

Microfluidic high-throughput encapsulation and hydrodynamic self-sorting of single cells

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We present a purely hydrodynamic method for the high-throughput encapsulation of single cells into picoliter droplets, and spontaneous self-sorting of these droplets. Encapsulation uses a cell-triggered Rayleigh–Plateau instability in a flow-focusing geometry, and self-sorting puts to work two extra hydrodynamic mechanisms: lateral drift of deformable objects in a shear flow, and sterically driven dispersion in a compressional flow. Encapsulation and sorting are achieved on-flight in continuous flow at a rate up to 160 cells per second. The whole process is robust and cost-effective, involving no optical or electrical discrimination, active sorting, flow switching, or moving parts. Successful encapsulation and sorting of 70–80% of the injected cell population into drops containing one and only one cell, with <1% contamination by empty droplets, is demonstrated. The system is also applied to the direct encapsulation and sorting of cancerous lymphocytes from a whole blood mixture, yielding individually encapsulated cancer cells with a >10,000-fold enrichment as compared with the initial mix. The method can be implemented in simple “soft lithography” chips, allowing for easy downstream coupling with microfluidic cell biology or molecular biology protocols.

droplets | Rayleigh–Plateau | size-fractionation | digital microfluidics

The postgenome era is stimulating a strong demand for high-throughput cell assays. Because genetically identical cells may display very heterogeneous behaviors (1, 2), bulk measurements on cell populations provide only partial information on cell metabolism, in particular from a dynamic point of view. Cell-to-cell variability is also of paramount importance for cancer research, developmental biology, drug screening (3), and stem cell research. Recent trends in cell biology thus put strong emphasis on studies at the single-cell level (4).

Microtechnologies raise the hope of dramatic breakthroughs in this field (5). For instance, compartmentalization of single cells in microchambers allows the analysis of stochastic protein expression at the single molecule level (6). In another approach, the capture of cells in microdroplets within double emulsions enables the screening of enzyme libraries with an unprecedented resolution and speed (7). In this perspective, combining encapsulation within droplets with microfluidic techniques may allow the observation and analysis of individual cells (e.g., with drugs or reagents) in a fully automated, time-resolved manner. These operations can be achieved using optical traps, but this approach remains complex and is hardly amenable to high throughput (8). In contrast, the use of classical continuous microdroplets generation techniques such as flow-focusing (9) or break-up at a T-junction (10) from a cell suspension offers potential for very high throughput, but could so far not warrant a controlled distribution of the cells in the drops (11). In these methods, the number of cells contained in the formed drops is dictated by the probability that a given volume of the initial cell suspension contains a given number of cells, following a Poisson distribution. It imposes a rather unfavorable compromise between the rate of encapsulation (total number of “positive drops,” containing one and only one cell, created per second) and the yield

(fraction of the initial cell population ending up in a positive drop). A suspension containing one cell per three drops volume on average is generally used, resulting in an $\approx 22\%$ rate of encapsulation for a 75% yield. To obtain reasonably pure populations of “positive” droplets, the steady-state generation of droplets from the suspension of cells is thus coupled with a detection and sorting of “positive” droplets by flow-cytometry-like technologies (12, 13). Active sorting can operate at very high frequencies, so that the presence of negative droplets does not raise serious problems regarding throughput, but it considerably increases the complexity and cost of the process. Also, in real systems, a perfectly random cell suspension is hard to achieve because of rapid sedimentation of cells in containers and pinning effects in microchannels (14). Therefore, the main issue when using these encapsulation devices in a routine mode lies in the number of droplets that contain no more than one cell. In summary, despite their strong interest for cell biology, current single-cell encapsulation processes remain delicate to tune and to maintain.

Here, we propose a different approach, relying entirely on passive hydrodynamic effects, in which picoliter droplets containing a single cell are prepared and self-sorted with high purity and yield. This system works even with highly concentrated suspensions, whatever the distribution of cells in the solution. It uses a triggered Rayleigh–Plateau instability in a jet flow (15), followed by shear-induced drift and excluded-volume-driven dispersion of the individual droplets. Its performance is characterized experimentally using a pure population of T-lymphocytes and described semi-quantitatively. We also demonstrate the robustness of the method for practical applications by directly encapsulating and sorting cancerous T-lymphocytes out of a whole-blood mixture. This method opens the route toward robust, low-cost and high-throughput molecular biology assays and diagnosis on single cells.

