Electronic Supplementary Material (ESI) for Lab on a Chip.

Droplet-based light-sheet fluorescent microscopy for high-throughput sample preparation, 3-D imaging and quantitative analysis on a chip

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Droplet microfluidic chip fabrication

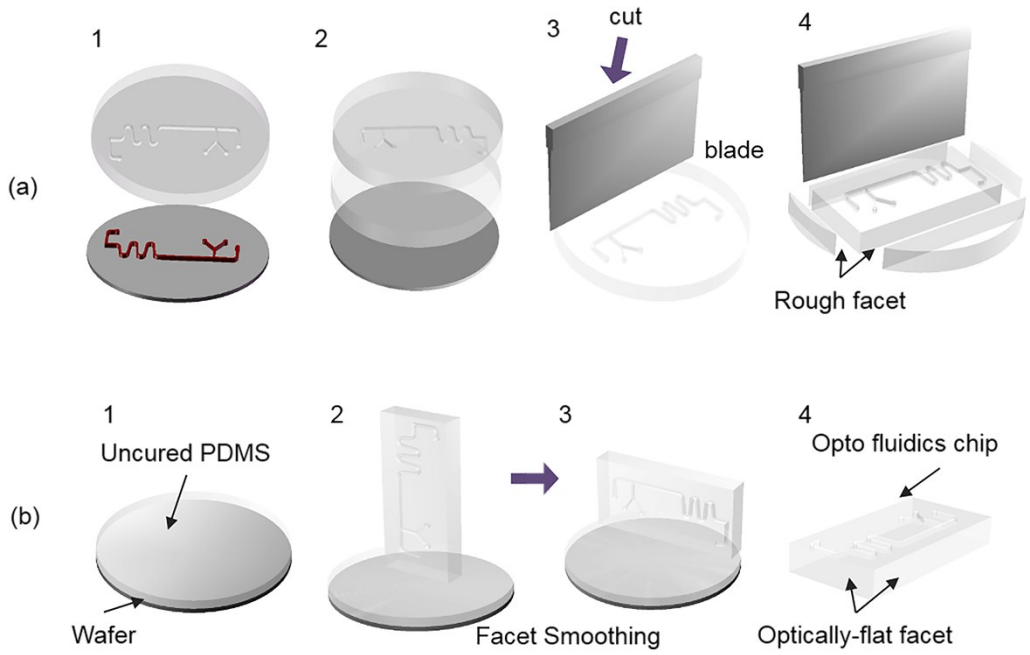

Fig. S1 The fabrication procedure of the droplet microfluidic chip. (a) We print the photo-masks on transparent films and made the mold through photolithography. The mold is made of positive relief patterns of SU-8 (MicroChem, Newton, MA, USA) photoresist on silicon wafers. We peel the cured PDMS replicas off the mold, punched the inlet holes (1), and bond them on a piece of blank PDMS substrate (thickness 1 mm) via oxygen-plasma surface treatment, sealing the channels inside a monolithic chip (2). We cut the PDMS slabs into rectangular chips using a razor blade in order to collect the plane fluorescence image through the side facets of the chip (3). The position of the side surfaces is important. Usually we choose to cut the facet very close to the L shape imaging region, leaving ~1mm space in between. In that way, first we are be able to use objective lenses with a short working distance to collect the light from the laser sectioned samples. Second, we could minimize the image aberration brought from the PDMS layer. However, the freshly cut surfaces are rough with blade marks, making the fluorescence collection inefficient due to the scattering loss (4). To reduce this loss at the air–PDMS interface, we perform an additional step to make the optically-flat facets in (b). We first spin-coat a thin layer (500 μm) of uncured PDMS on a silicon wafer (1). Then we place the PDMS chips with rough facets against the uncured PDMS, and cure at 80 ^{o}C for 30 min (3). We peel the whole piece off the wafer when the bonding PDMS layer is cured. A very flat layer is therefore bonded to the side facet seamless, making the whole surface optically flat (4). The chip is finally put into a desiccator for 1 hour gas salinization using TRIDECAFLUORO-1,1,2,2-TETRAHYDROOCTYL, to make the inner surface further hydrophobic for droplet formation.

Imaging through the side wall of PDMS chip before and after side-facet flattening

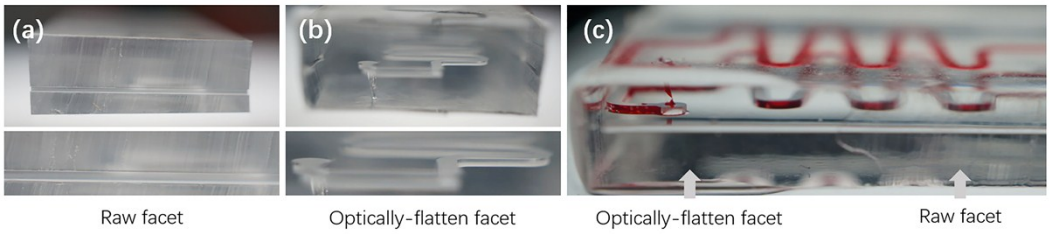

Fig. S2 Side facet flattening for clear imaging through the PDMS chip. (a) observing the channel through the raw facet (left) and optically-flatten facet (right). (b) Imaging the fluorescent micro-beads through the raw facet (left) and optically-flatten facet (right).

Pictures of our droplet light-sheet fluorescent microscopy platform

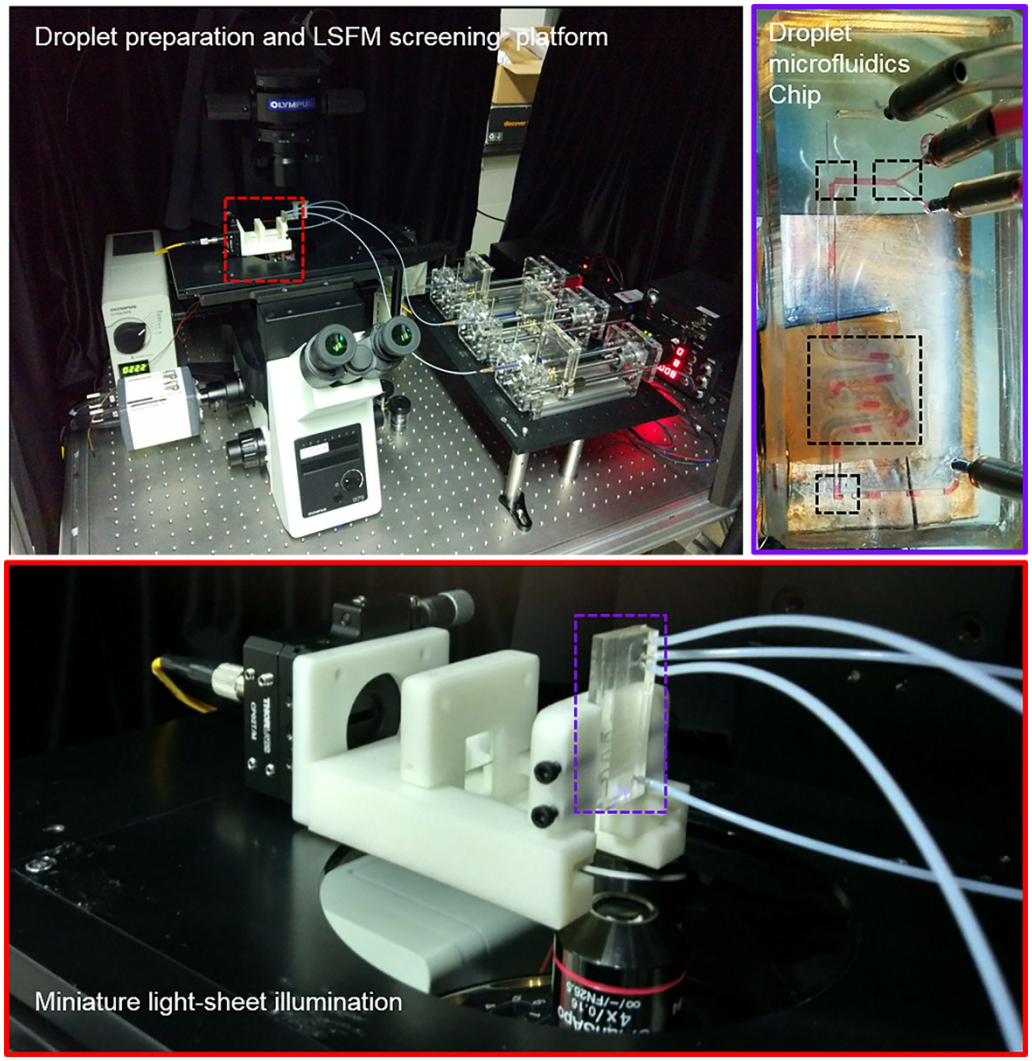

Fig. S3 High-throughput droplet light-sheet fluorescent microscopy platform. (a) The picture of the working system containing the portable droplet-LSFM device, inverted microscope and multi-channel syringe pump. (b) The miniature laser-sheet illumination part of the device consisting of a fiber laser/laser diode, a tunable slit and a cylindrical lens. The generated thin laser-sheet transversely penetrates the micro-channel of the chip which is vertically fixed on the device base. (c) the photograph of the fabricated droplet microfluidic chip.

