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# Monitoring of O<sub>2</sub> concentrations during Corneal Cross-Linking (CXL) by Phosphorescence Lifetime Imaging Microscopy (PLIM)

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Footnotes

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## Abstract

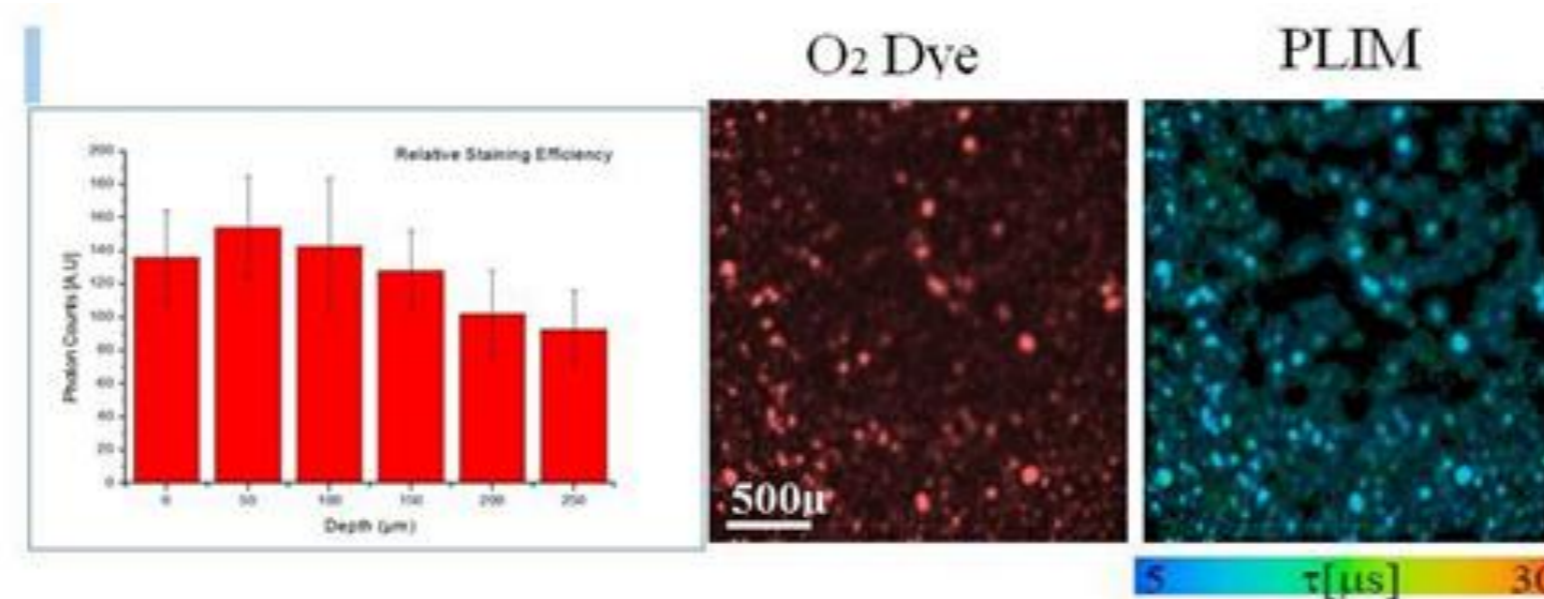
**Purpose :** The diffusion of riboflavin and oxygen is vital for efficient corneal cross-linking (CXL) with UV light. Previous studies found the biomechanical effect of CXL to be oxygen dependent. The purpose of this study is to investigate the role of O<sub>2</sub> and its distribution across the stroma before and during CXL through the use of phosphorescence based probes and imaging.

**Methods :** Porcine eyes were obtained from the local slaughterhouse 4 hours post mortem and kept at a temperature of 4°C. The epithelium was removed and the cornea was stained with a solution containing 0.5% riboflavin and infra-red emitting nanoparticles O<sub>2</sub> probe for 30 min to allow diffusion. The globe was then analysed at 37° C and 21% ambient O<sub>2</sub> on the confocal upright PLIM microscope (Zeiss, Becker & Hickl GmbH) using 5x/0.25 Fluar objective, excitation at 488 nm and emission collected at 750-810 nm. The cornea was imaged over 10 minutes at depths of 0, 50, 100, 150 and 200 μm. The cross-linking was achieved through periodic 20-30 cycles illumination of cornea with UV-A LED light (7 mW/cm<sup>2</sup>) whilst imaging. Photon distributions and phosphorescence decay curves were analysed after measurement, from which lifetime values and O<sub>2</sub> concentrations were calculated and presented as 2D and 3D maps.

**Results :** We optimised staining with the O<sub>2</sub> probe and measurement conditions for the cornea, and performed proof-of-principle PLIM experiments before and after CXL. We observed efficient and uniform in-depth staining of the cornea allowing us to generate high-resolution O<sub>2</sub> maps and monitor O<sub>2</sub> dynamics during CXL. Previous PLIM results scanning in the Z-direction revealed little to no change in lifetime decays during UV illumination, suggesting axial scanning may be a quicker and more efficient method in quantifying O<sub>2</sub> lifetimes during the CXL process.

**Conclusions :** The use of phosphorescent O<sub>2</sub> probes allows for efficient and a minimally-invasive method in measuring O<sub>2</sub> prior to, and during CXL. 2D and 3D maps of O<sub>2</sub> concentrations across the stroma during CXL will enable us to better understand the role of oxygen during CXL. Future work will focus on measurements under different O<sub>2</sub> environments to verify the CXL effect with O<sub>2</sub> probes, and to investigate the suitability of O<sub>2</sub> PLIM method for future in-vivo use.

This is an abstract that was submitted for the 2016 ARVO Annual Meeting, held in Seattle, Wash., May 1-5, 2016.



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Nanoparticle staining of the cornea at depths up to 250μm (ambient conditions only)

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