

User-defined local stimulation of live tissue through a movable microfluidic port

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Contents:

- CAD file for device design (Separate file)
- SI Movie: Animation of the Slipchip for local delivery to a tissue slice (Separate file)
- Supplemental Methods
- Supplemental Figures

CAD File for Device design:

The file contains designs for layers in both components. For the delivery component the delivery channel, delivery port layer, and inlet layers; while the chamber component's port array layer, rigid layer and perfusion chamber are all provided.

SI Movie: Animation of the Slipchip for local delivery to a tissue slice.

The window media video file is an animation of the movements of the movable port device. The video begins with a labeled schematic of the device in a cross-sectional view. The top of the chip is slipped to align a port in the array with the delivery port below, and pneumatic pressure is applied, causing fluid flow (green) through the device into the tissue. Next, flow is stopped and the device is slipped away to end the first delivery. Then, the device is reconfigured into a new position by slipping to a previously unused port, and delivery occurs again. Finally the device is slipped back to the original resting position.

Supplemental Methods

Preparation of agarose and tissue slices

Both male and female C57BL/6 mice (Jackson Laboratories or Taconic, USA) were used while 7-16 weeks old. On the day of the experiment, the mice were anesthetized with isoflurane and euthanized by cervical dislocation. Six peripheral (inguinal, brachial, and axillary) lymph nodes were removed and placed in ice-cold DPBS (without Calcium or Magnesium 17-512F/24, Lonza, Walkerville MD, USA), for a brief recovery. The lymph nodes were embedded in 6% low melting point agarose prepared in 1 x PBS and chilled on ice, then punched out using a 10-mm tissue punch (World Precision). A vibratome (Lecia VT1000S, Bannockburn, IL, USA), set at a speed of 3.90 and a frequency of 3, was used to slice the tissue 300 μ m thick in ice-cold 1 x PBS. The slices were immersed in warm culture media (RPMI supplemented with 10% FBS, 1% L-glutamine an 1% Pen/Strep, 1% Non-essential amino acids, 50 μ M beta-Mercaptoethanol, 1 mM pyruvate, 20 mM HEPES (Fisher Scientific, Hampton NH, USA). All tissue slices were placed in a sterile incubator at 37°C with 5% CO₂ and allowed to recover for 1 hour before the start of experiments.

Fresh lymph node slices were immunostained for B cells before being used in delivery experiments. The procedure will be described in an upcoming publication. Briefly, non-specific staining was blocked using antibody CD16/32 (clone 93, Biolegend, San Diego CA, USA), and B cells were labeled with FITC-labeled CD45R/B220 antibody (clone RA3-6B2, Biolegend). Stained slices were washed thoroughly with 1x PBS and immediately used for experiments.

Slices of 6% agarose were prepared in a similar fashion, by casting agarose in a dish and slicing as above into 1x PBS. Agarose slices were stored at 4°C.

R_fOEG synthesis

Triethyleneglycol mono[1H,1H-perfluorooctyl]ether, R_fOEG, was synthesized using published protocols.³³⁻³⁵ R_fOEG was stored at a stock concentration of 20 mg/mL in FC-40 (Sigma Aldrich, St. Louis MO, USA) at -20°C. For experiments, R_fOEG was diluted to a working concentration of 0.5 mg/mL in FC-40 unless otherwise specified.

Interfacial tension and contact angle measurements

Interfacial tensions and contact angles were measured on a ramé-hart goniometer (model 200-00, ramé-hart instrument co., Succasunna NJ, USA). Interfacial tension was measured by generating a hanging drop of FC40 oil (density 1.855 g/mL) in aqueous saline (density 1.000 g/mL). A 100- μ L syringe (Hamilton 1710 with 26s gauge needle) was prepared with PTFE tubing and filled with FC-40 oil containing R_fOEG at 0, 0.1, 0.25, 0.5, or 1 mg/mL. The tip of the tubing was inserted into a cuvette of aqueous solution, typically 1x PBS containing Bovine serum albumin at 0, 0.1, or 1 mg/mL (BSA, Fatty Acid-free, Thermo Fisher Scientific, Hampton

NH, USA). Hanging droplets of 3 – 5 μL were generated by driving the syringe and were imaged using the goniometer. Droplets were analyzed using a pendant drop program³⁶ in ImageJ to calculate interfacial tension ($n = 5$ droplets per condition).

