

APPLICATION OF FLUORESCENCE SECTIONED MICROSCOPY TO THE *IN-SITU* DIAGNOSIS OF ACANTHAMOEBA SP. OCULAR INFECTION

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Non-invasive detection of microscopic pathogens infecting the cornea has always been a challenge due to their small size and inherent transparency. *Acanthamoeba sp.* is an opportunistic protozoan that, if undetected, progressively invades deeper into the cornea causing irreversible scarring and impaired vision. A method for safe *in-situ* detection of the pathogen would be invaluable. We report on an imaging method based on optically sectioned corneal microscopy together with selective fluorescent staining of the pathogen that may allow its early identification and thus avoid the devastating consequences that often follow late diagnosis.

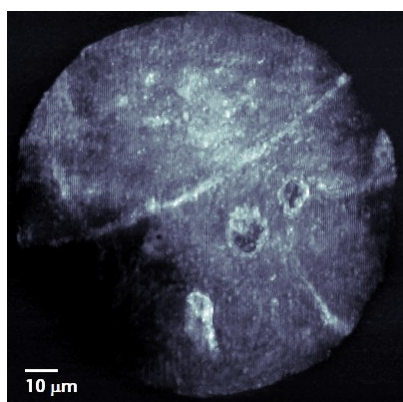


Figure 1: Fluorescence optically sectioned image of a porcine cornea populated with *Acanthamoeba sp.* and the filamentous fungus *Fusarium*.

A structured illumination based corneal microscope was built to conduct the inspection of *Acanthamoeba sp* superficially infected but otherwise intact porcine eyes. An ocular infection was mimicked transferring trophozoites of *Acanthamoeba sp* to the surface of freshly enucleated porcine eyes. A solution of a fluorescent analogue of the lipid hexadecyl-phosphocoline (Bodipy-MT) [1] was topically applied to both infected and uninfected control eyes. The integrity of the epithelium was tested on control eyes with a live/death fluorescent assay.

Optically sectioned microscopy conducted on the infected eyes demonstrated that the fluorescent compound was specifically internalised by *Acanthamoeba* trophozoites but stayed at the outer membrane of healthy squamous cells of uninfected corneas. Small specimens (~15 μm) of fluorescently stained *Acanthamoeba* trophozoites that populated the infected corneas were distinctly identified.

In conclusion, we have demonstrated that an imaging method based on an optically sectioned corneal microscope, together with a fluorescent analogue of hexadecylphosphocoline allows fast, minimally invasive, *in-situ* identification of *Acanthamoeba* specimens populating infected corneas of enucleated eyes. The microscopy strategies for the *in-vivo* application of this method will be discussed.

[1] V. Hornillos, E. Carrillo, L. Rivas, F. Amat-Guerri, A.U. Acuña. "Synthesis of BODIPY-labeled alkylphosphocolines with leishmanicidal activity as fluorescent analogues of miltefosine." Bioorg Med Chem Lett. 18, 6336-6339 (2008).