DETERMINING THE TIME AFTER DEATH OF THE EYE LENS BY MULTIPHOTON IMAGING

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Postmortem interval (PMI) estimates is a cornerstone in Forensic Science. Although some ocular structures might serve as a tool [1], the lack of objective classification parameters based on morphological changes make their use be complex and time-consuming. Since the crystalline lens is located between two humor chambers within the eye, then postmortem changes are assumed to take place later than in other structures of the human body [2]. Here we studied the changes in the lens at different PMIs using multiphoton (MP) microscopy, a robust imaging method able to evaluate tissues and biological structures at submicron scale.

A custom MP microscope [3] was used to image the healthy eye lens of an animal model (rabbit) as a function of time after death: 24, 48, 72 and 96 h (Fig. 1). The eyes were enucleated and the lenses excised and fixed. Slices (4-μm thick) were cut with a microtome, stained with hematoxylin and eosin, and mounted on glass slides for MP imaging. For each sample, three randomly chosen areas were imaged. Then, a texture-based analysis with the gray-level co-occurrence matrix [4] was carried out to evaluate morphological differences among the specimens as a function of PMI. The structure tensor [5] was also used to explore the changes in the structural dispersion (SD) of the visualized lens fibers. We found that both the MP signal (autofluorescence) and the entropy decreased with PMI. In addition, the SD was higher at longer PMIs. That is, as PMI increases tissue textures become more uniform (less delineated, lower entropy) and the fibrillar structure presents a larger non-organized pattern (i.e. higher SD).

In conclusion, our findings suggest that the combination of MP imaging and selected objective metrics for texture quantification is a useful tool to assess the gradual loss of structures occurring in the lens after death. This might provide complimentary information on the estimation of the PMI.

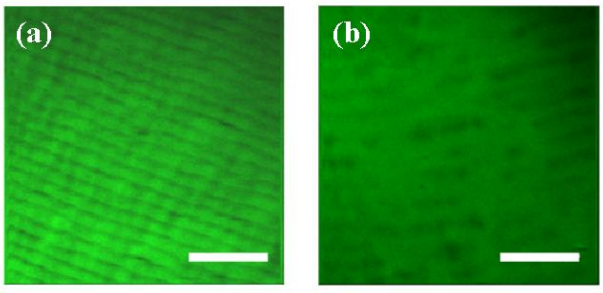

Fig. 1: MP images of the eye lens after 24 (left) and 96 h (right) of death.

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