Sorting by Interfacial Tension (SIFT): Label-Free Enzyme Sorting Using Droplet Microfluidics

Daniel G. Horvath[1], Samuel Braza[1], Trevor Moore[1], Ching W. Pan[1], Lailai Zhu, [3,4]
On Shun Pak[2], Paul Abbyad[1]*

1 Department of Chemistry and Biochemistry, Santa Clara University, Santa Clara, CA, 95053, USA

2 Department of Mechanical Engineering, Santa Clara University, Santa Clara, CA, 95053, USA

3 Department of Mechanical and Aerospace Engineering, Princeton University, Princeton, NJ 08544, USA

4 KTH Mechanics, Stockholm, SE-10044, Sweden

* corresponding author:
Paul Abbyad
500 El Camino Real
Santa Clara, CA 95053
(408) 554-6948
pabbyad@scu.edu

Supplemental Information

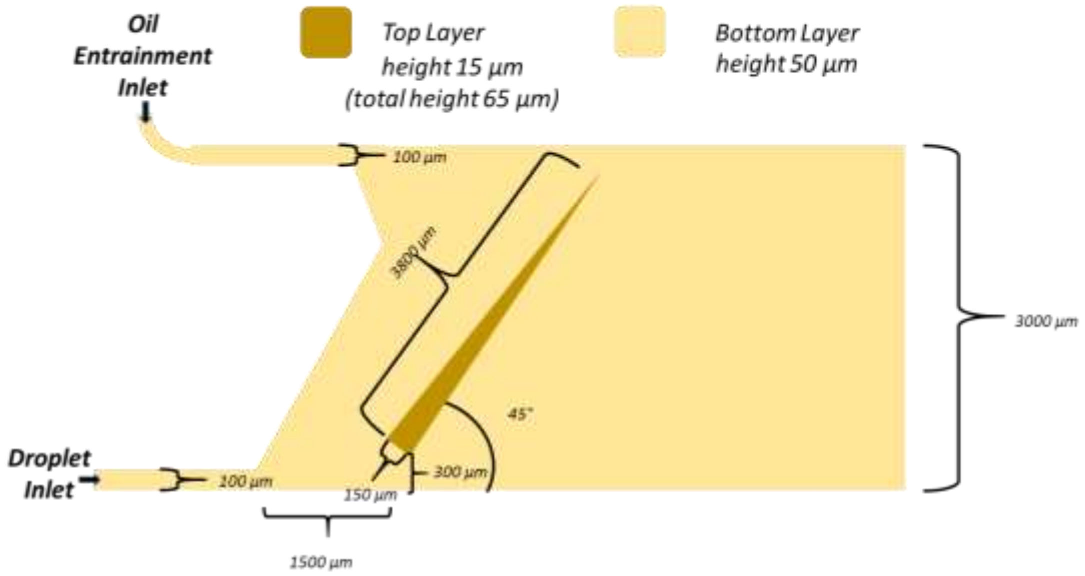
Table of Contents:

Video Caption:

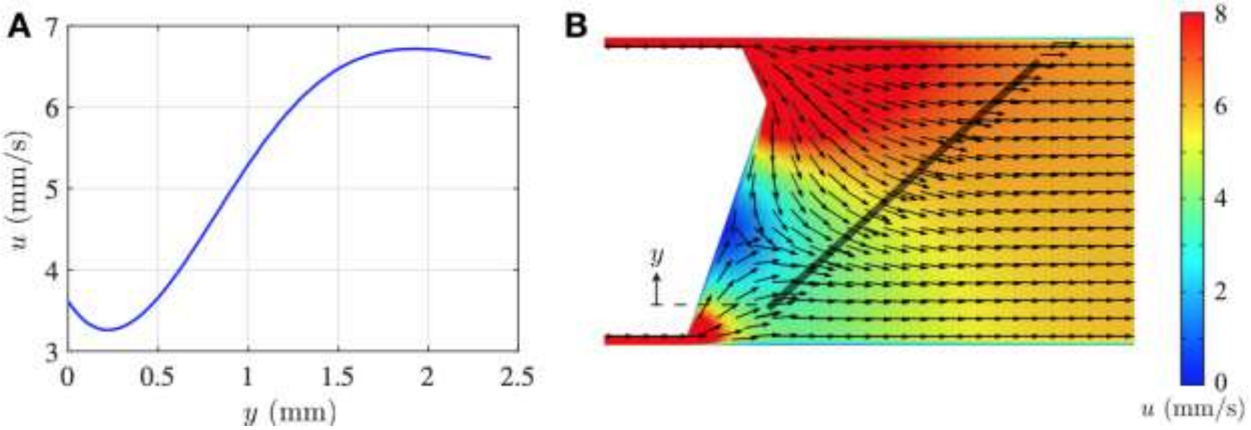
Video S-1. Selection of droplets of different pH using SIFT. White droplet at lower pH (pH = 6.90) of higher interfacial tension follow the rail upwards. Clear droplets at higher pH (pH = 7.47) are only slightly deflected by the rail. A small amount of fluorescein is added to the droplets at pH = 6.90 to identify the two droplet populations.

