med.* 2002; 47:634–638. [PubMed: 11948723]
5. Matsumoto K, et al. Pharmacokinetics of a triarylmethyl-type paramagnetic spin probe used in EPR oximetry. *Magnet Reson Med.* 2004; 52:885–892.
6. Krishna MC, et al. Overhauser enhanced magnetic resonance imaging for tumor oximetry: Coregistration of tumor anatomy and tissue oxygen concentration. *Proc. Natl Acad. Sci. USA.* 2002; 99:2216–2221. [PubMed: 11854518]
7. Williams BB, Pan XC, Halpern HJ. EPR imaging: The relationship between CW spectra acquired from an extended sample subjected to fixed stepped gradients and the Radon transform of the resonance density. *J. Magn. Reson.* 2005; 174:88–96. [PubMed: 15809176]
8. Deans, SR. The radon transformation and some of its applications. John Wiley & Sons; New York: 1983.
9. Ahn KH, Halpern HJ. Spatially uniform sampling in 4-D EPR spectral-spatial imaging. *J. Magn. Reson.* 2007; 185:152–158. [PubMed: 17197215]
10. Ahmad R, et al. Enhanced resolution for EPR imaging by two-step deblurring. *J. Magn. Reson.* 2007; 184:246–257. [PubMed: 17113800]
11. Maresch GG, Mehring M, Emid S. High-resolution electron-spin-resonance imaging. *Physica B & C.* 1986; 138:261–263.
12. Subramanian S, et al. Single-point (constant-time) imaging in radiofrequency Fourier transform electron paramagnetic resonance. *Magnet Reson Med.* 2002; 48:370–379.
13. Devasahayam N, et al. Strategies for improved temporal and spectral resolution in *in vivo* oximetric imaging using time-domain EPR. *Magnet Reson Med.* 2007; 57:776–783.

14. Matsumoto K, et al. Electron paramagnetic resonance imaging of tumor hypoxia: Enhanced spatial and temporal resolution for in vivo pO₂ determination. *Magnet Reson Med.* 2006; 55:1157–1163.
15. Mailer C, Sundramoorthy SV, Pelizzari CA, Halpern HJ. Spin echo spectroscopic electron paramagnetic resonance imaging. *Magnet Reson Med.* 2006; 55:904–912.
16. Epel B, Sundramoorthy SV, Mailer C, Halpern HJ. A versatile high speed 250-MHz pulse imager for biomedical applications. *Conc. Magn. Reson. B.* 2008; 33B:163–176.
17. Subramanian S, et al. Echo-based Single Point Imaging (ESPI): A novel pulsed EPR imaging modality for high spatial resolution and quantitative oximetry. *Journal of magnetic resonance (San Diego, Calif. : 1997).* 2012; 218:105–114.
18. Epel, B.; Halpern, HJ. Comparison of transverse and spin-lattice relaxation based electron paramagnetic resonance oxygen images; *Biomedical Imaging: From Nano to Macro, 2011 IEEE International Symposium on Biomedical Imaging*; Chicago: 2011. p. 754-757. IEEE Xplore

Figure 1.
Chemical structures of OX063 trityl.

Figure 2.
Filling of two dimensional k -space by projection-based and single point imaging methods.

Figure 3.
Acquisition of 4D images.