



Supporting Information

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One Cell, One Drop, One Click: Hybrid Microfluidics for
Mammalian Single Cell Isolation

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Supplementary Note 1: Device channel and electrode design

Optimizing single-cell trapping and encapsulation was done by designing the channel microfluidics device following hydrodynamic resistance (R_h)^[1] and using streamline based design rules^[2].

$\Delta P = R_h Q$, with R_h the hydrodynamic resistance, Q the volumetric flow rate and ΔP the pressure drop in the channel. The hydrodynamic resistance can be described as follows:

$$R_h = \frac{12\mu L}{wh^3(1 - 0.63\frac{h}{w})}$$

We define three hydraulic resistances: The trap constriction R_2 , the trap R_3 , and the bypass channel R_1 (Supplementary Figure 2).

Determining the width of the opening of the trap, the depth of the trap, the height of the channel, and the length of the bypass, we can calculate the three resistances. Before the cells are loaded, we aim for $R_2 + R_3 > R_1$, to encourage cell trapping. When a cell is trapped, we want $R_2 + R_3 \leq R_1$, to encourage $Q_1 > Q_{2+3}$ (or $Q_{bypass} > Q_{trap}$) while taking into account R_3 increases when a cell is trapped. Taking this into account, we looked at the velocity streamline profile and positioned the trap near the curvature of the main channel (i.e. the end of a serpentine channel) along with narrowing the width of channel near trap to improve cell trapping.

For the electrode design, four electrodes were sufficient to perform desired operations. A common ground electrode wire going through the center of the trap was chosen to act as a ground in case all four electrodes receive a potential. Interdigitation was used and electrodes have a minimum gap of 14 μm . The wiring has a thickness of 20 μm . The wiring density through channels is minimized, as actuations through wiring can manipulate the flow. This limited us to only equip 6 out of 12 traps with 4 electrodes each.

Supplementary Note 2: Computational fluid dynamics and electric field simulations

The model portraying the channel geometry with flow velocity profile, velocity streamlines and pressure was stationary simulated using COMSOL Multiphysics 2D creeping flow physics. Inertia and turbulence were neglected, and no slip was set for channel walls. The fluid within the channels was PBS, the boundaries were PDMS.

Conditions used for modeling:

$P = 0$ Pa for outlet pressure

$\mathbf{u} = \begin{cases} x = 0 \\ y = 0 \end{cases} \text{ m s}^{-1}$ for initial velocity profile

$V_0 = 0.0008 \text{ } \mu\text{L s}^{-1}$ for volumetric laminar inflow

The model portraying the electric field generated by different actuation patterns, was simulated in COMSOL Multiphysics with a static electricity module (Supplementary Figure 6). Electrodes were modeled as 2D surfaces without thickness, under a 7 μm SU-8 surface, covered with an HFE oil of 30 μm thickness (Figure 1). The top PDMS features on the device were omitted from the simulation.

Conditions used for modeling the electric field above the dielectric:

$T = 293.15$ K is the temperature

As potential V_0 :

1.8 $V_{pp} = 142 V_{RMS}$ for droplet generation

1.6 $V_{pp} = 126 V_{RMS}$ for droplet release, keep and encapsulate

And with ground potential and initial potential $V = 0$ V

The model was static, stationary and materials were continuous. For each model, the not actuated electrodes were selected as ground.

Under static conditions, the electric potential, V , is defined by the relationship

$$\mathbf{E} = -\nabla V$$

and the electric displacement or flux density \mathbf{D} is defined by the relationship

$$\mathbf{D} = \epsilon_0 \mathbf{E} + \mathbf{P}$$

with the electric constant ϵ_0 , electric field \mathbf{E} , and \mathbf{P} .

Following Gauss' Law:

$$-\nabla(\epsilon_0 \nabla V - \mathbf{P}) = \rho$$

With electric field \mathbf{E} , tangential to the xy-plane (3D) this becomes:

$$-\nabla d(\nabla V - \mathbf{P}) = \rho$$

with d and ρ the total electric charge density.

In COMSOL, the magnitude of the electric vector field (V m^{-1}) is calculated with:

$$E = \sqrt{E_x^2 + E_y^2 + E_z^2}$$

Table 1. Parameters for multi-physics modeling

Property	Value
SU8-5 Relative Permittivity	4.5
PDMS Relative permittivity	2.75
PDMS density	970 [kg m ⁻³]
PBS density	1000 [kg m ⁻³]
PBS dynamic viscosity	0.0008882 [Pa s]
HFE 7500 oil relative permittivity	5.8
HFE 7500 oil boundary size	500x 500 [μm ²]
Dielectric thickness SU-8 5	7.0 [μm]
SU-8 5 boundary size	500 x 500 [μm ²]
Electrode sizes	110 x 110 and 110 x 40 [μm ²]
Ground electrode size	10 [μm]
Electrode gap width	14 [μm]

Supplementary Note 3: Automation setup and GUI operation for trapping, encapsulation and release

Software that we used for this work can be found here: <http://bitbucket.org/shihmicrolab>

The microfluidics device was placed inside a 3D printed pogo pin PCB board holder of which the base plate fits on the stage of an inverted microscope (Olympus IX7) ^[3]. The flow was observed under a 4X or 10X objective under bright-field illumination. A neMESYS Low Pressure pump (Cetoni) with five syringe units, and an Arduino Uno were connected to a PC, and operated through Python 2.7 GUI (see Supplementary Figure 3C). The Arduino Uno was connected to a stack of 120 optocouplers, powered by a 5 V_{DC} power supply. A 15 kHz sinusoidal signal (function generator) was amplified by 100 times. All droplet operations were controlled using the GUI.

For trapping, encapsulation, and release, below describes the automation/manual controls that were used:

1. Priming → enter flow rate and press Start. Stop flow by pressing Stop.
2. Manually remove tubing that was used for priming and insert tubing for cell solution.
3. Flowing cell solution → enter flow rate and press Start. Stop flow by pressing Stop.
3. Manually insert tubing between I1 and O3 (see Figure 2) in preparation for encapsulation and release.
4. User observes mCherry expression in single clones under the microscope by excitation (XLED power source)
5. Encapsulation → enter flow rate for oil and press Start. User observes oil flow until the flow reaches the trap. Once reached, enter trap number and click on 'Encapsulate' on GUI.
6. Release → enter trap number, check 'forward' tickbox and click 'Release'.

