

Tomographic Phase Microscopy

Quantitative 3D Imaging of Living Cells

In visualising transparent biological cells and tissues, the phase contrast microscope and its related techniques have been a cornerstone of nearly every cell biology laboratory. However, phase contrast methods are inherently qualitative and lack in 3-D imaging capability. We introduce a novel tomographic microscopy for quantitative three-dimensional mapping of refractive index in live cells and tissues using a phase-shifting laser interferometric microscope with variable illumination angle.



[BU Gruppenbild kommt mit Korrektur]

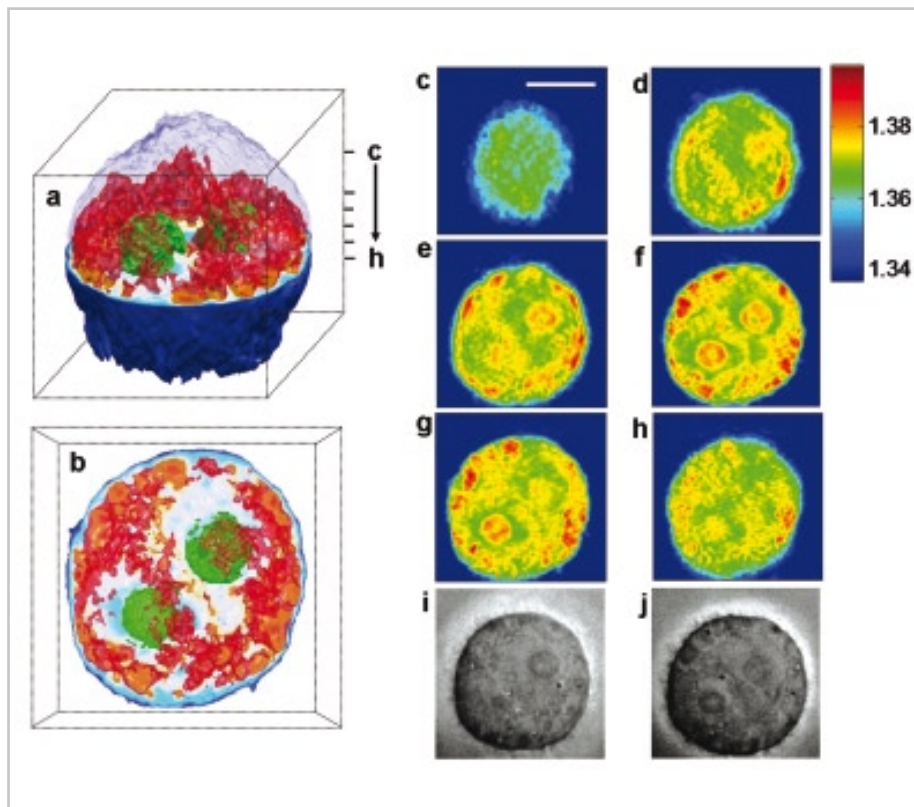


Fig. 1: Refractive index tomogram of a HeLa cell. (a) 3-D rendered image of a HeLa cell. The outermost layer of the upper hemisphere of the cell is omitted to visualise the inner structure. Nucleoli are coloured green and parts of cytoplasm with refractive index higher than 1.36 are coloured red. The dotted box is a cube of side 20 μm . (b) Top view of (a). (c)–(h) Slices of the tomogram at heights indicated in (a). Scale bar, 10 μm . The colour bar indicates the refractive index at $\lambda = 633 \text{ nm}$. (i) and (j) Bright field images for objective focus corresponding to (e) and (f), respectively.

Refractive Index as an Intrinsic Source of Contrast in Light Microscopy

Most biological cells and tissues exhibit negligible absorption under visible light illumination while there are still distinctive refractive index differences among organelles, which amount to several percents relative to the mean refractive index. For this reason, the refractive index has been served as a much better source of intrinsic contrast than absorption in biological studies.

Local variations of refractive index in the specimen induce different phase delays from point to point in the field of view. Phase microscopy techniques such as phase contrast microscopy, differential interference contrast (DIC) microscopy and quantitative phase microscopy are used to image such specimen-induced phase delay to visualise the biological structures [1–3]. These techniques are quite sensitive enough to easily resolve the phase delay induced by a single cell, which is typically around half a radian. However, the phase delay is proportional to the product of refractive index and path length or, more generally, the convolution of the refractive index with the point spread function of the optical system. Thus, phase microscopy techniques provide neither a 3-D image of the cell nor a 3-D map of the refractive index distribution.

CT Scan with Biological Cells and Multicellular Organisms

In this letter we present a technique for quantitative, high-resolution 3-D refractive index measurements of suspended or substrate-attached cells and multicellular organisms with no need for sample perturbation or immersion in special media [4].

For near-plane wave illumination of a thin sample with small index contrast, the phase of the transmitted field is to a good approximation equal to the line integral of the refractive index along the path of beam propagation. Therefore, the phase image can simply be interpreted as the projection of refractive index, analogous to the projection of absorption in x-ray tomography. If we take many angular projection phase images over wide range of angles, we can reconstruct a 3-D map of refractive index of the sample with similar algorithm used in x-ray tomography.

In obtaining a set of angular projection phase images, we change the direction of illumination beam rather than rotate the sample. This leaves the sample unperturbed during the measurement, which is critical for biological specimen, and enables a fast dynamic study as well. It takes about 10 seconds to obtain a set of angular projection phase images for a single tomogram. To reconstruct a 3-D refractive index tomogram from the projection phase images, we applied a procedure based on the filtered back-projection method [5], which is typically used in x-ray tomography. We validate that our instrument can determine the refractive index with an accuracy of 0.001 by imaging 10 μm polystyrene beads (Polysciences #17136, $n=1.588$ at $\lambda=633$ nm). The estimated spatial resolution of our tomography technique to be approximately 0.5 μm in the transverse (x - y) directions and 0.75 μm in the longitudinal (z) direction.

We imaged single HeLa cells in culture medium. Cells were dissociated from culture dishes and allowed to partially attach to the coverslip substrate. A 3-D index tomogram for a single cell (fig. 1a,b) and x - y tomographic slices of the same cell at heights of $z = 12, 9.5, 8.5, 7.5, 6.5$ and 5.5 microns above the substrate (fig. 1c-h) show that the index of refraction is highly inhomogeneous, varying from 1.36 to 1.40. Bright field images for objective focus corresponding to figure 1e-f are shown in figure 1i-j, respectively. There is a clear correspondence between the tomographic and bright field images in terms of cell boundary, nuclear boundary, and size and shape of the nucleoli.

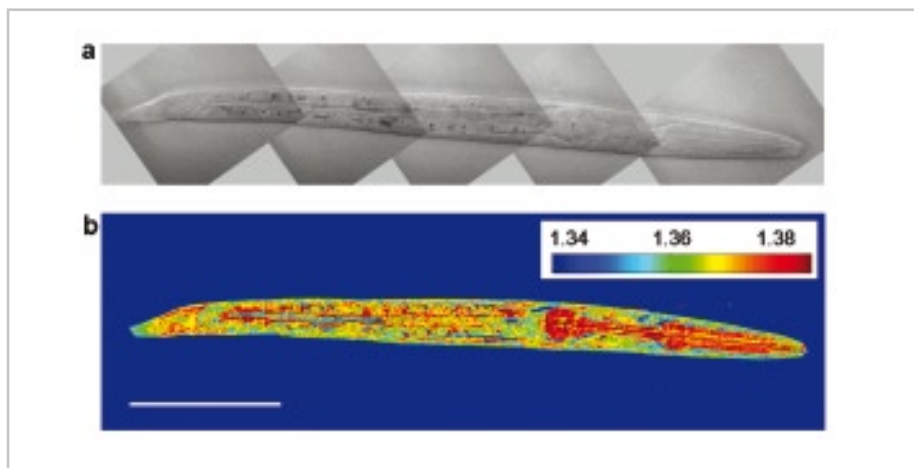


Fig. 2: Bright field image (a) and slice of index tomogram (b) of the nematode *C. elegans*. Anterior is to the right. Scale bar, 50 μm . The colour bar indicates the refractive index at $\lambda = 633$ nm.

Note that the refractive index of the nucleus ($n \approx 1.36$), apart from the nucleolus, is smaller than some parts of the cytoplasm ($n \approx 1.36$ - 1.39) and that the refractive index of the nucleoli, $n \approx 1.38$, is larger than that of the rest of the nucleus. This is contrary to the widely cited claims that the refractive index of the nucleus as a whole is higher than that of the rest of the cell [6]. Similar results were obtained for cultured HEK 293 cells, B35 neuroblastoma cells, and primary rat hippocampal neurons. All cells imaged contained many small cytoplasmic particles with high refractive index, which may be lipid droplets, lysosomes, vacuoles, or other organelles.

To demonstrate tomographic imaging of a multicellular organism, we imaged the nematode *C. elegans*. Worms were paralyzed with 10 mM sodium azide in NGM buffer and imaged in the same solution. Overlapping tomograms were created and the resulting data assembled into a mosaic (fig. 2). Several internal structures are visible, including a prominent pharynx and digestive tract.

In summary, we have developed a technique for quantitative refractive index tomography of living cells and tissues. We note that the 3-D structure mapped by tomographic phase microscopy can complement the images revealed by techniques such as hematoxylin and eosin staining. The refractive index is determined by the type and concentration of the molecules. From the quantitative index map, we may be able to quantify the concentrations of molecular species. This can lead to new quantitative biological studies.

Acknowledgements

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References

- [1] Zernike F.: *Physica* 9, 686 (1942)
- [2] Barty A. *et al.*: *Optics Letters* 23 (11), 817 (1998)
- [3] Ikeda T. *et al.*: *Optics Letters* 30 (10), 1165 (2005)
- [4] Choi W.: *et al.*: *Nat Methods* (2007)
- [5] Kak A.C. and Slaney M.: *Principles of Computerized Tomographic Imaging*, Academic Press, New York, 1999
- [6] Brunsting A. and Mullaney P.F.: *Biophys. J.* 14 (6), 439 (1974)

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