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# Improved 3D Imaging of Zebrafish Larvae Microcirculation by Digital Holography

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**Abstract:** A microscopic technique based on digital holography is proposed to investigate blood microcirculation and vascular development in model organisms such as zebrafish larvae. Recent achievements in 3D imaging of blood flow in vessels are presented.

**OCIS codes:** (090.1995) Digital holography; (170.0180) Microscopy; (170.1470) Blood or tissue constituent monitoring; (290.5850) Scattering, particles.

## Citation

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## 1. Introduction

Blood flow imaging is important for biological and medical purposes. It enables to assess physiological or pathological processes such as angiogenesis or tumor vascularization [1] and allows early detection of diseases [2] like age-related macular degeneration [3]. Since vascular systems are generally tridimensional structures, 3D visualization of microcirculation is of a great interest.

The presented label-free method, based on digital holography, generates 3D images of moving red blood cells (RBCs) in the vascular microsystem. The technique is validated in the case of a zebrafish larva (2-5 days of development). Compared to previous results [4, 5], a better z-resolution is achieved here due to the use of a higher numerical aperture (NA) microscope objective.

## 2. Materials and methods

The experimental setup is similar to the one used in previous works [5, 6]. It's a modified upright microscope (Olympus® CX41) where the illumination light source is replaced by a laser diode (HL6545MG: 60 mW@ $\lambda=660$  nm) attenuated to not saturate the camera. The sample is a zebrafish larva (*Danio rerio*) of 2-5 days exposed to tricaine and fixed in agarose in a petri dish. The observation is performed with a Zeiss® (x20 /0.5 NA) water immersion microscope objective (MO).  $G = 41.2$  is the overall magnification of the setup.

The optical field scattered by the sample is collected by the MO and is combined with a coherent reference beam on the camera sensor (Mikrotron Eosens CL: 1280x1024 pixels, 14 $\mu$ m square pitch, 200 Hz, 10 bits). The beam splitter that recombines the scattered ( $E_S$ ) and reference ( $E_R$ ) fields is angularly tilted to achieve off-axis holography. An interference pattern (the hologram) is produced at the sensor plane. The camera records the corresponding intensity  $I_{CAM}(x, y) = |E_R + E_S|^2$ . In order to eliminate the light scattered by the immobile objects and keep only the moving RBCs, the hologram  $H_{CAM}$  is calculated by:

$$H_{CAM}(x, y) = \sum_{n=1}^6 I_{CAM,n}(x, y) \sin(n \frac{\pi}{3})$$

Where  $I_{CAM,n}$  with  $n = 1..6$  are successive camera frames.

The optical field can be numerically back propagated from the sensor and reconstructed in a plane near the object.  $E_S(x, y, z)$  can then be computed on either side of the sample plane using the reconstruction method described in [7]. A cube of data containing  $E_S(x, y, z)$  is thus obtained, giving the complex field in a volume around the object. From this cube of data, the positions of the red blood cells (RBCs) are extracted using a cleaning algorithm. The reconstruction procedure and the cleaning algorithm are detailed in [5, 7].

### 3. Results

The cleaning algorithm determines the positions of the RBCs in a grid of  $640 \times 640 \times 128$  pixels with pitches  $\Delta x = \Delta y = 0.54 \mu\text{m}$  and  $\Delta z = 1.08 \mu\text{m}$ . Note that the optical lateral resolution is smaller. The nominal resolution of the MO is  $r = 0.6\lambda / ON = 0.79 \mu\text{m}$ . The calculations are made on a Nvidia GTX TITAN Graphics Processing Unit (GPU) using CUDA.

The results of the reconstruction are presented in Fig. 1. It was made on 400 successive frames recorded at 200 Hz. The averaging of the RBCs positions on 100 images gives the form of the blood vessels. The internal structure of the vascular system can be visualized. The rendering is performed using the OpenGL interface of Nvidia CUDA, which allows a convenient translation and rotation of the 3D image.

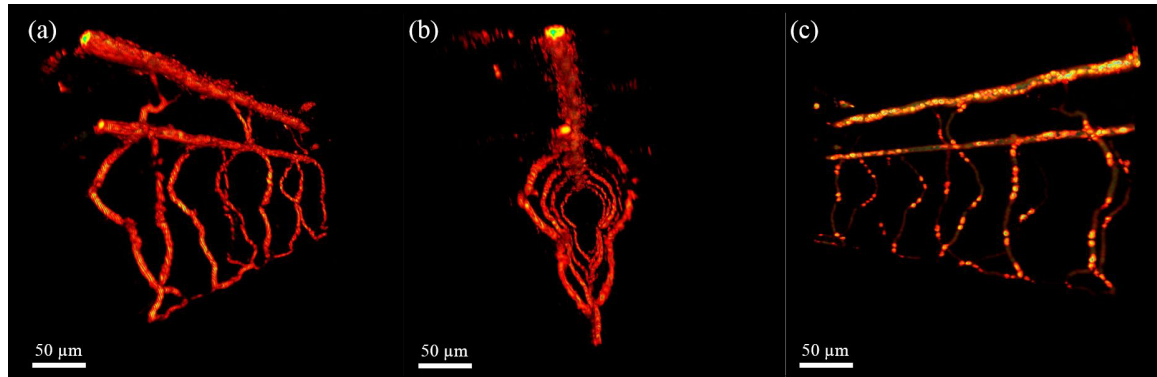


Fig. 1. 3D reconstruction of a 5-days-aged zebrafish vascular system. Average of the RBCs positions over 100 frames gives the form of the vessels. (a,b) Two different points of view. (c) RBCs on the 50th frame superposed to the averaged image.

### 4. Conclusion

As compared with previous results, an improved 3D imaging is obtained. The use of a higher NA objective and the CUDA-OpenGL rendering enables a complete rotation around the object. The technique can be adapted to any microscope and can be combined with other conventional microscopic techniques, providing new tools for blood flow studies.

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