QUANTITATIVE ANALYSIS OF THE CHANGES IN THE POST-MORTEM CRYSTALLINE LENS USING MULTIPHOTON MICROSCOPY

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INTRODUCTION

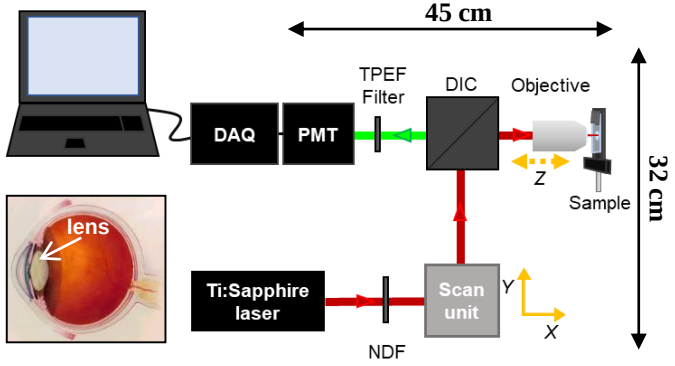
Postmortem interval (PMI) is the time elapsed since a person's death. When the actual time of death is unknown, an estimation is required for different purposes. The procedures used for this are of critical importance in **forensic sciences**.

In the eye, some structures can be used to estimate the PMI, but the lack of the objective classification parameters make this process difficult and time consuming [1]. Due to the location of the **crystalline lens within the eye**, the postmortem changes can be shown later than other structures of the body [2]. On the other hand, **multiphoton (MP) microscopy** has been shown to be useful in the analysis and evaluation of ocular structures such the cornea, the sclera and, even the crystalline lens.

The purpose of this work is to evaluate **postmortem changes in the lens** as a function of the PMI using MP microscopy**.**

METHODS

Experimental setup: Compact MP microscope [3]



References:

[1] Nioi et al., *Exp. Eye Res.* **169**, 20-27 (2018).
[2] Prieto-Bonete et al., *Leg. Med.* **17**, 437-442 (2015).
[3] Ávila et al., *Sci.Rep.* **9**, 10121 (2019).
[4] Avila & Bueno, *Appl. Opt.* **54**, 9848-9854 (2015).
[5] Haralick et al., *IEEE* **SMC-3**, 610-621 (1973).

Support:

SéNeCa (+)

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Samples

Samples (H&E):
Rabbit crystalline lenses
PMIs: 24, 48, 72, 96 h

Laser power = 150 mW
Imaged Area = 180x180 μm²
Resolution = 256x256 px²

Image analysis (metrics)

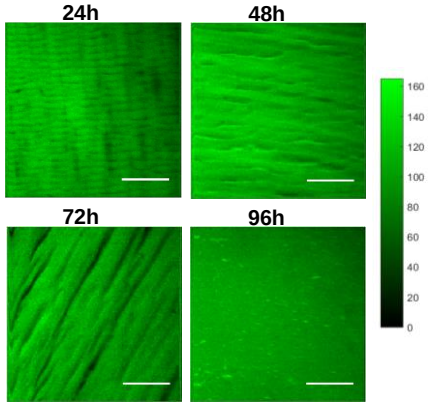
→ **Entropy**
It is a measure of the organization of the structures within the image.

→ **Structural dispersion (SD) [4]**
Measurement of the arrangement of the fibers in the image by calculating the partial derivatives along the image.

→ **Fiber density (FD)**
Estimation of the fiber density through the correlation parameter of the Grey Level Co-occurrence Matrix [5] along the image.

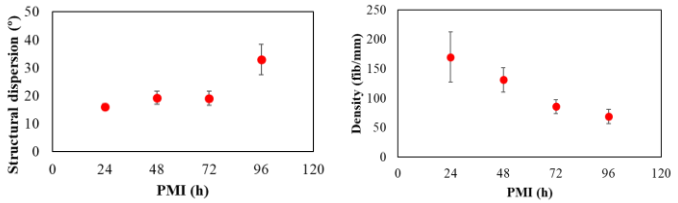
RESULTS

MP images: Two-photon excitation fluorescence



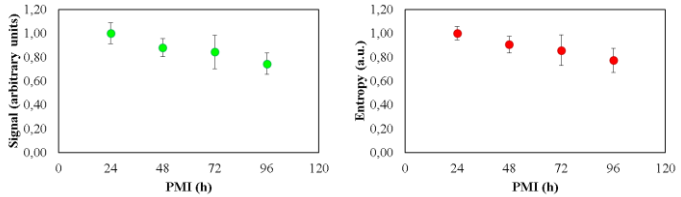
MP images of the rabbit lens acquired at different PMIs. Scale bar: 50μm

Metrics for Structural Features



Mean values of SD and FD of the tissues as a function of the PMI.

Metrics for Texture Analysis



Mean values of the MP signal and Entropy as a function of the PMI.

CONCLUSIONS

MP images of the crystalline lens at 24, 48, 72 and 96 h after death were successfully acquired with a compact home-made MP microscope.

MP images show noticeable differences with PMIs as confirmed when using metrics for structural analyses
1. SD reveals a decrease in the organization of the fibers (maximum values at 96h).
2. FD reduces, what leads to a more homogeneous tissue arrangement (fibers lose their tubular entity).

Texture analysis reveals a reduction in both MP (autofluorescence) signal and Entropy when increasing PMI. This means that the tissue is becoming more uniform, with a fibrillar arrangement less delineated.

The combination of MP imaging and selected objective metrics for texture and structure quantification is a useful tool to assess the gradual loss of structures occurring in the lens after death, providing complimentary information on the PMI estimation.