

Laser Scanning Microscope Based Digital Holography for biomedical application

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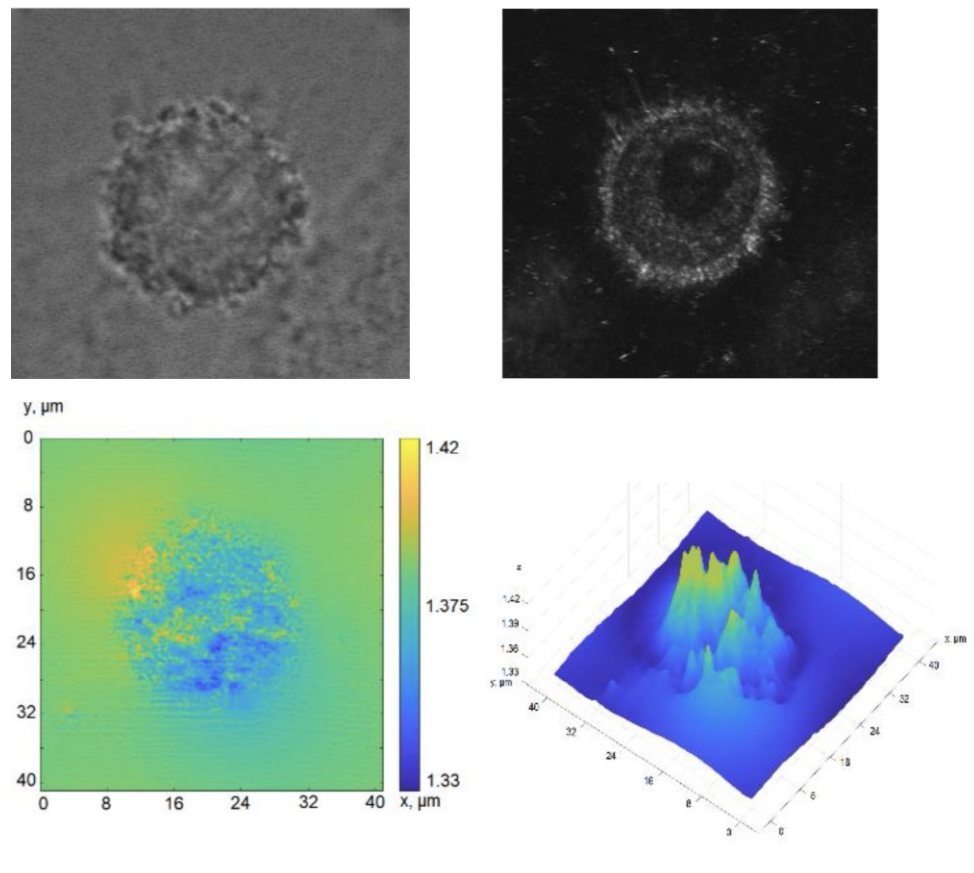
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Abstract: Digital holographic system is built on the base of Zeiss LSM-510 microscope. Using created algorithm and software for correct hologram reconstruction and quantitative phase imaging, reflection and phase map of cell cultures and tissues are obtained with other regular microscopic images.

The application of HSM [1] with developed reconstruction procedure [2] was performed on the cancerous line HeLa (human cervix epidermoid carcinoma): live and apoptosis. Cells were cultivated in the Dulbecco's modified Eagle medium (DMEM) supplemented with fetal bovine serum and streptomycin in incubator (37°C , $5\%\text{CO}_2$). The cell hologram was recorded at room temperature (21°C).

Phase maps obtained using HSM depend on refractive index distribution paying in attention that optical path difference become formed within confocal microscopy optical slice. In our case optical slice thickness was $2.5\text{ }\mu\text{m}$ (confocal aperture is 3 Airy at $20\times/1.0\text{NA}$ water immersion objective). Reconstructed phase map and respective transparent and



reflectance images of live and apoptotic HeLa cells are presented in Fig. 1 and Fig.2.

