# Resolution of Heterogeneity of Oxygen in Tissues Using EPR Oximetry with Particulates

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**Introduction:** The presence of heterogeneity in oxygen concentration is a crucial issue when considering methods to measure $pO_2$ in tissues because of the physiological and pathophysiological effects of such heterogeneity. Because of the nature of functioning biological systems there MUST be heterogeneity extending into dimensions smaller than cells as most of the consumption of oxygen occurs in mitochondria. Therefore, methods to measure oxygen *in vivo* cannot fully resolve heterogeneity; however, it is essential that their resolution be understood. We have developed methods to increase the resolution of EPR oximetry significantly, enabling us to take full advantage of the favorable characteristics of this method: the ability to make repeated and accurate non-invasive measurements from the same site.

**$pO_2$ Heterogeneity:** To characterize oxygen heterogeneity in tissues, methods of linear and nonlinear regression analysis of a single EPR spectrum recorded without a magnetic field gradient were developed. If the linewidth is specified at equidistant values with separation $\delta$ and a minimum linewidth $\Delta_0$, $\Delta(n)=\Delta_0+\delta*n$, where n=0,1,…,(N-1) and N is the number of distribution bins. If L(n) is the relative contribution of the $n^{th}$ Lorenzian line, the resulting EPR spectrum is a linear combination $\Sigma[a(n)*L(n)]$+noise, where n=0,1,…,(N-1). From 1024 data points of the EPR spectrum, we recover frequency coefficients a(0),a(1),…,a(N-1) by least-squares fitting. After normalizing, we treat the coefficients as histogram frequencies. We apply methods of statistical significance testing to identify the number of bins and to test the hypothesis H0: a=0. Numerical simulations have shown that this method allows accurate estimation of the distribution coefficients a(0),a(1),…,a(N-1). Figure 1 shows a simulated EPR spectrum of LiPc crystals with an N=5 component $pO_2$ distribution as specified in the right upper corner and with additive random noise. The true and estimated parameters are shown in Table 1, demonstrating the accuracy and precision of this technique.

**Overmodulation:** In order to increase spatial resolution of multi-site EPR oximetry, spectra with two gradients are recorded [1]. One limitation of the technique is the decrease in the signal-to-noise ratio (SNR) of the measured data as the strength of the applied gradient increases. Typically, the Zeeman modulation amplitude has been set to 1/3 of the minimum observed spectral linewidth to avoid distortion of the measured spectra. Spectra simulated under this condition are shown in Figure 2a. We have investigated the effect of increasing the Zeeman modulation amplitude as a means to improve the SNR at high gradient magnitudes, as demonstrated in Figure 2b. Through theoretical analysis and simulations, we have found that when the modulation amplitude is increased in proportion to the applied gradients, accurate $pO_2$ estimates can be made even with very large modulation amplitudes. With a true linewidth of 0.5 G, under conventional conditions the linewidth was estimated to be 0.55 G with a standard error of 0.08 G (n=100); with larger modulation amplitudes, the accuracy and precision improved to 0.50 G and 0.01 G (n=100). Figure 2 demonstrates that overmodulation allows a significant increase in SNR.

| True $pO_2$ (mmHg) | True Fraction | Estimated Fraction | Standard Error |
|---|---|---|---|
| 0 | .3 | 0.298 | 0.002 |
| 10 | .1 | 0.100 | 0.017 |
| 20 | .1 | 0.098 | 0.069 |
| 30 | .4 | 0.419 | 0.110 |
| 40 | .1 | 0.080 | 0.060 |

Table 1 True $pO_2$ distribution used to generate the simulated spectrum of Figure 1.

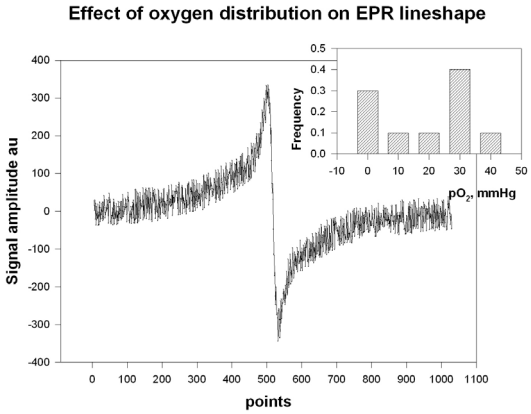


Figure 1 Simulated LiPc spectrum for a sample with heterogeneous $pO_2$ distribution.

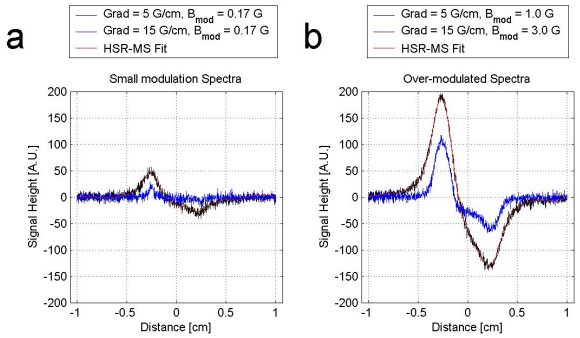


Figure 2 Spectra were simulated for a sample with a uniform 0.5 G linewidth and linearly varying spin intensity for typical gradient strengths and noise level. The multi-site EPR technique was used to estimate the true linewidth of the sample under (a) conventional, low modulation conditions and (b) with large modulation amplitude. The precision of the estimated linewidth improved when large modulation amplitudes were applied due to the dramatic increase in the SNR.

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**References**: [1] Grinberg OY, Smirnov AI, Swartz HM. High Spatial Resolution Multi-site EPR Oximetry. Journal of Magnetic Resonance 2001;152(2):247-58.