4D DIGITAL HOLOGRAPHIC MICROSCOPY OF MICROCIRCULATION

Alexey Brodoline*, Daniel Alexandre, Michel Gross
Laboratoire Charles Coulomb - UMR 5221 CNRS-Université Montpellier,
Place Eugène Bataillon 34095 Montpellier, France
***alexey.brodoline@umontpellier.fr**

KEY WORDS: digital holographic microscopy, 4D imaging, zebrafish microcirculation

The presented 4D Digital Holographic Microscopy (4D-DHM) technique is able to perform 3D imaging of moving sparse objects. It is well suited to image microcirculation and has been validated imaging red blood cells in a zebrafish embryo in vivo [1-3].

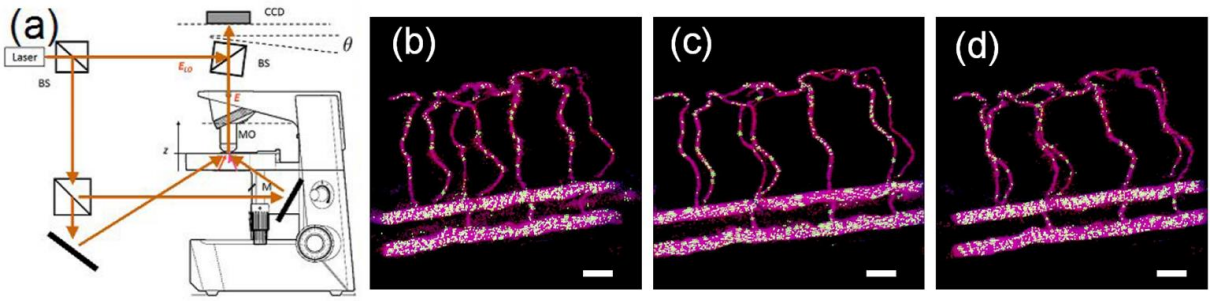

Fig. 1 (a) Holographic setup. (b, c and d) Images of blood flow within a zebrafish embryo: blood vessel (purple) and RBCs (white). The displayed 2D images correspond to the projections along z of the 3D images rotated along the *y* direction by -30° (b), 0° (c) and +30° (d). Bar is 50 µm.

The setup, illustrated in Fig.1 (a), is an upright microscope that has been modified to perform DHM in transmission. The sample placed under a Microscope Objective (MO) is illuminated by two laser beams from different directions. To perform holographic detection, a reference arm (E_{LO} field) is added on the sensor side, and interferes on the camera with the field E scattered by the object. The recorded sequences of frames are used to calculate the holograms, and to reconstruct in 3D the field scattered by the sample. By analysis of this data, 3D images of the blood vessels, and 4D images of the moving red blood cells (RBCs) can be obtained (see Figs.1 (b) to (d)).

To get these images, we first calculate the holograms as differences of frames and select by this way the signal from the moving components of the object (i.e. the RBCs). For each RBC, and each illumination, the field can be reconstructed by holography as a narrow angularly tilted cone of light. The location of RBCs is then obtained by considering the intersection of the two cones. To prevent the signals of the different RBCs to mix together, the positions of the RBCs that give the largest signals are calculated first. The contributions of these RBCs are then removed from the hologram, and the positions of the following RBCs are calculated from the hologram thus cleaned [2, 3].

4D-DHM is thus capable of performing 3D reconstruction from a single hologram, but is restricted to sparse objects that occupy only small part of the volume.

We acknowledge Labex Numev (convention ANR-10-LABX-20) for funding.

REFERENCES

[1] Verrier, N., Alexandre, D., & Gross, M. (2014). Laser Doppler holographic microscopy in transmission: application to fish embryo imaging. *Optics express*, *22*(8), 9368-9379

[2] Brodoline, A., Donnarumma, D., Alexandre, D., & Gross, M. (2016, July). 4D Holographic Reconstruction of Embryonic Blood Flow by Greedy Algorithm. In *Digital Holography and Three-Dimensional Imaging* (pp. DTh1E-1). Optical Society of America.

[3] D. Donnarumma, A. Brodoline, D. Alexandre, and M. Gross. 4d holographic microscopy of zebrash larvae microcirculation. Optic Express 24(3):26887-26900, 2016