Supplementary Note 4 : Device assembly and channel treatment

Novec 1720 contains a fluorosilane polymer surfactant dissolved in an ether solution. It is volatile solvent and ideal to avoid remaining surfactant clogs in the traps. ^[4] This solvent swells PDMS, and care should be taken to follow this procedure accordingly. Clean PDMS channel slabs are treated with oxygen plasma for 30 s and directly aligned on top of a clean dielectric coated electrode patterned glass, under a microscope (4X). The device is sealed with transparent adhesive tape and pressure is applied. Immediately, treat the device channels for 20 min with Novec 1720 and dry for 20 min at room temperature. Bake device at 150 °C for 30 min, while applying 750 g weight on the device. For the droplet bridge, a 2 cm piece of PEEK tubing is treated with Novec 1720 for 20 min by flowing it through.

To reuse of devices, flush the device with Fluorinert FC-40 to remove the oil containing surfactant in the traps, and then bake at 100 °C for 2 h. If actuations took place, wash the device with FC-40, treat the device with Novec 1720 as before. Chips not used for 14 days should be treated again with Novec 1720. If needed, chips can be washed with a 1% Triton X-100 solution to flush out debris, and then continue with an FC-40 wash.

Table 2. Additional device statistics

Dispersed	Continuous	Contact angle (°)	sd	N
PBS	HFE 7500 + 2% Ran	139.55	4.90	10
DI Water	Air	128.97	4.53	10
Droplet volume		Volume (pL)	sd	N
After single-cell encapsulation		150.25	55.58	11
On-demand droplet-generation		250.90	39	44
Before entering droplet bridge		76.19	9.41	10
After exiting droplet bridge		72.97	4.59	10

Table 3. Cells and plasmids

Cells Lines	Transgene integration	Source
NCI-H1299	Luciferase, eGFP, KanR	Genecopeia SL001
MCF-7	N/A	Donated by Piekny Lab
Plasmids	Relevant characteristics	Source
pCRISPR_eGFP_497	AmpR, Neo/KanR	Addgene #111824
pCRISPR_RAF1_94	AmpR, Neo/KanR	Addgene #111824
All_in_one_CRISPR/Cas9_LacZ	AmpR	Addgene #74293

Table 4. Primers for genomic cleavage detection

Targete d gene	Name	Orientation	Primer Sequence (5' to 3')
eGFP	P1	Forward	TGACCACCCTGACCTACGG
eGFP	P2	Reverse	ATGTGATCGCGCTTCTCGTT
RAF1	P3	Forward	CGATTACCGAGTGCCTCTCC
RAF1	P4	Reverse	CCGCATCGTAGCAAACGC

Table 5. Expected cleavage band size for GCD.

Target Gene	Primer pair	Amplicon	Amplicon Size	Expected cleavage band size
eGFP	P1 P2	TGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTA CCCCACCACATGAAGCAGCAGCACTTCTTCAAGTCCGCC ATGCCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCA AGGACGACG GCAACTACAAGACCCGCGCCG AGGTGAA GTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAA GGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCA CAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATAT CATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTT CAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCT CGCCGACCACTACCAGCAGAACACCCCATCGGCGACGG CCCCCTGCTGCTGCCCCGACAACCACTACCTGAGCACCCAG TCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCAC AT	472	132, 340
RAF1	P3 P4	CGATTACCGAGTGCCTCTCCTGAAAGCAAGTCAGCGTCGC CTAACCTCTTCAGCTTCGAAATGGCGGCCACCAGATCGCT AGGCCACGCCCCGGGGGCGGGGCTGAGTTCAGGCCAGA GCGATGGATGCCGAGCCAAGTTAGAAGTCGACTGCCAG TAGGGCTCGCGCAGAATCGGAGAGCCGGTGGCGTCGCAG GTCGGGAGGACGAGCACCGAGTCGAGGGCTCGCTCGTCT GG GCCGCCGAGAGTCTTAATCG CGGGCGCTTGGGCCG CCATCTTAGATGGCGGGAGTAAGAGGAAAACGATTGTGA GGCGGGAACGGCTTTCTGCTGCCTTTTTTGGGCCCCGAAA AGGGTCAGCTGGCCGGGCTTTGGGGCGCGTGCCTGAGG CGCGGAGCGCGTTTGCTACGATGCGG	418	241, 177

Table 6. Three parameter logistic regression model. Parameters and the Hosmer-Lemeshow goodness of fit statistic of each fitted model.

$$y = \frac{A}{1 + e^{\frac{B-x}{C}}}$$

Data	Asymptote (A)	Inflection point (B)	Scale (C)	P (Goodness of fit)
Forward flow on-demand droplet release	1.1646	3.23	-1.92	0.9888
Reverse flow Hydrodynamic droplet release	0.96059	5.68	1.73	0.9616
Reverse flow on demand droplet keeping	0.80613	64.01	-5.87	0.9967

References

- [1] M. Zimmermann, H. Schmid, P. Hunziker, E. Delamarche, *Lab Chip* **2006**, 7, 119.
- [2] E. Brouzes, M. Medkova, N. Savenelli, D. Marran, M. Twardowski, J. B. Hutchison, J. M. Rothberg, D. R. Link, N. Perrimon, M. L. Samuels, *PNAS* **2009**, 106, 14195.
- [3] F. Ahmadi, K. Samlali, P. Q. N. Vo, S. C. C. Shih, *Lab Chip* **2019**, 19, 524.
- [4] M. Sauzade, E. Brouzes, *Lab Chip* **2017**, 17, 2186.

