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Abstract: An holographic laser Doppler scheme using two illumination beams is proposed to image blood vessel in fish embryo. The coincidence of the reconstructed images made for each illumination orientation leads to an accurate z sectioning.

OCIS codes: (090.1995) Digital holography, (110.6150) Speckle imaging, (170.6480) Spectroscopy, speckle.

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1. Introdution

Blood flow imaging techniques are widely used in biomedical studies, since they can assess physiological processes or can be used for early detection of disease. However, many blood flow studies require, for imaging purposes, the use of a contrast agent, making the blood flow characterization invasive [1]. Combination of laser Doppler holography [2] with transmission microscopy has been proposed to image blood flow in fish embryos [3]. To enhance the slicing along z, we have used two illumination beams oriented in two different directions. We have then selected in the Fourier space the field corresponding to each of the illuminations, and we have reconstructed the blood vessels images. By adjusting the reconstruction distance so that the reconstructed images for each illumination coincide, a precise measurement of the vessel z position can be obtained.

2. Material and method

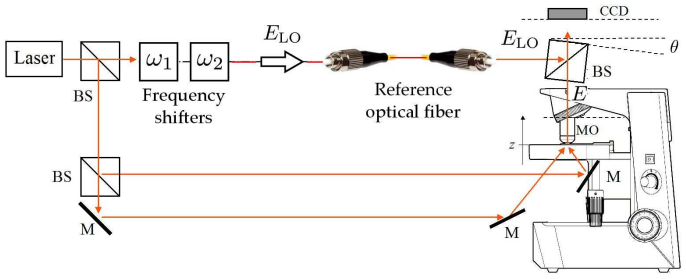

Fig. 1. Holographic microscopy setup: BS: beam splitter; M: mirror; ω_1, ω_2: Acousto optics modulators (Bragg cell) that shift the frequency of the local oscillator beam. MO: microscope objective; CCD: CCD camera; E and E_{LO}: signal and reference complex fields.

The set-up (see Fig. 1) is an upright microscope that has been modified for making heterodyne holography [4]. The main laser (frequency ω_I) is split into two arms (illumination and reference) by a beam splitter. Two acousto optic modulators (AOM) at $\omega_{1,2} \simeq 80$MHz control the frequency $\omega_{LO} = \omega_I + \omega_1 - \omega_2$ of the reference arm (E_{LO}). The illumination arm (E) is split into two branches so as to illuminate the sample in two directions. The sample is a zebrafish embryo (48 hours-old) between slide and cover slip. It is imaged with a microscope objective MO (NA= 0.25,

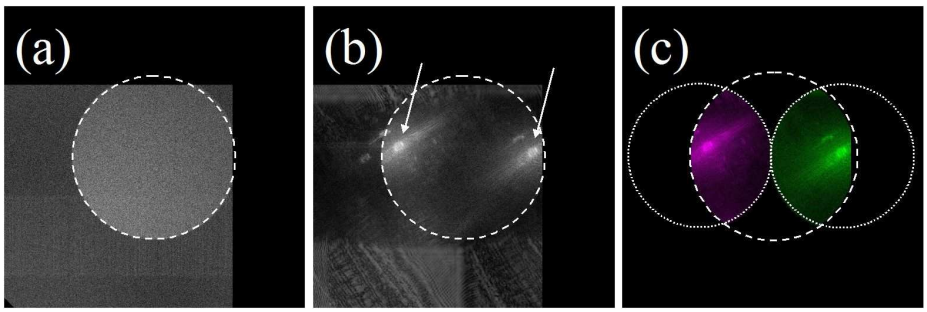

Fig. 2. Pupil images reconstructed with 4 phases holograms $H = (I_0 - I_2) + j(I_1 - I_3)$, and with $\omega_1 - \omega_2 = \omega_{CCD}/4$. Sample is ground glass (a) or zebrafish sample (b,c).

G=10). To achieve off-axis holography, the beam splitter that recombines the signal (E) and reference (E_{LO}) fields is angularly tilted: $\theta \neq 0$. The CCD camera (1280×1024 pixels, $\omega_{CCD} = 110$ Hz, 10 bits) record the interference pattern $I = |E + E_{LO}|^2$. Sequences of 300 frames are recorded: I_0 ... I_{299}. Recorded data are cropped into a 1024×1024 calculation grid to perform holographic calculations like Fast Fourier Transforms (FFT).

To select the signal corresponding to each illumination, we have reconstructed the image of the field E scattered by the sample in the MO back pupil plane. Reconstruction is made by the 1 FFT method with 4 phases holograms $H = (I_0 - I_2) + j(I_1 - I_3)$, and with $\omega_1 - \omega_2 = \omega_{CCD}/4$. Images are displayed in intensity by averaging data over 80 frames. We have imaged a ground glass (Fig. 2 (a)) and a zebrafish sample (Fig. 2 (b,c)). The pupil image, which is located in the upper right hand size of the calculation grid, has been displayed in the center of Fig. 2 (a...c). The pupil image is especially bright with the ground glass. It corresponds to the bright circular zone of Fig. 2 (a), whose edges are sharp (white dashed line circle). We have select the useful holographic information by cropping the pupil zone (and by filling the rest of the calculation grid with zeros).

