Digital Holography Enables Quantitative Phase Evaluation during Cellular Microsurgery

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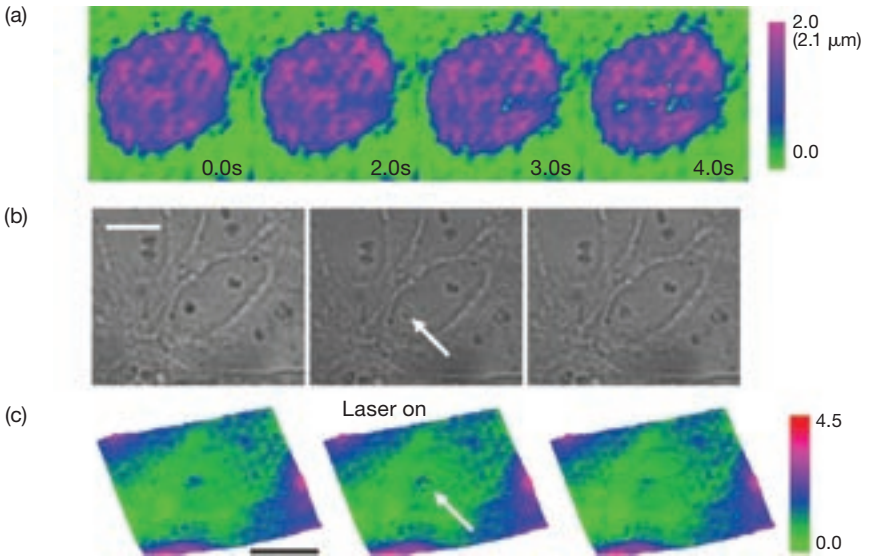
Laser scissors have become an important tool that allow cell biologists to alter cellular structures in order to study their structure-function relationship.[1] Because it is difficult to visualize transparent specimens being manipulated by a microbeam with conventional bright-field microscopy, phase-contrast or differential-interference-contrast microscopy is most widely used. However, the phase-to-amplitude conversion is nonlinear, and, therefore, quantitative phase imaging is not feasible with these techniques.

Earlier, we developed a quantitative phase laser microsurgery system using short-coherence interference microscopy.[2] Unlike phase-shifting interferometry,[2] digital holographic microscopy can be performed in real time, and it allows one to determine dynamic changes in the optical thickness profile of a transparent object with sub-wavelength accuracy.[3] In order to evaluate quantitative high speed phase measurement of sub-cellular changes due to laser micro-irradiation, we incorporated digital holographic microscopy on a laser microbeam system.[4]

We recorded a hologram that consists of the interference between the object and reference beams using a CCD camera. Then we numerically reconstructed the holographic image using the results of diffraction theory. Calculation of the complex optical field allowed direct access of both amplitude and phase information about the optical field, and, by numerical focusing, the images could be obtained at any distance from a single recorded hologram.

Another advantage of digital holography is that it allows numerous digital processing techniques for manipulating the optical field information in ways that are difficult or impossible in real-space processing. For example, optical system aberration can be numerically corrected and multi-wavelength interferometry can be accomplished with precise control of the reconstruction wavelengths.



Time-lapse quantitative phase maps of red blood cells during laser microirradiation (n=1.43 assumed for crenate RBCs). (b) Bright-field images of PTK2 cell nucleolus before, during and after laser micro-dissection. (c) Time-lapse sequence of quantitative phase images during laser micro-irradiation of the nucleolus. Scale bar: 10 μm.

For our experiments, we used the micro-focused 532-nm green laser beam from a nanosecond Nd:YVO_4 laser as laser scissors. At the same time, we split the red light from a 675-nm laser diode into a Mach-Zehnder interferometer for digital holography. We then directed two red beams through the object and reference arms toward a CCD camera. A 20X objective was placed in the reference arm of the interferometer. The curvature of the interference fringes was digitally compensated and controlled via software. A slight angle was introduced between the object and reference beams for off-axis holography.

Quantitative phase imaging by digital holographic microscopy provided high spatial as well as temporal resolution to dynamic changes in cell thickness. In dynamic phase imaging during microsurgery of red blood cells, the damage to the cell membrane was not uniform, owing to the non-uniformity in spot-scanning. A quantitative phase laser micro-irradiation system was even capable of evaluating dynamic changes in the sub-cellular structure (nucleolus) of a cell. When the nucleolus was irradiated by the laser microbeam, one could observe a change in phase (in radians). Label-free digital holographic microscopy may enable scientists to better study damage to cellular structures, including the nucleus, chromosomes and even DNA.

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