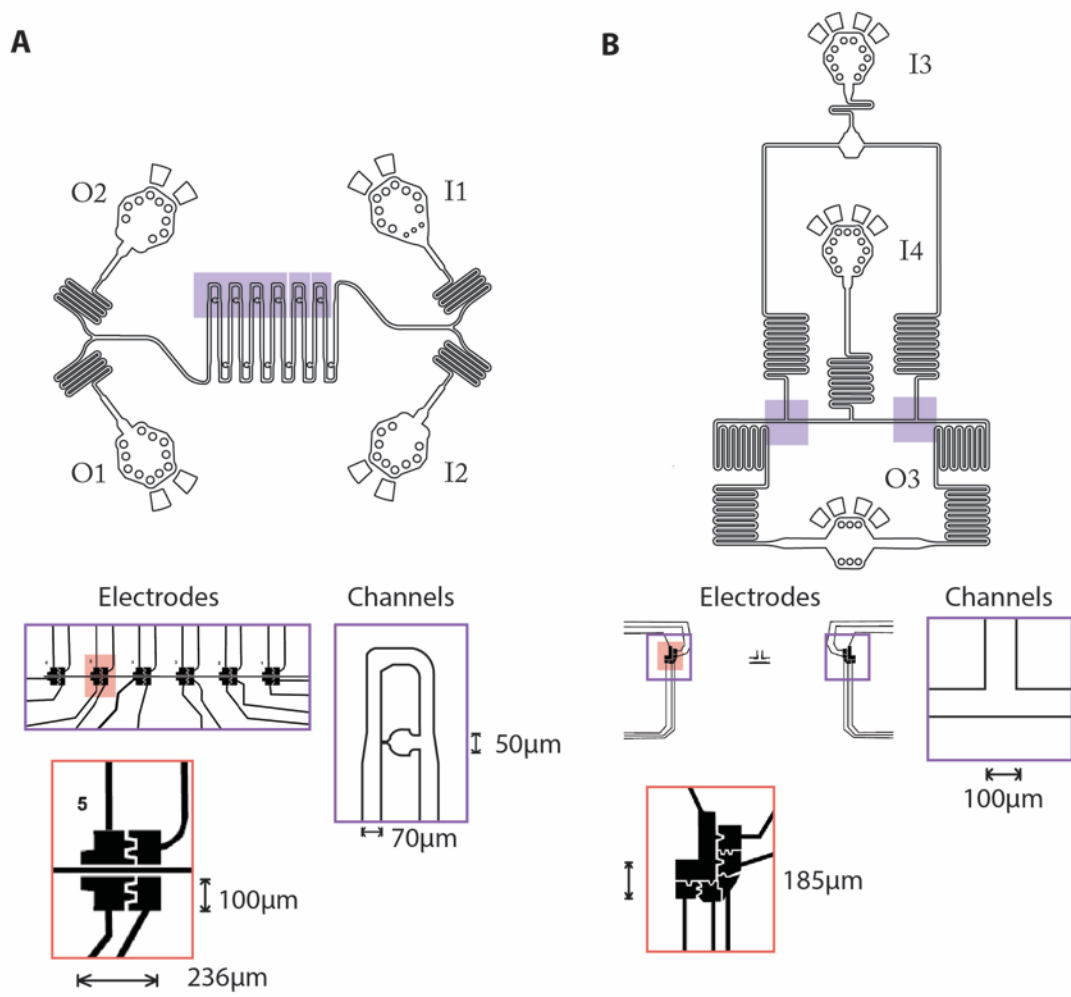


Figure 1

Device electrode and channel geometry. The device consists two separate PDMS layers, a serpentine trapping channel and a droplet generator. A) The serpentine trapping channel contains two inlets (I1, I2) and two outlets (O1, O2). The channel width is 70 μm with bypass channel width of 50 μm , and a height of 35 μm . Highlighted top area of 6 traps is aligned with electrodes (enlarged). B) The droplet generator has two inlets (I3, I4) and one outlet (O3). The channel width is 100 μm , and height is 35 μm . Highlighted area is lined with electrodes (enlarged).

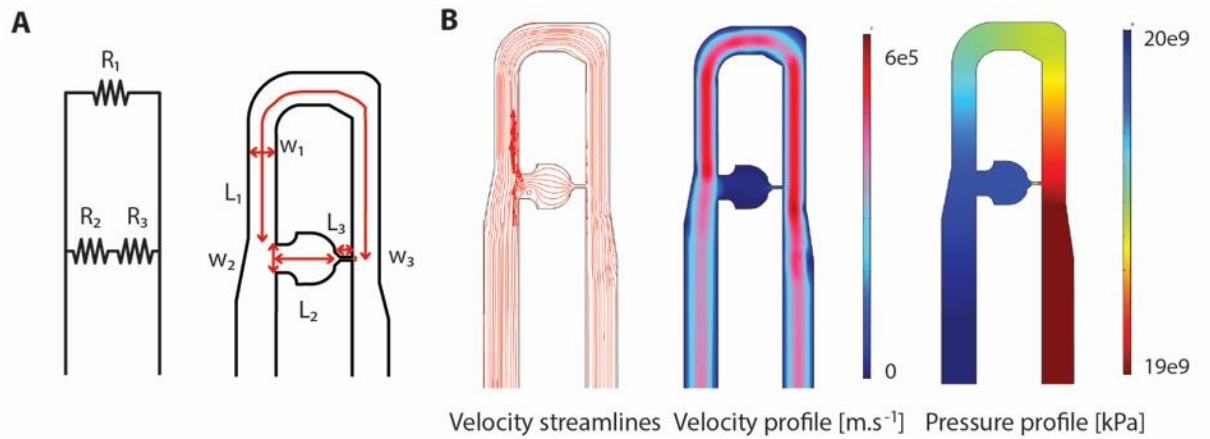


Figure 2

Resistance based channel design. A) Overlay of a resistor diagram on the trapping device. Hydrodynamic resistance in the cell trap (R_3) will increase upon trapping a cell. The flow prefers the path of least resistance and the bypass path (R_1) is usually preferred. The length and width of the bypass ($L_1 = 1000 \mu\text{m}$, $W_1 = 50 \mu\text{m}$) and trap ($L_2 = W_2 = 50 \mu\text{m}$; $L_3 = 30 \mu\text{m}$, $W_3 = 8 \mu\text{m}$) were modified such that it satisfied the conditions for trapping cells (see Supplemental Note 1). Dimensions are as indicated. B) Numerical simulations with COMSOL Multiphysics show flow velocity pattern across the trapping array.

