Supplementary Information

Profiling individual human red blood cells using common-path diffraction optical tomography

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Inventory of Supplementary Information

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Experimental setup

A diode-pumped solid-state (DPSS) laser ($\lambda = 532$ nm, 50 mW, Cobolt, Solna, Sweden) was used as an illumination source for an inverted microscope (IX73, Olympus Inc., Center Valley, PA, USA). The laser beam was first spatially filtered by a pinhole with a diameter of 25 µm. The collimated laser beam was steered by a two-axis galvanometric mirror (GM1, GVS012/M, Thorlabs, USA), and then projected onto a sample plane via a 4-*f* telescopic lens system comprised of a lens (L1) and a condenser lens (CL, UPLSAPO 60×, numerical aperture (NA) = 0.9, Olympus, Japan). A sample was prepared and sandwiched between two cover glasses separated by a thin spacer of double side tape. At the sample plane, the illumination angle of the beam can be rapidly scanned by GM1. The diffracted beam from the sample was collected by a high-NA objective lens (OL, UPLSAPO 60×, NA = 1.42, Olympus, Japan).

To maintain an optical axis for common-path interferometry, the second two-axis galvanometric mirror (GM2) was synchronized with GM1. The mirror of GM2 rotates exactly as much as rotated by GM1 but in an opposite direction, such that the beam reflected from GM2 kept an optical axis regardless of the illumination angle at the sample plane. The optical field of the diffracted beam is quantitatively and precisely measured by a commonpath interferometry setup. Here, the common-path Diffraction Optical Tomography (cDOT) employs the principle of diffraction phase microscopy to construct a common-path interferometry. The beam from a sample is diffracted by a transmission grating (70 grooves mm⁻¹, #46-067, Edmund Optics Inc., NJ, USA). Among several orders of diffracted beams, only the 0th and 1st orders of the diffracted beams are used and the others are blocked. The 0th diffraction beam is spatially filtered by a 4-f lens system with a spatial filter such that it serves as a reference plane-wave at the image plane. The first order beam is directly projected onto the image plane. At the image plane, the sample and reference beams interfere with a small angle difference defined by a spatial period of the grating and the 4-f lens system, forming the spatially-modulated interferograms. The interferomgrams are recorded by a scientific complementary metal-oxide semiconductor camera (Neo sCMOS, ANDOR Inc., Northern Ireland, UK) having 528×512 resolution with a pixel size of 6.5 µm and x199 of total magnification of the imaging system. The detailed information on the instrument and validations can also be found in elsewhere¹.

Reference

1. Kim, Y., *et al.* Common-path diffraction optical tomography for investigation of three-dimensional structures and dynamics of biological cells. *Optics Express* **22**, 10398-10407 (2014).