Digital In-Line Holographic Microscopy in 4-D

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Abstract: Digital in-line Holography with spherical waves has been developed into a new microscopy for microfluidic, biological and marine applications, that routinely achieves both lateral and depth resolution at the submicron level in 4-D imaging.

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1. Background

Unlike conventional compound light microscopy, which can give high-resolution information about an object only in the focal plane, digital in-line holographic microscopy (DIHM) offers a rapid and efficient approach to construct high-contrast 3-D images of a sample volume from a single hologram. Details of the method and a thorough discussion of its history and potential have been presented in a number of publications together with earlier results in such diverse areas as cell biology, marine biology, microfluidics, particle imaging and tracking [1-3]. In digital holography the hologram is captured by a CCD camera and stored in a computer. Reconstruction of a three-dimensional image of the object is achieved via a Kirchhoff--Helmholtz transform [4]

$$K(\vec{r}) = \int_S d^3\xi \widetilde{I}(\vec{\xi}) \exp[2\pi i \vec{\xi} \cdot \vec{r} / \lambda\xi] \tag{1}$$

in which the integration extends over the two-dimensional surface of the screen with coordinates coordinates $\xi=(X,Y,L)$, where L is the distance from the source (pinhole) to the center of the screen (CCD chip) and $\widetilde{I}$ (ξ) the is the contrast image (hologram) on the screen, obtained by subtracting the images with and without the object present. The function K(r) is significantly structured and differs from zero only in the space region occupied by the object. By reconstructing the wave front K(r) on a number of planes at various distances from the source in the vicinity of the object, a three-dimensional image can be built up from a single two-dimensional hologram. K(r) is a complex function and one usually plots its magnitude to represent the object, although phase images are also available. For the numerical implementation of the transform we have developed a fast algorithm that evaluates K(r) without any approximations: it employs a coordinate transformation that transforms the integral into a scalable convolution as implemented in the DIHM software [5]. As an example we show in Figure 1 the reconstructed image of a diatom.

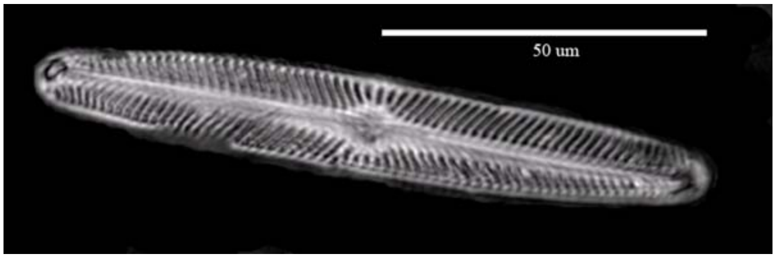

Figure 1: Reconstructed image of a diatom.

2. 4-d particle tracking

In many fields of science and technology the microscopist is faced with tracking many objects such as particulates, bubbles, plankton or bacteria as they move in space, asking for an efficient way of 4-d particle tracking. DIHM is the perfect tool to do this job [2]: 4-D tracking is achieved by (1) recording digitally, e.g. on a CCD chip, a film of N holograms $h_1, h_2, h_3, \ldots$ at times $t_1, t_2, t_3, \ldots$ (2) One constructs digitally a difference hologram $h_1-h_2+h_3-h_4+h_5-h_6+\ldots$; thus retaining only those features in the holograms that correspond to moving objects; the recording speed must be adjusted accordingly. This construct also reduces the size of the data set by a factor of N. (3) Lastly, one numerically reconstructs a stack of images in a sufficient number of planes throughout the sample volume so that a

3-D rendering displays the tracks of all the moving particles. One could also reconstruct stacks of images from each frame in the film of holograms and would then be able to produce a 3-D movie of the motion itself.

3. Underwater DIHM

To allow observations with DIHM in ocean or lake environments a submersible version of DIHM has been developed to perform submicron imaging and particle tracking in situ underwater [6]. The microscope consists of two pressure chambers one of which contains the laser and the other the CCD camera (plus power supply). The two chambers are kept at a fixed distance to each other to allow water to freely circulate between them. In the center of the chamber plates facing each other are small windows with the one on the laser chamber having the pinhole. The signal from the CCD camera is transmitted via an underwater USB cable to a buoy or a boat above from where a satellite link can be established for data transmission to a laboratory. Depending on the design of the pressure chamber water depths of several hundred meters should easily be possible. Underwater DIHM has been performed in the North Atlantic off Halifax at a depth of 15m depth, in an extensive study of biofouling of ships, and in waterholes in the high arctic on Axel Heiberg Island. For the arctic expedition a small lightweight instrument was designed that can be further reduced in weight to less than one pound without forfeiting any performance characteristics; such an instrument would be ideal for exobiology looking for life forms on other planets [7].

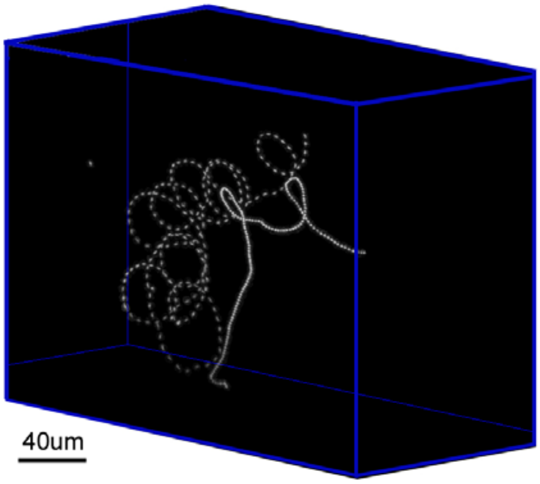

Figure 2: 3-D rendering of bacteria from an arctic spring on Axel Heiberg Island. Holography with blue light (λ=408 Å), pinhole diameter 0.5 µm, pinhole-object distance 0.8 mm, pinhole-CCD distance 21 mm, CCD chip size 1.28x1.28 cm^2, NA=0.3. Reconstruction volume 500x500x300 µm^3.

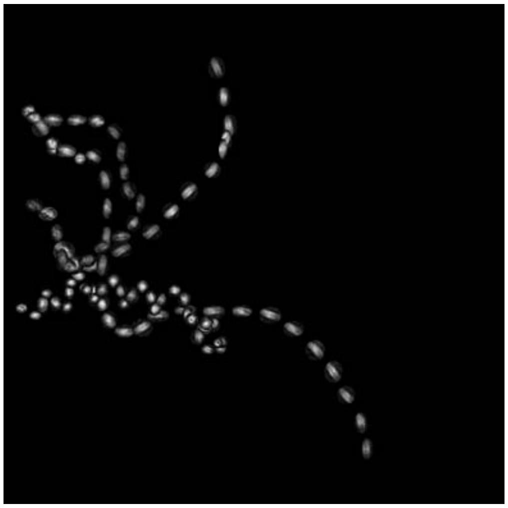

Figure 9: Algae moving in arctic springs. Reconstruction area 400x400 µm^3.

4. Outlook

We want to conclude underlining some remarkable characteristics of DIHM and underwater DIHM:

1. Simplicity of the microscope: DIHM as well as underwater DIHM, is microscopy without objective lenses. The hardware required for the desktop version is a laser, a pinhole and a CCD camera. For the underwater DIHM version we need the same elements contained in a submersible hermetic shell.
2. Maximum information: A single hologram contains all the information about the three-dimensional structure of the object. A set of multiple holograms can be properly added to provide information about 4D trajectories of samples.
3. Maximum resolution: Optimal resolution, of the order of the wavelength of the laser can be obtained.
4. Resolution can be increased dramatically by a simple procedure called immersion holography [8]. It is based on the observation that the hologram is obviously formed in the space between the object and the CCD camera. If one therefore fills this space by oil (n=1.5) in a beaker or by a block of sapphire glass (n=1.8) one effectively performs holography in the ultraviolet, i.e. using a blue laser (λ = 408 Å) the hologram is formed with effective wavelengths of λ = 272 Å and λ = 226 Å for oil or sapphire glass, respectively. Thus resolution is increased by 50 % and 80 %, respectively, without the need for expensive UV lasers or cameras and much smaller pinholes[8].
5. Simplicity of sample preparation, particularly for biological samples where no sectioning or staining are required, so that living cells and specimens can be viewed. Indeed, for the underwater DIHM there is no sample preparation at all, and real time information of living organism can be retrieved.
6. Speed: The kinetics of the sample, such as particle motion or metabolic changes in a biological specimen, can ultimately be followed at the capture rate of the image acquisition system.
7. 4-D tracking: a large number of particles can be tracked simultaneously in 3-D as a function of time.

Films on 4-D tracking and more examples can be viewed at http://www.physics.dal.ca/~kreuzer and also at http://www.resolutionoptics.com together with technical details.

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