The contact angle for the three phase system of oil, aqueous, and fluorinated acrylic surface was measured using DROPimage Advanced software. A silianized piece of acrylic with a port attached by tubing to a syringe was submerged in FC-40 containing various concentrations of R_fOEG. Then a 3-5 μL aqueous droplet containing various amounts of BSA was formed in the oil by applying manual pressure to the syringe. Contact angle was measured for four droplets per condition.

Measurement of gap height

The height of the gap was measured using a ratiometric approach described previously.³⁰ BSA was labeled with DyLight 488 NHS ester (Thermo Fisher Scientific, Hampton NH, USA) according to manufacturer recommendations. The device was assembled and the channel was filled with 1 mg/mL labelled BSA in 1x PBS. The chamber component was slipped into a closed position, with the delivery port away from any array port, and the pump was started to force fluorescent BSA solution to spread into the gap. Images were collected of both the gap and the channel upstream of the chamber component, using a AxioZoom microscope (Carl Zeiss Microscopy, Germany) with an AxioCam 506 Mono camera (14-bit images). Assembly, delivery, and imaging were repeated 3 times.

Images were analyzed using Zen 2 software (Blue Edition, Zeiss) to determine the fluorescent intensity in the channel (FI_{ch}) minus background (FI_{cb}) and the gap, using linescans to average the intensity in each region. For each delivery image, 3 linescans were drawn across the interface of the BSA solution and FC-40, to measure the background fluorescent intensity (FI_{gb}) on the oil side of the interface and the fluorescent intensity of the gap (FI_g) on the BSA side. The channel depth was independently measured to be 155 μm (H_{ch}). The height of the gap (H_g) was calculated to be $14.1 \pm 2.3 \mu\text{m}$ ($n = 3$ independent measurements) from equation 1:

$$H_g = \frac{(FI_g - FI_{gb}) * H_{ch}}{(FI_{ch} - FI_{cb})} \quad [1].$$

Validation of tissue viability after delivery

To assess the viability of tissue slices after delivery on the movable port device, slices were analyzed by flow cytometry. Analysis was performed on lymph node slices from 1 female mouse and 1 male mouse, analyzed on consecutive days. For treatment on the chip, each slice was placed in the device and treated with a 5-s delivery of labeled 10-kDa dextran, then cultured for 3 hr at 37 °C at 5% CO₂ in complete media ($n = 12$). Untreated control slices were maintained in culture for the same period, but were never treated on the chip ($n=12$). Slices were crushed through a 70- μm filter and centrifuged (Sorvall ST40R Centrifuge, Fisher Scientific) at

400G for 5 min. Cells were resuspended in PBS with 2% serum (VWR). Cells from an additional subset of untreated control slices were killed with 35% ethanol for 10 mins (n = 12), to serve as a negative control. All cell suspensions were stained with two live-dead discriminator dyes, 0.01 μM Calcein AM (BD USA/564061) and 0.5 $\mu\text{g/mL}$ 7-AAD (Biolegend USA/420404). Cell viability was analyzed using a FACS Calibur flow cytometer (Table S3), acquiring data using BD CellQuest Pro 5.1 software. Suspensions of fresh murine splenocytes were prepared at 1 million cells/mL and stained with the same concentrations of Calcein AM or 7-AAD, to serve as single-stained and unstained controls. Compensation was performed using FCS Express 6 software after gating the cells on scatter. Positive and negative ranges of signal for each dye were set as shown in Figure S7. Compensation matrices were calculated based on median fluorescent intensity of controls.

Dependence of the spread of analyte on time and flow rate

To fit the curve of the spread of analyte with respect to time and flow rate in Figure 4, we considered the volume delivered per unit time:

$$\left(\frac{1}{4}\right)\pi w^2 h = Q\Delta t \quad [2],$$

we approximated the delivery volume to be a cylinder, where w [μm] is the width of the delivery, h [μm] is the height of the sample, Q [$\mu\text{L}/\text{min}$] is the volumetric flow rate and Δt [sec] is the length of time of delivery. Solving for width gives Eq. 3:

$$w = 2\sqrt{\frac{QT}{\pi h}} \quad [3].$$

Supplementary Figures and Tables:

Table S1: Capillary pressure calculations for conditions tested in Figure 3b. To vary the capillary pressure, both the concentration of RfOEG in FC-40 oil and BSA in 1x PBS was varied. ΔP_c was calculated from Eq. 1 in main text.

| Concentration of RfOEG (mg/mL) | Concentration of BSA (mg/mL) | Interfacial tension γ (mN/m) | Contact angle θ (degrees) | Calculated ΔP_c (Pa) ^a |
|--------------------------------|------------------------------|-------------------------------------|----------------------------------|---|
| 0 | 0 | 46.0 | 137.9 | -4.85×10^3 |
| 0.25 | 0 | 45.9 | 139.0 | -4.91×10^3 |
| 0.5 | 0 | 41.6 | 129.8 | -3.78×10^3 |
| 1 | 0 | 27.2 | 107.3 | -1.15×10^3 |
| 0.5 | 0.1 | 13.7 | 132.8 | -1.32×10^3 |
| 0.5 | 1 | 11.4 | 127.6 | -9.90×10^2 |

^a The gap height was set to 14.1 μm for this calculation.