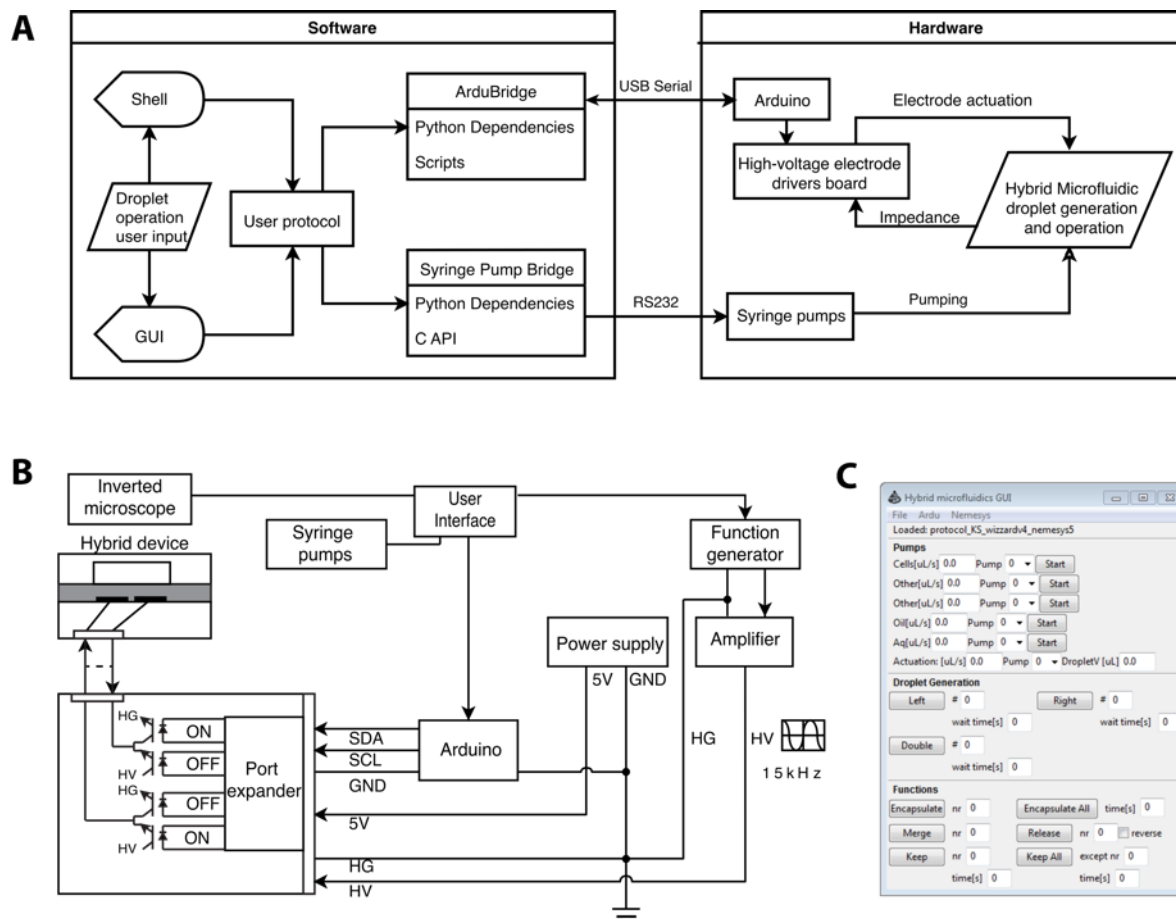


Figure 3

Software and hardware diagram. A) Diagram showing communication lines between software and hardware, with integration of pump and electrode operation using Python 2.7. Through a graphical user interface (GUI), the user can perform on-demand droplet operations such as droplet generation, encapsulation, keeping or releasing a droplet. For example, the user can create 'x' droplets with set time interval or can perform on-demand encapsulation in trap number 5, release or keep a specific droplet, by a 'click-of-a-button'. The GUI accesses a bridge that writes to an Arduino or a syringe pump system. The software is accessible on <http://bitbucket.org/shihmicrolab>. B) Hardware setup. Arduino controls an I²C communication protocol to address specific optocouplers. Automation system hardware setup is similar to previously reported ^[3] C) Screenshot of the graphical user interface (GUI). GUI consists of three sections. The 'Pumps' section allows the user to set flow rates for six pumps, start and stop the continuous flow. The 'Droplet Generation' section allows the user to set the number of droplets to be generated from a double T-junction generator, and the time between the droplets. The 'Functions' section contains pre-programmed electrode actuation sequences that a user can execute. 'nr' indicates the trap number for which trap the function will be applied to. 'time' indicates the duration of the applied electric potential.

