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Label-free functional and structural assessment of in vitro tissues by computational optical coherence tomography

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1. Introduction

In vitro cell cultures are increasingly valuable models for drug testing, both in ophthalmology and other medical fields. Three-dimensional (3D) *in vitro* samples with structures and functions mimicking native tissues, such as spheroids and organoids, have become particularly important. In ophthalmology, *in vitro* corneal and retinal tissues including pigment epithelium are crucial for drug testing and hold potential for transplantation. For these applications, non-invasive visualization and assessment of tissue viability are essential.

This paper introduces dynamic OCT (DOCT), a label-free, 3D imaging modality designed to visualize and assess the structure and function of in vitro samples. DOCT combines sequential OCT acquisition with subsequent analysis of the temporal properties of OCT signal fluctuations. This approach makes DOCT sensitive to intracellular and intratissue motions, allowing it to visualize and assess tissue functionality through these signal fluctuations.

In this presentation, we first introduce our 3D DOCT methods: authentic LIV (aLIV) and Swiftness [1–3]. These methods are sensitive to the occupancy of dynamically moving light scatterers in tissue and the speed of these scatterers, respectively. For DOCT, the same sample location is scanned 32 times using a repeating raster scan protocol, and two DOCT contrasts—aLIV and Swiftness—are computed. With standard-speed OCT devices (50,000 to 100,000 A-lines/s), 3D DOCT volumes can be acquired in less than a minute. We then introduce a neural network-based acceleration of DOCT, which reduces the required number of OCT frames per location from 32 to a minimum of 4. This significantly shortens the volume acquisition time to a few seconds.

2. Methods and results

We demonstrate the DOCT methods for investigating cancer spheroids, alveolar organoids, and cultured human corneal epithelial cells (HCE-T). The HCE-T samples, approximately 10 mm in diameter, were cultured on a collagen-Vitrigel membrane insert. They were placed in a 12-well plate and stored in a compact incubator integrated with the OCT microscope. DOCT images were acquired through the incubator's glass window.

During measurements, two samples were maintained in cultivation media, while a third was deprived of nutrients by being placed in Dulbecco's phosphate-buffered saline (DPBS). Samples were measured every 30 minutes for up to 14 hours to monitor their degradation process. As shown in Fig. 1, time-dependent alterations in morphology, such as sample thickness, and cellular dynamics were visualized, revealing phasic alterations of the tissue under nutrient deprivation.

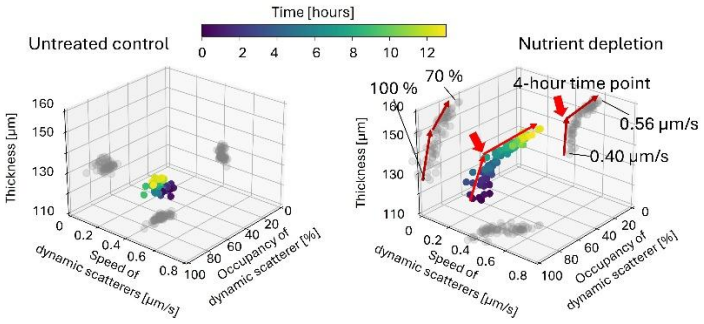

Fig. 1. The alteration of the layer-cultured human corneal epithelium. The nutrient depleted sample (right) is thickened and the intracellular dynamics altered by nutrient depletion, and the alteration was found to be phasic as showing the deflection point at 4-hour time point. On the other hand, the untreated sample are stable both in morphology and the intracellular dynamics for 14 hours.

3. References

[1] R. Morishita, P. Mukherjee, I. A. El-Sadek, T. Seesan, T. Mori, A. Furukawa, S. Fukuda, D. Lukmanto, S. Matsusaka, S. Makita, and Y. Yasuno, "Dynamic optical coherence tomography algorithm for label-free assessment of swiftness and occupancy of intratissue moving scatterers," arXiv arXiv:2412.09351 (2024).

[2] S. Fujimura, I. A. El-Sadek, R. Morishita, Y. Feng, and Y. Yasuno, "Intracellular activity-type and parameter estimation from dynamic optical coherence tomography signals," Prof. SPIE **13305**, 133050P (2025).

[3] Y. Feng, S. Fujimura, Y. Lim, T. Seesan, R. Morishita, I. A. El-Sadek, P. Mukherjee, S. Makita, and Y. Yasuno, "Dynamic-OCT simulation framework based on mathematical models of intratissue dynamics, image formation, and measurement noise," Biomed. Opt. Express **16**, 2875–2897 (2025).