Design Principles

Fluidic Design. The device consists in (i) a flow focusing region (9); (ii) a narrowed straight channel; and (iii) an expansion finally splitting into two symmetric outlets (Fig. 1A). By convention, “right” and “left” sides of the microfluidic channels are referenced to the flow direction. The stream of culture medium containing the cells is introduced in the center channel and is focused into the narrow channel downstream by two co-flowing streams of oil. The flow rate of the left oil stream Q_o is twice that of the right oil stream $Q_o/2$, and the flow rate of the central

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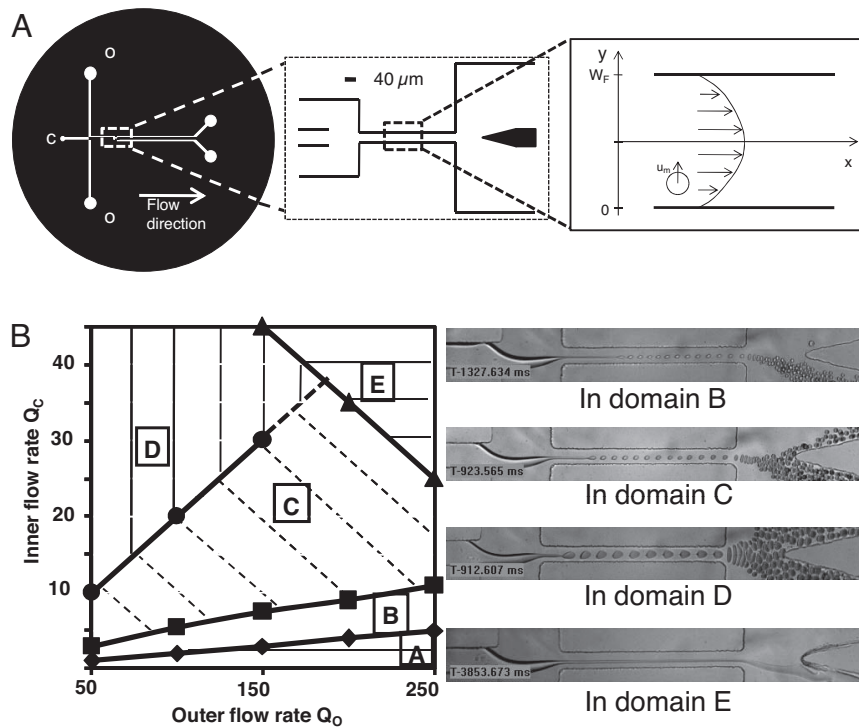


Fig. 1. Characterization of the experimental setup. (A) Device geometry (top view). Rectangular channels with a uniform height of $30\ \mu\text{m}$. (Left) Complete circuit, c and o correspond to the cell suspension and oil inlet respectively. (Center) Zone of interest. Scale bar is $40\ \mu\text{m}$. (Right) Central channel with the coordinates used for calculating droplet migrations. Inferior (y-coordinates between 0 and $W_F/2$) and superior (y-coordinates between $W_F/2$ and W_F) regions of these schematics are denoted as right and left, respectively, in the text. The inlets are located on the low value side of the x axis, whereas outlets are located on its high value side. (B) Experimental phase diagram for droplet formation in the absence of cells as a function of oil and cell culture medium flow rates, Q_o and Q_c ($\mu\text{l/h}$). For $Q_o < 50\ \mu\text{l/h}$, the device functions in dripping mode and individual droplets are formed without jetting. Domain A corresponds to unstable jetting. Above the filled triangles line (domain E), the cell medium stream is stable across the whole system. In the remainder of the diagram, the jet breaks at a stable position in the strait. The lines separating domains A–D correspond to different fixed droplet diameters: filled diamonds, $\Phi_N = 7\ \mu\text{m} \pm 10\%$; filled squares, $\Phi_N = 10\ \mu\text{m} \pm 10\%$; filled circles, $\Phi_N = 17\ \mu\text{m} \pm 10\%$. Micrographs in Right exemplify the flow stream's behavior in the different zones of the diagram.

stream, Q_c , is always kept lower than Q_o . As discussed below, the asymmetry of the oil flow is a key feature of the system, instrumental in the hydrodynamic sorting of positive droplets.

