Four dimensional motility tracking of biological cells by digital holographic microscopy

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Abstract: We utilize quantitative phase microscopy by digital holography to analyze intracellular fluctuation and track cellular motility in four dimensions. The three-dimensional trajectories have been measured as a function of time at sub-second and micrometer level.

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1 Introduction

Quantitative phase microscopy by digital holography (DH-QPM) is a particularly important feature of digital holography [1, 2]. It allows measurement of optical thickness with nanometer-scale accuracy by single-shot, wide-field acquisition, and it yields phase profiles without some of the complications of other phase imaging methods. The phase image is immediately and directly available on calculating the two-dimensional complex array of the holographic image, and the phase profile conveys quantitative information about the physical thickness and index of refraction of cells and the substrate [1, 2]. DH-QPM has been widely used in biological field. We have utilized DH-QPM to visualize wrinkle formation of biological cells on silicone rubber film [3]. Non-wrinkling elastics substrate PAA was used as the flexible membrane for cell attachment [4]. Accurate determination of Young's moduli of polyacrylamide gel by DH-QPM was essential for obtaining exact responses of the cells to the substrate flexibilities [5]. Quantitative measures of surface deformation have been extracted and the cellular traction force has been estimated in a direct and straightforward manner [3, 4]. Here we utilized DH-QPM to statistically analyze the intracellular fluctuation of amoeba and the amplitude of fluctuation was measured quantitatively. Also, three dimensional trajectories of moving chilomonas which served as food of amoeba in the cells environment were visualized. DH-QPM was shown to be able to track three-dimensional motion of cells with temporal and spatial resolution at the sub-second and micrometer level.

2 DHM setup

The DHM setup used in this work is illustrated in Fig. 1. It consists of a Mach-Zehnder interferometer illuminated with a HeNe laser [1, 2]. The object arm contained a sample stage and a microscope objective lens (MO1) that projected a magnified image of the object onto a CCD camera. The reference arm similarly contained MO2, so that the holographic interference pattern contained fringes due to interference between the diffracted object field and the off-axis reference field. The numerical aperture (NA) of the microscope objectives is 0.25 and the magnification is 10×. The specification of CCD is 1024×768 pixels, and the pixel size is 4.65μm. The QPM images were reconstructed from the captured holograms by the angular spectrum method [1, 2]. Aberrations and background distortions of the optical field were minimized by available DHM techniques [1, 2].

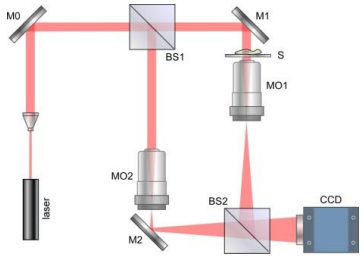

Fig. 1: DHM setup. M's: mirrors; BS's: beam splitters; MO's: microscope objectives (10×); S: sample object.

3 DHM analysis of cellular sample

Cellular sample consists of amoeba and chilomonas on a glass slide, Fig. 2. Figure 3 illustrates DHM analysis of the cells sample. Interference of the diffracted object field and off-axis reference field resulted in the hologram, Fig. 3a). In Fig. 3b), the angular spectrum shows the zero order and a pair of first order components. One of the first-order components was separated with a numerical band-pass filter when the

off-axis angle of the reference beam was properly adjusted. The corresponding amplitude and phase profiles after correct centering of the filtered angular spectrum and numerical propagation to the object focus distance are shown in Fig. 3c) and Fig. 3d). Figure 3e) shows unwrapped phase due to the phase discontinuity in Fig. 3d).

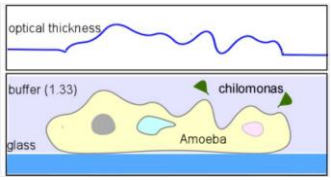

Fig. 2: Schematic of the cell sample (lower) and the corresponding optical thickness profile (upper).

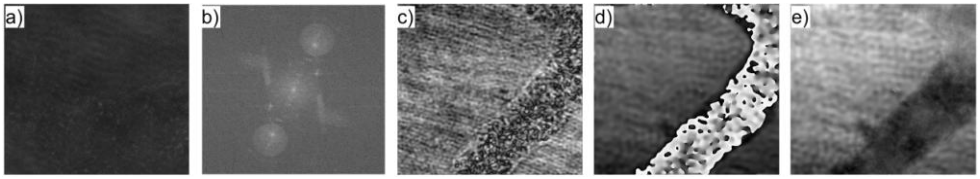

Fig. 3: DHM analysis. The field of view is 216×216 μm^2 with 464×464 pixels. a) Hologram; b) Angular spectrum; c) Amplitude image; d) Wrapped quantitative phase image; e) Unwrapped phase image.

4 Statistical analysis of intracellular fluctuation of amoeba

A hologram movie of moving amoeba was recorded at the frame rate 30fps, up to 10 seconds. The average and standard deviation profiles of all the 300 corresponding phases and the pseudo-color 3-D rendering are shown in Fig. 4. Figure 4c) and d) indicates that intracellular fluctuation amplitudes significantly larger than the background noise level. Temporal phase variations of one pixel inside the cell and in the background are shown in Fig. 5. The measured standard deviations of the pixel inside the cell and in the background are 0.38 rad and 0.03 rad, respectively. The amplitude of intracellular fluctuations was defined as the standard deviation of the phase profile of a pixel in the image within the cell compared with a pixel in the background during a recording time period [6]. The temporal standard deviation of each pixel in the cell was evaluated as: $SD(\varphi_{cell}) = \sqrt{\left[SD\left(\varphi_{cell}+\varphi_{BG}\right)\right]^2 - \left[SD\left(\varphi_{BG}\right)\right]^2} \approx 0.41 rad$. The amplitude of intracellular fluctuation was then estimated as: $\frac{SD(\varphi_{cell})\cdot\lambda}{2\pi(n_{cell}-n_{medium})} = 824nm$, where λ is the wavelength of the light source (633nm), n_{cell} and n_{medium} are the refractive indices of cell and medium (1.38 and 1.33).

