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Case Report

3D Diffraction – Limited Imaging with a Laser Fourier Holographic Microscope

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Abstract: A laser holographic microscope (LHM) is investigated experimentally. The standard slide of Parascaris Univalens Iarva (ascaris) is studied. Comparison of the pictures of the same ascaris cell, observed by the LHM and high-performance Nikon conventional optical microscope (COM) 10×100/1.25 with immersion oil and green filter indicates that the both microscopes provide diffraction – limited 3-D spatial resolution, but dramatically different contrast. Thus, the LHM gives much more subcellular information.

Keywords: Speckle-Noise, Fourier Holography, Mach – Zehnder Scheme, CCD Detector, Digital Image Reconstruction

1. Introduction

The fundamental property of holography is creation of 3D pictures from 2D holograms [1 - 3]. The LHM is no exception. Moreover [4], it is shown both theoretically and experimentally that LHM is free from the speckle-noise and provides ultrahigh contrast.

Unfortunately, high contrast images obtained by the electron microscopes [5, 6] contain 2D information only.

From the standpoint of digital holography [7, 8] two various programs were applied. The first one is the fast Fourier transform (well known FFT). The second one is stigmatic. FFT has taken several minutes, but image quality was not ideal (carried coma aberration). Stigmatic has taken several hours. It should be noted that only stigmatic images were declared here and in [4] as obtained with the LHM. Anyway, FFT was also useful as first rough approximation.

2. Experimental Study of LHM of Visible Range λ=0.514μm

An experimental setup was described also in [4, 9, 10].

Results of the study of a real biological sample, specifically, a standard slide of Parascaris Univalense Iarva (ascaris) with an LHM are presented. An experimental setup, based on the Fourier holography, is shown in Fig. 3 [4]. Here, a cw Ar^+ - ion laser 1 provides a continuous, linearly polarized, single transverse and longitudinal mode beam of wavelength $\lambda = 0.514\ \mu m$. A shutter 2 creates a pulse with controlled duration. A beamsplitter 3 divides the beam into two parts, specifically, reference (transmitted) and sample (reflected). Intensities of both beams are controlled. The reference beam after reflection from a plane mirror 4 is focused by an objective 5. The waist W_1 can be considered like a point source of a spherical wave, which after reflection from a beamsplitter 6 reaches the CCD detector 7. The sample beam is reflected by a mirror 8 and then focused by an objective 9. A sample 10, which is a standard slide with a thin section of ascaris, is placed in a focal waist W_2. A scattered wave is a result of interaction between the sample and sample wave. A transmitted unscattered beam is blocked by an absorbing blocker 11. The scattered light transmitted the beamsplitter 6 incidents the CCD detector 7. The scattered field interference pattern with the reference wave (a Fourier hologram) is captured by the detector. The hologram is recorded, digitized, and stored by a personal computer 12. The holographic data are then transferred to a Stardent GS

2000 Supergraphic Workstation 13, where numerical image reconstruction is performed. The reconstructed image can then be displayed by a monitor 14, or printed by a Tektronix Copy Processor 15.

A picture of one certain ascaris cell, chosen for particular study, obtained with a high-performance Nikon COM 10×100/1.25 with immersion oil and green filter is given in Fig. 4 [4]. Figure 5 [4] shows the image of the same cell and approximately equal magnification obtained with the LHM. Figures 4 and 5 [4] present an image of the cell as a whole.

A picture of ascaris cell, obtained with the LHM is shown in Fig. 1. A set of the images of the piece of the cell, distinguished in Fig. 1, including a part of the nucleus and cytoplasm for various z-crossections are shown in Figs. 2a – 2c. Distances between the slices a – b and b – c are 3.6 μm. A part of ascaris nucleus magnified by the computer is presented in Fig. 3. Here, the length of a side of a square, corresponding to a step of the computer calculation, is 0.16 μm.

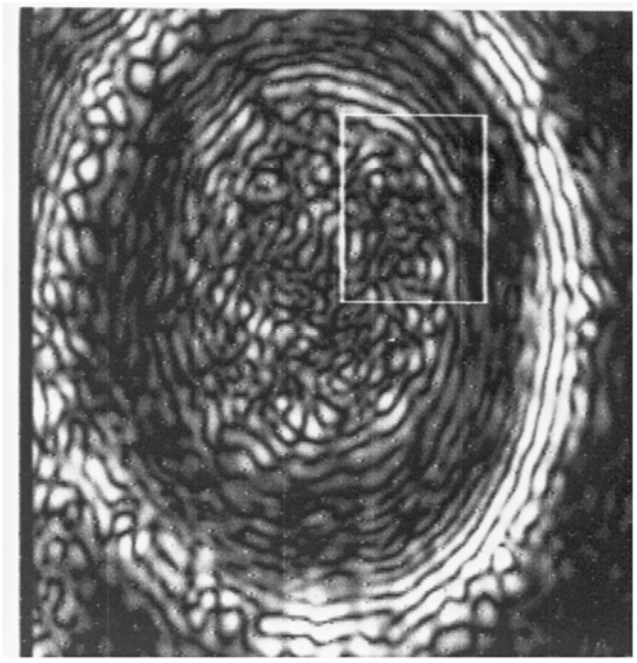
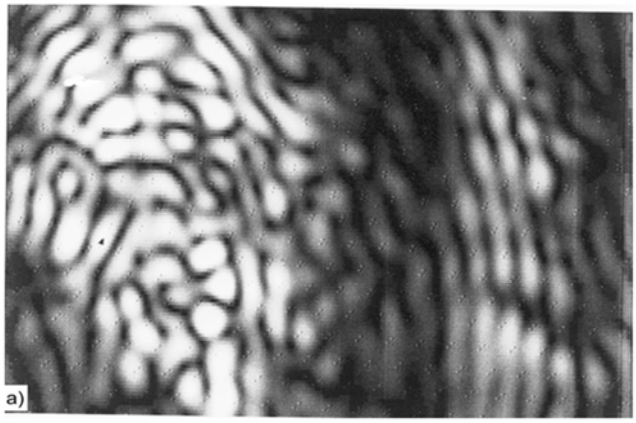


Figure 1. *Image of ascaris cell, chosen for particular study in [4] obtained with the LHM.*



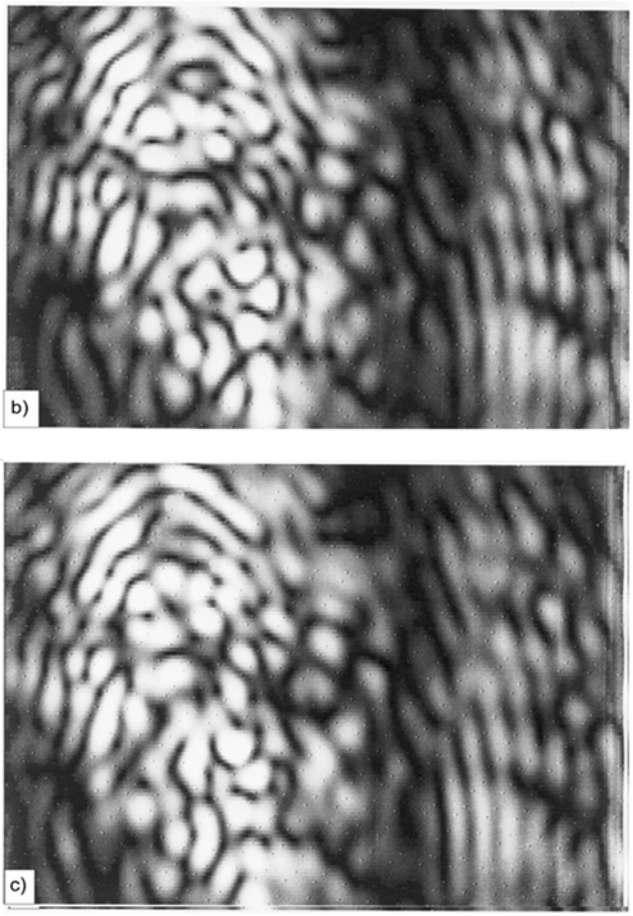


Figure 2. *Images of the piece of ascaris cell marked in Fig. 1 obtained with the LHM for various Z-crossections (longitudinally spaced transverse slices). Distances between the slices a – b and b – c are 3.6 μm.*

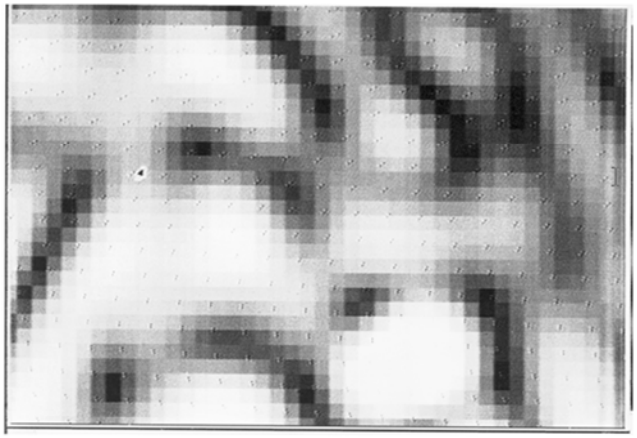


Figure 3. *Image of the part of the ascaris nucleus obtained with the LHM. The length of a side of the square, corresponding to a step of the computer calculation, is 0.16 μm.*

3. Conclusions

Transverse and longitudinal resolutions of the LHM discussed are $\Delta_T \approx 1$ μm and $\Delta_L \approx 3$ μm, respectively. Taking into account the experimental geometry (NA = 0.35) and wavelength $\lambda = 0.514$ μm, this values are consistent with the diffraction limits of 3D spatial resolution [1]. (The values of

Δ_T and Δ_L have been checked also by an independent experiment of a point spread function determination.)

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