Four dimensional motility tracking of biological cells by digital holographic microscopy

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

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Digital holography microscopy (DHM) has been widely utilized in the biomedical field [1, 2, 3]. It allows measurement of optical thickness with nanometer-scale accuracy by single-shot, wide-field acquisition, and is able to produce a single hologram contains all the information about the three dimensional structure of the object. Here we utilized DHM to image microspheres and microfibers in three dimensions, visualize the trajectory of moving cell and tracking the cells motility through microfibers in four dimensions. DHM is shown to be able to track three dimensional motions of cells with temporal and spatial resolution at the sub-second and micrometer level.

DHM setup used in this work is illustrated in Fig. 1. DHM images were reconstructed from the captured holograms by the angular spectrum method [3, 4]. Aberrations and background distortions of the optical field were minimized by available DHM techniques [3, 4].

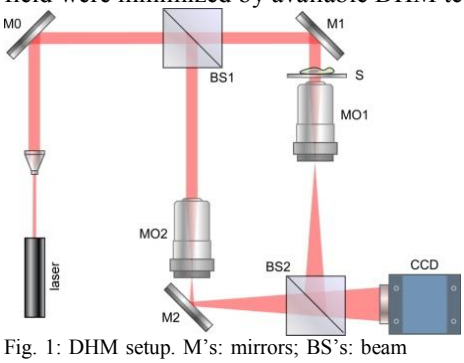

Fig. 1: DHM setup. M's: mirrors; BS's: beam splitters; MO's: microscope objectives (10×); S: sample object.

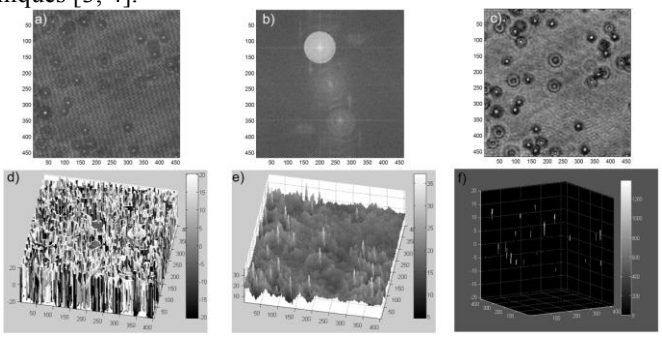

Fig. 2: 3D profiles of microspheres by DHM. The field of view is 90×90 μm^2 with 464×464 pixels. a) Hologram; b) Angular spectrum; c) Amplitude image; d) XYZ profiles; e) XYA profiles; f) 3D profile.

Three-dimensional profiles of microspheres

We present three-dimensional profiles of microspheres in Fig. 2. A single shot hologram of microspheres is captured in Fig. 2a) and then DHM analysis is perform. The amplitude image Fig. 2c) is reconstructed from Fig. 2a) by angular spectrum method, Fig. 2b) [3, 4]. A microsphere is seen to have a maximum intensity at its center in the focal plane. The hologram is reconstructed numerically in different planes (Z) from 20 μm to -20 μm, in -2 μm step. Maximum amplitude (A) of each pixel along Z direction and its corresponding location are chosen, Fig. 2d) and e). We combine Fig. 2d) and e) and a threshold of intensity ($P=A^2$) is set to distinguish in focus objects from other elements. This algorithm of numerical focusing is able to determine focal planes for all the objects in the reconstructed volume. The 3D profile of microspheres is show in Fig. 2f). Z coordinates are the reconstructed planes and the grey scale represents the intensity P.

Three-dimensional profiles of microfibers

Three-dimensional profiles of microfibers are shown in Fig. 3. Hologram Fig. 3a) shows two microfibers overlap each other. Angular spectrum method is utilized (Fig. 3b)) and amplitude (image Fig. 3c)) is extracted. We produce an axial projection by adding up reconstructed images in different planes of the intensities, Fig. 3d). The projected image is converted to binary Fig. 3e) using threshold and segmentation. The 1 and 0 represent microfibers and background respectively. The binary image is taken as a mask and is multiplied by each reconstructed image. Then we followed the same algorithm to determine the focal planes for all the objects in the reconstructed volume, Fig. 3f), g) and h). Z coordinate provides the depth information of real image of microfiber in the reconstruction volume. The orientation and length of microfibers in 3D volume can be determined from x, y, and z coordinates of real image of microfibers.