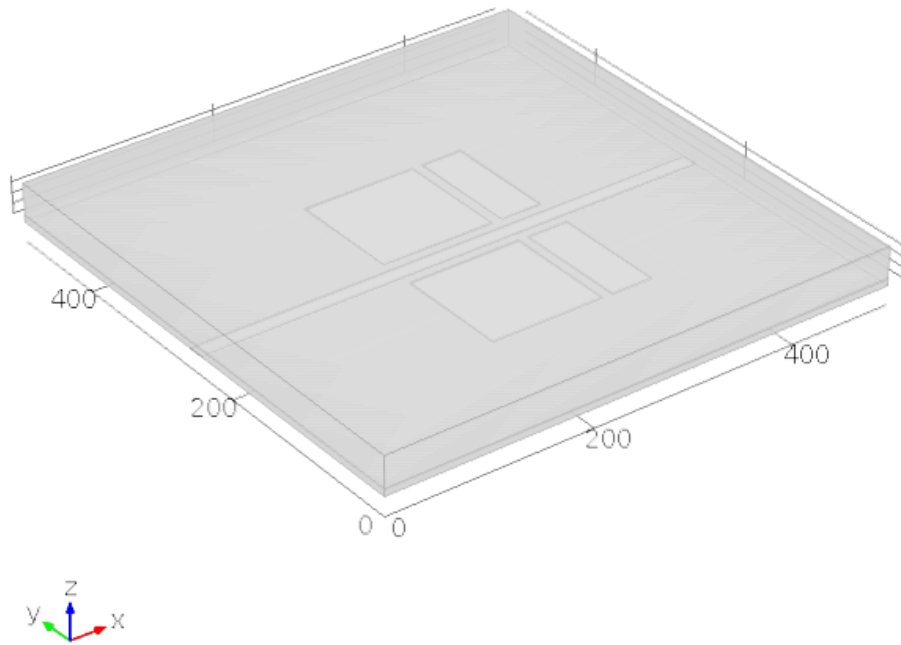


Figure 4

Geometry used for electrostatic field calculations. Geometry used for electric potential (V) and electric field (V m^{-1}) modeling on 7 μm SU-8 5 dielectric layer above co-planar electrode surfaces using COMSOL Multiphysics electrostatics numerical modelling. Dimensions are in microns. Top layer is a 35 μm HFE 7500 oil layer, under which a 7 μm thick SU-8 5 layer is positioned with defined areas of potential or grounding.

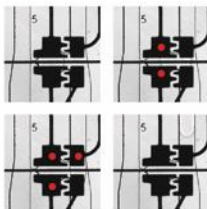
Actuation sequence

Electric potential [V_{RMS}]

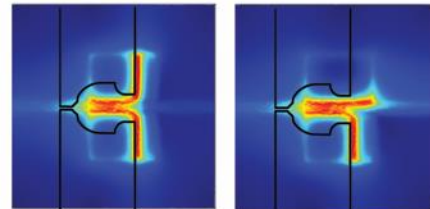
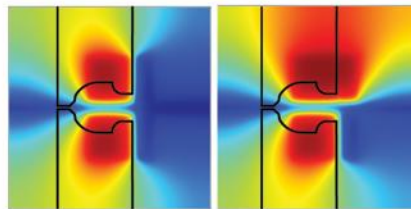
Electric field density [V_{RMS}/m]



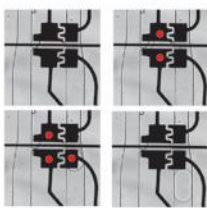
Release (forward)



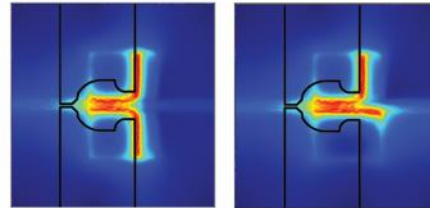
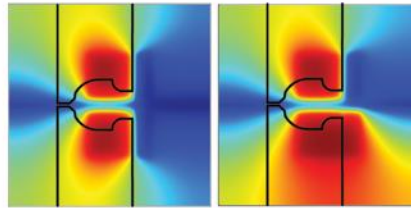
onTime 1.1s
Period 1.3 s



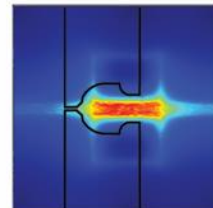
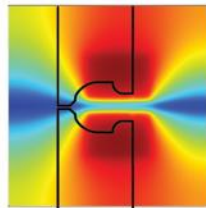
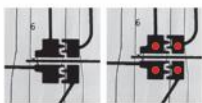
Release (reverse)



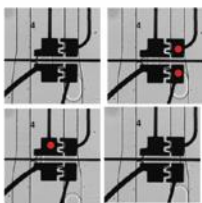
onTime 1.1s
Period 1.3 s



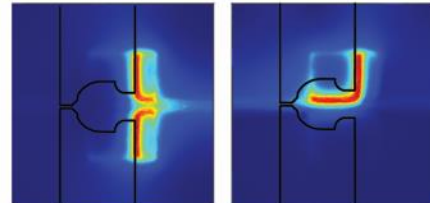
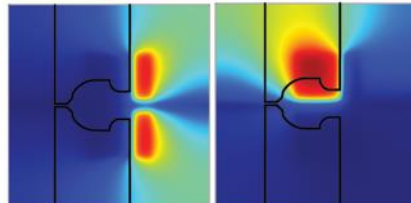
Keep



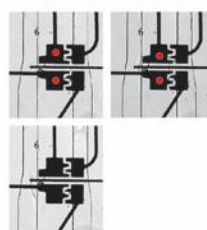
Merge



onTime 1.1s
Period 1.3 s



Encapsulate



onTime 0.9 s
Period 1.1 s

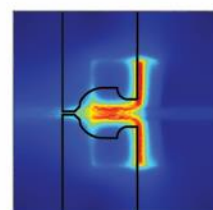
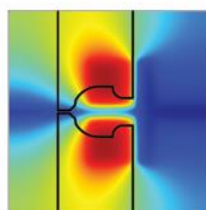


Figure 5

Electrode sequences for on-demand droplet operations. Electric potential (V) and electric field (V m^{-1}) on $7\mu\text{m}$ SU-8 5 dielectric layer above co-planar electrode surfaces were modelled using COMSOL Multiphysics electrostatics numerical modelling. Actuated electrodes are marked by a red dot (bright field image, 15X). All droplet operations (encapsulation, release, merging, and keeping) were simulated using a potential of $126 \text{ V}_{\text{RMS}}$ at a frequency of 15 kHz with varying pulse width (0.9 – 1.1s) and period (1.1-1.3s).