Phase Diagram for the Jet Instability. The hydrodynamic behavior of the system in the absence of cells (but with culture medium as the aqueous phase) is depicted in the phase diagram shown in

Fig. 1B. We attribute the unstable jetting in region A of the diagram to flow oscillations due to discrete steps in the functioning of syringe pumps at very low flow rates, combined with PDMS elasticity. This domain is not useful for encapsulation and will not be discussed further. In regions B–D of the diagram, monodisperse droplets are generated at rates $>1,000\ \text{s}^{-1}$ by jet break-up in the focusing channel. In regions B and C, the

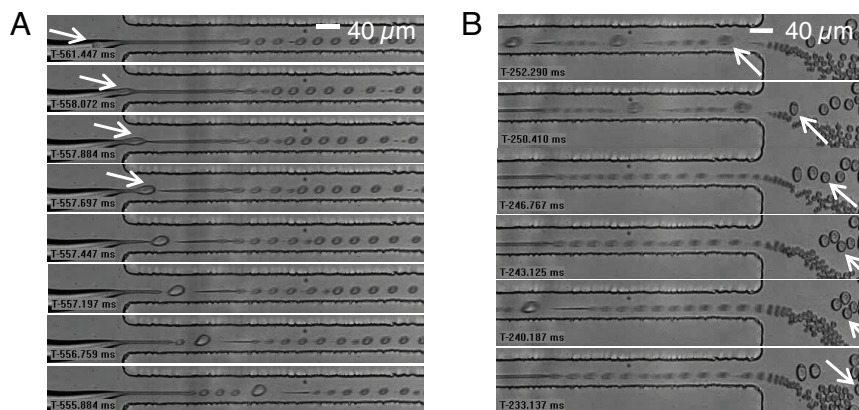


Fig. 2. Images from video sequences of cell encapsulation obtained by high-speed videomicroscopy (negative timescale). Scale bar for each image sequence is $40\ \mu\text{m}$. $Q_o = 150\ \mu\text{l/h}$; $Q_c = 8\ \mu\text{l/h}$. (A) Jet neck breaking by the passage of a cell (white arrow), and consecutive destabilization of the jet (triggered Rayleigh–Plateau instability, see also SI Movie 1). (B) Sorting of positive droplets. The white arrow points to the mechanism by which some positive drops (generally those smaller than the average size of positive drops) can be “lost” when a series of closely spaced positive drops exits the focusing region: the positive droplet marked by the arrow is pushed down by the train of larger ones that follows it (see also SI Movie 2).

2B). These interactions lead to an extra dispersion of their position in the exiting flow, and to the delivery of some of them toward the wrong outlet. This effect is increased when the cells concentration increases, but remains marginal. Indeed, even at a concentration of 10^8 cells per ml, positive droplets are most often separated by enough negative ones to render direct interaction between positive drops infrequent.

The recovery of positive drops is also affected by the inherent variability in size of the cell population: because the size of the final drop (and its consequent migration speed) is correlated with the size of the cell that initiated the Rayleigh–Plateau instability, smaller cells tend to exit the expansion in streamlines closer to the right side than larger ones. In the next section, we demonstrate how this effect can be used to sort the prepared droplets according to the size of the cells they contain. Specifically, we use it to sort cancerous cells out of a whole blood cell mix.

A further increase in throughput could be expected by simultaneously increasing the oil and cell medium flow rates. However, at oil flow rates above $200 \mu\text{l/h}$, the sorting efficiency decreases due to increased deformations of positive drops, which perturb droplet–droplet interactions in the channel expansion. In addition, these strong deformations may damage certain cell types. For instance, subjecting T lymphocytes to high shear stress (although for periods of time much higher than the typical few tens of ms involved here) has been demonstrated to affect their proliferative response (20). Therefore, we avoided in the present study flow regimes likely to induce such high shear stresses.

Cells were checked for integrity and viability during and after encapsulation using a $40\times$ objective. When using culture medium containing trypan blue, the fraction of cells collected in the outlet channel with a stained cytoplasm was equal, within experimental error, to that in the inlet (10%). This demonstrates that the cell membrane was not affected during the sorting. However, as compared with previous cell encapsulation methods, the present one leads to a small droplet volume. This is a definite advantage for the application of molecular biology protocols, for which dilution is detrimental. However, it is not advantageous for long-term cell viability, because the encapsulated cell must find in the aqueous drop the nutrients necessary for its survival. For combining this method with downstream protocols requiring live cells, it is important to add culture medium immediately after encapsulation. For this purpose, the present method can be associated with known droplet fusion methods (21). The surfactant used here (see *Materials and Methods*), which possesses an acid hydrophilic tail, may also be detrimental to the cell viability and interfere with some downstream cell biology or molecular biology protocols. In this case, it should be easy to transpose the technology to other biphasic systems, using e.g., a fluorinated oil with a PEG-terminated fluorosurfactant in a fluorosilanzed system, to ensure more extensive biological compatibility (22). As demonstrated below, the mechanisms at play in our system are purely physical and should depend on the viscosities and interfacial tension of the oil and water phase only. Thus, they can be transposed to different fluids by adapting these two parameters.