All the parts used for our droplet-LSFM device are compact and cost-effective. Their complete information, including function, specification, price and vendor, are listed in Table. S1. The total cost of a fiber laser configuration is only around $800, which is affordable to many resource-limited environments.

Parts	Number	Unit price	Part note	Vendor	Total
Base track	1	$142.86	3D printing	Sogaa	$142.86
Lens holder	1				
Chip holder	1				
Screw mounting base	1				
Adjustable slit	1	$325.62	VA100C/M,Slit Width: 0 to 0.24" (0 to 6 mm)	Thorlabs	$325.62
Collimator	1	$181.60	F280FC-A, f = 18.07 mm, NA=0.15 FC/PC Fiber Collimation		$181.60
Cage plates	1	$26.30	CP02T/M,Threaded 30 mm Cage Plate, 0.50" Thick, 2 Retaining Rings, 8-32 Tap		$26.30
SM1-Threaded colllimator adapter	1	$36.07	AD12F,SM1-Threaded Adapter for Ø12 mm Cylindrical Components		$36.07
Construction rods	2	$6.19	ER1,1 inch long,dia 6 mm		$12.37
Cylindrical lens	1	$91.43	LJ1810L2-A,f = 25.00 mm		$91.43
Total					$816.25

Table. S1 The manufacturing parts for droplet-LSFM device. Table includes the parts' specifications, prices, manufacturing methods and vendors.

Image a flowing sample droplet using LSFM, wide-field epifluorescence microscope and wide-field epifluorescence plus 3D deconvolution

