

Detection of singlet oxygen using fluorescent chemical traps in sub-cellular domains of a single cell

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Singlet oxygen ($^1\text{O}_2$), the lowest excited electronic state of molecular oxygen, plays a major role in many chemical and biological processes, e.g. in photodynamic therapy.

Our group, the Center for Oxygen Microscopy and Imaging, is concerned with the development and use of optical tools to study processes that involve $^1\text{O}_2$ [1, 2].

At present, we are unique in being able to selectively create and directly detect $^1\text{O}_2$ at a single cell level with time and sub-cellular resolution by measuring its weak near-IR phosphorescence at 1270 nm [3-7].

Such measurements respond to the inherent heterogeneity of a cell and provide valuable insight into a variety of $^1\text{O}_2$ -dependent phenomena, including the photoinitiated death of cells [8].

In figures 1-3, I show representative images pertinent to our research.

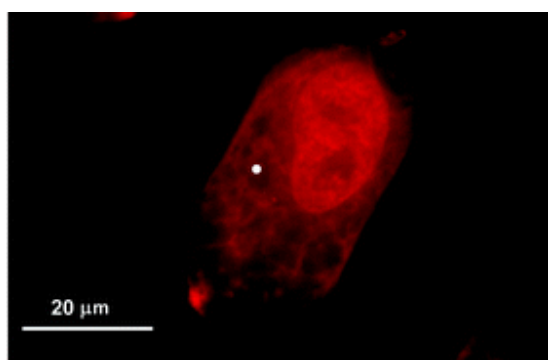


Figure 1: Image of a HeLa cell created using the fluorescence of a cationic porphyrin-based $^1\text{O}_2$ sensitizer. Under the conditions of this experiment, the sensitizer localizes mainly in the nucleus. The white spot in the cytoplasm represents the approximate cross-sectional spatial resolution obtained from a focused laser beam in our $^1\text{O}_2$ experiments.

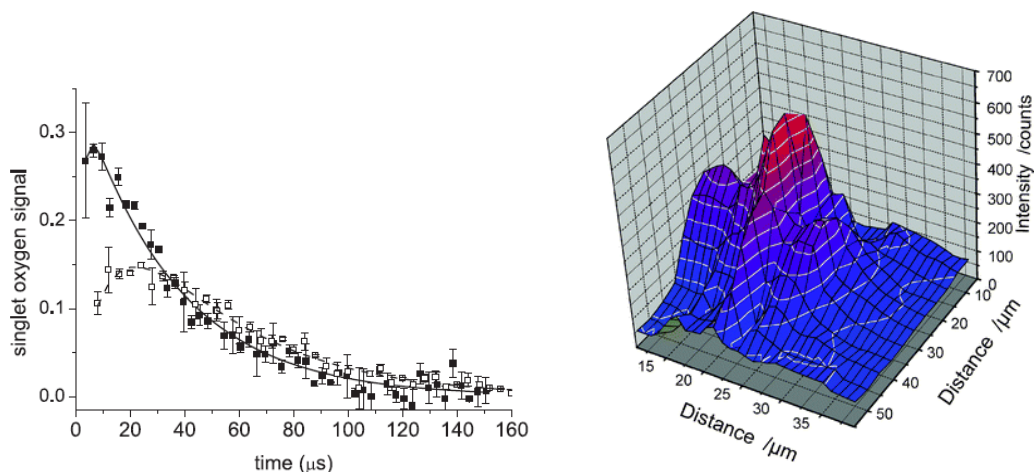


Figure 2: (a) Time-resolved ¹O₂ phosphorescence signals recorded upon irradiation of a ¹O₂ photosensitizer incorporated into the nucleus of HeLa cells. These experiments were performed with cells in which the intracellular H₂O had been replaced with D₂O, hence the long ¹O₂ lifetime. Data were recorded from cells exposed to an atmosphere containing 50% oxygen (□) and 100% oxygen (■). (b) Contour plot that shows the intensity of photosensitized ¹O₂ phosphorescence from a portion of a D₂O-incubated HeLa cell. The data were generated by raster-scanning the sample through the focused laser beam used to excite the sensitizer.

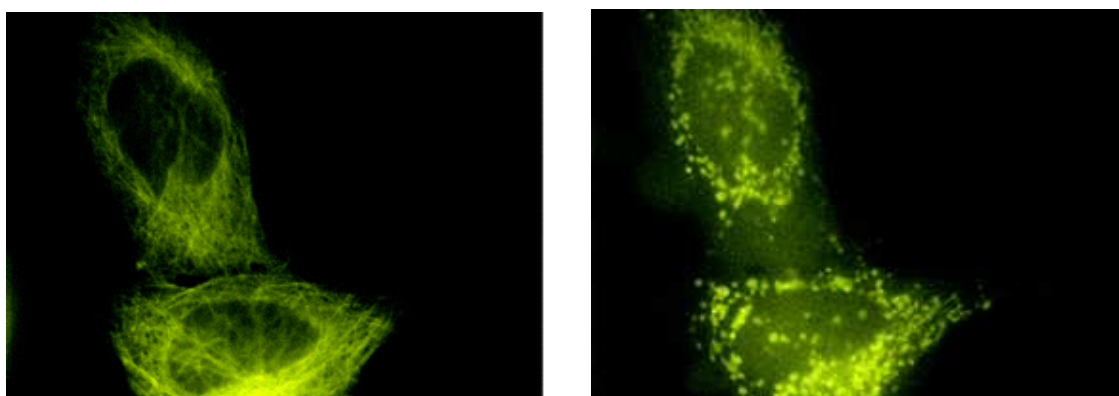


Figure 3: (Left) Image based on a yellow fluorescent protein (YFP) in the tubulin cytoskeletal network of HeLa cells. The YFP was genetically encoded *via* transient transfection. (Right) YFP-based image that shows the collapse of the cytoskeletal network upon 420 nm irradiation of a ¹O₂ sensitizer in the cell.

Although our direct time-resolved spectroscopic probe has provided invaluable information about the intracellular behavior of ¹O₂, it nevertheless has its drawbacks. For example, our method suffers from weak signals attributed to the low quantum efficiency of ¹O₂ phosphorescence ($\Phi_{\text{ph}} = 10^{-6} - 10^{-7}$). Therefore, we would like to complement these direct studies with independent work based on the use of fluorescent probes specific for ¹O₂.

I have just started my PhD project on the 1st of February 2009 with the focus on the development of a complementary strategy to detect intracellular ¹O₂ with a higher sensitivity.

I want to introduce fluorescence probes which are chemical traps for detecting ¹O₂ in single cells [9]. Fluorescence probes are known to be excellent sensors for biomolecules, being sensitive, fast-responding and capable of affording high spatial resolution via microscope imaging.

Chemical traps which are normally almost non fluorescent, can react with ¹O₂ forming a fluorescent endoperoxide [9]. Measuring its fluorescence or monitoring the decrease in the amount of the trap provides a powerful tool to detect ¹O₂ with a higher sensitivity. Another promising advantage of this indirect method is that lower amounts of ¹O₂ are required to detect a signal. The fluorescence quantum yield of the endoperoxides are much higher than the phosphorescence quantum yield of ¹O₂. Therefore, the cells are less in risk of being damaged under unnecessarily harsh experimental conditions.

The next step of the project will be to specifically localize a photosensitizer and a trap into different parts of a single cell using, for example, protein labeling techniques [10]. With this method we want to achieve a greater control of the generation and decay of ¹O₂ inside a cell.

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