

2D Time-resolved singlet oxygen luminescence scanning on microorganisms on surfaces

and singlet oxygen kinetics *in vivo*

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The concept of monitoring the distribution of photosensitizers (PS) in biological systems is a well-known and common procedure but it is limited to obtaining information about its localization only. Even evaluating the PS fluorescence does not necessarily correlate one-to-one with singlet oxygen ($^1\text{O}_2$) generation efficiency.

It is well accepted that $^1\text{O}_2$ is the main mediator of the photodynamic effect.¹ For this reason, numerous efforts have been made to detect $^1\text{O}_2$ *in vitro* as well as *in vivo*. The detection methods of $^1\text{O}_2$ range from indirect methods using monitor molecules, other indirect methods that do not require additional drugs, to direct $^1\text{O}_2$ detection via its weak near-infrared (NIR) phosphorescence at around 1270 nm.^{2,3,3-8} Beside using the photodynamic effect in photomedicine for the treatment of different diseases it is also important for the inactivation of microorganisms like bacteria, phototrophic organisms and fungi.

For the first time the kinetics of $^1\text{O}_2$ luminescence of photodynamically treated microorganisms on surfaces was detected. A clear correlation between photodynamic activity and $^1\text{O}_2$ generation could be shown.

The time resolved registration of $^1\text{O}_2$ phosphorescence could be a potent PDT-dosimetry technique, which may be used for improving the treatment efficiency. Furthermore, it could be used for quantitative investigations of the mechanism of $^1\text{O}_2$ generation and its interaction with the micro-environment of the PS.^{9,10} Elevating the ability of $^1\text{O}_2$ NIR detection to an imaging level will greatly improve knowledge about basic processes.

Up to now, an evaluation of kinetics was not possible due to insufficient signal-to-noise ratio. Here we present high signal-to-noise ratio $^1\text{O}_2$ luminescence kinetics obtained in the CAM and mouse tumor model under PDT relevant conditions. We will present as luminescence kinetics with unsurpassed signal to noise ratio gained from tumor bearing nude mice *in vivo* upon topical application, subcutaneous injection as well as intravenous injection of different photosensitizers. Singlet oxygen kinetics in appropriate model systems are discussed interpreting the complex kinetics obtained from *in vivo* tumor tissue.

This is the first study addressing the complexity of $^1\text{O}_2$ luminescence kinetics in tumor tissue. At present further investigations are needed to fully explain the processes involved. Nevertheless, the high signal to noise ratio proves the applicability of direct time-resolved $^1\text{O}_2$ luminescence detection as a prospective tool for monitoring PDT.

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