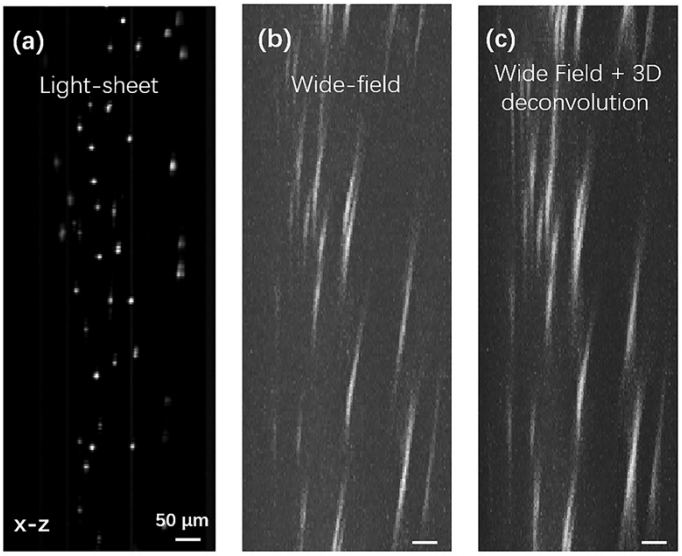

Fig. S4 Compare the imaging of a flowing droplet using LSFM (a), wide-field epifluorescence (b), and wide-field epifluorescence plus 3D deconvolution (c). The reconstructed x-z plane indicates the axial resolution, contrast and signal distortion of different methods.

Droplet-based LSFM provides motion-free, 3-D imaging of flowing droplet encapsulated with fluorescent micro-beads.

During imaging, to reduce the undesired motion of fluorescent beads caused by the convective flow's interference. We design a sufficiently long buffering channel after mixing, to slow down the convective flow before LSFM imaging. We also use an upright flow design (fix the chip vertically) to minimize the blurring of acquired image from gravity. Finally, the optimization of chip design, in conjunction with the high acquisition rate typically at 300-500 fps, can readily freeze the movement of the fluorescence beads in the droplet, providing clear sectional images as well as accurate 3-D reconstruction that is free from distortion. To verify this, we further image small fluorescent beads (which is more sensitive to motion blurring) using our droplet LSFM. The flow rate is 1000 μL/hr. The recorded raw image sequence (x-y, 300 fps) and the reconstructed plane (x-z) are provided in the additional figure below, proving a clear image capture and 3-D visualization of the flowing droplet. Of course, the convective flow and fluctuation of bead still physically exists. As we just use wide-field epifluorescence to image the droplet (equal to a much thicker optical sectioning), the beads are thereby exposed to the illumination for a much longer time (around 8 times longer compared to light-sheet illumination). Then we do observe slight movement/distortion of the bead (right part of the figure), hence confirming the need of sharp light-sheet illumination and high-speed acquisition here.

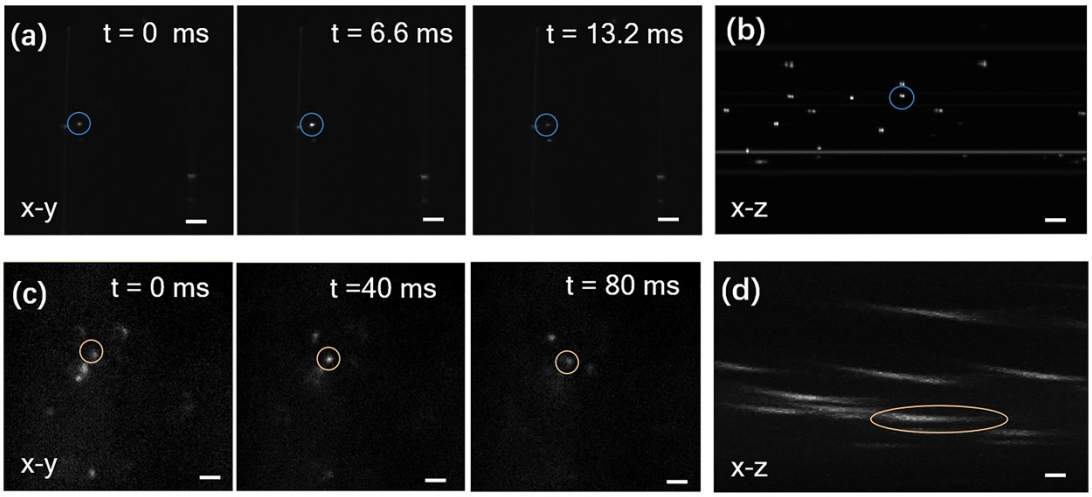

Fig. S5 Motion-free, 3-D imaging of flowing droplet by rapid Droplet-based LSFM (a) 3

consecutive frames of the recorded image sequence, proving a clear, motion-free capture of micro-beads. (b) the reconstructed x-z plane of the droplet, indicating an accurate 3-D reconstruction without distortion. (c)-(d) The comparison from wide-field epifluorescence. With much longer exposure to light illumination, slight motion blurring can be observed, confirming the need of sharp light-sheet illumination and high-speed acquisition here. Scale bars are 10 μm.

