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# Fish embryo multimodal imaging by laser Doppler digital holography

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**Abstract:** A laser Doppler imaging scheme combined to an upright microscope is proposed. Quantitative Doppler imaging in both velocity norm and direction, as well as amplitude contrast of either zebrafish flesh or vasculature is demonstrated.

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

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## 1. Introduction

Combination of laser Doppler holography [1] with transmission microscopy is proposed to analyze blood in fish embryos. We have adapted a laser Doppler holographic setup to a standard bio-microscope by carrying the two beams (illumination of the object and reference) with optical fibers. Multimodal acquisition and analysis of the data is made by adjusting the reference versus illumination frequency offset and the shape of the filtering zone in the Fourier space. Considering the same set of data, we have extracted amplitude contrast of the whole fish embryo, or only the moving blood vessels. Images where the flow direction is coded in RGB color are obtained.

## 2. Experiment

Our digital holography set-up consists in a Mach-Zehnder interferometer in which the recombining cube beam splitter is angularly tilted. The interferometer is split into an injection (a) and a recombination (b) part, which are connected, using optical fibers, to a classical upright microscope (Fig. 1). The injection part (Fig. 1(a)) is used to build both object illumination (field  $E_1$ ) and reference (or local oscillator (LO)  $E_{LO}$ ) beams. Phase shifting interferometry and frequency scanning of the holographic detection frequency are made possible using the heterodyne holography method [2]. The reference arm is thus frequency shifted by using two acousto-optic modulators AOM1 and AOM2, operating at a controlled angular frequency difference  $\Delta\omega$ . Reference and object fields are injected into two mono-mode optical fibers. The object is imaged by a microscope objective (MO: NA= 0.25, G=10), mounted on an upright microscope (Fig. 1(b)). The illumination field ( $E_1$ ) is scattered by the studied fish embryo (zebrafish) yielding the signal field ( $E$ ). Signal ( $E$ ) and reference ( $E_{LO}$ ) fields are combined using an angularly tilted beam splitter cube (BS). Interferences (i.e.  $E + e^{j\Delta\omega t} E_{LO}$ ) between both fields are recorded on a  $1360 \times 1024$  pixel ( $6.45 \mu\text{m}$  square pitch) 12-bits CCD camera operating at  $\omega_s/(2\pi) \leq 10$  Hz. Recorded data are cropped to  $1024 \times 1024$  for FFT (Fast Fourier Transform) calculations. All the driving signals are synchronized by a common 10 MHz clock.

Figure 2 illustrates our reconstruction procedure [4, 5] with non-Doppler shifted images. We have considered here four phase holograms:  $H = (I_0 - I_2) + j(I_1 - I_3)$  (see Fig. 2(a)) where  $I_0..I_3$  are 4 successive camera frames.  $H$  is refocused in the objective pupil plane leading to  $H_1(k_x, k_y) = \mathcal{F} \left[ H(x, y) e^{j|k|(x^2+y^2)/2d} \right]$ , where  $\mathcal{F}$  is the FFT operator, and  $d$  is the propagation distance to refocus over the pupil (Fig. 2(b)). The pupil image is then

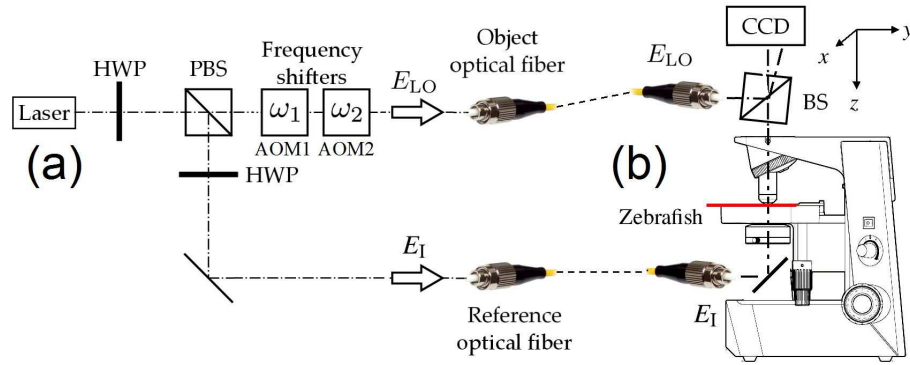


Fig. 1. Heterodyne digital holographic microscopy experimental arrangement (a) Injection part of the interferometer. HWP: half wave plate; PBS: polarizing beam splitter; AOM1, AOM2: acousto optic modulators (Bragg cells). (b) Classical upright microscope used for the off-axis recombination of reference and object beams. BS: cube beam splitter; CCD: CCD camera.

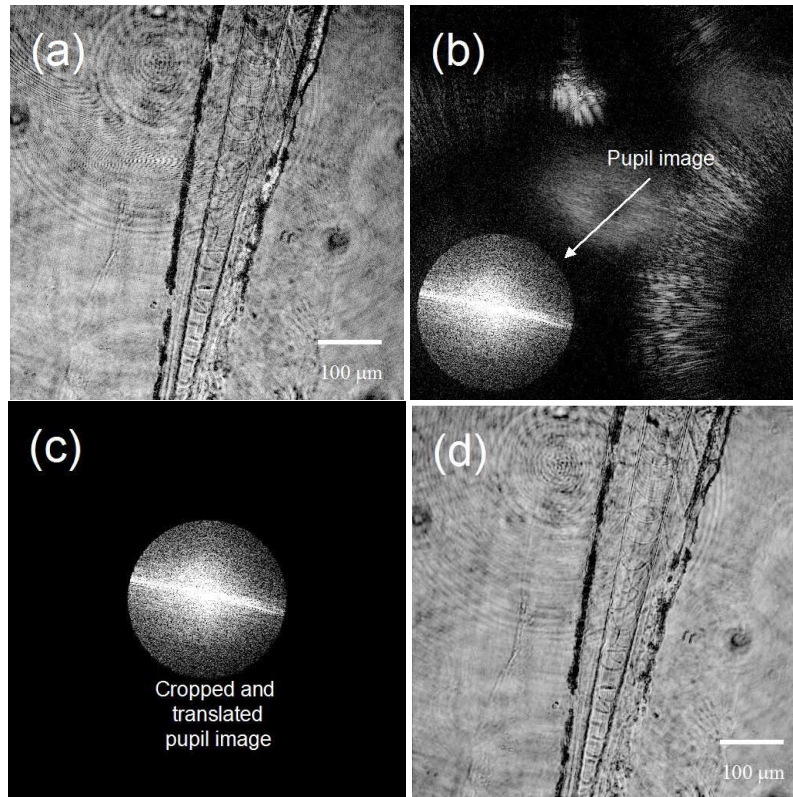


Fig. 2. Spatial filtering and reconstruction principle. Holograms  $H(x, y)$  (a),  $H_1(k_x, k_y)$  (b),  $H_2(k_x, k_y)$  (c) and  $H_3(x, y, z = 0)$  (d). The display is made in arbitrary Log scale for the average intensity  $\langle |H_X|^2 \rangle$ .

cropped and recentered leading to  $H_2(k_x, k_y)$  (Fig. 2(c)) and the complex object field  $H_3(x, y, z)$  is extracted by  $H_3(x, y, z) = \mathcal{F}^{-1} \left[ H_2(k_x, k_y) e^{j(k_x^2 + k_y^2)/2z} \right]$ .

