

Improved Temporal and Spatial Resolution In Vivo Oxymetry Using Time-Domain EPR Imaging

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Abstract

Time-domain Electron paramagnetic resonance imaging (EPRI) is being developed at 300 MHz for the purpose of developing quantitative in vivo oxymetry in animal models. Unlike other imaging modalities, EPR can provide direct measurements of tissue oxygen concentration in a manner that is independent of complex biological processes such as ligand binding specificity or tracer metabolism. We describe the implementation of single-point imaging (SPI) a purely phase-encoded imaging modality in mouse tumor models and present the techniques for performing rapid quantitative oxygen imaging in phantoms and *in vivo*. Results from mouse tumor experiments as the mouse breathed alternately air or carbogen® (95% O₂, 5% CO₂). The reconstructed images demonstrate that the SPI EPR imaging technique readily distinguishes between the normal and tumor legs and can track the changes in tissue oxygen concentration in response to percentage of oxygen in breathing gas.

Introduction

Since EPR spectroscopy has the ability to perform direct and noninvasive detection, characterization, and quantification of paramagnetic species, it promises to become a prominent clinical diagnostic tool. Although closely related to nuclear magnetic resonance (NMR) spectroscopy, EPR is still under development as an imaging modality. In contrast to MRI, in which water protons (being in high concentration *in vivo*), are readily imaged, endogenous paramagnetic species that are present *in vivo* are well below the detection levels of the EPR technique. In order for EPR imaging to be performed, stable free radicals must be introduced into or generated in the living system. With the recent availability of biologically compatible spin probes with optimal toxicologic, pharmacologic, and spectroscopic properties, it has become possible to implement *in vivo* EPR imaging and extract valuable physiological information, noninvasively, in small animals. The time-course of spin distribution in organs and metabolism of drugs tagged with spin labels can be observed directly via EPR. The most attractive characteristic of EPR is its ability to image *in vivo* oxygen concentration in tissue noninvasively. In radiation oncology, it is known that hypoxic tumor fractions are at least three times more resistant to radiation compared to normoxic regions. The only accepted way of assessing *in vivo* pO₂ is to use Clarke electrodes which are invasive. As an alternative and non-invasive method, EPR can be of great benefit to radiation therapy in the scheduling of radiation dose to maximize killing of cancer cells at minimal dose.

Experimental, results & discussion

The single-point imaging (SPI) scheme is essentially a phase-encoding technique that operates by acquiring a single data point in the free induction decay (FID) after a fixed delay (the phase encoding time), in the presence of static magnetic field gradients. A single point of intensity data is collected as a function of gradients (in ΔG steps from $-G_{\max}$ to $G_{\max}$) at a constant delay τ from the pulse.

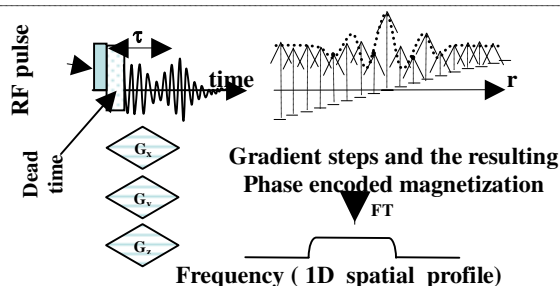


Fig. 1. Schematic illustration of the principles of SPI.

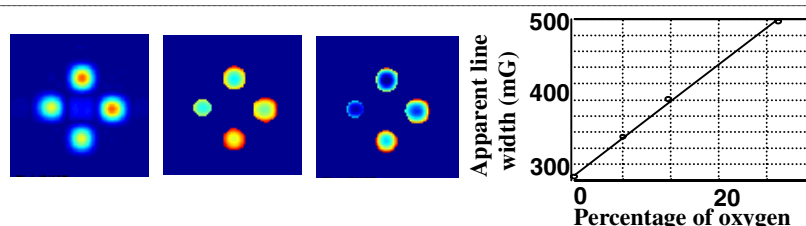


Fig. 2. Images of the calibrated 4-tube study. Left: spin density image. Center: spectral/spatial image. Right: Oxymetric image after correction. The O₂ concentrations are clockwise from left to right 0, 5, 9.9 and 21%. The graph on the extreme right shows the perfect linearity between $(1/T_2^*)$ and % O₂.

The gradients are applied along the three Cartesian axes in a nested loop depending on the dimensionality of the image. On the right, the formation of the pseudo-echo as a function of the gradient ramping and the corresponding profile that results on Fourier transform is shown for a 1D experiment. To acquire a 2-D image using 25×25 steps, the time required is ~17 sec. and A 3-D image with 25×25×25 steps requires ~7 min. 3D images of a spiral phantom is shown in Fig. 2. The SPI technique lends itself well to imaging the oxygen concentration. Although a single time-point from each FID is needed to estimate a spin-concentration image, in fact a set of full FIDs is collected. Thus, an FID decay curve is in principle available for every voxel in the image. The semi-log plot of voxel intensity versus τ should be linear with a slope of $1/T_2^*$. The presentation will illustrate the extraction of oxygen-dependent T_2^* from other contributions such as the intrinsic line width (from T_2 and unresolved hyperfine coupling), probe concentration and gradient induced susceptibility shifts. The linear dependence on oxygen concentration of corrected line width is clear from the results of a phantom study using tubes with different known equilibrated concentrations of O₂, as depicted in Fig. 2. 3D images of normal and SCC tumor-bearing legs of mice were subjected to T_2^* based oxymetric imaging (as described above), while the mouse was breathing alternatively air and carbogen. The switching from air to carbogen brought a distinct increase in tumor oxygenation (Fig. 3)

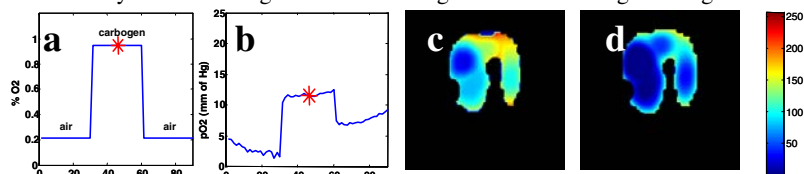


Fig. 3. (a) The time course of air/carbogen/air breathing, (b) the measured change in oxygen in the tumor leg (the larger left part in the image), (c) oxygen map during carbogen breathing and (d) that during air breathing. The distinct increase in pO₂ can be seen in (b) and (c). 3D imaging time was approx. 90 sec.

Conclusions: Time-domain EPR imaging based on SPI and T_2^* has the potential to provide rapid quantitative in vivo oxymetric information in small animal tumor models.

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