Video S-2. Movement of droplet from the enzymatic hydrolysis of phenyl acetate (0.102M) by porcine liver esterase (0.36µM) in PBS. Enzymatic formation of the product leads to an acidification of the droplet and a larger lateral displacement relative to the flow direction.

Video S-3. Selection of isozymes based on enzymatic activity for the hydrolysis of phenyl acetate (0.244M). White droplets contain Esterase Isoenzyme 4 while clear droplets contain Esterase Isoenzyme 5 (both at 3.75 µM). Esterase Isoenzyme 5 has greater activity producing more acidic product, leading to a lower droplet pH and higher interfacial tension. Esterase 5 droplets follow the rail and have a different lateral position in the channel as compared to droplets containing Isoenzyme 4 with lower enzyme activity. A small amount of fluorescein is added to the droplets containing Esterase Isoenzyme 4 to identify the two droplet populations.



Supplemental Figure S1. Sorting rail dimensions and geometry. Exact position of rail is approximate as layers are positioned by eye.



Supplemental Figure S2. a) Local flow speed *u* at varying locations along the rail. **b)** Distribution of the local flow speed *u* over the flow domain in the chip. A thick black line indicates the rail. The color represents the magnitude of the local flow speed. The flow speed presented here is at the mid-plane of the channel height and simulated with COMSOL Multiphysics (see Materials and Methods).

Estimation of Interfacial Tension to Hold Droplet on Rail: The magnitude of interfacial tension γ required to hold a droplet of radius R on the rail of depth H may be estimated by a simple balance of the magnitudes of the confinement force F_γ due to surface energy gradient and the fluid drag force F_D acting on the drop (Dangla *et al.*, 2011). We first approximate the magnitude of the confinement force as $F_\gamma \approx \Delta E/D$, which depends on the change in surface energy $\Delta E = \gamma \Delta A$ and the distance over which the energy changes, which we take as the drop diameter $D = 2R$ (Rashid *et al.*, 2019). To estimate the change in drop surface area $\Delta A = A_{out} - A_{in}$ inside and outside the rail, we assume the drop to be non-wetting and approximate its surface area as $A \approx H\pi(H + B\pi) + 2B^2\pi$, where $B = (D - H)/2$; the surface area of the drop inside the rail can be calculated similarly with the condition of conservation of drop volume. As an example, a drop has a typical diameter $D = 100$ µm when it is outside the rail in a channel of depth $H = 50$ µm; its diameter reduces to $D \approx 91.5$ µm when it resides inside a rail of depth H =65 µm. The change in surface area gives rises to a confinement force of magnitude $F_\gamma \approx \gamma \Delta A/D$, where $\Delta A/D = 13.5$ µm. To obtain the required interfacial tension γ to balance the fluid drag given a flow speed U, we estimate the drag on the drop in a Hele-Shaw flow as $F_D \approx \xi U$, where $\xi = 24\pi\mu \frac{R^2}{H}\left[1 + 2\frac{K_1(q)}{K_0(q)}\right]$ is the drag coefficient, $q = 2\sqrt{3}R/H$, and K_0 and K_1 are the modified Bessel functions of the second kind (de Ruiter *et al.*, 2014; Pit *et al.*, 2016). With a fluid viscosity of $\mu = 1.3$ mPa·s, we obtain the magnitude of drag as $F_D \approx \xi U$, where $\xi = 6.23$ µN·s/m. For a flow speed from $U = 3$ to 7 mm/s (supplemental Figure 1), the balance of $F_\gamma = F_D$ allows us to estimate that the minimum magnitude of interfacial tension required to hold the droplet on the rail ranges from $\gamma = 1.8$ to 3.2 dyne/cm. As a remark, this estimation assumes that the drop entirely resides inside the rail (i.e. the drop diameter is smaller than the width the rail). As the drop moves along the rail, which has a decreasing width, the change in drop surface area decrease and hence larger interfacial tensions will be required to continue to hold the drop on the rail.

Dangla, R., Lee, S., Baroud, C. N. Trapping microfluidic drops in wells of surface energy. *Phys. Rev. Lett.* **107,** 124501 (2011).

de Ruiter, R., Pit, A. M., de Oliveira, V. M., Duits, M. H. G., van den Ende, D., Mugele, F. Electrostatic potential wells for on-demand drop manipulation in microchannels. *Lab Chip* **14**, 883–891 (2014).

Pit, A. M., Bonestroo, S., Wijnperle, D., Duits, M. H. G., Mugele, F. Electrode-assisted trapping and relase of droplets on hydrophilic patches in a hydrophobic microchanel. *Microfluid Nanofluidics* **20**, 123 (2016).

Rashid, Z., Erten, A., Morova, B., Muradoglu, M., Jonas, A., Kiraz, A. Passive sorting of emulsion droplets with different interfacial properties using laser-pattern surfaces. *Microfluid Nanofluidics* **23**, 65 (2019).