
3D lensfree microscopy for 3D cell cultures

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Résumé

New microscopes are needed to help realize the full potential of 3D organoid culture studies by gathering large quantitative and systematic data over extended period of time while preserving the integrity of the living sample. Current 3D techniques achieve a good resolution but on small volumes and need labelled samples or can present toxicity, forbidding to temporal study.

Lensfree video microscopy is addressing these needs in the context of 2D cell culture, providing label-free and non-phototoxic acquisition of large datasets over time. The system is minimalist: the sample is placed on the sensor chip, diffracting a semi-coherent incident light. Knowing the light propagation physics, the absence of physical lens can be replaced by a numerical focusing. As scientists routinely adopt 3D culture techniques, the new challenging task is to extend lensfree microscopy techniques to 3D structures. The objectives of the current work are to port lensfree techniques toward 3D cell culture acquisitions and reconstructions while preserving its advantages.

We developed several experimental benches to test different acquisitions modalities, both on inert and living biological samples. Unlike 2D lensfree imaging, where only one image is required for retrieving the 2D object, the reconstruction of a 3D object from lensfree acquisitions requires multiple viewing angles. The object is placed on top of a CMOS sensor at a distance of 1 to 3 mm, the illumination by a semi-coherent source being tilted relative to the sensor, a configuration well adapted to standard containers such as Petri dish or well plates. The incident wave is diffracted by the sample and the sensor records the resulting hologram.

*Intervenant

If different alternative methods were developed during our work, the last techniques are based on the Fourier diffraction theorem, linking the 2D Fourier transforms of the diffracted wave-front to the 3D Fourier space of the sample. But one needs the diffracted wave whereas only the intensity of the total wave is recorded by the sensor. This lack of phase added to the limited number of available angles makes the 3D sample retrieval difficult. Different algorithms were developed to overcome this limitations, mainly based on regularised inverse problem techniques.

The first results on sparse biological data are really convincing. 3D scenes such as cells embedded in Matrigel® capsules or vast cellular network assembly were successfully reconstructed on volumes as large as 20 mm^3 , establishing the proof of concept of 3D lensfree microscopy.

Mots-Clés: Lensfree microcopy, Diffractive tomography, Videomicroscopy : Inverse problem