# Opto-acousto-fluidic microscopy for three-dimensional label-free detection of droplets and cells in microchannels
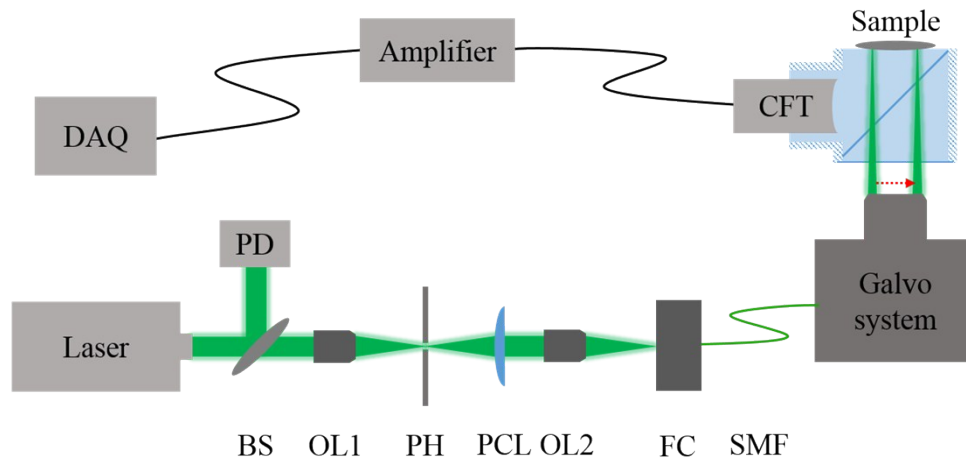
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PD: photo diode; OL: object lens; PH: pinhole; PCL: Plano-convex lens; FC: fiber coupler; CFT: cylindrically focused transducer; LT: lens turret, SMF: single mode fiber; DAQ: data acquisition.

Figure S1 The layout of optoacoustic imaging system. The 532 nm laser beam emitting out of a Q-switched pulsed laser has a 7 ns pulse duration and a maximum repetition rate of 50 kHz, and is split by a beam splitter (BS025, Thorlabs Inc.). The reflection beam is delivered into a fast photodiode (PD818-BB-21, Newport Corp.) records partial pulse energy to remove the fluctuation of laser pulses. The transmission beam, reshaped using an iris (GCM- 5702M, Daheng Optics Ltd.), pass through an optical spatial filter system (KT310/M, Thorlabs Inc.) to form a clean, spatially uniform, Gussian beam, enter a single mode fiber assisted with a free-space to fiber coupler (APFC-5T-FC, Zolix Instruments Ltd.), and is collimated via a fiber collimator (F220FC-532, Thorlabs Inc.). The collimated beam is delivered into a one-dimensional (1D) galvanometric scanner (GVS001, Thorlabs Inc.) and scanned on the back surface of an objective (RMS4, Thorlabs Inc.). The scanner is driven by a multifunctional data acquisition card (PCI-6731, National Instrument). To avoid the acoustic scanning and record the induced optoacoustic signals, a cylindrically transducer (V324-SU, Olympus IMS) with a focal length of 38 mm, a center frequency of 25 MHz, an active area of 6 mm, and a bandwidth of 75% is used. The optoacoustic signals are amplified by ~39dB using an amplifier (5073PR, Olympus IMS) and digitized by a data acquisition card (ATS-9325, AlazarTech Inc.) at a sampling rate of 200 MS/s. Depending on the optical and acoustic components used in this study, the system has a lateral resolution of 3.2 μm, an axial resolution of 60 μm, and a maximal B-scan rate of 200 Hz.
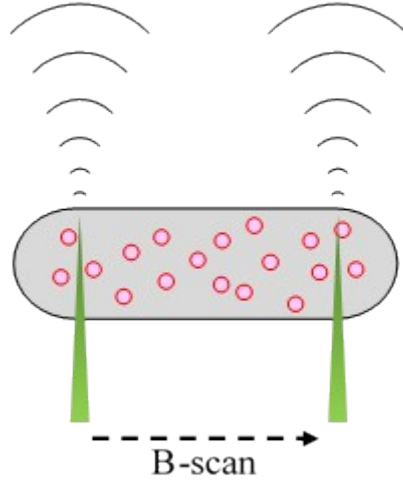
Figure S2 When laser beam penetrates the sample, it can be scattered and absorbed. The absorbed laser energy is converted into heat by vibrational and collisional relaxation. This produces an initial pressure increase and the subsequent emission of acoustic waves which propagate to the surface and can be detected by an ultrasound transducer. The signal generation mechanism can be regarded as one in which the optically induced initial pressure distribution $p_0$ is encoded onto a propagating acoustic wave which, upon detection by an ultrasound receiver located on the surface, can be converted to a time-resolved electrical signal [1]. If the laser pulse duration is much shorter than the thermal diffusion time, thermal diffusion can be neglected, and consequently, the thermal equation becomes [2]:

$$\rho Cp\frac{\partial T(r,t)}{\partial t} = H(r,t) \qquad (1)$$

where $\rho$ is the mass density; $C_p$ is the specific heat; $T(r,t)$ is the temperature rise due to the energy pumping pulse; the absorbed optical energy distribution $H(r,t)$ is effected by the local absorption coefficient $\mu_a(r)$. The two basic acoustic generation equations in an acoustically homogeneous medium are the linear inviscid force equation:

$$\rho\frac{\partial 2u(r,t)}{\partial t^2} = -\nabla p(r,t) \qquad (2)$$

and the expansion equation:

$$\nabla \cdot u(r,t) = -\frac{p(r,t)}{\rho c^2} + \beta T(r,t) \qquad (3)$$

Where $\beta$ is the isobaric volume expansion coefficient; $c$ is the sound speed; $u(r,t)$ is the acoustic displacement; and $p(r,t)$ is the acoustic pressure.

Combining (1)–(3), the pressure $p(r,t)$ produced by the heat source obeys the following equation:

$$\nabla^2 p(r,t) - \frac{1}{c^2}\frac{\partial 2}{\partial t^2}p(r,t) = -\frac{\beta}{C_p}\frac{\partial}{\partial t}H(r,t) \qquad (4)$$

[1] Paul Beard, "Biomedical photoacoustic imaging", *Interface Focus*, 1, 602-631,

2011.

[2] Minghua Xu and Lihong V. Wang, "Time-Domain Reconstruction for Thermoacoustic Tomography in a Spherical Geometry", *IEEE Transactions on Medical Imaging*, 21, 7, 814-822, 2002.

Supplementary videos:

Video S1: The flowing droplets at a flow ratio of $Q_d/Q_c$=1:9.

Video S2: The flowing droplets at a flow ratio of $Q_d/Q_c$=3:2.