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Opto-acousto-fluidic microscopy for three-dimensional label-free detection of droplets and cells in microchannels

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Abstract This paper reports a novel method, opto-acousto-fluidic microscopy, for label free detection of droplets and cells in microfluidic networks. Leveraging the optoacoustic effect, the microscopic system possesses capabilities of visualizing flowing droplets, analyzing droplet contents, and detecting cell populations encapsulated in droplets via the sensing of acoustic waves induced by intrinsic light-absorbance of matters.

Introduction

Recently, droplet-based microfluidics has shown its vast potential in the fields of biology, chemistry, environment monitoring, nano-material synthesis and etc. due to its high-throughput compartmentalization of nano or picoliter-sized environments for biochemical assays^{1, 2}. To date, droplet manipulation techniques have been intensively investigated and developed, such as control of droplet size³, coalescence of droplet⁴, droplet splitting⁵, injection of droplet⁶ and etc. Meanwhile fluorescence-based detection is still mainly used for analysis of droplet contents due to its exquisite sensitivity of detection, high specificity of targeting and flexible compatibility with high throughput microfluidic systems⁷⁻¹⁰. Although the fluorescence-based techniques have been highly developed and widely used, some inherent limitations are still the bottlenecks for their applications. For example, the use of fluorophores always complicates the assay procedures and inevitably intervenes with the intrinsic chemical analytes or biological cells under study. Therefore, the research communities are expecting to see novel detection tools with

comparable sensitivity and throughput that could offer more physical and chemical information¹¹.

Absorbance-based spectroscopy emerged as an alternative to investigate the microfluidic mass transfer in a label-free way with high specificity by measuring the light-absorbance of analytes. Imposed by the challenge of short light-matter interaction length in microscale systems, the application of absorbance-based spectroscopy to microfluidics always involves in-plane light-matter interaction with assistance of integrated optofluidic elements¹²⁻¹⁴. Such architectures do not offer the feasibility of single droplet or cell detection with high spatial resolution, but only yield the measurement results in a spatially averaged manner. Recently, Maceiczky *et al.* reported an absorbance-based spectroscopic method for high-throughput single-point detection of droplet by overlapping one excitation beam to generate absorbance-based thermal effect with one interferometric probe beam to measure the thermal-induced optical path variation¹⁵. Yet the configuration cannot be used for occasions when both spatial and temporal information are important, such as the full-field measurement of concentration gradient in microchannels, or distribution of cells within a flowing droplet.

Optoacoustic imaging techniques emerged as biomedical diagnostic tool to investigate a variety of endogenous chromophores in bio-samples¹⁶⁻¹⁸. Owing to the chromophores' characteristic absorption of electromagnetic (EM) energy at specific wavelength, optoacoustic techniques can offer detection of targets with uniquely high sensitivity and specificity. To date, various optoacoustic imaging systems with high temporal and spatial resolution have been demonstrated in both clinical and fundamental applications, such as breast cancer detection, arthritis diagnosis, brain investigation, nanomaterial evaluation and *et al.*¹⁹⁻²¹

To the best of the authors' knowledge, no published literature has documented the utilization of optoacoustic imaging technique for the analysis of contents in drop-based microfluidics. In this paper, we report the label-free detection of droplets and their encapsulated red blood cells (RBCs) in microchannels by adapting optoacoustic microscopy to the

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inspection of microfluidics, named opto-acousto-fluidic microscopy. Particularly, we demonstrate that this method with a high spatial-temporal resolution can be used to monitor the dynamics of droplet formation and the process of internal mixing in a droplet. We also study the feasibility of using the microscopic technique for quantitative measurement of molecule species concentration and cytometric measurement of encapsulated RBCs.

Materials and method

Preparation of opto-acousto-fluidic chips

The opto-acousto-fluidic chip was fabricated using standard soft-lithography with a channel width of 200 μm and height of 120 μm . To minimize the acoustic attenuation for the detection of optoacoustic signal, a PDMS membrane (thickness $\sim 100 \mu\text{m}$) was used for bonding and sealing of the PDMS replica. Fluid samples were injected into chips using syringe pumps (TYD02-01, Lead Fluid) via plastic tubings. A T-junction was used to produce aqueous droplets which consisted of light-absorbing molecule species or cells and buffer fluids, Fig. 1 (b). A serpentine channel was employed for the fully-mixing of the contents under study and buffer fluid.

Optoacoustic microscopy for microfluidics

The detailed description on the hardware of the optoacoustic microscopic system was reported in our previous work¹⁹ or can be referred to Fig. S1. Fig. 1 (a) illustrates the adaption of the microscopic system to the inspection of microfluidics. In particular, the opto-acousto-fluidic chip was placed on a cuvette filled with optoacoustic coupling fluid. The cuvette allows a pulse laser beam (repetition rate 50kHz) steered by a galvanometer mirror and focused by an objective (4X) to

transmit through and subsequently to converge with a focal spot size of 3.2 μm in the microfluidic channels. Upon the absorption of the pulse laser by the contents in microchannels, acoustic waves can be initiated, Fig. 1 (c). Penetrating the PDMS membrane, the backward-irradiating acoustic wave can be coupled into the cuvette, reflected by a thin cover glass and detected by an ultrasound transducer, Fig. 1 (c). The time-resolved optoacoustic signal collected by the transducer is referred to as A-line that can reveal depth information along the laser propagation direction (z-axis), Fig. 1 (d). One-dimensional scanning of laser beam along the transverse direction (y-axis) of microchannels generated a series of A-lines which can be spatially combined and referred to as a B-scan, Fig. 1 (e). For each B-scan, 250 sampling was made with a spatial interval of 1 μm across the channel with a width of 200 μm in the experiment, and thus the 50kHz repetition rate of the laser provides a 200Hz B-scan rate. Assisted by the creeping flow in microchannels, continuous B-scans can be generated by automatic fluid scanning to capture the information along the flow direction (x-axis). The resolution along the x-axis depends on the velocity of the flow and the B-scan rate. Given a 200Hz B-scan rate and a velocity of 1mm/s, the resolution would be 5 μm . The off-line image reconstruction was performed using Matlab (MathWorks, Inc.) and Amira (Visage Imaging). Volumetric images of droplet were reconstructed by back-projecting each depth-resolved A-lines in the rectangle coordinate and exhibited in maximum amplitude projection (shown in Fig. 1 (f)).

Results and Discussions

Firstly to verify the capability of 3-D visualization of droplet

Figure 1 (a) Schematic of the opto-acousto-fluidic imaging module. Pulse laser beam is steered via reflective mirror (RM), galvanometer mirror (GM) and objective (4X) to orthogonally illuminate the opto-acousto-fluidic chip; (b) The microfluidic network with a T-junction to produce droplet and serpentine channel for passive-mixing; (c) Layout of the probe for optoacoustic signal generation and detection; (d) The schematic of time-resolved optoacoustic signals collected by ultrasound transducer and represented as A-lines; (e) A series of B-scans as a combination of A-lines; (f) The reconstructed 3-D visualization of droplet based on B-scans.

formation with the proposed microscopic module, the opto-acousto-fluidic chip was operated at a small capillary number ($Ca=0.001$), and the B-scan was implemented at the downstream after droplet breakup (illustrated in the inset of Fig. 2 (a)). Previous studies show that the formation of droplets with a Ca much smaller than the critical capillary number ($Ca^*\approx 0.015$) lies in the squeezing regime, where the squeezing pressure serves as the dominant force responsible for droplet breakup, and the size of the droplet plugs can be controlled by varying the flow rate ratio between the dispersed and continuous phases^{22, 23}. In the experiment, ponceau 4R-water solution (with strong EM absorbance spectrum around 510nm) and mineral oil served as the dispersed and continuous phases, respectively. The total flow rate of the two phases was fixed at 240 $\mu\text{L/h}$. By varying the flow rate ratio between the two phases, we managed to produce droplets with different sizes. The 2-D and 3-D visualizations of the droplets were reconstructed from the time-resolved optoacoustic signals (A-line) and illustrated as figure 2 (b)-(e). The fitting curve of the experimentally measured droplet sizes presents a linear relationship (slope $\alpha\approx 2$) with flow rate ratio (Fig. 2 (a)), which agrees well with previous experimental and theoretical studies²²⁻²⁴. This

Figure 2 Employing the opto-acousto-fluidic imaging modality, the size of droplets is experimentally validated to have linear relationship (slope of the fitting curve $\alpha\approx 2$ in (a)) with flow rate ratio when droplets are formed at a capillary number of 0.001 and a channel width ratio ($\Lambda = w_d/w_c$) of 1. The 3-D visualizations and 2-D projections of droplets obtained at conditions of $\phi=0.05$, 0.33 , 0.82 , and 1.5 are illustrated as (b)-(e), respectively.

validation provides the confidence to apply this method to the study of droplet formation, manipulation and its dynamic morphology. However, it should be noted that the axial resolution (along the light propagation direction) is approximately 60 μm which is dependent on the bandwidth of the currently employed ultrasound transducer (25MHz), and it is expected to have an improved axial resolution up to several microns when wide bandwidth transducer ($>100\text{MHz}$) is used for observing smaller features²⁵. In addition, each substance, even pure fluid, has its characteristic absorption spectrum, and hence a pulse laser with the corresponding emission spectrum can be selected to produce optoacoustic signals. As currently we have an in-house 532nm pulse laser with high repetition rate, we purposely select ponceau 4R as a contrast agent for the proof-of-concept.

In this work, we also demonstrate that the proposed opto-acousto-fluidic microscopy can be used to inspect the performance of a droplet-based micro-mixer by experimentally observing the mixing dynamics. Specifically in the experiment, parallel streams (ponceau 4R solution $Q_{\text{pon}}=60\mu\text{L/h}$ and DI-water $Q_{\text{wat}}=60\mu\text{L/h}$) with identical flow rate were dispersed by mineral oil ($Q_c=120\mu\text{L/h}$) at a T-junction, and we used serpentine microchannel as a passive mixer (illustrated in the inset of Fig. 3 (a)). B-scans were implemented at different locations: the entrance, halfway positions and the exit of the serpentine channel (P1-P8). As the magnitude of the laser-induced acoustic wave (optoacoustic signal) depends on the absorbance coefficient β and the laser power, the reconstructed images of the droplets (shown in Fig. 3 (b)) represent the distribution of absorbance coefficient which is actually an inherent physical property of a substance. Owing to the unique molecular structure, the analytes under study present a specific absorbance spectrum. Therefore, the optoacoustic signal can provide a detection of high specificity. The images in Fig. 3 (b) qualitatively reflect the advection dynamics inside the droplets for monitoring mixing processes. We observed that the mixing occurred right-after the

Figure 3 (a) The profiles of normalized optoacoustic signals across the channels at positions of P1, P4 and P8; (b) 2-D projected image of droplets obtained at positions from P1 to P8 (illustrated in the inset of (a)).

formation of the droplet due to the shear-stress induced inner circulating flows. During the transportation of droplet in the straight channel, the mixing was enhanced (Fig. 3 (b):P1) due to the internal symmetric circulation. After passing through the winding sections of the serpentine channel, the asymmetric circulation expedited the mixing process (Fig. 3 (b):P2-P7) and finally flattened the distribution of the two agents (Fig. 3 (b):P8). We plotted the signal profiles along the transverse direction of the droplet (shown in Fig. 3(a)), which shows a good agreement with previous studies²⁶⁻²⁸.

We also investigated the feasibility of this opto-acousto-fluidic microscopy technique for quantitative measurement of molecular concentration in microfluidics. Two parallel streams, DI-water and ponceau 4R solutions, were squeezed at T-junction by mineral oil to form aqueous droplets flowing toward the serpentine channel for fully mixing of the contents in droplets. By changing the fraction ratio between the two aqueous streams, droplets with different molecular concentration can be generated. The B-scan was carried out at the tail of the serpentine channel to interrogate the droplets. Reconstructed images of droplets with different concentrations were shown in Figure 4 (b), and the spatially-averaged magnitudes of optoacoustic signals were plotted against the fraction ratio between the two aqueous flows, Fig. 4 (a). The experimental results show that initially the optoacoustic signal is linearly increasing with the concentration level and eventually becomes saturated. As the concentration proportionally determines the absorption coefficient β (the fraction of incident energy absorbed per unit thickness of an absorber), our experimental observation agrees well with the theoretical characterization of optoacoustic signal as function of absorption coefficient²⁹.

Based on the study of the relationship between the magnitude of optoacoustic signal and the concentration of molecular species in microfluidics, it is envisioned that this method can be used to quantitatively evaluate the

Figure 4 (a) The magnitude of optoacoustic signal as a function of the concentration of light-absorbing molecules. Droplets with different concentrations can be obtained via varying the fraction ratio of two aqueous fluids (ponceau 4R solution and water). Optoacoustic imaging of the droplet is implemented at the exit of serpentine channel. 2-D projections of optoacoustic images are illustrated in (b) at varied fraction ratios.

endogenous chromophores in bio-samples for lab-on-chip applications, such as the on-chip measurement of hemoglobin carried by red blood cell. As a tentative attempt, we encapsulated RBCs in aqueous droplets with different sizes, and made cytometric measurement using the opto-acousto-fluidic imaging module.

In detail, arterial blood was draw from a rat and buffered with saline. All the animal and cell operations were approved by the Southern University of Science and Technology (SUSTech) and in compliance with the animal care and biosafety guidelines of Guangdong province. The buffered blood sample was dispersed into droplets flowing through the passive mixer for evenly distributing RBCs inside the droplets in order to avoid opto-acoustic signal saturation due to agglomeration of RBCs. Since the hemoglobin carried by RBCs has intrinsically strong absorption of 532nm laser, the scanning pulsed laser beam can excite acoustic waves when a RBC enters the focal spot of the laser beam. Via implementing the B-scan of laser beam with high pulse repetition rate, the RBCs in the droplets can be "lightened", and the off-line reconstruction of signals yields the full-field visualization of RBCs in the droplet, Fig. 5 (b)-(e). As the diameter of the beam waist is about $3.2 \mu\text{m}$ that is smaller than that of RBCs (diameter $\sim 10 \mu\text{m}$), each bright spot in the reconstructed images represents an event of a RBC's engaging with laser beam. To test the reliability of the method, droplets with different volumes but same density of cell population were generated via varying the flow rate ratios between the aqueous and oil phases, and the cytometric measurement shows that the number of detected RBCs increases

Figure 5 The number of RBCs encapsulated in a droplet is found to be proportional to the volume of droplet in a cytometric measurement (a). Reconstruction of time-resolved optoacoustic signals gives the 3-D and 2-D visualization of RBCs in a flowing droplet (b)-(e).

proportionally with the volume of the droplet (illustrated as the linear curve-fitting in Fig. 5).

Conclusions

This work demonstrates a novel method of opto-acousto-fluidic microscopy for on-chip three-dimensional label-free detection of droplets and their encapsulated cells via their intrinsic absorbance of pulsed EM energy and subsequently triggered acoustic waves. The microscopic system shows a capability of full-field visualization of droplets with high spatial-temporal resolution, which can be used to study the dynamics of droplet formation. As the magnitude of opto-acousto signal is a direct reflection of absorption coefficient that depends on the concentration of analytes under study, this method can be used to map the distribution of analyte concentration within a droplet, which can further serve as a tool to analyse the droplet contents, such as cells with endogenous chromophores. As a proof-of-concept, red blood cells encapsulated in flowing droplets are studied with cytometric measurements through the detection of haemoglobins using this opto-acousto-fluidic microscopy technique.

Compared to conventional high-speed optical imaging techniques, this proposed method can be used for on-chip quantitative analysis of specific substances from bio-tissues such as lipid, fat, water, oxy- and deoxy- haemoglobin etc., based on their finger-print EM absorbance rather than simply a visualization of the samples. We also envision that on-chip bio-assays can greatly benefit from deriving functional cell parameters, such as concentration of oxy-haemoglobin, concentration of deoxy-haemoglobin, saturation of oxygen, and oxygen consumption rate etc., using multispectral strategy of optoacoustic imaging. Additionally, through analysing the spectrum of generated optoacoustic signals, mechanical parameters of cells, such as elasticity/rigidity, can also be derivable.

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Conflicts of interest

There are no conflicts to declare.

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Table of content and graphical abstract

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