Table S2: Calculation of pressure due to flow resistance for varied flow rates in Figure 3b. ΔP_r was calculated according to Eq. 2 and 3 in the main text.

| Flow rate Q (m^3/s) | Calculated ΔP_r (Pa) ^b |
|---|---|
| 8.33×10^{-12} | 0.68 |
| 1.67×10^{-11} | 1.36 |
| 3.33×10^{-11} | 2.72 |
| 6.67×10^{-11} | 5.43 |

^b The viscosity used in this calculation was 1.00×10^{-3} Pa s, the port length was 200 μm , and the port radius was approximated as 100 μm .

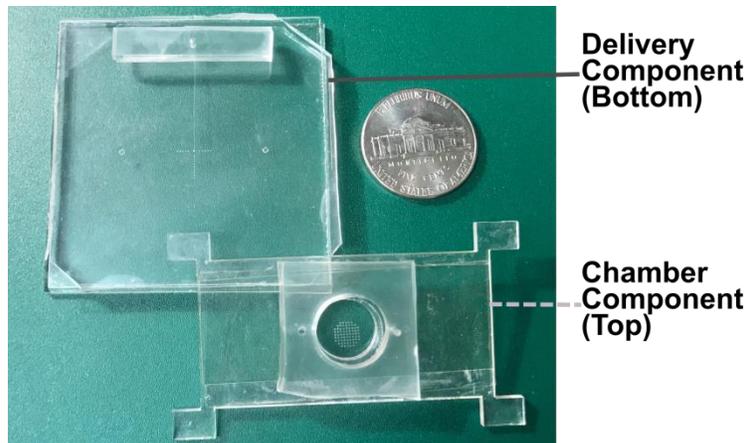


Figure S1: An image of a disassembled device. The two components (delivery and chamber components) are labeled in the above image. The chamber component is resting on top of the corner of the delivery component. A United States nickel was used for size reference.

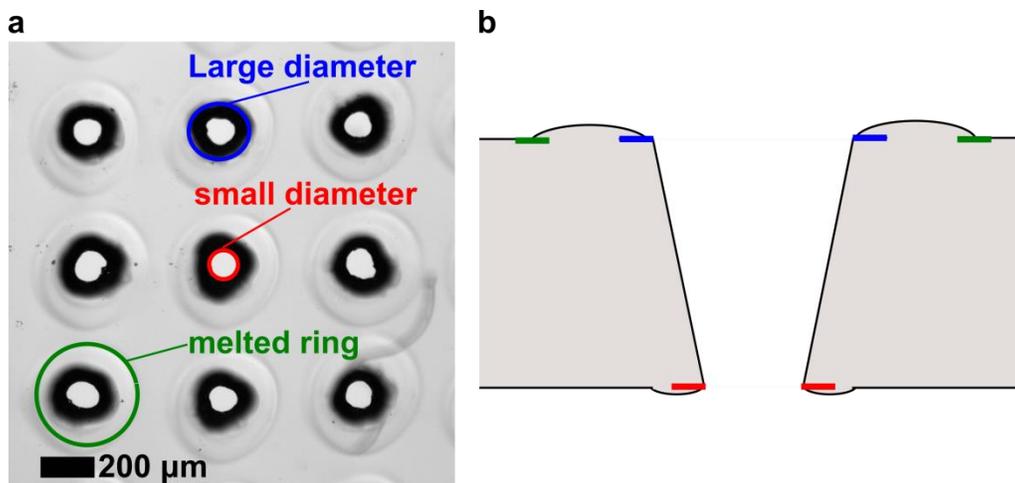


Figure S2: Acrylic port array after laser etching. a) A micrograph shows that each port is surrounded by a ring of melted acrylic (outlined in green). The ports have a conical cross section; the large diameters (blue) were closest to the laser during etching, and ranged in size from 90-110 μm . The smaller diameters (red) ranged from 70-90 μm . b) A side-view depicting the three highlighted regions of the conical port in the respective colors.

