Tomographic imaging of a suspending single live cell using optical tweezer-combined full-field optical coherence tomography

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We propose a label-free depth-resolved tomographic scheme for imaging a single live cell in fluid. This approach utilizes a modified time-domain full-field optical coherence tomography (FF-OCT) system combined with an optical tweezer technique. The optical trap for holding a moving specimen is made by tightly focusing a 1064 nm Q-switching pulsed laser beam with a 1.0 NA microscope objective in the sample arm of the FF-OCT part. By cohering the probe for both systems, the optical actions of trapping and cellular resolution tomographic imaging could be achieved simultaneously. Feasibility of the combined system is demonstrated by imaging micron-sized polystyrene beads and a living suspension cell in medium. © 2012 Optical Society of America

Optical coherence tomography (OCT) is a low-coherence interferometric technology that produces optically sliced cross-sectional images of a turbid specimen with micrometer resolution [1]. In the past decade, OCT has become a powerful tool for three-dimensional imaging of biological tissues and has opened up new possibility for applications in biomedical and clinical fields [2,3]. In cell biology, this technique has shown its promising potential of imaging live cells without the aid of any exogenous agents. For example, Tan et al. have evaluated dynamic behaviors of cells in a tissue model under external stimuli with highly scattered OCT intensities [4]. Individual single cells also have been investigated with quantitative phase-contrast images of cells adherent on a culture dish using full-field OCT (FF-OCT) in various domains, which employed a broadband light source like a halogen lamp and high numerical aperture (NA) microscope objectives (MOs) [5,6]. Moreover, en-face tomograms of single cells in suspension have been achieved with Fourier domain optical coherence microscopy [7]. Eventually, these cell studies using OCT have provided valuable insights into complex biological interactions [4] or relation between the single cell and human disease states [6]. However, the cells in flow have yet to be explored in the OCT community. Most OCT systems have not been well-adapted to control and image the fast flowing or moving cells in fluid. This issue may restrict application of the OCT technique to micromechanical systems, like a lab-on-chip that deals with the behavior of single cells in microfluidic channels.

In this letter, we first report the feasibility of label-free cell imaging in microflow using a modified OCT system. The key of the proposed scheme is introducing an optical tweezer (OT) technique into an FF-OCT microscope system. OT is a well-known scientific instrument for laser-based optical trapping [8]. This technique uses light radiation pressure in the form of a single-beam gradient force trap to physically freeze moving micron-size dielectric particles, which uses the momentum transfer of the incident photons at the dielectric interface of the sample [8]. Such trapping ability of OT has made it attractive for micromanipulating biological specimens, such as live cells, bacteria, and even DNA [9,10]. In this experiment, the OT system is combined with the FF-OCT system. This approach can offer the advantage of position-drift suppression by spatially fixing the moving cells, which ensures more stable subcellular OCT imaging.

Figure 1 shows the schematic of our combined FF-OCT/OT platform, consisting of a typical standard time-domain FF-OCT system [11] and a single-beam OT unit (dotted box). A 7 fs ultra short pulsed laser, centered at 800 nm (Rainbow, Femtosource) was used as the FF-OCT light source. Its effective spectral bandwidth
was ~134 nm at FWHM of the source spectrum (see inset figure). The laser output beam (300 mW) was heavily attenuated using a linear polarizer to adjust the average power (a few hundred micron watt) of the beam incident on the sample and was condensed into a 22 m long multimode fiber (MMF) having a core diameter of 400 μm. The MMF in the illumination part allows rejection of crosstalk artifacts in the scattering media by reducing the spatial coherence of the laser beam [12]. The beam was passed through a Kohler illuminator to form an extremely even illumination plane and then was delivered into a Linnik interferometer. After splitting the laser beam by a beam splitter into two arms, the reference arm and the sample arm, each beam was strongly focused onto the object in the sample arm and the reflector in the reference arm by a pair of water-immersion 100× MOs with 1.0 NA, respectively. A sample (microparticle solution) was mounted on an XYZ translation stage in the sample arm for en-face OCT imaging.

In the OT unit, a 1064 nm Q-switching pulsed laser (SNP-08E, 60 mW, Teem photonics) was used as the trapping laser source. The effectiveness of the optical trap in the short pulse regime has been confirmed in previous literature [13]. The 3 mm diameter of the output beam was first passed through a neutral density filter and expanded to 4.5 mm with a 1.5× telescope beam expander. The beam was transmitted into the back aperture of the same MO in the sample arm of the FF-OCT system through a 45 deg dichroic mirror (T > 99% at λ = 1064 nm, Korea Electro Optics). The ratio of the beam diameter (at intensity point fallen to 1/e^2 of the central peak) to the entrance aperture diameter D = 7.9 mm of the MO (filling factor) was 0.71, which was similar to the optimal value (0.67) of the strongest trap for a micron-sized particle under the Mie regime [14]. Thus, one of the moving microparticles inside the solution could be optically trapped with the laser beam at the region where the scattering force and the restoring force are balanced [8]. Spatial fluctuation of the particle in an optical trap was within 200 nm. The particle manipulation was real-time monitored with a near-infrared CCD camera (CCD1020, 12 bit, 512 × 512 pixels, 20 frames/s, Allied Vision Technologies) and light illumination of the OCT system. It is well known that optical interference occurs only when the optical path lengths of two interferometer arms are well-matched within the coherence length. Thus, the OCT system with blocking or detuning the reference arm can work as a monitor system.