Due to the red blood cell (RBC) motion, the light passing through the zebrafish undergoes a Doppler shift  $\omega_D = (\mathbf{k}_S - \mathbf{k}_I) \cdot \mathbf{v}$ , where  $\mathbf{k}_I$  and  $\mathbf{k}_S$  are the illumination and scattered wavevectors, and  $\mathbf{v}$  is the velocity. To measure the Doppler shift due to scatterer motion, we have swept the detection frequency  $\Delta\omega$  from 0 to 120 Hz, and calculated two-phases holograms  $H = I_0 - I_1$  for each frequency point. To assess for the norm of the velocity vector, the signal

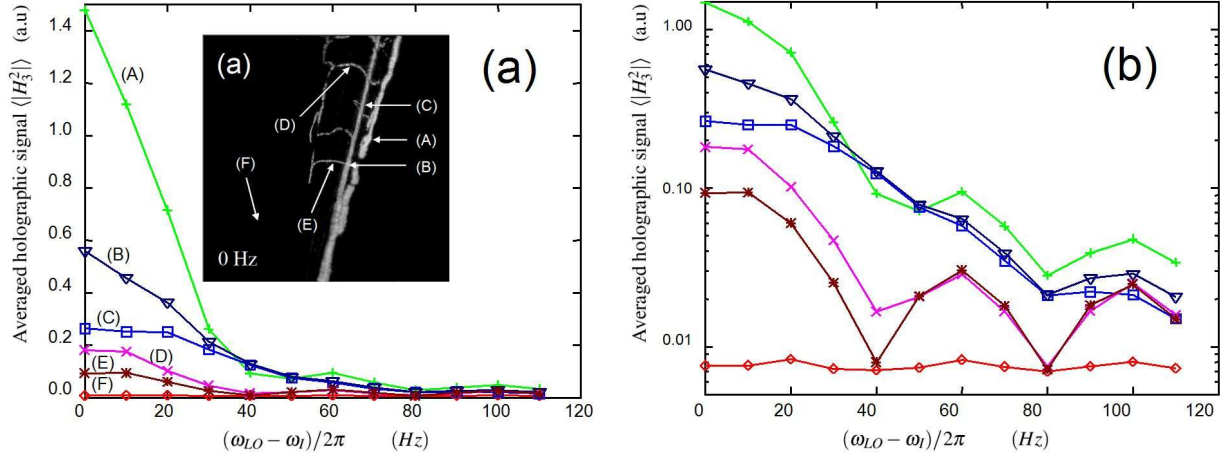


Fig. 3. Dependence of the Doppler holographic signal  $\langle |H_3(x, y)|^2 \rangle$  with the frequency offset  $\Delta\omega = (\omega_{LO} - \omega_I)/(2\pi)$  for different location A to F in the insert (a). Curves are drawn with linear (a) and logarithmic (b) scales.

is averaged over different zones the reconstructed image. Obtained results are depicted Fig. 3 for zones labeled in the insert of Fig. 3(a). By estimating the half-width at half-maximum of each spectra, it is possible to obtain the norm of the velocity vector.