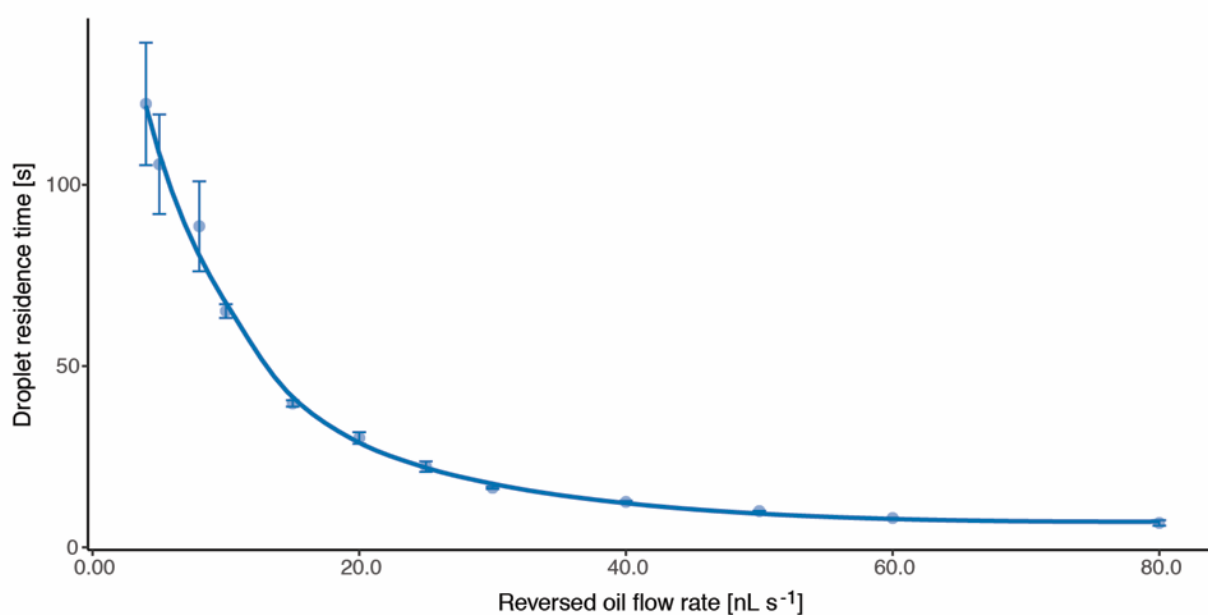


Figure 6

Droplet residence time. The time for the last droplet (from trap 6) to leave the trapping array under different flow rates. At low flow rates ($4 - 8 \text{ nL s}^{-1}$), residence time is highly variable. At high flow rates ($> 20 \text{ nL s}^{-1}$), all droplets are usually released within $\sim 30 \text{ s}$. Droplet residence time is an important parameter for *sorting* droplets under flow reversal and for *keeping* desired droplets such that an automation sequence can be executed for these operations. One standard error, $N = 3$ displayed.

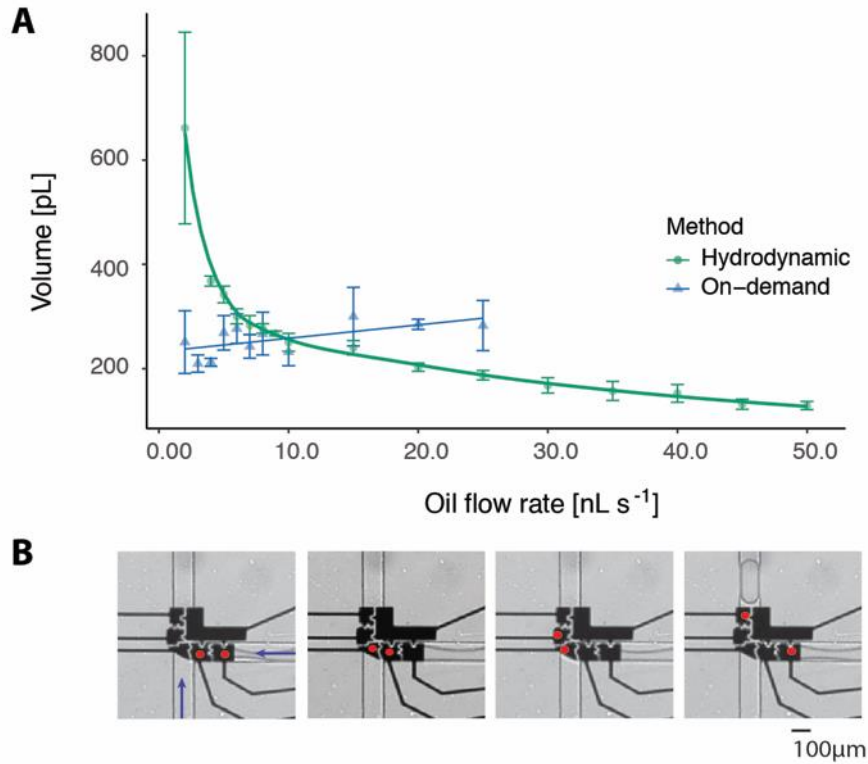


Figure 7

Characterization of on-demand droplet generation. A) Average droplet volume (in pL) for on-demand and hydrodynamically generated droplets. Droplets are generated with a double T-junction of 100 μm width. On-demand droplet-generation shows a linear relationship with respect to oil flow rates. An average droplet volume of 207.5 pL was achieved for on-demand droplet generation. Droplet volume was calculated by multiplying droplet area and a channel height of 35 μm using Fiji (Image J). All error bars are one standard deviation ($N = 4$). B) A series of images showing electrodes are serially actuated to generate droplets on-demand at a potential of 126 V_{RMS} and a frequency of 15 kHz (bright field image, 10X). Red circles indicate the electrodes that are activated.

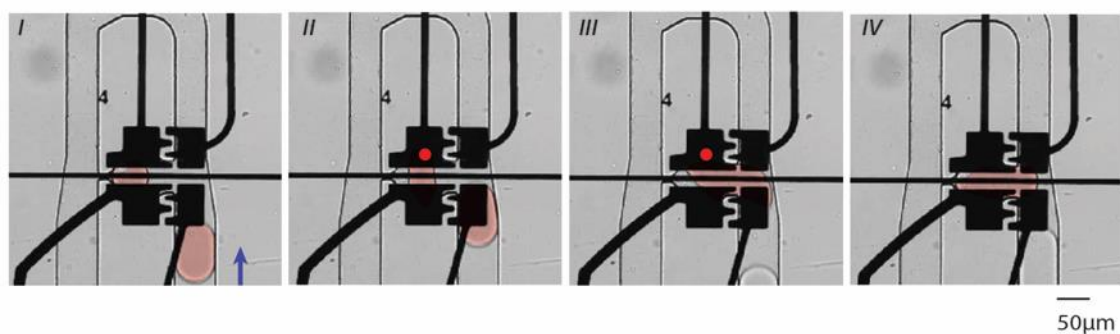


Figure 8

Droplet merging. A series of four frames from a movie showing on-demand merging of two droplets at an oil flow rate of 4 nL s^{-1} . The trapped droplet contains a single MCF-7 cells. A red false color is shown to highlight the droplet.