Theoretical Estimation of the Lateral Distance Traveled by Droplets of Different Sizes. A simple calculation for droplets shear induced migration can help understanding the striking positioning difference between positive and negative droplets in the focusing region. The behavior of deformable drops in a shear flow was described theoretically by Chan and Leal (16) for two simple geometries: a slit between two infinite walls and a circular pipe. The geometry of our strait does not correspond perfectly to any of these “solvable” cases, but we preferred to use an analytical solution clearly showing the physical processes at play rather than using difficult numerical simulations for biphasic flows (23). Thus, we model the focusing region as a slit between two infinite walls, separated by $W_F = 40 \mu\text{m}$. As shown in *SI Text*, using the

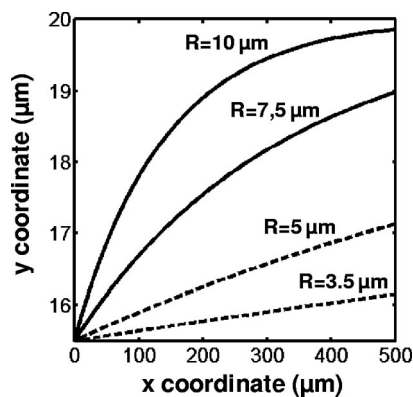


Fig. 4. Trajectories of droplets with various radii in the focusing channel as predicted by ref. 16, assuming a 2D parabolic flow, the droplet center of mass starting from $y = 15.5 \mu\text{m}$.

other analytical solution and modeling the strait by a cylinder produces a comparable behavior.

Assuming a laminar Poiseuille flow, mass conservation applied to the two oil streams implies a location of their interface at $\approx 3/8$ of the channel width (precisely $15.5 \mu\text{m}$ as measured from the right wall). Experimentally, droplets resulting from jet break-up are indeed initially located at this coordinate ($15 \pm 0.5 \mu\text{m}$, Fig. 2).

Following the steps outlined by Chan and Leal (ref. 16 and *SI Text*) and using the coordinates of Fig. 1A, one gets for the droplets trajectory in the slit:

$$16\alpha \frac{\eta_o}{\gamma} V_M \frac{a^3}{W_F^4} (x - x_o) = \frac{y^2 - y_o^2}{W_F^2} - \frac{y - y_o}{W_F} - \frac{1}{2} \left(1 - \kappa \frac{a^2}{W_F^2} \right) \ln \left(\frac{1 - 2\frac{y}{W_F}}{1 - 2\frac{y_o}{W_F}} \right), \quad [1]$$

where V_M is the maximum speed (at $y = W_F/2$), $\alpha \approx 0.66$ and $\kappa \approx 0.004$ are dimensionless coefficients depending on the dispersed and continuous phase viscosity ratio, η_o is the oil viscosity, γ is the interfacial tension between oil and aqueous solution, and a is the drop radius.

Numerical results of Eq. 1 for lateral distances traveled by droplets starting from $y_o = 15.5 \mu\text{m}$ and for $(x - x_o) = 500 \mu\text{m}$ are summarized on a plot of droplet trajectories in Fig. 4, for various drop radii and an oil flow rate $Q_o = 100 \mu\text{l/h}$ ($\gamma = 5 \text{ mN/m}$; $\eta_o = 120 \text{ mPa}\cdot\text{s}$).

These results correlate qualitatively well with our experimental observations. A clear-cut distinction appears between the positioning of negative drops (with a typical radius in the range of $3.5\text{--}5 \mu\text{m}$), and that of positive ones (with a typical radius between 7.5 and $10 \mu\text{m}$). Positive droplets are predicted to travel almost all of the way to the focusing region’s center, whereas negative ones are predicted to travel no more than half of this distance. Moreover, Eq. 1 highlights the dependence of the droplet migration amplitude upon the maximum speed of the oil in the channel, and therefore upon the oil flow rate. That might explain the improved efficiency of our system at high oil flow rates (i.e., $150 \mu\text{l/h}$) as compared with lower ones ($50 \mu\text{l/h}$).

It should be kept in mind that the theoretical model in ref. 16 assumes that the droplet dimension is small as compared with the microchannel’s size, and that droplet deformations are small. In our experiments, the largest droplets can reach $\approx 1/2$ of the channel’s dimension, and they visibly deform in the narrow

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