Opto-Electronic Advances

News & Views

June 2023, Vol. 6, No. 6

DOI: 10.29026/oea.2023.230083

In-flow holographic tomography boosts lipid droplet quantification

Michael John Fanous[1] and Aydogan Ozcan[1,2,3,4*]

A 3D microscopy instrument using flow tomography significantly advances the screening and quantification of intracellular lipid droplets.

Fanous MJ, Ozcan A. In-flow holographic tomography boosts lipid droplet quantification. *Opto-Electron Adv* **6**, 230083 (2023).

In their recently published paper in *Opto-Electronic Advances,* Pietro Ferraro and his colleagues report on a new high-throughput tomographic phase instrument that precisely quantifies intracellular lipid droplets (LDs)[1].

LDs are lipid storage organelles found in most cell types and play an active role in critical biological processes, including energy metabolism, membrane homeostasis, and biomolecular signaling[2]. They have also been linked to numerous diseases, such as diabetes[3] and cancer[4]. Recent evidence also indicates that monocytes from COVID-19-infected patients exhibit above-average levels of LDs[5]. The precise measurement of LD features can thus provide invaluable insights into the molecular mechanisms underlying these processes and holds promise for developing new therapeutic strategies. In particular, LD number, size, and interplay with other organelles have been significantly correlated with many cellular processes[2]. However, achieving both the non-destructive and efficient analysis of these parameters remains challenging, particularly in assessing LD spatial organization in 3D.

Transmission electron microscopy (TEM) and fluorescence microscopy (FM) are the two most commonly employed techniques for investigating LDs[6]. However, TEM analysis is limited to small sample areas, which hinders the overall study of LDs inside cells. It also requires highly skilled operators. As for FM, it is a more accessible method that can make use of a growing number of fluorescent lipophilic dyes. These labels, however, do not provide information related to the sample's 3D spatial distribution. Furthermore, fluorescent dyes are subject to photobleaching and may interfere with cellular functions by inducing phototoxicity, especially during long exposure times.

To address these limitations, label-free methods, such as Tomographic Phase Microscopy (TPM), have been increasingly adopted as a viable imaging alternative, especially for live cells. Moreover, coupled with deep learning-based computational postprocessing, label-free modalities have been successfully used to circumvent many traditional chemical preparations of biological specimens and avoid the related cost, labor, duration, sample damage and inconsistencies of such procedures[7–9].

TPM is a non-invasive imaging technique that combines digital holography and tomography to provide three-dimensional (3D) images of transparent samples

[1]Electrical and Computer Engineering Department, University of California, Los Angeles 90095 CA, USA; [2]Bioengineering Department, University of California, Los Angeles 90095 CA, USA; [3]California NanoSystems Institute (CNSI), University of California, Los Angeles 90095 CA, USA; [4]Department of Surgery, David Geffen School of Medicine, University of California, Los Angeles 90095 CA, USA.

*Correspondence: A Ozcan, E-mail: ozcan@ucla.edu

Received: 15 May 2023; Accepted: 17 May 2023; Published online: 25 June 2023

based on their refractive index (RI) contrast[10]. TPM involves the acquisition of a series of two-dimensional (2D) holographic images of the sample at different viewing angles, which are then processed to reconstruct a 3D map of the sample's RI distribution. This enables the visualization and quantification of the internal structures and dynamics of cells, including LDs, organelles, and cytoskeletal elements, without the need for staining or labeling.[11] This technology belongs to the broader category of quantitate phase imaging (QPI)[12–16] techniques, and can be used to generate precise phase maps, which serve as an intrinsic biomarker, bypassing the need for exogenous labels. TPM thus offers enormous potential in biomedical and life sciences, as it can provide a non-invasive means of early disease detection and contribute to the advancement of personalized medicine.

