Electron Spin Resonance Microscopy of Oxygen Concentration near Live Species with Photosynthetic Activity

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Abstract

Electron Spin Resonance Microcopy (ESRM) is an imaging method aiming at the observation of stable free radicals in samples with a spatial resolution of ~1 micron. One of the important potential applications of ESRM is the accurate measurement of oxygen concentration levels. Such measurements take advantage of the fact that the ESR linewidth (and the inverse of the relaxation time, T_2) is linearly proportional to the oxygen concentration at all levels from 0 to 1 atm.

This study is aimed at demonstrating for the first time ESR micro-imaging of oxygen concentration with live species, namely the common type of cyanobacteria (a model system with photosynthetic activity). Pulsed ESRM experiments were performed with a unique "home-made" high resolution system that was recently developed at the Technion. The cells were placed in special glass sample holders with stable paramagnetic species (trityl) water solution. The spatially-resolved spin-spin relaxation time, T_2, was imaged at high resolution (15-30 microns) in a 3D manner in the volume around the cells with and without light irradiation.

The advantages of ESRM over other oxygen measurement techniques such as Clark oxygen microelectrodes or optical methods are that it can provide improved resolution and accuracy, enable complete 3D mapping of the oxygen concentration, and has the potential to measure O_2 both outside and inside the cells in a noninvasive manner.

Reference Samples

In order to examine the capability to map T_2 at high resolution a special reference sample was prepared with trityl solution in water/glycerol mixtures with varying glycerol content. A simple Hahn echo imaging pulse sequence with varying pulse separation, τ, is employed. We perform a fit to a simple exponential decay curve for each voxel, based on the voxel signal for different values of τ, to determine the amplitude of the exponent (extrapolate the spatially resolved echo amplitude to $\tau = 0$) and its decay time constant (T_2). It is evident that T_2 decreases with increasing viscosity due to incomplete motional averaging of anisotropic hyperfine interaction (Fig.1c).

Fig.1

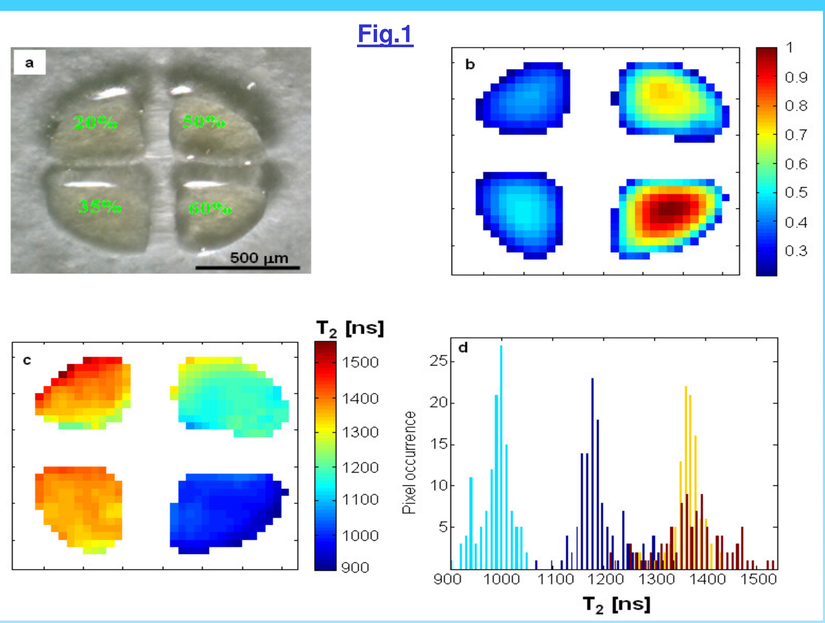


Fig. 1: Images of the reference sample.
(a) Optical photo, showing the liquid sample of 1 mM Triarylmethyl (trityl) in the four quadrants. The numbers mark the percentage of glycerol in water for each quadrant.
(b) Amplitude ESR image (normalized).
(c) Image of the fitted T_2.
(d) Histogram of the fitted T_2 values for the four different quadrants. The resolution of the ESR images is ~30×30 μm.

Calibration of the $[O_2]$ vs $1/T_2$ ratio

Fig.2

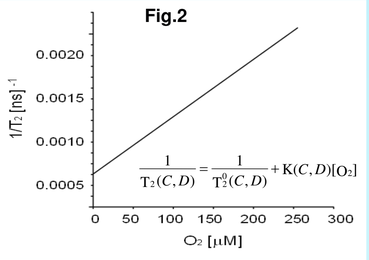


Fig.2 : the calibration graph of oxygen *vs* T_2

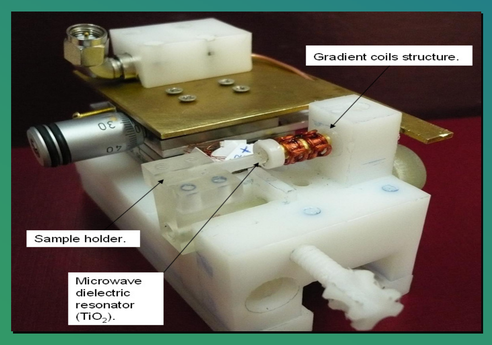
T_2^0 is the spin-spin relaxation time of the probe under anoxic conditions (depending on the probe's concentration, C, and its diffusion coefficient, D), and k is a proportionality constant. For trityl solution of ~1.25 mM in buffer at 25° C the values are:

T_2^0 = 2186ns = 0μm O_2

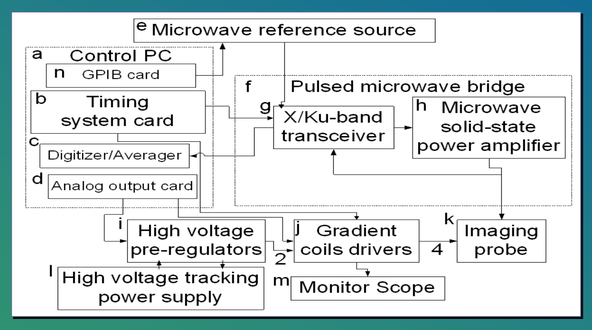
T_2^{atm} = 468ns = 255μm O_2

ESR Microscopy @ the Technion

A photo of the CW imaging probe:



Block diagram of the pulsed ESR microscopy system:



Oxygen imaging of *cyanobacterium* Synechocystis *PCC 6308* with photosynthetic activity

Fig.3 : Three dimensional ESR Micro-images of oxygen concentration.

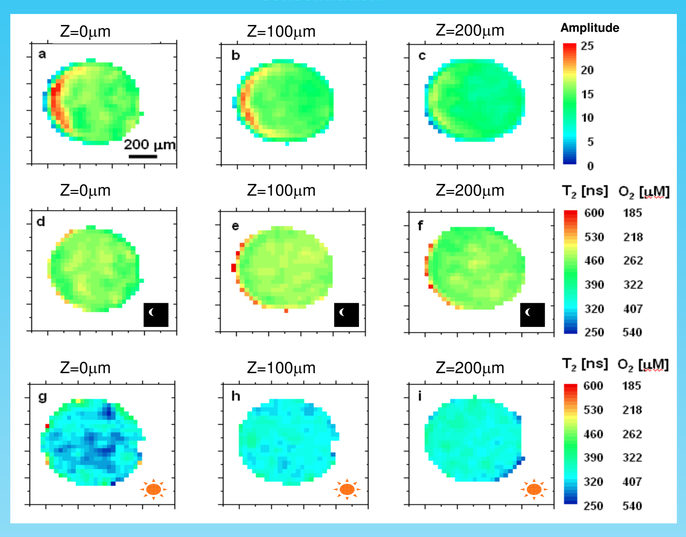


Fig.3: Amplitude = radical concentration for three different z slices (z=0 ,z=100 and z=200mm).
T_2 images and the corresponded oxygen concentration in the Cyanobacterium sample with

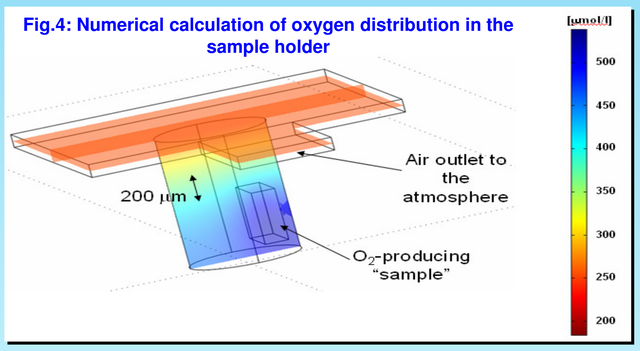
no illumination

with illumination

Table 1: Summery of the results of Fig. 3

Slice position in the "cup-like" sample holder [μm]	T_2 [ns]	O_2 [μM]
Z=0, 100, 200 (light off)	470±18	255±10
Z=0 (light on)	329±24	394±34
Z=100 (light on)	342±16	376±18
Z=200 (light on)	352±18	364±22

Fig.4: Numerical calculation of oxygen distribution in the sample holder



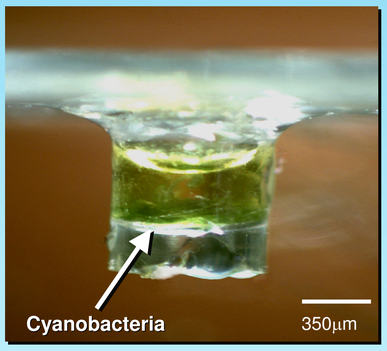


Fig.4: Numerical calculation of the oxygen concentration due to oxygen-producing mass located at the bottom (was carried out with the aid of COMSOL Multiphysics, ver. 3.5, Chemical Engineering Diffusion Module).
The dimensions of our sample holder were included in the simulation with insulating boundary conditions in all sides except for a small air outlet with atmospheric oxygen concentration boundary conditions.

The parameters used in the simulation were:

Size of the O_2 production region - 0.4×0.4×0.15 mm (the box inside the tube in Fig.4). Diffusion constant of O_2 in the buffer – 2.19×10^{-5} m^2/s; Diffusion constant of O_2 in air – 1.97×10^{-9} m^2/s; O_2 concentration in air - 9.35 mol/m^3; O_2 production rate – 0.0072 $mol\cdot m^{-3}\cdot s^{-1}$; O_2 concentration in the buffer under normal atmosphere – 0.255 $mol\cdot m^{-3}$.