Holographic methods for 3D imaging of phase microscopic objects

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Most of microscopic objects are phase objects that do not change the intensity of radiation transmitted through them and are inaccessible to direct observation. They are cells and tissues of human organism, thin films, micro-crystals etc. To observe such phase microscopic objects it is necessary to convert phase changes inserted by them into the light wave passed through them into intensity changes. Though, special methods must be used for phase microscopic objects study. The two main methods were proposed for phase microscopic objects visualization: phase-contrast and interference contrast methods. For the first time the problem of realizing phase-contrast was solved by F. Zernike in 1934. For the phase-contrast method and the phase-contrast microscope he won Nobel Prize in Physics in 1953. Thought the problem of 3D imaging of phase microscopic objects has not been solved in classical microscopy. Until now the method of electron microscopy was the single method which allows obtaining 3D images of the microscopic objects.

The appearance of holography opened up new possibilities in the microscopy of phase microscopic objects and resulted in the appearance of holographic methods of phase microscopic objects visualization [1, 2]. The problem of 3D imaging of phase microscopic objects was solved by combining holographic methods with the methods of computerized image processing. This resulted in creating the digital holographic interference microscope (DHIM) allowing 3D imaging of phase microscopic objects and measurement of their morphological parameters. First 3D images of native blood erythrocytes were obtained using the DHIM.

The DHIM consists of three main units: holographic interference microscope, digital video camera and computer. DHIM allows realizing the main holographic methods for obtaining 3D images of phase microscopic objects in a single device. They are methods of holographic phase-, interference and polarization contrast.

The holographic method of phase contrast is the method of holographic addition and subtraction in an interference fringe. This method allows obtaining bright images of phase microscopic objects on the dark background (subtraction of waves) in the dark interference fringe and dark images on the bright background (addition of waves) in the bright interference fringe. The contrast of the images is maximal. The intensity distribution in the image of the microscopic objects is modulated by the phase increments inserted by the microscopic object into the wave passing through it.

In the interference- contrast method the system of interference fringes is modulated by the phase increments inserted by the microscopic object under study into the wave passing through it. In this case deviations of the interference fringes are observed on the interferogram of the microscopic object.

The phase increments and thicknesses of the microscopic objects in every point can be calculated under computer processing of phase-contrast images and interferograms, and 3D image of the microscopic objects can be reconstructed.

The polarization contrast method can be used for visualization of microscopic objects that possess the anisotropy property. The holographic polarization-contrast images of anisotropic microscopic objects can be obtained when linear polarizations of the object and reference beam are orthogonal. The polarization-contrast method improves quality of images in comparison with the phase and interference contrast methods due to removing coherent noise and filtering all others noise microscopic objects. Contrast images of anisotropic microscopic objects can be obtained with the maximal resolution. The intensities of the images are modulated by the thicknesses of the microscopic objects. This allows one to reconstruct theirs 3D image under computer processing of the polarization-contrast images.

So, application of the holographic methods in microscopy allows optical 3D imaging of phase microscopic objects, native cells and tissues of living organisms.

References

[1] T.V. Tishko, V. P. Titar, D. N. Tishko, Journal of Optical Technology, **72** (2005),p.203.
[2] Tatyana Tishko, Tishko Dmitry, Titar Vladimir “Holographic microscopy of phase microscopic objects. Theory and practice” (World Scientific) 97p.

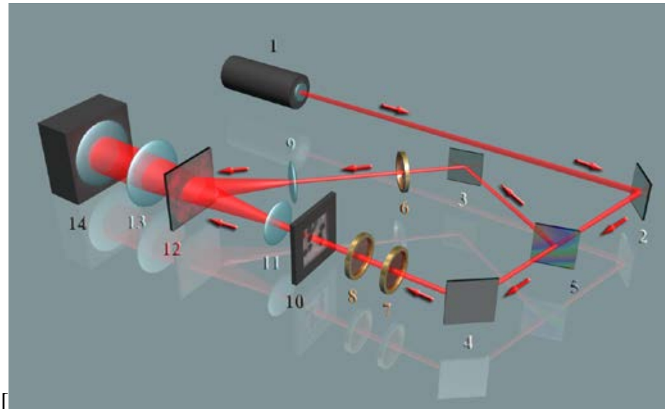

Figure 1. *Optical layout of the DHIM. 1-laser; 2-4 –mirrors; 5- semitransparent mirror; 6,7 and 8 - polarisers; 9- collimator; 10- test specimen; 11-objective; 12-hologram; 13-eyepiece; 14 – image-recording unit.*

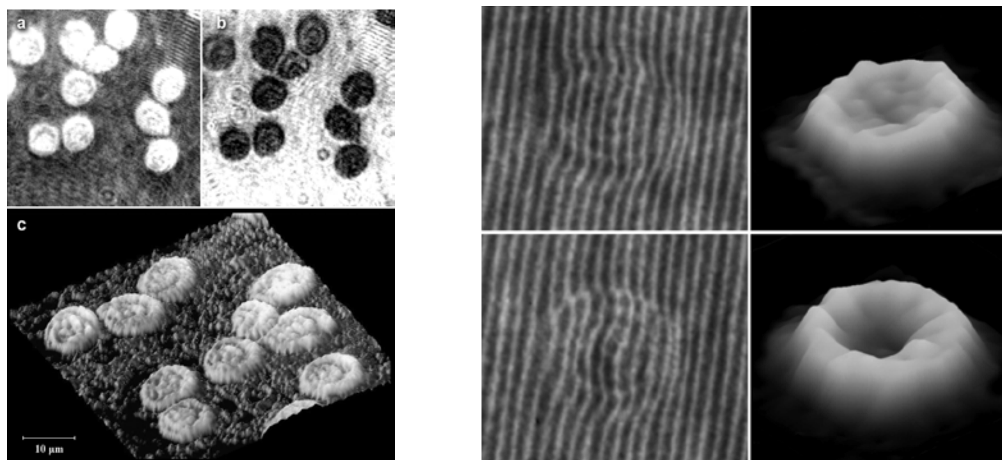

Figure 2. *Holographic phase-contrast images of human blood erythrocytes (a, b) and their 3D images (at the left), iInterferograms of individual erythrocytes and their 3D images (at the right) obtained using the DHIM.*

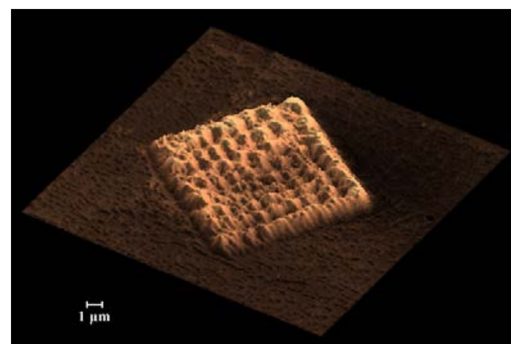

Figure 2. A *The 3D image of the microcrystal with the cellular structure reconstructed from the holographic polarization-contrast image.*