In this recent work by Ferraro et al., TPM was applied for visualizing and characterizing LDs in stain-free suspended cells flowing through a microfluidic channel. The devised in-flow TPM system was proven reliable by reconstructing the 3D refractive index distribution of LDs in ovarian cancer (Fig. 1) and monocyte cells.

These measurements enabled the extraction of important parameters related to spatial correlations and three-dimensional (3D) positioning of LDs at high-volume rates. Collecting such information from numerous cells yields statistically rich data, revealing additional quantitative properties, such as the RI, equivalent radius, dry mass, sphericity, and concentric LD zones. Beyond these metrics, the 3D spatial localization of LDs inside suspended cells was also delineated, which is advantageous to understanding the interactions of LDs with other organelles. Also, this opto-fluidic TPM configuration is highly conducive to monitoring various simulated physiological scenarios, such as circulating tumor cells.

Prospectively, this technology establishes the groundwork for a plethora of exciting imaging opportunities with lipid droplets, especially if used in conjunction with deep learning models. For instance, the average in-flow TPM experiment involves recording holographic sequences comprising hundreds of images with 3D RI reconstructions typically requiring about 15 minutes. With fewer frames, this step can potentially be accelerated by implementing appropriate neural networks. In addition, this microfluidic system propels hundreds of cells past the sensor per minute, at 50 nL/s, and is an ideal setup for achieving substantially higher throughput with the aid of machine learning. For example, its throughput may be increased using specialized deblurring neural networks trained on data acquired at different flow rates[17].

Furthermore, such comprehensive characterization of LDs may assist in their function as endogenous microlenses[18], possibly relaxing the requirement of optical tweezers. By monitoring and manipulating the orientation of LDs, it may be feasible to dispense with optical tweezers and fine-tune the optics accordingly using, e.g., an LED array. Also, deliberately oscillating cells in a controlled manner could potentially be used to engage the LDs in a type of fluorescent scanning operation for improved imaging of targeted intracellular components.

Overall, this QPI-based TPM approach has the potential to promote greater accessibility in lipid droplet research and enable the development of more advanced LD-assisted biomedical tools.

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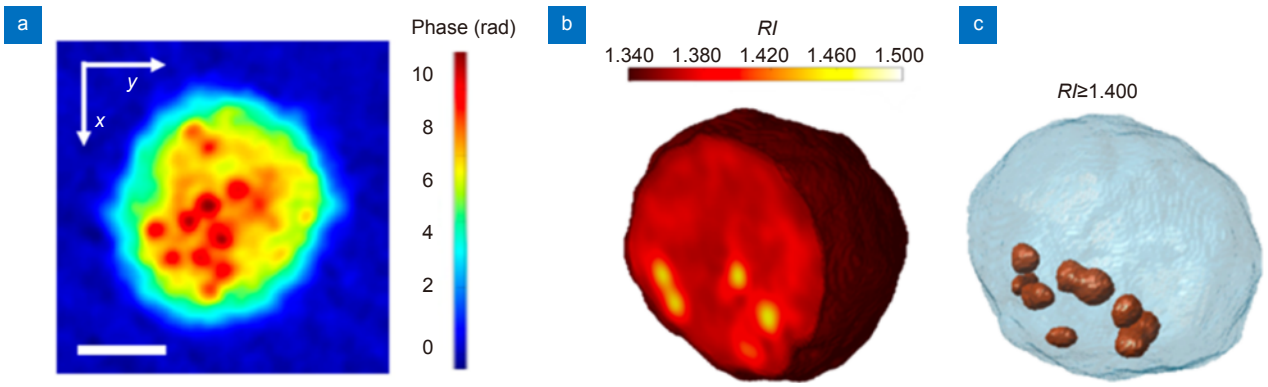

Fig. 1 | (**a**) TPM QPM of an ovarian cancer cell containing LDs, scale bar 5 μm. (**b**) Derived RI distribution of a similar cell. (**c**) A 3D rendering of the individual LDs based on an RI threshold.

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