Hybrid photoacoustic and fluorescence label-free microscopy for the investigation and identification of malignancies in ocular biopsies

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ABSTRACT

Uveal melanoma is a highly aggressive and potentially life-threatening intraocular cancerous tumor, which if not urgently treated, it can metastasize rapidly in several organs such as liver, lungs and bones, leading to a 5-year mortality rate of more than 50% [1]. The early and accurate differentiation between melanomas and benign tumors (e.g. nevi) constitutes a long-sought target in ocular oncology, as it can significantly improve the prognosis of these patients. Nevertheless, the routinely used standard opthalmoscopes usually provide a coarse picture of tumor morphology, not permitting the extraction of quantified data linked to a specific molecular background, which could act as an objective indicator for malignancy.

To that end, we demonstrate the first application of a hybrid microscopy system integrating autofluorescence and photoacoustic label-free contrast modalities [2,3] for the investigation of ocular tumors in human surgical biopsies, involving 5-μm thick sections of conjunctive nevi and choroidal melanomas. The acquired complementary imaging contrast allowed for the delineation of melanin's spatial distribution and relative concentration, as well as, the detection of several well-known autofluorescent tissue components such collagen, elastin and lipofuscin. Furthermore, by estimating the linear correlation coefficient between autofluorescence intensity and maximum photoacoustic amplitude, we have discovered a clear negative relation between the two signals in the case of choroidal melanomas, in contrast to benign nevi tissues, presenting a respective positive correlation.

The proposed dual-mode diagnostic approach could find applications in the discrimination of malignant ocular tumors in surgical biopsies at high spatial resolution and in a completely label-free approach, simplifying and expediting the relevant procedures (i.e. immunostaining), advancing technology towards fully automated digital pathology. The findings of this study could be further utilized towards the development of a hybrid contrast opthalmoscope for the *in vivo* early diagnosis of ocular malignancies in a clinical setting, significantly upgrading the capabilities of existing instruments.

REFERENCES

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