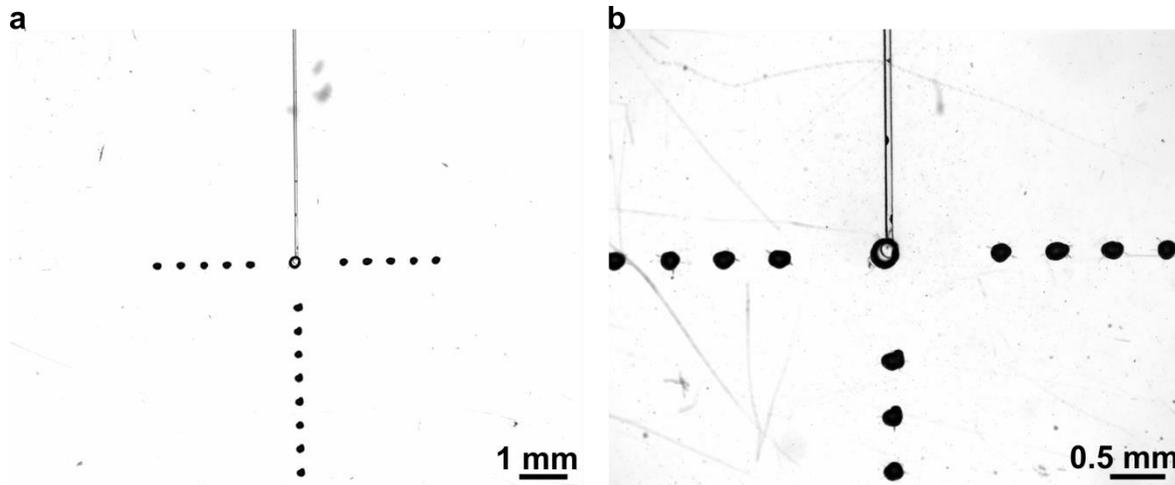


Figure S3: Micrographs of the alignment marks in the delivery port layer. a) A micrograph showing the entire cross hair of alignment marks. These marks are pitched 500 μm apart so that they align with adjacent ports in the port array layer. b) The alignment marks were designed to be partially etched through the acrylic. This design prevents air from becoming trapped within the alignment marks during assembly of the device.

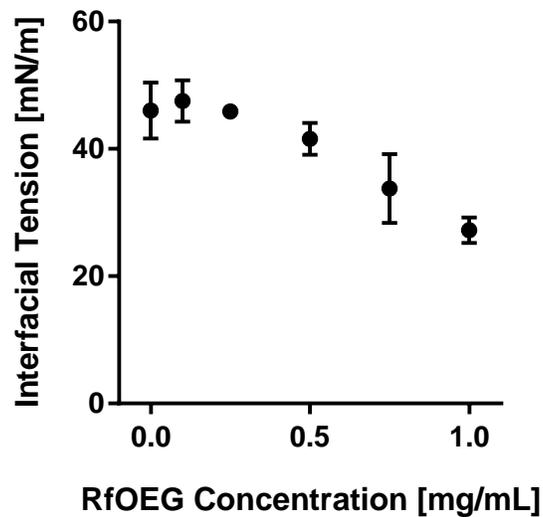


Figure S4: Interfacial tension of 1xPBS and FC-40 oil as a function of the concentration of surfactant, R_fOEG. The mean \pm std dev is plotted, showing that as R_fOEG concentration increased, the interfacial tension decreased as expected. (n = 5)

