

# High-resolution tomographic phase microscopy

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## Technology Overview

Refractive index (RI) of biological specimens is a source of intrinsic contrast that can be explored without any concerns of photobleaching or harmful effects caused by extra contrast agents. The refractive index can be related to the speed of light wave inside a material [1]. Therefore, wavefront distortion due to a specimen represents the total phase (time) delay of the light wave induced by the specimen. This wavefront distortion can be measured using a Shack-Hartman wavefront sensor [2], interferometry [3], or inline holography (also called propagation-based methods) [4]. The measured phase delay is proportional to the specimen's optical path length, the integral of refractive index along the light propagation direction. The tomographic refractive index measurement is typically performed with a collimated laser beam whose angle of incidence onto the sample is varied as in X-ray computer tomography [5-7]. It has been also demonstrated that one can obtain the refractive index map with a spatially-incoherent beam and scanning the objective focus through the sample [8].

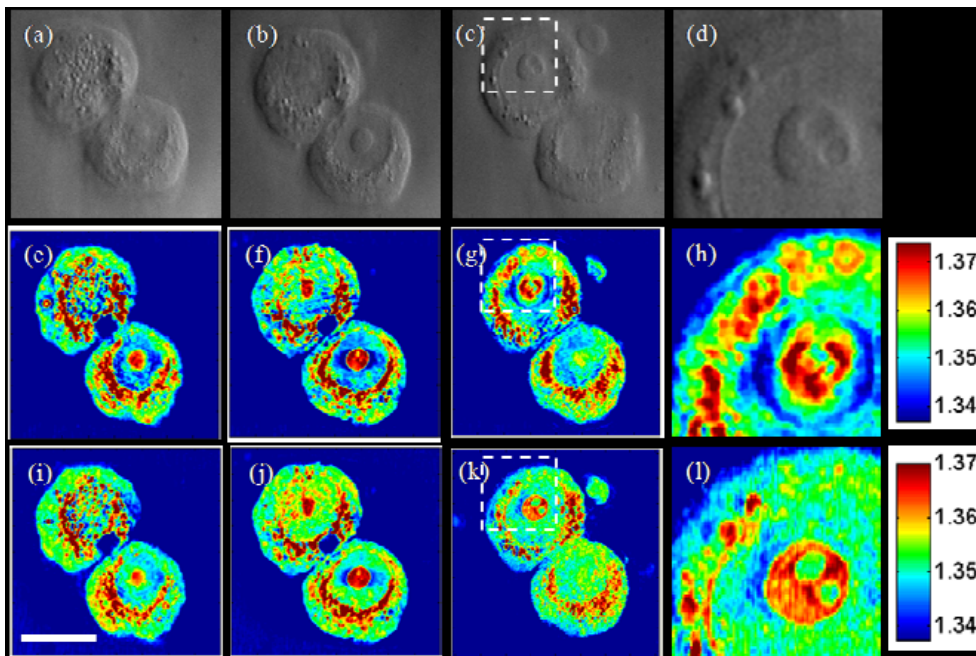
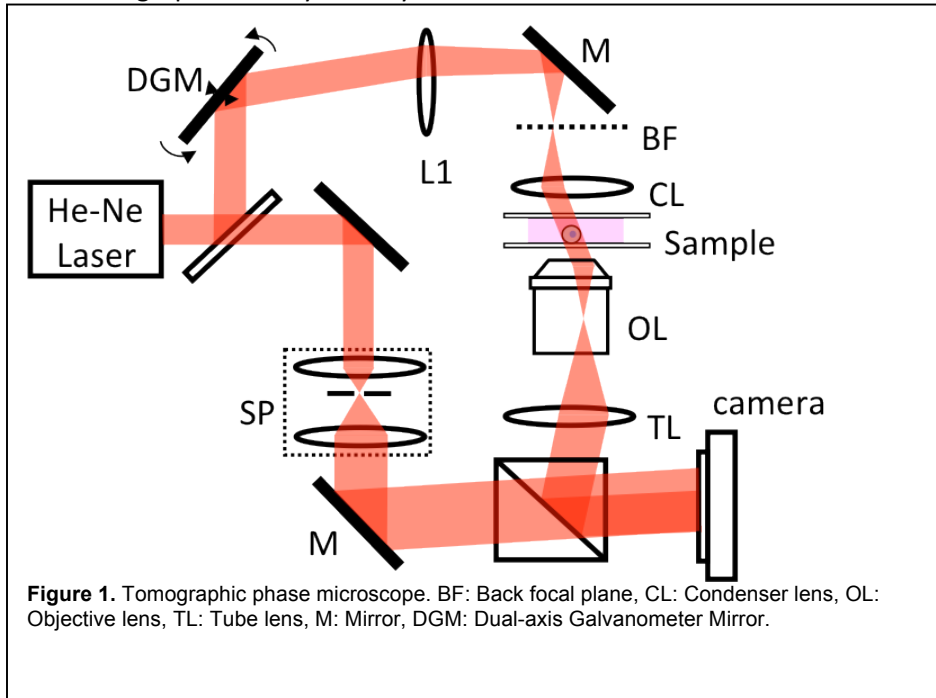
At the LBRC, we have utilized a Mach-Zehnder interferometric approach (Fig. 1) where we systematically vary the incident beam angle and measure the amplitude of the forward scattered light in the detector plane. The illumination angles are typically limited to  $-60 < \theta < 60$  degrees by appropriately selecting the numerical apertures of condenser and objective lenses. For thin samples with small refractive index contrast, the measured phase images can be interpreted as the projections of refractive index at the detector plane. One can then reconstruct a 3-D refractive index tomogram using filtered back-projection method [9]. However, for thick biological samples where light undergoes scattering, the diffraction effects cannot be ignored. We have therefore implemented optical diffraction tomography algorithm based on the Rytov approximation to obtain diffraction-free tomographic reconstruction [10] (Fig. 2). As mentioned above, the angular coverage is defined by the numerical apertures of condenser and objective lenses. The limited angular coverage also affects the achievable spatial resolution. We have minimized the effects of limited angular coverage by implementing regularization algorithms during post-processing step [11] (Fig. 3).

## Biomedical Application Potential

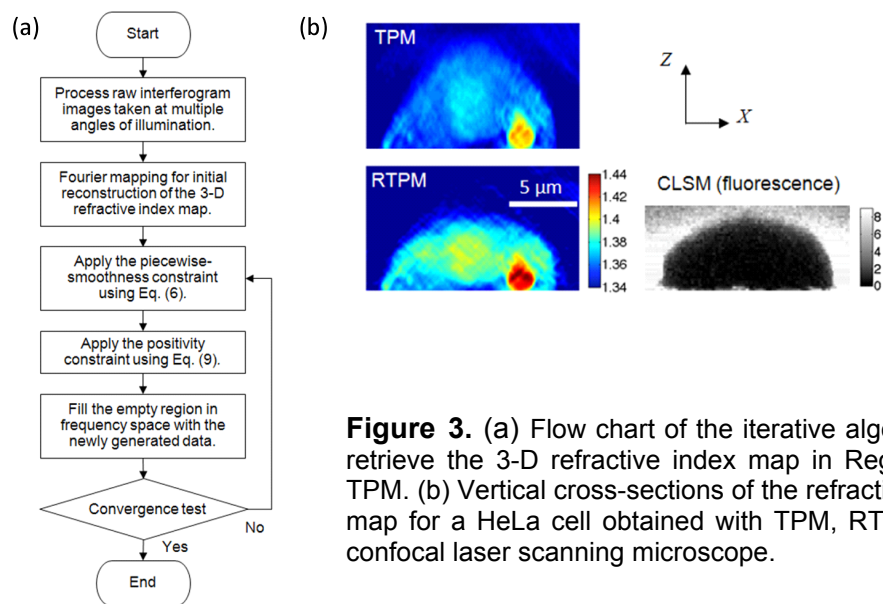
The volume integral of the refractive index can provide the total amount of non-aqueous content in a cell [12-14] or sub-cellular organelles [11]. Changes in the refractive index of cells have been also linked to carcinogenic transformations [15]. At LBRC, we have utilized this capability to study cell growth and division [14] as well as quantification of different stages of growth in chondrocytes [16]. The approach may also be used to study the structural and functional changes in individual cells during drug action.

Ongoing Projects

a. 3-D holographic flow cytometry



**Figure 2.** Refractive index tomogram of a HeLa cell. (a) 3-D rendered image. The dotted box is a cube of side  $20\ \mu\text{m}$ . (b) Top view of (a). (c)-(h) Slices of the tomogram at heights indicated in (a). Scale bar,  $10\ \mu\text{m}$ . The color 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.



## Background Publications

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5. Lauer, V., *New approach to optical diffraction tomography yielding a vector equation of diffraction tomography and a novel tomographic microscope*. *Journal of Microscopy*, 2002. **205**(2): p. 165-176.
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9. Choi, W., et al., *Tomographic phase microscopy*. *Nature Methods*, 2007. **4**(9): p. 717-719.
10. Sung, Y., et al., *Optical diffraction tomography for high resolution live cell imaging*. *Opt Express*, 2009. **17**(1): p. 266-77.
11. Sung, Y., et al., *Stain-free quantification of chromosomes in live cells using regularized tomographic phase microscopy*. *PLoS One*, 2012. **7**(11): p. e49502.
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13. Popescu, G., et al., *Optical imaging of cell mass and growth dynamics*. American Journal of Physiology-Cell Physiology, 2008. **295**(2): p. C538.
14. Sung, Y., et al., *Size homeostasis in adherent cells studied by synthetic phase microscopy*. Proceedings of the National Academy of Sciences, 2013: p. 201315290.
15. Zysk, A.M., E.J. Chaney, and S.A. Boppart, *Refractive index of carcinogen-induced rat mammary tumours*. Physics in medicine and biology, 2006. **51**: p. 2165.
16. Cooper, K.L., et al., *Multiple phases of chondrocyte enlargement underlie differences in skeletal proportions*. Nature, 2013. **495**(7441): p. 375-8.

#### Representative Center Publications

1. Choi, W., et al., *Tomographic phase microscopy*. Nature Methods, 2007. **4**(9): p. 717-719.
2. Sung, Y., et al., *Optical diffraction tomography for high resolution live cell imaging*. Opt Express, 2009. **17**(1): p. 266-77.
3. Sung, Y., et al., *Size homeostasis in adherent cells studied by synthetic phase microscopy*. Proceedings of the National Academy of Sciences, 2013: p. 201315290.
4. Cooper, K.L., et al., *Multiple phases of chondrocyte enlargement underlie differences in skeletal proportions*. Nature, 2013. **495**(7441): p. 375-8.

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