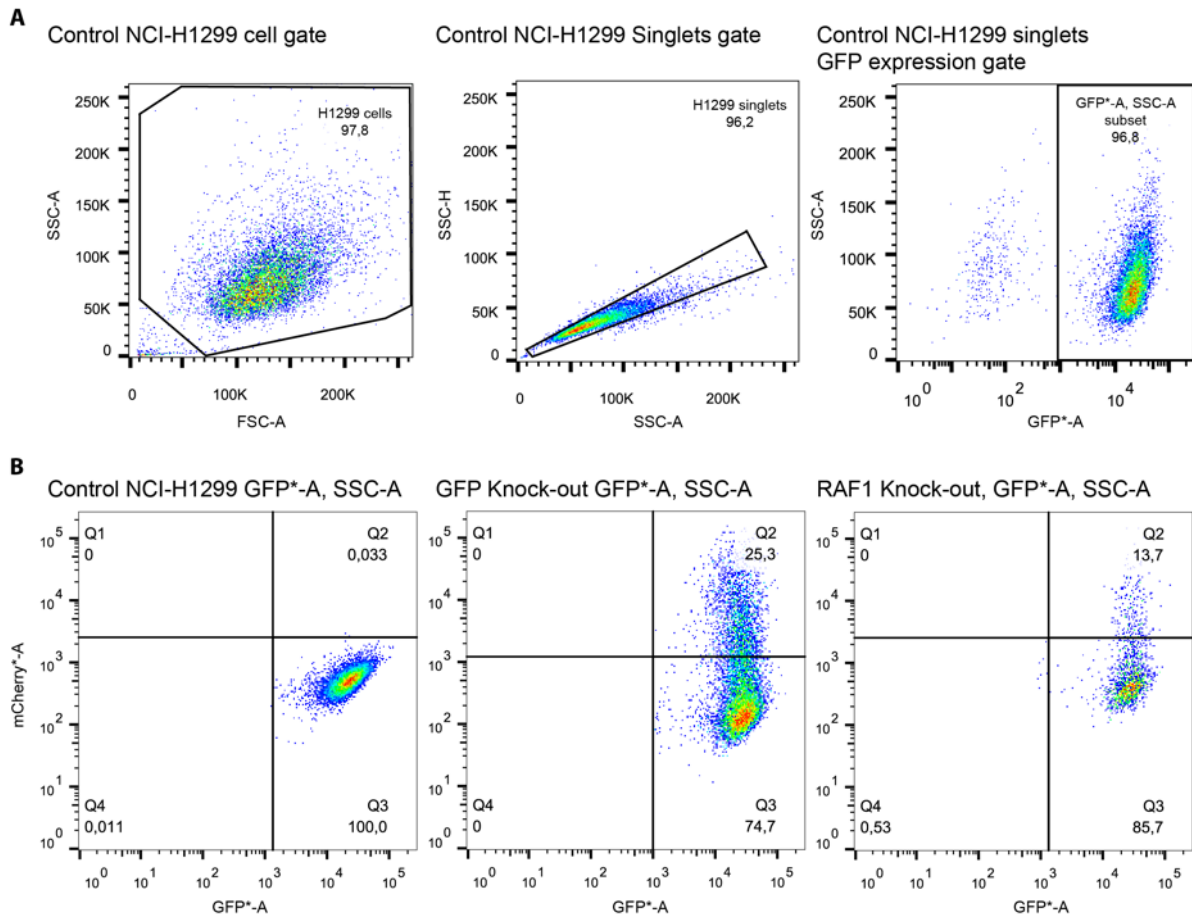


Figure 9

Transfection efficiency determined with flow cytometry A) Gating strategy demonstrated with non-transfected NCI-H1299 cells. B) Left: mCherry vs. eGFP of control NCI-H1299 lung squamous cell carcinoma singlets. Middle: mCherry vs. eGFP of NCI-H1299 lung squamous cell carcinoma singlets transfected with a plasmid expressing an sgRNA directed to knock out the stably integrated eGFP cassette. The determined transfection efficiency is 25.3%. Right: mCherry vs. eGFP of NCI-H1299 lung squamous cell carcinoma singlets transfected with a plasmid expressing an sgRNA directed to knock out the RAF1 oncogene. The determined transfection efficiency is 13.7%.

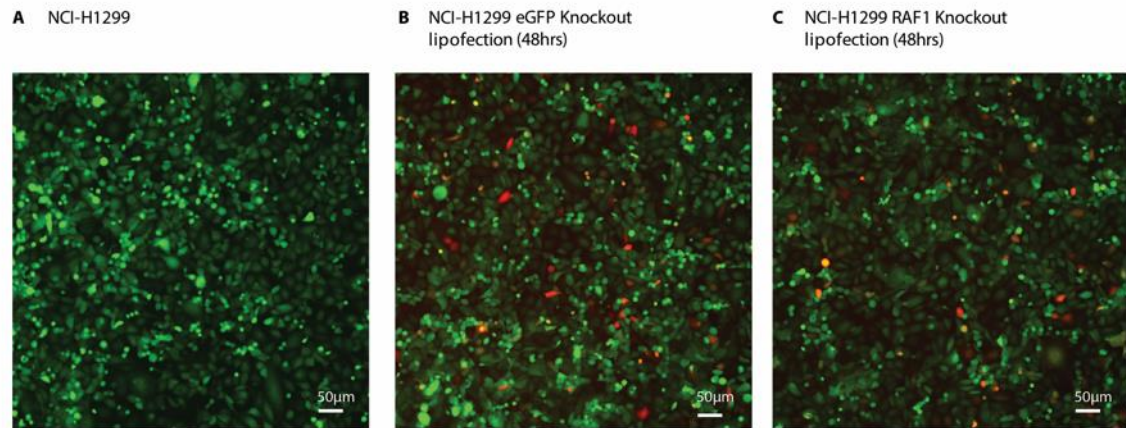


Figure 10

Fluorescent microscopy images of NCI-H1299 lung squamous carcinoma cells. A) NCI-H1299 cells containing eGFP integrated at the AAVS1 site. B) NCI-H1299 cells 48 h after lipid-mediated transfection with eGFP targeting sgRNA encoding plasmid. C) NCI-H1299 cells 48 h after lipid-mediated transfection with RAF1 targeting sgRNA encoding plasmid. For all images, mCherry is shown in red (λ_{Ex} : 585 nm / λ_{Em} : 608 nm) and native eGFP is shown in green (λ_{Ex} : 488 nm / λ_{Em} : 509 nm).

