Quantitative image-analysis of organoids with high-throughput digital holography microscopy

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Organoids imitate organ physiology better than any previously described *in vitro* tissue model, which makes them ideal candidates to capture our increasingly complex diseases. Their biological relevance raised high expectations in drug development and in regenerative medicine.

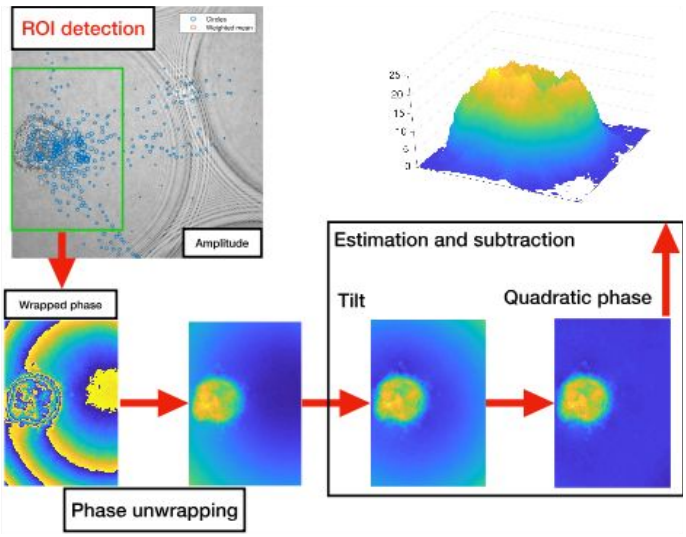
However, the existing readouts are simplistic and provide limited information. In this study, we show that more phenotypically accurate measures can be extracted from intestinal organoids using digital holographic microscopy (DHM). This is done in U-shape microwell that we recently designed. DHM provides quantitative phase information in a non-invasive manner, thus enabling time-lapse analysis of organoids and providing quantitative information on their morphology and intracellular content.

The holographic images (phase + amplitude) present inhomogeneity in the amplitude, a phase delay coming from the image formation model, a deformation due the microwell, and misalignment coming from the robotized system.

To tackle these problems, we developed an automated pipeline (Fig. 1) which notably features:

- Detection of organoids from partial view of microwell based on a circular Hough Transform;
- Phase unwrapping that relies on a robust non-iterative algorithm [1];
- Flattening the background to correct the phase induced by the meniscus.

Our automated image-analysis pipeline enables the extraction of the quantitative phase information from a large number of organoid cultures. As results, we succeeded to quantify the time evolution of the growth of the organoids over long period of time.



[1] H. Takajo and T. Takahashi, "Noniterative method for obtaining the exact solution for the normal equation in least-squares phase estimation from the phase difference," J. Opt. Soc. Am. A 5, 1818-1827 (1988).