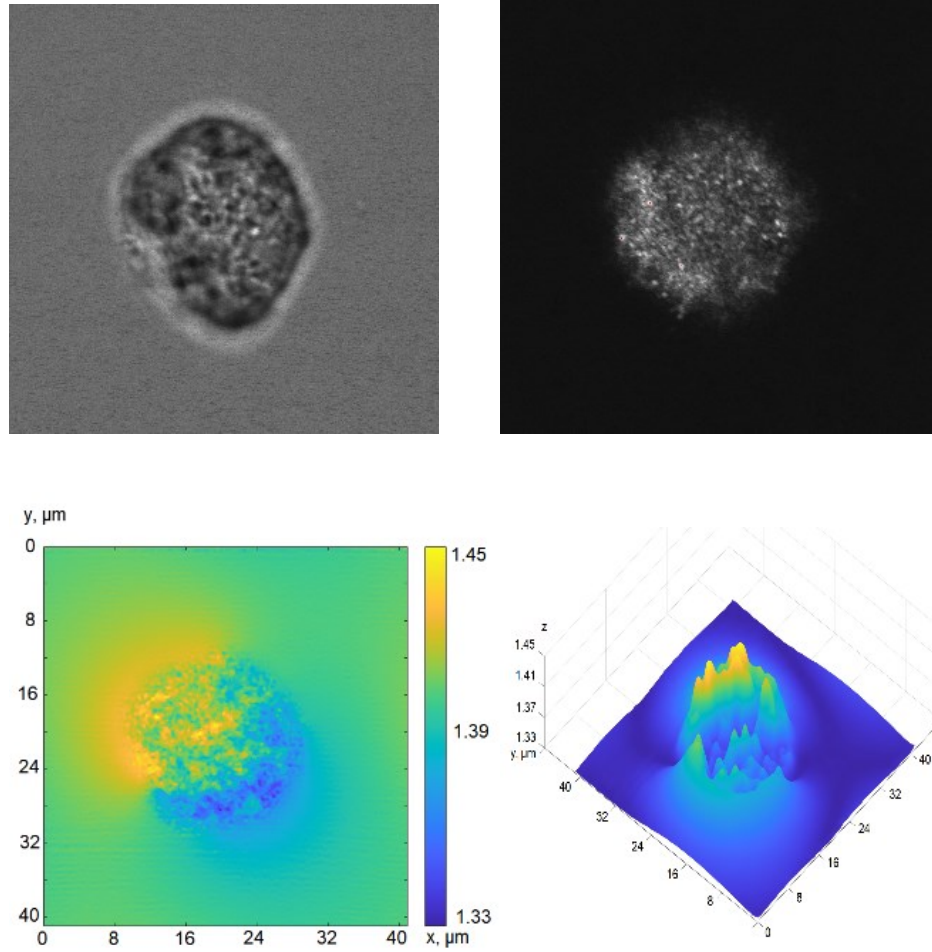


Fig. 1 Microscopic images and phase map of live HeLa cell. a Transparent DIC image. **b** Reflectance confocal image. **c , d** Reconstructed phase map of the cell optical slice (color bar (**c, d**) and z-axis (**d**) represent refractive index)

Fig. 2. Microscopic images and phase map of apoptotic HeLa cell. a Transparent DIC image. **b** Reflectance confocal image. **c , d** Reconstructed phase map of the cell optical slice (color bar (**c, d**) and z-axis (**d**) represent refractive index)

Reconstructed phase map of the cancerous cells clearly show inhomogeneity of cell structure and remarkable difference of refractive index in live and apoptotic cells. A comprehensive analysis of biological processes in cells and diagnostic criteria requires more statistics and is beyond the scope of this paper.

[1] Y. Zakharov, M. Muravyeva, U. Khan, L. Zhang, V. Turzhitsky, E. Vitkin, I. Itzkan, L. Qiu, and L. T. Perelman, "Wavefront Reconstruction in Holographic Scanning Microscopy," in Imaging and Applied Optics 2018 (3D, AO, AIO, COSI, DH, IS, LACSEA, LS&C, MATH, pcAOP), OSA Technical Digest (Optical Society of America, 2018), paper DTh2C.7..

[2] M. S. Muravyeva, I. V. Mukhina, and Y. N. Zakharov, "Phase distortion in holographic scanning microscopy: source and elimination," in Imaging and Applied Optics Congress, OSA Technical Digest (Optica Publishing Group, 2020), paper JTh2A.27.