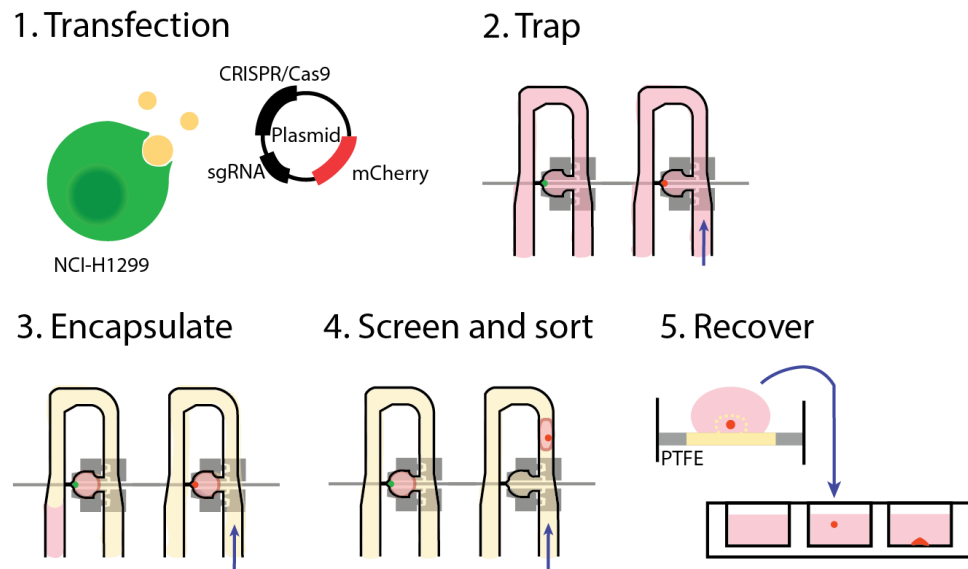


Figure 11

Overview of hybrid microfluidics assisted isoclonal recovery pipeline. After transfection (1), the device is loaded with a heterozygous transfected cell suspension and single isoclones are trapped (2). An HFE-7500 2% Ran surfactant oil flow is flown through the device and electrodes are actuated to encapsulate single-cells in droplets (3). Single isoclone containing droplets can be selected and released on demand. After capillary recovery and centrifugation, isoclones are recovered in 96-well plates and maintained for expansion (4).

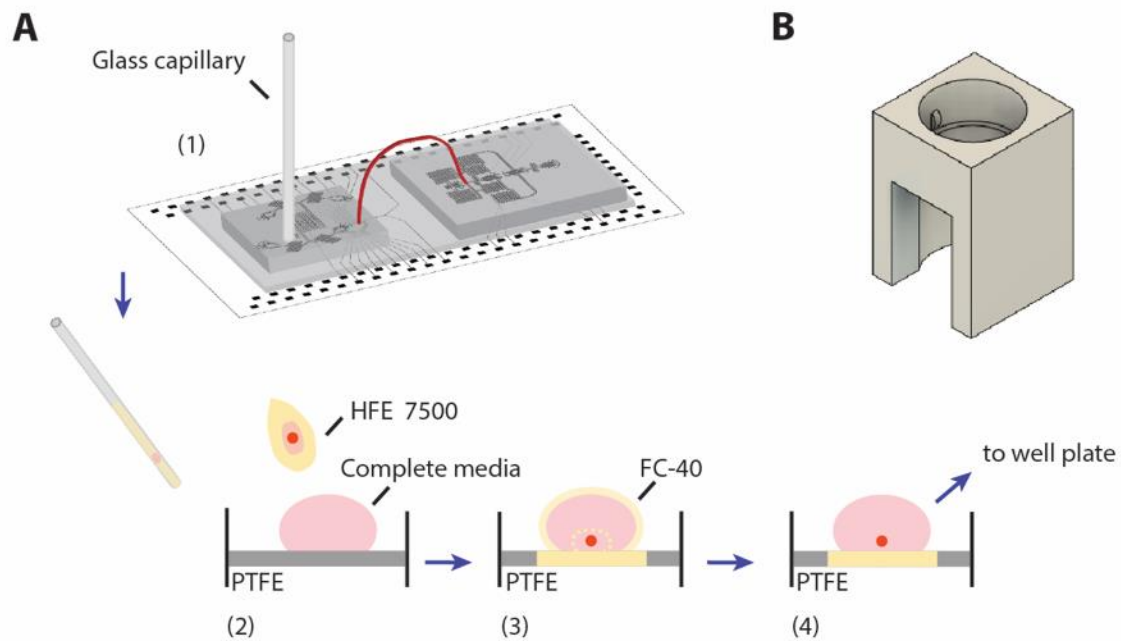


Figure 12

Overview of single-clone recovery method. A) A four-step droplet isclonal recovery procedure. (1) After on-demand release of an isoclone containing droplet from the device, an HFE 7500 filled glass capillary is held on top of the outlet. (2) The capillary content is flushed out using FC-40. A droplet of complete media is situated on top of a PTFE membrane. (3) The FC-40 further destabilizes the surfactant held emulsion, and the oils are absorbed by the membrane. Droplet containing the single cell and the droplet of complete media are merged. (4) The remaining media and the single cell containing droplet can be transferred (via pipette) to a culturing platform. B) 3D printed PTFE membrane holder to allow airflow for fast oil evaporation in a biosafety cabinet.