위상이동 디지털 홀로그래피 현미경을 이용한 살아있는 섬유아세포의 3차원 형상측정

Three-dimensional shape measurement of a living fibroblast cell with in-line phase-shifting digital holographic microscopy

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Three-dimensional shape measurement of microscopic objects is important in many fields such as biology, micro-electronics industry, and MEMS engineering. Scanning confocal microscopy has been used for this purpose, but the process of the three-dimensional scanning is rather time-consuming.(1) Microscopic electronic speckle pattern interferometrycan provide the three-dimensional information with a whole-field measurement, but it requires a focusing mechanism to record speckle patterns at various field depths.(2) This problem has prevented its application to the observation of objects with a considerable thickness or axial movement.

In digital holography, many holograms can be recorded with a CCD in a matter of sub-seconds and the images of the object are reconstructed numerically afterwards. Because the focusing can be adjusted in the reconstruction process, digital holography is free from the process of mechanical focusing and can be used to monitor the dynamic change of objects.

Digital holographic system can be configured in off-axis or in-line setup. In off-axis setup, about a half of the CCD pixels are filled with the carrier fringes and the distance between the object and the CCD must be long enough to separate the reconstructed object image from the meaningless zero-order. As a result, the numerical aperture of the imaging optics is reduced and the reconstructed image is of low resolution. These problems can be solved by configuring the holographic system in in-line setup.(3) Furthermore, in this case phase-shifting methods can be employed to measure the phases of the complex object field on the CCD plane, in addition tc its amplitudes, providing superior axial resolution.

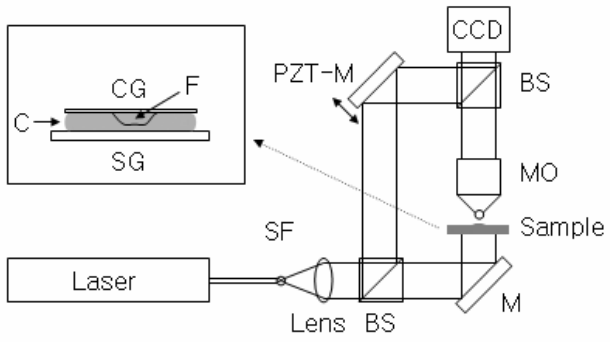

Fig. 1. In-line phase-shifting digital holographic microscopy setup: SF, spatial filter; BS, beam splitter; M, mirror; PZT-M, PZT-attached mirror; MO, microscope objective. Inset, prepared sample: SG, slide glass; CG, cover glass; C, culture solution; F, fibroblast cell attached on the cover glass.

We have setup an in-line phase-shifting digital holographic system and used it to measure the three-dimensional shape of a living fibroblast cell. Although three-dimensional shapes of some living cells were already measured with digital holographic microscopic systems, they were reconstructed from a single hologram taken with off-axis setups.[4,5]

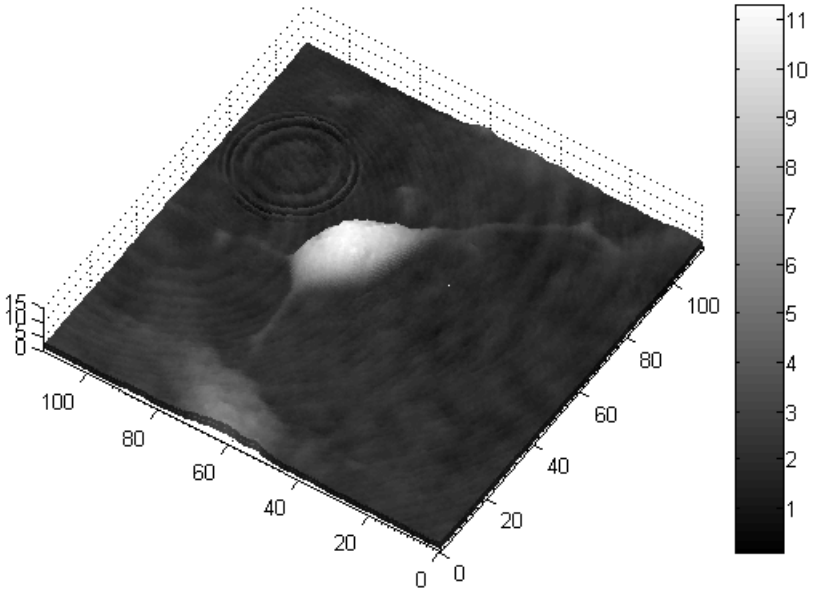

Fig. 2. Three-dimensional shape of the fibroblast cell. All numbers are in micrometers.

The axial accuracy of our system, estimated by using the glass flatness (0.06μm) and the field mismatch (0.04μm), was better than 0.1μm. The use of the phase-shifting method with a phase-shift-error self-calibration algorithm made the thickness error due to the phase-estimation error much smaller than 0.1μm. The lateral resolution of our system was 0.6μm corresponding to that of conventional optical microscopes, 0.61λ/NA.

In summary, we have developed an in-line phase-shifting digital holographic microscopic system and measured the three-dimensional shape of a living fibroblast cell. The use of an in-line configuration made it possible to estimate the complex object fields on the CCD plane with a phase-shifting method. A self-calibration algorithm was used to enhance the phase accuracy by eliminating the phase-shift error. The complex object fields on the sample plane reconstructed numerically from the complex object fields on the CCD plane had higher lateral and axial resolutions than that could be obtained with off-axis digital holographic systems. This system can be applied to the precise three-dimensional shape measurement of any transparent microscopic object such as a micro-lens array and to the observation of the dynamic change of microscopic objects.

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