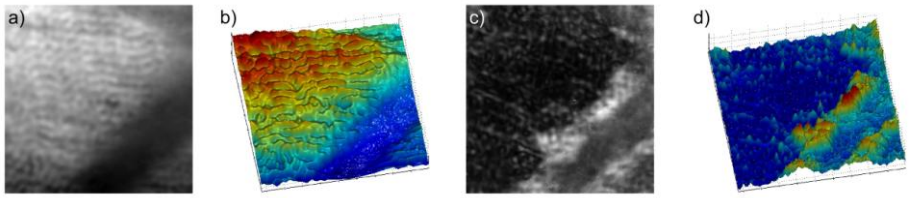

Fig. 4: Average and standard deviation of 300 phase frames. The field of view is 216×216 μm^2 with 464×464 pixels. a) Average; b) average, rendered in 3-D; c) Standard deviation; d) standard deviation, rendering in 3-D.

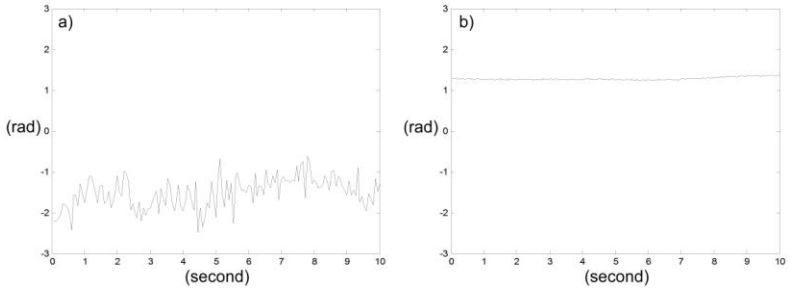

Fig. 5: Temporal phase variations at 30fps up to 10s. a) a pixel within the cell; b) a pixel in the background.

5 Four-dimensional motility tracking of chilomonas

A sequence of holograms (h_1, h_2… h_n) of a moving chilomonas in the amoeba environment was recorded. Subtraction of two consecutive holograms (h_2-h_1, h_4-h_3…h_n-h_{n-1}) was performed to eliminate background structure. The difference holograms were summed (h_2-h_1+h_4-h_3+h_n-h_{n-1}) to be a single hologram and reconstruction in angular spectrum method was applied, Fig. 6. The 3-D image representing the trajectory of the moving chilomonas was built up by reconstructing the 2-D hologram at various distances, from 3.2mm to 3.5mm, in 0.1mm step. A segmentation algorithm was employed to separate the chilomonas with amoeba and background noise, Fig. 7. Arrow indicates the moving direction.

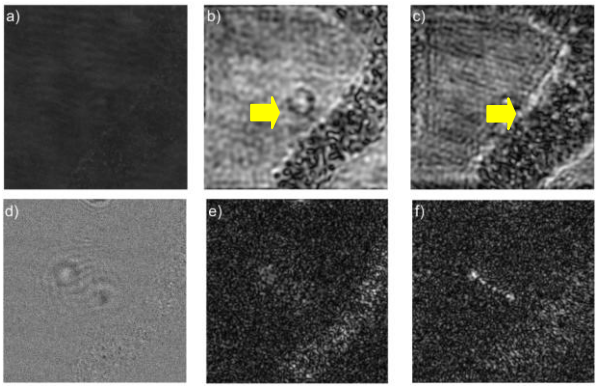

Fig. 6: Holograms and reconstructed trajectories of a chilomonas in the amoeba enviromment. The field of view is 216×216 μm^2 with 464×464 pixels. a) Single hologram of a chilomonas; b) Single amplitude of a chilomonas out of focus (arrow); c) Single amplitude of a chilomonas in focus (arrow), reconstructed by a distance 3.5mm; d) Sum of 4 pairs difference holograms (from a total of 8) of the chilomonas in a); e) Out of focus amplitude reconstructed from d); f) In focus amplitude, reconstructed by a distance 3.5mm.

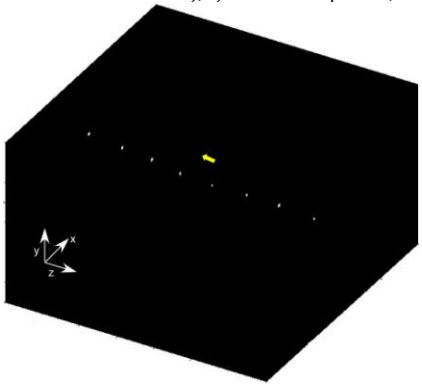

Fig. 7: Three- dimensional trajectory of a moving chilomonas. Arrow indicates the moving direction. The field of view (x-y) is 186×186 μm^2 with 400×400 pixels. Reconstructed distances in z are 3.2mm-3.5mm, step by 0.1mm.

Conclusion

DH-QPM has been applied to analyze intracellular fluctuation and track cells motility in four dimensions. The approach is sensitive to cellular or intracellular motility and it can detect and quantify variations inside or out of a cell over time. DH-QPM is shown to be an effective approach to study motility of biological cells with temporal and spatial resolution at the subsecond and micro level.

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