The OCT interference image was captured by the CCD camera through a tube lens (f = 250 nm). An edge short-pass filter (FF01-945/SP-25, Semrock) placed in front of the CCD totally blocked the residual trapping beams coming from the MO and the sample. A single en-face (XY) OCT image (113(X) × 113 (μm(Y)) of the trapped sample was obtained in 1 s (five images averaged) by employing the conventional continuous phase-shifting interferometry [11]. The incident power on the sample was about 230 μW. The axial resolution was estimated to be 2.0 μm with FWHM of the autocorrelation function envelope of the OCT source, slightly worse than the theoretical one of 1.66 μm. This difference might be due to the non-Gaussian spectral shape of the source spectrum and the residual optical dispersion in the system. For optical sectioning, the coherence-gating plane of OCT was axially (Z) shifted for the trapped sample by slightly controlling the length of the reference arm.

To demonstrate the capability of the concurrent trapping and OCT imaging, we have imaged dielectric microspheres (Spherotech) of several sizes in suspension with the combined system. A water chamber containing 5 μm diameter polystyrene beads solution was loaded on the sample stage and the MO was positioned for trapping a bead adjacent to the bottom of the chamber. Then, the coherence-gating plane of the OCT was situated near the bottom of the trapped bead (see left inset in Fig. 2). Figures 2(a)–2(d) show FF-OCT images of the polystyrene beads within the chamber that was under transverse motion. In the figure, only the bead A was optically seized with an average trapping power of 4 mW, and the others including the control bead B were free. It was observed that the untrapped beads in suspension were transported with the chamber (see the trajectory of B), whereas the tweezeed bead A was adhered to the same position. Some of the untrapped beads (cell 1 and cell 2) are fluctuated during the chamber movement. The total moving distance of the chamber was 50 μm.

Under the same experimental condition, the same measurement was made but with larger 11 μm polystyrene beads and vertical (Z) motion of the chamber. This time, the coherence-gating plane of OCT was positioned at the top surface of the beads lying onto the bottom of the chamber (see illustration in Fig. 3). Figure 3(a) is a
bright-field image of the polystyrene beads located usually at the bottom of the chamber, where bead A was optically trapped, and Fig. 3(b) is the corresponding en-face OCT tomogram. And then, slowly moving down the chamber along the Z direction with a step of 0.5 μm, a series of en-face OCT tomograms were taken. We could see that the untrapped beads were moved down with the chamber and then faded out of the imaging plane as shown with the control bead B in Figs. 3(c)–3(g). On the other hand, the OCT images of the trapped bead A remained at the same location despite the chamber movement. It is remarkable to see the enlarged views of the trapped bead A and the untrapped bead B (insets in each image). These experimental results suggest that the combined FF-OCT/OT system can be effective to actively manipulate and image dielectric particles under the microflow environment.

To confirm the system performance for a biological specimen, FF-OCT imaging of an unmodified cancer cell in suspension was carried out. Human acute monocytic leukemia cells (THP-1), as a general suspension cell, were cultured in RTMI-1640 medium at 37 °C and the cultured cell plate was placed on the sample stage for experiment. Stable optical trap of a single cell was also confirmed while agitating the culture plate as in Fig. 4.

Figure 5(a) is a microscope image of three individual THP-1 cells in which cell 2 was optically trapped at 10 μm above others (cell 1 and cell 3) with an average trapping power of 8 mW. En-face OCT image of the trapped cell is shown in Fig. 5(b). Its depth-resolved OCT images were obtained with a step of 2.5 μm in Figs. 5(c)–5(e), revealing internal cellular structures.

In summary, we have proposed and implemented a novel optical imaging system combined with an FF-OCT microscope and an OT for subcellular OCT imaging of a micro-object in microflow. Cochanneling of OCT beam and trapping beam has allowed simultaneous optical confinement and optical slicing of the specimen in motion. The feasibility of the proposed system has been experimentally verified with trapping and imaging of polystyrene beads and a live suspension cell. It is expected that this FF-OCT/OT system would pave the way for single cell study in microfluidic field.

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References: