Ultrafast high-contrast microfluidic cellular imaging by asymmetric-detection time-stretch optical microscopy (ATOM)

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Abstract: We demonstrate asymmetric-detection time-stretch optical microscopy which delivers high-contrast (simultaneous enhanced phase-gradient and absorption contrasts) microfluidic imaging with subcellular resolution and in-line optical image amplification (20dB), at a record imaging flow speed of 10 m/s. **OCIS codes:** (170.0180), (170.7160)

Having the ability of accessing to the morphological information of the cells and thus facilitating better cellular identification/classification with high statistical accuracy, optical microscopy is of great value when it is incorporated in high-throughput cell screening – a routine utilized in clinical diagnostics and basic life science research. Specifically, high-speed optical imaging techniques are essential in order to maintain the required high-throughput. Yet, current approaches have largely been restricted by a trade-off between throughput and image quality (i.e. screening accuracy). Very often, scaling the imaging speed in optical microscopy is achieved at the expense of image quality, primarily dictated by image contrast, image resolution, and detection sensitivity – a common predicament for advancing high-speed and high-throughput cellular imaging. It is exemplified by that the current state-of-the-art imaging flow cytometers have to scale down the *imaging* throughput to ~1000 cells/sec, compared with the throughput of ~100,000 cells/sec of the classical non-imaging flow cytometers [1].

In this regard, a new optical imaging modality called time-stretch microscopy, which bypasses the intrinsic speed limitation of classical light microscopy, has recently been developed for ultrafast imaging. It is achieved by ultrahigh-speed retrieval of image information (MHz frame rate) encoded in the spectrum of a broadband and ultrashort optical pulse (fs – ps) by converting it into a serial temporal data in real time. This technique has shown to be able to perform high-throughput image-based cell screening, which could be an ideal complementary tool to typical multiparametric flow cytometry. However, time-stretch microscopy has so far mostly been operated in bright-field (BF) imaging mode in longer wavelength range [2-4], and is thus not capable of revealing high-contrast and detailed morphology of the transparent cells – hindering accurate *image-based* cell recognition and screening. As a result, effective use of time-stretch imaging to-date only has been limited to microparticle or cell screening in high-speed flow with trivial size and shape differences, especially when the targeted cells are labeled with contrast agents [3]. Similar to the classical optical imaging modalities, the time-stretch image quality is thus still compromised by the higher imaging speed. To this end, we present a solution by a new imaging approach called *asymmetric-detection time-stretch optical microscopy (ATOM)*, for capturing label-free, high-contrast image of the live cells at ultrahigh speed, in the context of microfluidic flow imaging at a record high *imaging* flow speed of 10 m/s. ATOM reported here enables, for the first time, time-stretch operation in the short near-infrared wavelength range with in-line optical image amplifiers – achieving subcellular resolution and high detection sensitivity (optical image gain of 20dB) at an ultrahigh imaging speed using a single-pixel photodetector. The achieved flow speed is equivalent to an imaging throughput of ~ 100,000 cells/sec – unreachable by any state-of-the-art image sensors. ATOM thus represents a significant advancement in bringing the essential imaging metrics – high resolution and high contrast – to high-speed time-stretch imaging, making it a genuinely appealing platform for realizing high-throughput image-based cellular assay.

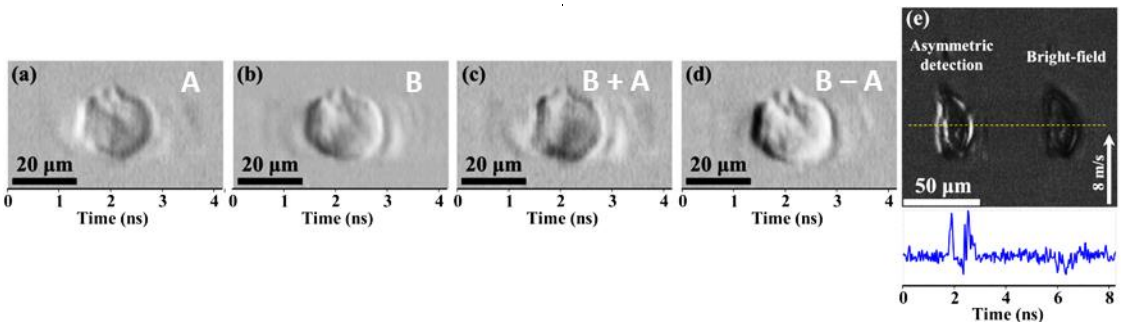

Fig. 1: (a,b) Two ATOM images of a normal hepatocyte cell (MIHA), which show the opposite phase-gradient contrasts, respectively. (c) Sum of the two images (i.e. B+A) yields an absorption-contrast image. (d) Subtraction of the two images (i.e. B-A) yields a differential-phase-gradient contrast image. Note that each line scan of the image is captured within ~ 4 ns. (e) Demonstration of the contrast enhancement in ATOM by comparing the asymmetric detection scheme with the bright-field (BF) scheme in ultrahigh-speed flow imaging (~8 m/s) of the MIHA cells in a PDMS microfluidic channel (see also the line profile in the bottom inset). Note that both images of the same cell are obtained simultaneously by the time-multiplex scheme.

The central idea of ATOM is to generate the enhanced-contrast time-stretch images by asymmetrically detecting the spectrally-encoded pulses prior to the time-stretch process. It is done by off-axis light coupling into the optical fiber core, which acts as a confocal pinhole of the ATOM system – resembling the contrast-enhancement effect in Schlieren imaging [5]. More interestingly, the spectrally-encoded pulses can be split and time-multiplexed into the multiple replicas, each with different off-axis coupling angle – yielding different phase-gradient contrast. We show that by time-multiplexing two ATOM images which have opposite phase-gradient contrasts, we further obtain two different and decoupled contrasts from the same cell: one with differential (enhanced) phase-gradient contrast and another with absorption contrast, simultaneously. Our ATOM system is operated in the 1-μm wavelength regime based on a home-built ytterbium-doped mode-locked laser (repetition rate: 26 MHz; center wavelength: 1064 nm) with a bandwidth of ~10 nm. It employs standard spectral shower illumination for time-stretch imaging [2-4], with a holographic grating (1200 lines/mm) and an imaging objective lens (NA = 0.66). The time-stretch process is enabled by a dispersive fiber module with a GVD of 0.35 ns/nm and an in-line fiber-based semiconductor optical amplifier (SOA). This allows the image-encoded spectrum of each ATOM pulse (line-scan) to be mapped to the time and amplified with an optical gain is of 20 dB. Finally, the signal is detected by a photo-detector (10 GHz) and a real-time oscilloscope (40 GS/s).

Figs. 1(a)-(b) show the ATOM images of the unstained normal hepatocyte cell (MIHA) with two opposite phase-gradient contrasts. Note that the shadow cast of the images can be switched to the opposite side of the MIHA cell. By using the two time-multiplexed opposite-contrast ATOM images, we simultaneously obtain both absorption and differential (enhanced) phase-gradient-contrast images of the MIHA cell at ultrahigh speed (line scan time ~4 ns). It is simply done by computing the sum and difference of the two images, respectively (Figs. 1(c) and (d)). Fig. 1(e) shows the contrast enhancement in ATOM (asymmetric detection scheme) compared with the typical time-stretch imaging (BF scheme), under high-speed flow imaging (~8 m/s) in a PDMS microfluidic channel. Such high flow speed is achieved by inertial microfluidic flow. The 2D images are here acquired by continuous *real-time* 1D line-scan at a rate of 26 MHz (governed by the repetition rate of the laser), which is naturally provided by the unidirectional cell flow.

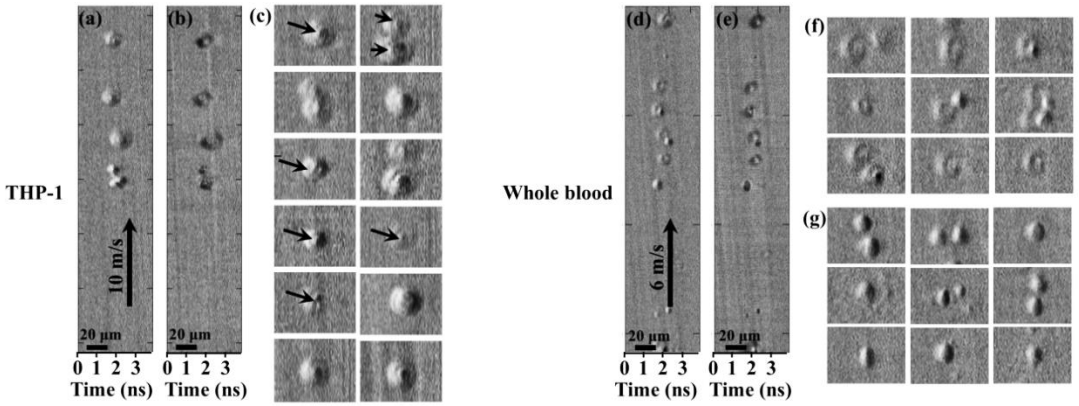

Fig. 2: Imaging acute monocytic leukemia cells (THP-1, a-c) and normal human blood cells (from whole blood, d-g) by ATOM in ultrafast flow (10 m/s and 6 m/s respectively). (a) Differential (enhanced) phase-gradient-contrast ATOM image. (b) Absorption-contrast ATOM image. (c) More differential phase-gradient-contrast ATOM images of THP-1 cells. The enhanced contrast enables visualization of the nuclei of the cells (see the arrows). (d) Differential (enhanced) phase-gradient-contrast ATOM image. (e) Absorption-contrast ATOM image. (f) The biconcave disk shape of red blood cells can be clearly visualized. (g) The swelled RBCs in the whole blood can also be identified by ATOM.

We also captured the ATOM images of the acute monocytic leukemia cells (THP-1) (Figs. 2(a) – (c)) as well as normal human blood cells, from fresh blood obtained from a healthy donor (Figs. 2(d) – (g)), flowing in the microfluidic channel at a speed as high as 10 m/s – equivalent to *an imaging throughput of ~ 100,000 cells/sec*. It is worth noting that the differential phase gradient contrast in the ATOM images of the THP-1 cells enables us to visualize the nuclei (Fig. 2(c)) – imaging such sub-cellular structure without label is yet to be demonstrated in ordinary BF time-stretch imaging. In addition, ATOM is able to identify the biconcave disk shape of the red blood cells (RBCs) (Fig. 2(f)) and can differentiate them from the swelled RBCs in the flow, which are in either spherical or elliptical shapes (Fig. 2(g)). The results of ATOM presented here are of great significance for advancing time-stretch imaging for high-throughput imaging flow cytometry with high statistical precision (enabled by high-contrast images). Such unique platform is particularly envisaged for rare cell screening in early metastasis detection, or post-chemotherapy detection of the residual cancer cells.

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