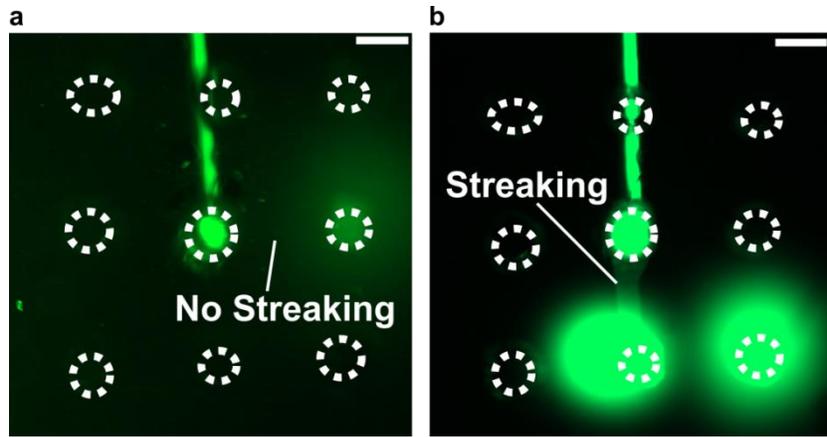


Figure S5: At low concentrations of BSA in the aqueous phase, the top surface of the gap was not wetted, while at high concentrations, the top surface was wetted. a) At 0.05 mg/mL BSA, no wetting of the surfaces was observed, and there was no streak of fluorescent signal after slipping. Here, the delivery port was just slipped to the center port from the port to its right. b) At 1 mg/mL of BSA, a streak of fluorescence appeared when the port was slipped from the previous port below it. The contrast in the two images is not scaled the same, in order to make the streak visible. The scale bar is 200 μm .

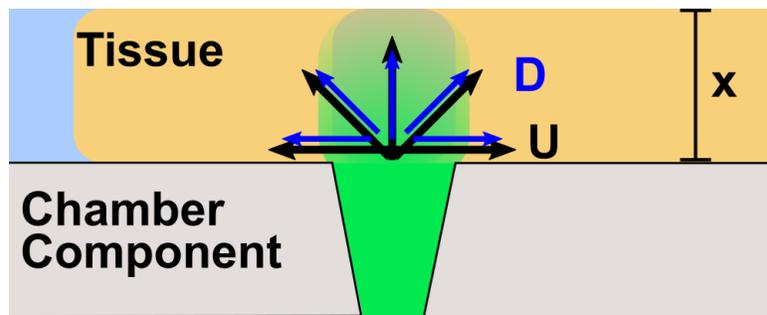


Figure S6: An illustration of the geometry used to calculate the Peclét number. D [m^2/s] is the diffusion coefficient of the analyte through the tissue matrix. U [m/s] is flow velocity through the tissue. The diffusion distance, x [m], was set to the thickness of the tissue (300 μm).

Table S3. Laser and emission filter configuration used for flow cytometry analysis of viability for crushed slices (Figure 5). Instrument: BD FACS Calibur.

| Fluorochrome | Laser line | Emission filter |
|--------------|------------|-----------------|
| Calcein AM | 488 nm | 530/30 |
| 7-AAD | 488 nm | 670LP |

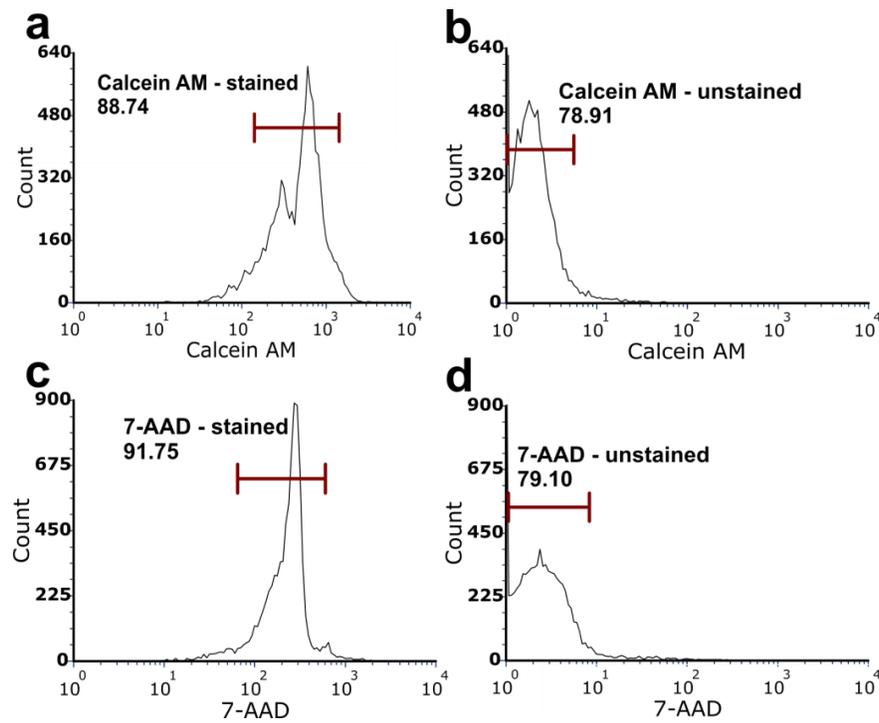


Figure S7: Histograms for software compensation of flow cytometry samples. Histograms were manually adjusted for software compensation to be centered on the single-stained positive (Figure S7a, c) and unstained negative (Figure S7b, d) populations for each dye.