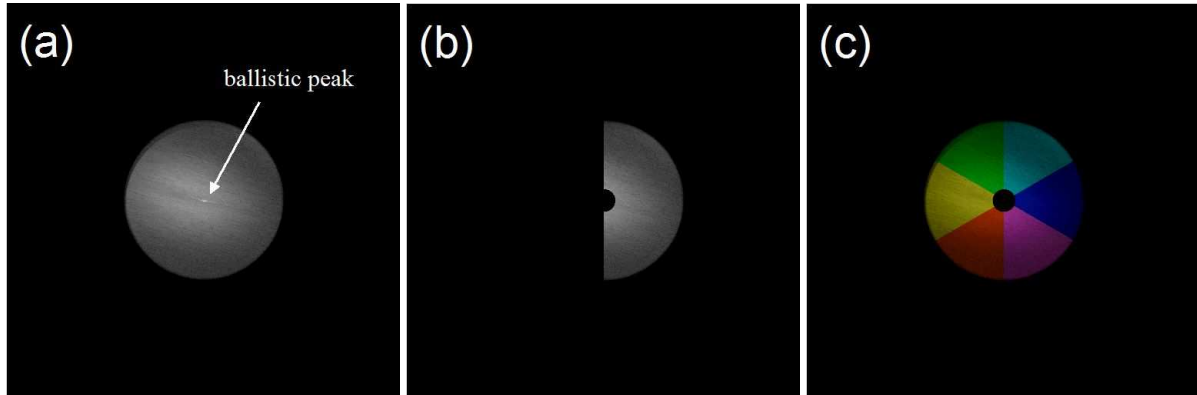


Fig. 4. Fourier space reconstructed hologram  $H_2(k_x, k_y)$  made without (a) and with (b,c) selection of the scattered wave vector  $\mathbf{k}_S$ . In (b) the selected zone is oriented in x direction. In (c), three zones obtained by rotating (b) by 0 (blue),  $2\pi/3$  (green) and  $4\pi/3$  (red) are displayed. Display is made in arbitrary log scale for  $\langle |H_2|^2 \rangle$ . Frequency shift is  $(\omega_{LO} - \omega_I) = 0$ .

In our experiment, the reference arm frequency offset  $\Delta\omega$  (which is known) is close to the Doppler shift:  $(\mathbf{k}_S - \mathbf{k}_I) \cdot \mathbf{v}$ , where  $\mathbf{k}_I$  is known ( $\mathbf{k}_I$  is parallel to the z axis), and where  $\mathbf{k}_S$  can be selected in the Fourier space (by applying mask to  $H_2(k_x, k_y)$ ). Quantitative information on the velocity  $\mathbf{v}$  can thus be obtained. This point is illustrated by Fig. 4 and Fig 5. By filtering half of the Fourier space (Fig. 4(b)), one can discriminate RBC motion along the x direction. By

rotating the filtering zone by 0 (blue),  $2\pi/3$  (green) and  $4\pi/3$  (red), one can map in false color the reconstruction space (see Fig. 4(c)). Combining the 3 reconstructed holograms (displayed in blue, green and red) for the 3 rotations

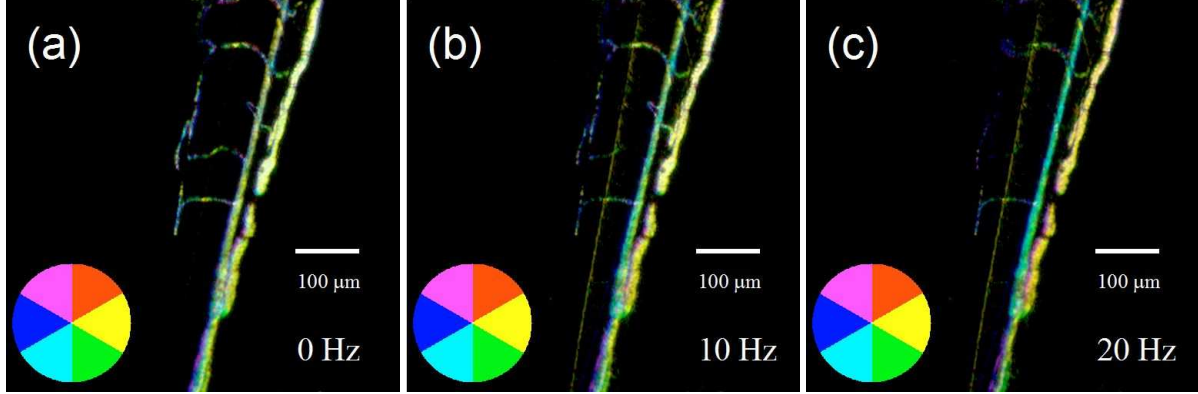


Fig. 5. Colored reconstructed hologram  $H_3(x, y, z = 0)$  made for  $(\omega_{LO} - \omega_I)/(2\pi) = 0$  Hz (a), 10 Hz (b), 20 Hz (c) displayed with a color RGB Log scale for the average intensity  $\langle |H_3|^2 \rangle$ .

of the filtering zone, one can obtain an image where the flow-direction is coded in RGB colors. This point is illustrated Fig. 5 where blood flow direction between veins and arteries can be discriminated.

### 3. Conclusion

We have proposed an holographic laser Doppler set-up based on heterodyne digital holography working in transmission configuration, and fitted to an upright-microscope. Upon classical holographic modalities, coupling spectral broadening measurement with RGB fluid flow-direction discrimination has made possible to obtain a quantitative Doppler measurement.

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