Validation of the quantification accuracy of droplet-based LSFM

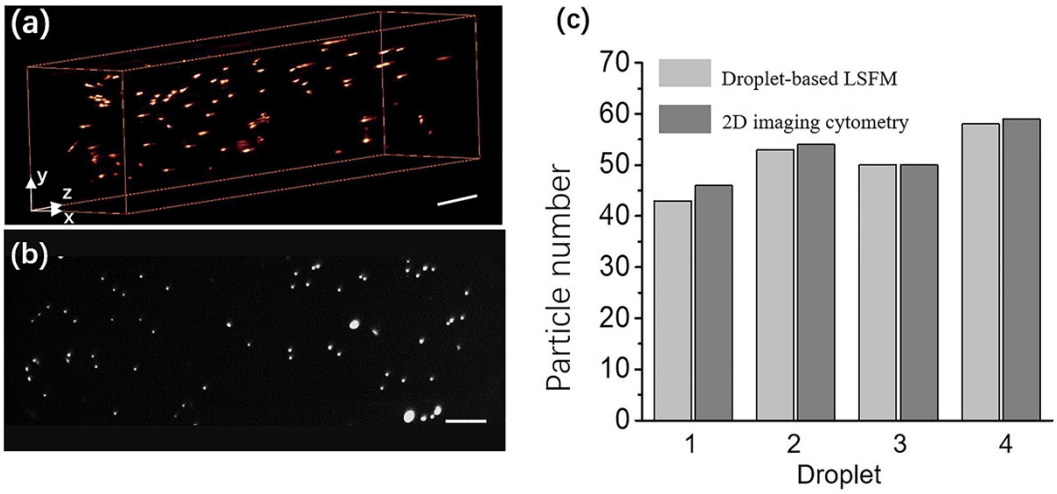

Fig. S6 Validating the counting accuracy of droplet-based LSFM through the comparison with standard 2-D imaging-based cytometry. (a) LSFM reconstruction of the droplet in 3 dimensions (b) standard wide-field imaging of the same droplet (c) Comparing 4 droplets' counting results by 2 approaches. They are all very close, with averaged variation rate measured ~3%. Scale bars are 100 μm.

Setting 1 Oil: 600 / Sample: 250 / Dilution:750 (μL/hr)		Setting 2 Oil: 600 / Sample: 500 / Dilution: 500(μL/hr)	
Status	Particle Number	Status	Particle Number
	77		146
	66		132
	46		207
	48		141
Average	59		156

Fig. S7 Tuning droplet sample conditions. In setting 1, The flow rates of FC-40 oil (carrier fluid), particle solution (sample) and diluting solution (red dye) are 600, 250 and 750 μl/hr, respectively. The resulting droplet sizes are stably at ~2.5 mm with 59 average particles encapsulated. In setting 2, the flow ratio between particle solution and diluting solution has been changed from 1:3 into 1:1 while keeping the total rates of oil and water phase at 600 and 1000 μl/hr. The size of the droplet remains unchanged because of a constant oil: water ratio. At the same time, the average number of particles packed inside each droplet greatly increases to 156, which means a close to three-times higher sample concentration. Through the control over infusion rate, we prepare droplet samples with desired size and concentration.

Supplementary Videos:

Video. S-1	The Implementation of high-throughput droplet-LSFM.
Video. S-2	Fast optical sectioning of droplets based on fluid self-scan.
Video. S-3	3-D reconstruction/visualization of serial sample droplets under flow control