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Digital holography provides novel capabilities for biological microscopy

Myung K. Kim

By reconstructing and manipulating amplitude and phase of the optical field in virtual numerical space, digital holography generates unique microscopic images.

Although holography was invented by Dennis Gabor in 1948, more than a decade passed before the invention of the laser made it a practical and powerful tool. Today, holograms and the principles of holography find vast areas of application in such fields as metrology, data storage, and even the fine arts. Yet, the conventional process of holography using photographic plates is time-consuming and cumbersome. Real-time processing is not feasible without photorefractives and other nonlinear optical materials.

Recently, the field has been undergoing a paradigm shift with the advent of digital holography, in which the holographic interference pattern is digitally sampled by a CCD camera and the image numerically reconstructed by applying results from diffraction theory. This approach offers a number of significant advantages, including the ability to acquire images rapidly, the availability of both amplitude and the phase information, and the versatility of the processing techniques that can be applied to the complex field data acquired. In addition, advances in digital imaging devices, such as the CCD and CMOS cameras, and in computational and data storage capacities, have been central to broadening applications of digital holography.

We have used microscope objectives in various interferometers to capture magnified images of holographic fringes. In addition, a number of optical and numerical techniques have been developed to obtain high-fidelity and resolution images of biological specimens using these holographic microscopes.

Quantitative phase-contrast imaging

Imaging of mostly transparent microscopic biological specimens presents a special challenge. Existing techniques such as Zernike phase-contrast microscopy and differential interference contrast microscopy do not offer direct quantitative evaluation of the phase information. In digital holography, however, the phase information is directly available from the calculated optical field.

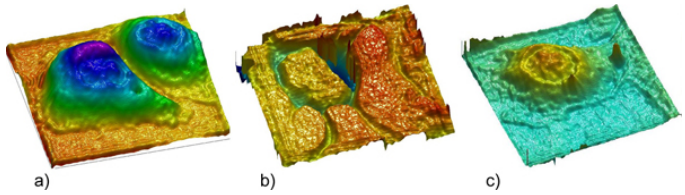

Figure 1. *Quantitative phase contrast images of SKOV3 ovarian cancer cells in various physiological states. Notice the subcellular features such as nuclear membranes, chromosomes, and lamellipodia.*

Figure 1 presents phase contrast images of SKOV3 ovarian cancer cells obtained by digital holographic microscopy.[1] The field of view is about $60\times60\mu m^2$ in each image and the pseudocolor axis of the images represents profiles of optical thickness of several microns. The phase images from digital holography containing 2π-discontinuities have been unwrapped using a software algorithm. These images allow direct observation and accurate interpretation of morphological changes in the specimen. For example, Figure 1(b) exhibits the peculiar textural changes of confluent cancer cells. This effect has been observed in electron microscopy but never, to the best of our knowledge, in optical microscopy with such quantitative accuracy.

Optical tomographic imaging

Although the hologram produces a 3D image of the optical field, this does not by itself yield the tomographic distance information, other than by focusing and defocusing of image points. The distance information can be obtained by counting the number of actual or effective wavelengths, the latter in the case of

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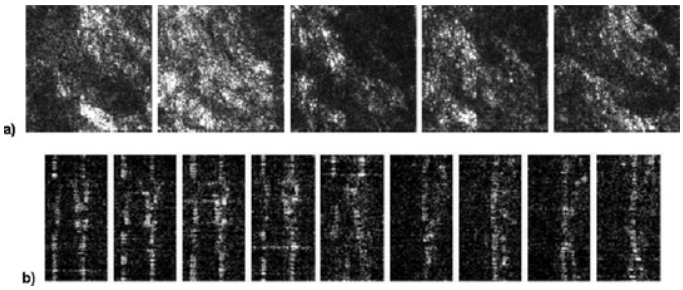

Figure 2. *Digital interference holographic tomography of beef tissue.*

multi-wavelength interference systems. One can also determine fractions of a wavelength, as in the phase-contrast holography described above. A well-known technique is the interference of two holograms recorded at two different wavelengths, resulting in a contour interferogram with the axial distance between the contour planes inversely proportional to the wavelength difference.

In digital interference holography (DIH), it is possible to extend the process to recording and reconstruction of many holograms without introducing wavelength mismatch. In effect, the short coherence length of optical coherence tomography (OCT) is synthesized by the multiplicity of wavelengths. Though DIH does not involve pixel-by-pixel mechanical scanning of three-dimensional volume, it achieves comparable resolutions to OCT. Figure 2 is the result of a DIH imaging experiment on a $2.62 \times 2.62 \times 0.75 \text{mm}^3$ volume of a piece of beef tissue. It shows images of tissue layers taken at several depths below the surface.[2] Figure 2(b) shows variations of the tissue layers in a few XZ cross-sectional images.

We are preparing to apply phase-imaging digital holography to the study of the cell-substrate contact layer in cell locomotion, where the only currently available technique is total internal reflection microscopy that suffers from poor resolution. Digital interference holography will also be used for tomographic imaging of epithelial structures. In general, this technique is promising in biomedical microscopy, and certain to find a wide range of applications in years to come.

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Author Information

Myung K. Kim
Professor of Physics
University of South Florida
Tampa, FL
http://shell.cas.usf.edu/~myungkim/

M. K. Kim directs the Digital Holography and Microscopy Laboratory in the Department of Physics at the University of South Florida. After receiving his Ph.D. from the University of California, Berkeley, in 1986, he undertook postdoctoral research at SRI International and then joined the faculty of Wayne State University. He currently serves on the SPIE Membership Committee.

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