Since the zebrafish sample is quite transparent, the two illumination beams yield two bright spots corresponding to the white arrows of Fig. 2 (b). Since these spots are well separated in the Fourier space, we have cropped 2 circular zones of radius 240 pixels centered on each spot (dotted white circle of Fig. 2 (c)) in order to perform reconstruction with one or other of the illumination beams (i.e with the purple and green zone of Fig. 2 (c)).

3. Results

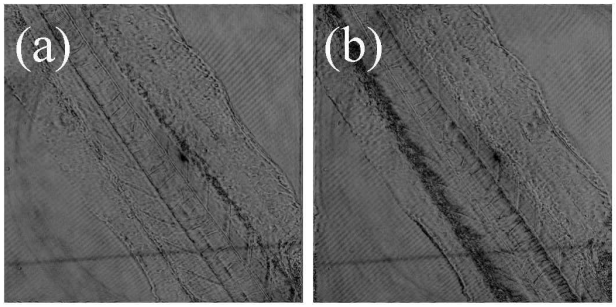

Fig. 3. Zebrafish images reconstructed with 4 phases holograms $H = (I_0 - I_2) + j(I_1 - I_3)$, and with $\omega_1 - \omega_2 = \omega_{CCD}/4$ with the purple (a) and green (b) zone of Fig.2 (c).

Figure 3 (a) and (a) shows the zebrafish reconstructed images obtained at illumination frequency ω_I i.e. with 4 phases holograms $H = (I_0 - I_2) + j(I_1 - I_3)$, and with $\omega_1 - \omega_2 = \omega_{CCD}/4$). Reconstruction is made by the 2 FFT method from the FFT^{-1} of the Fourier space selected data [5]. The images of Fig. 3 (a) and (b) are obtained by selecting the purple (a) and green (b) zones of the Fourier space (i.e. with one or the other illumination beam). The images of Fig. 3 (a, b) are quite different, since the illumination directions are different.

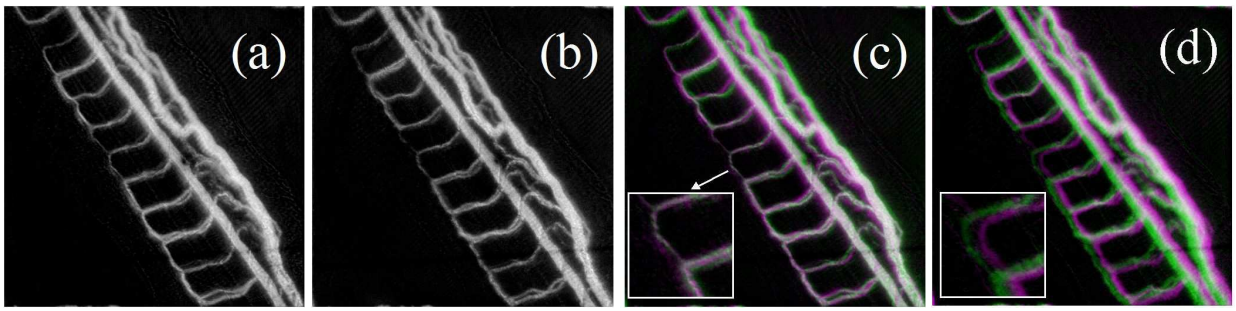

Fig. 4. Recontructed image of zebrafish blood vessels made with 2 phases holograms $H = I_0 - I_1$) and with $\omega_1 - \omega_2 = 0$. Reconstruction is made by selecting the purple (a) or the green (b) or both zone (c,d). In the last case the 2 images are displayed in purple and green. Reconstruction is made with $z = 0$ (a...c) and $z \simeq 370\mu$m. Images are obtained by averaging reconstructed signal energy over 80 frames.

We have then made the 2 beams reconstruction by using a different holographic modality. To image the moving red blood cells (and thus the blood vessel by averaging the reconstructed images over 80 frames), we have consider 2 phases holograms $H = I_0 - I_1$ with $\omega_1 - \omega_2 = 0$. By the way, the signal which is scattered at the illumination frequency ω_I is not detected, while the signal of the moving cells, which is Doppler shifted is detected. The reconstructed images obtained by selecting the purple and green zones are displayed on Fig. 4 (a) and (b). They correspond to a single illumination beam, and are very similar. To better visualise the small differences of the two images, we have displayed them in purple and green on Fig. 4 (c). For the vessels that are located in the reconstruction plane $z = 0$, the purple and green images coincide , as seen in the zoom of Fig. 4 (c)). Figure 4 (d) show the purple and green images obtained for $z \simeq 360\mu$m. The coinciding vessels are now well separated, and the separation is much larger than the slight blur of the vessel seen on the zoom of Fig. 4 (d). The coincidence of the purple and green images yield thus a much better determination of the vessel z position than the blurring of the reconstructed images.

Exploiting the z information of the coincidence of purple and green image obtained with double illumination beam open the way to 3D imaging of the structure of the embryos blood flow. Motions of the individual reed blood cell with possible coincidence of images can be also studied from sequence of reconstructed images similar to the one displayed on Fig. 4. Experiment is under progress.

All animal experiments that are described in the present study were conducted at the University Montpellier 2 according to European Union guidelines for the handling of laboratory animals (http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm) and were approved by the Direction Sanitaire et Vétérinaire de l'Hérault and Comité d'Ethique pour l'Expérimentation Animale under reference CEEA-LR-13007.

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