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Imaging of oxygen levels in cancer spheroids

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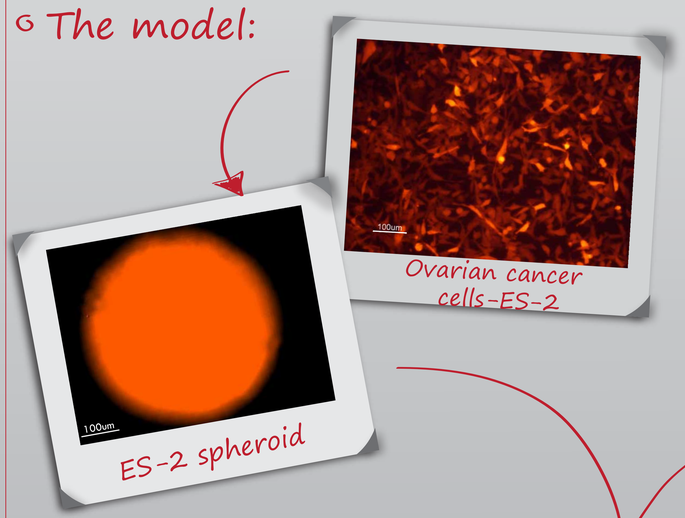
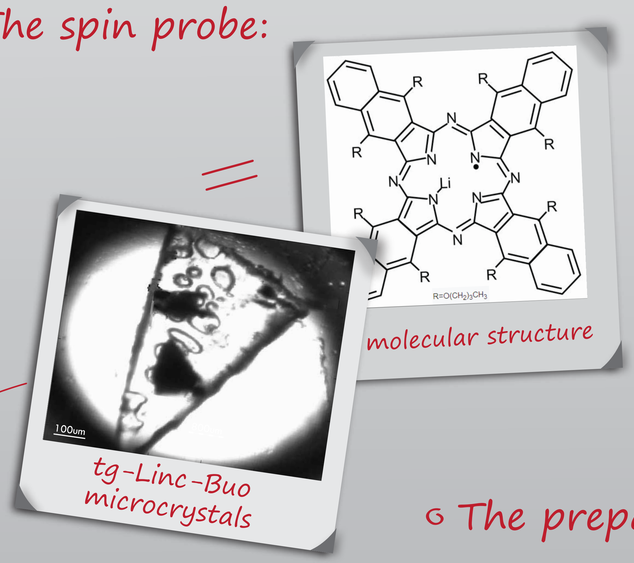
Abstract:

Oxygen (O_2) plays a central role in most living organisms. The concentration of O_2, or more accurately, its activity, is important in physiology and pathology. Despite the importance of knowing accurately the O_2 levels, there is very limited capability to measure it in small live biological samples. Therefore, it is important to find new methods for measuring the oxygen concentrations at high spatial resolution. This work is focused on the use of electron spin resonance (ESR) micro-imaging technique for accurate mapping of the oxygen concentration in small biological samples.The model used in this research is spheroids of Ovarian Cancer cell lines, ES-2. The new ESR oximetry method we use here overcomes many of the problems of the other oximetric methods such as micro-electrodes and fluorescence lifetime imaging. The advantages of this method lie in being noninvasive, sensitive at physiological levels, and easy to calibrate. Furthermore, it can be used for repetitive measurements without cell damage. The ESR technique requires the incorporation of a suitable stable and inert paramagnetic spin probe into the desirable object. In this work we used microcrystals of paramagnetic spin probes of tg-Linc-Buo.These paramagnetic species interact with the paramagnetic oxygen molecules which cause spectral line broadening that is linearly proportional to the oxygen concentration. The linewidth of these spin probes is highly oxygen-sensitive and thereby capable of providing noninvasive repetitive measurements. The results show the capability to grow the spheroids with the spin probe and map the O_2 levels inside the spheroids with high accuracy and spatial resolution.

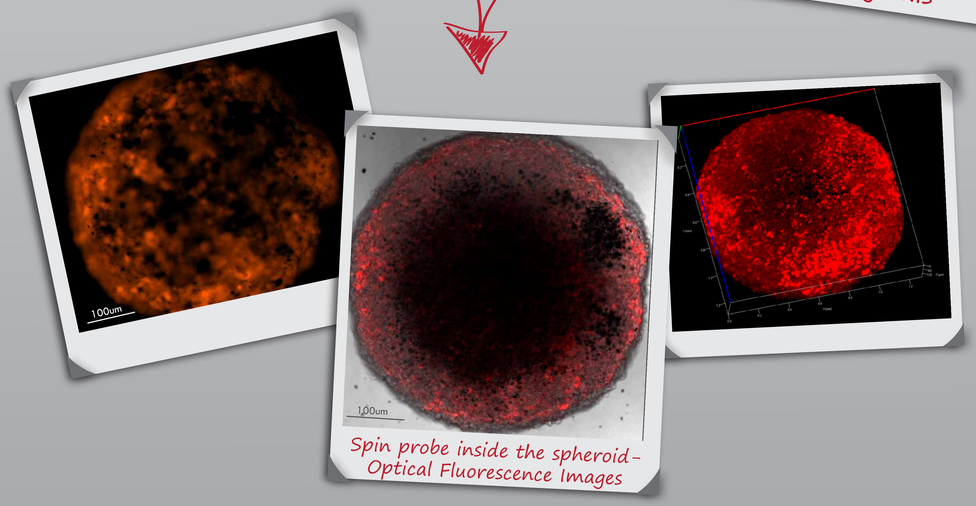
The model:

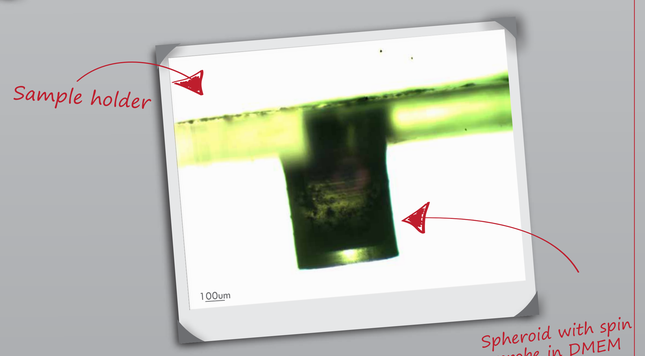


The spin probe:



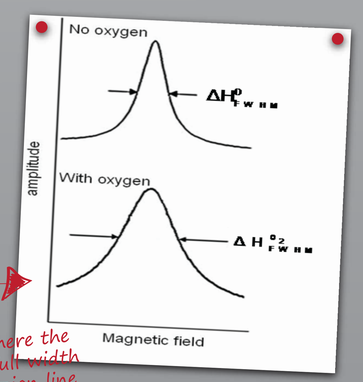
The prepared sample:



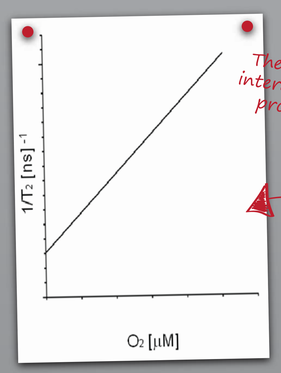


From ESR spectrum to O_2 concentration

ESR oximetry is based on the paramagnetic properties of molecular oxygen. The interaction between O_2 and the spin probe causes an increase in the relaxation rate of the spin probe, which results in spectral line broadening, with the oxygen - broadened linewidth (or T_2 relaxation time) directly proportional to oxygen concentration.

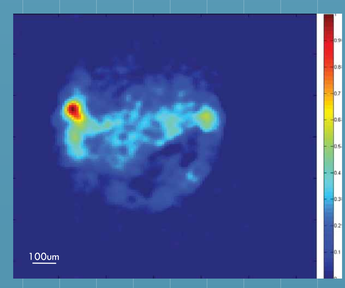


The spectral ESR line shape where the ΔH^{O}_{FWHM} and $\Delta H^{O_2}_{FWHM}$ are the full width at half maximum of the Lorentzian line without O_2 and with O_2, respectively.

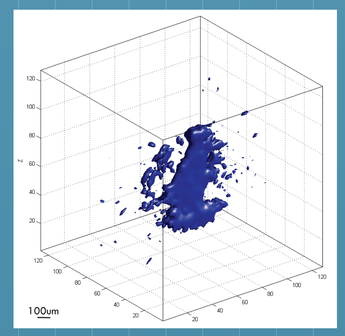


The paramagnetic oxygen molecule interacts with the ESR paramagnetic probe and shortens its spin-spin relaxation time, T_2.

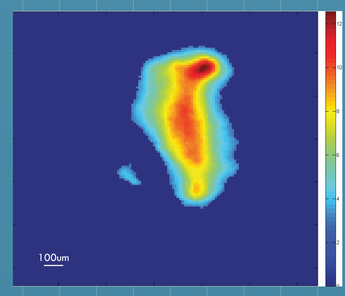
ESR micro-images of the ES-2 spheroids



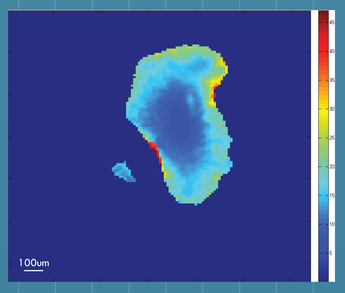
A 2-D CW-ESR spatial image of the spheroid with the spin probe inside.



A three-dimensional ESR image of the microcrystals in the spheroid.



Amplitude map. Different colors show different signal amplitudes in the spheroid.



Linewidth map. The map shows a differentiation between areas with relatively narrow line (blue) and areas with broader line (red).

Results show spectra with different linewidths, which indicate that the distribution of O_2 is not uniform in the spheroid. As expected, cells that are in the middle of the spheroid have a lower concentration of O_2 than cells that are in the periphery of the spheroid.