論文の内容の要旨

Three-dimensional refractive index reconstruction by coherent diffraction tomographic microscopy

「コヒーレント回折トモグラフィー顕微鏡法を用いた３次元屈折率の再構成」

ベルツ　スタニスラス　ギョム

Microscopic techniques have rapidly evolved to meet new requirements of contemporary biomedical science for improved spatial resolution and improved accuracy in three-dimensional image reconstruction. Particularly, super resolution microscopes such as 4Pi and stimulated emission depletion microscopes have offered tremendous improvements with regard to spatial resolution and are now being used in research laboratories to image fluorescent specimens. In the area of three-dimensional imaging, diffractive tomographic microscopy has recently attracted interest because of its potential ability to deliver accurate three-dimensional images of phase objects much needed in imaging low-contrast transparent biological specimens without staining or fluorescent labeling. In comparison to other microscopic techniques, diffraction microtomography allows for the measurement of the three-dimensional distribution of the complex optical refractive index within the sample by mapping a three-dimensional region in the spatial frequency domain. The frequency mapping is realized by varying the sample illumination direction, a method which is known to generate a strong anisotropy in the recorded frequencies with missing information along the optical axis. This anisotropy precludes efficient three-dimensional reconstruction of the sample.

The author proposes to perform the frequency mapping by rotating the sample perpendicularly to the optical axis. The reconstruction of the sample refractive index in the case of diffraction microtomography with sample rotation is presented for the first time in his thesis. It was found that the mapping of the sample frequencies is nearly isotropic, but still exhibits a missing region along the axis of rotation. The effects of this missing region on the sample reconstruction are quantified by simulations of objects with different morphologies and sizes. The main effect is observed as a blurring of the object surfaces perpendicular to the axis of rotation. For membrane-like objects resembling biological cells, the effect translates into a slight elongation of the membrane along the axis of rotation. Significant object deformation is observed for object sizes smaller than 10 microns (wavelength of 633 nm and numerical aperture of 1). In comparison to the illumination variation method, reconstructions achieved by sample rotation method show a less pronounced deformation along the missing information axis, thus making theoretically possible a nearly three-dimensional isotropic reconstruction of the sample.

On the basis of this theoretical analysis, a tomographic microscope with sample rotation was designed and built. The author has modified a brightfield transmission microscope to form a Mach-Zehnder interferometer that is used to generate phase-shifted holograms recorded in image plane. Semi-transparent objects mixed with an index matching medium are inserted into a microcapillary and holograms of these objects are taken under different view angles by rotating the microcapillary. Precise rotation of the microcapillary is accomplished by clipping the microcapillary in a precisely machined V-groove. The measured scattered wave is Fourier transformed and projected in the spatial frequency domain taking into account the curvature of the detected frequency space. A final inverse Fourier transform gives the complex refractive index. The refractive index distribution of pollen grains and glass beads as small as 10 microns are reconstructed in three dimensions with a nearly isotropic resolution, thus offering a significant improvement over the illumination variation technique.