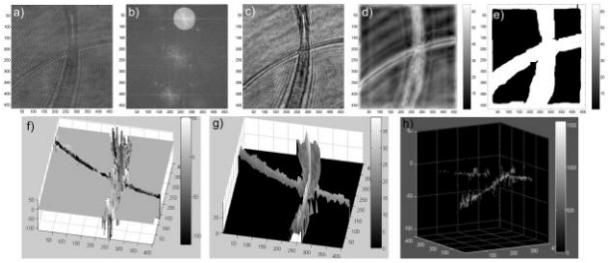

Fig. 3: 3D profiles of microfibers by DHM. FOV is 90×90 μm^2 with 464×464 pixels. a) Hologram; b) Angular spectrum; c) Amplitude image; d) Projected image; e) Binary image; f) XYZ profiles; g) XYA profiles; h) 3D profile.

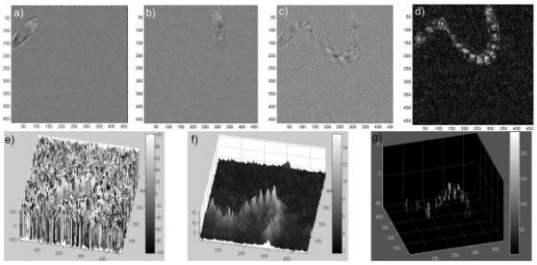

Fig. 4: 4D tracking of chilomonas by DHM. FOV is 90×90 μm^2 with 464×464 pixels a) h2-h1; b) h18-h17; c) Sum of 9 difference holograms; d)Amplitude image; e) XYZ profiles; f) XYA profiles; g) 3D profile.

Four-dimensional motility tracking of chilomonas

Four-dimensional motility tracking of chilomonas is demonstrated here. A holograms sequence (18 frames in total) of a moving chilomonas is recorded. Subtraction of two consecutive holograms (h_2-h_1,…h_{18}-h_{17}) is performed to eliminate background structure and interference effects. The 9 difference holograms (from a total of 18) are summed (h_2-h_1+h_4-h_3+…+h_{18}-h_{17}) to be a single hologram which provides time evolution of the trajectory of chilomonas and reconstruction in angular spectrum method is applied. The 3-D image representing the trajectory of the moving chilomonas is built up by reconstructing the 2-D hologram at various distances the same numerical focusing algorithm was applied, Fig. 4.

Four dimensional tracking of paramecium through fibers

Here shows the four dimensional tracking of paramecium through fibers. A hologram movie of paramecium through fibers is recorded at frame rate 17fps. A paramecium cell is seen to moving through the fibers which are then pulled to an obvious displacement. We extract two frames, showing the paramecium coming close to the fibers and running away after fibers are hit, Fig. 5a), b). A difference hologram is obtained and reconstructed in different planes, Fig. 5c). The numerical focusing algorithm is then applied to determine the focal planes for all the objects in the reconstructed volume and the profile of 4D tracking of paramecium through fibers is well present, Fig. 5f), g) and h).

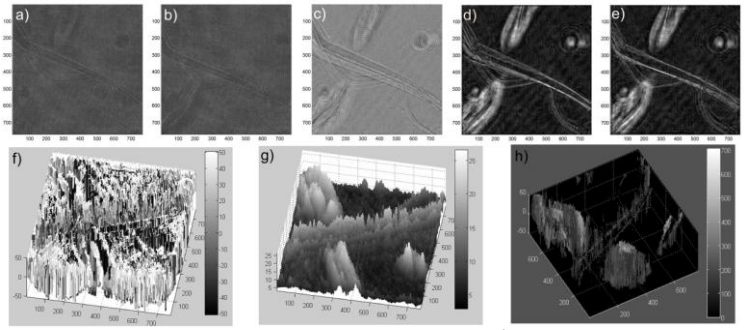

Fig. 5: 4D tracking of paramecium by DHM. The field of view is 200×200 μm^2 with 768×768 pixels. a) hologram 1, paramecium approaching fibers; b) hologram 2, paramecium leaving fibers after hitting; c) Difference holograms h1-h2; d)Amplitude image reconstructed at Z=-2μm; e) Amplitude image reconstructed at Z=-8μm; f) XYZ profiles; g) XYA profiles; h) 3D profile.

Conclusion

DH-QPM has been applied to image microspheres and microfibers in three dimensions, and track cells motility in four dimensions. The approach is sensitive to cellular 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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References

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