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

OPTOFLUIDIC ULTRAHIGH-THROUGHPUT DETECTION OF FLUORESCENT DROPS

Minkyu Kim[1], Ming Pan[2], Ya Gai[3], Shuo Pang[4], Chao Han[5], Changhuei Yang[5], and Sindy K.Y. Tang[1*]

[1]*Department of Mechanical Engineering, Stanford University, CA 94305, USA*
[2]*Department of Material Science and Engineering, Stanford University, CA 94305, USA*
[3]*Department of Aeronautics and Astronautics, Stanford University, CA 94305, USA*
[4]*College of Optics and Photonics (CREOL), University of Central Florida, FL 32816, USA*
[5]*Department of Electrical Engineering, California Institute of Technology, CA 91125, USA*

*sindy@stanford.edu

Electronic Supplementary Information

Note S1. Preparation of CMOS chip and integration with PDMS microfluidic channel.

I) Removing glass coverslips from CMOS chips

1. Pour ~ 30 mL of sulfuric acid (reagent grade, 95%-98%) into a glass beaker.
2. Heat the acid on a hotplate to a temperature of about 300 ˚C.
3. Place CMOS chips (with glass coverslips on) into the acid for 3 min using stainless steel or polymer tweezers that are resistant to the acid. Be careful not to splash any acid out of the beaker!
4. Remove the chips from the acid.
5. Rinse the chips twice in two beakers of DI water at room temperature.
6. Blow dry the chips with an air gun.
7. Use a razor to peel off the glass coverslip from the CMOS sensor. Note: The coverslips are attached to the chip (brown outer housing) by an adhesive. The heated acid dissolves the adhesive. The amount of adhesive is left can be estimated by checking the outer edges of the chip where the coverslips are attached. Initially, the outer edges show no color, but when the adhesive is dissolved by the acid, the edges will turn dark blue. The thickness of the dark blue frame at the outer edges of the chip indicates the extent to which the adhesive has been dissolved. If the coverslips cannot be removed due to the presence of too much adhesive, place the chips into the heated acid for 1-2 extra minutes. Be careful not to let any acid touch the sensor surface. This destroys the sensor. If the chips are left in the acid for too long, the coverslips will detach and the acid will destroy the sensor surface.
8. Repeat steps 5-9 for as many chips as needed (only 3 at a time).
9. Clean the chips with acetone, DI water and then blow dry with compressed air.

II) Spin-coating liquid-phase dye filters onto CMOS chip

1. Clean a CMOS chip with compressed air.
2. Center the chip on a chuck of the spin-coater.
3. Set the spin-coater to spin at 1500 rpm for 45 s with an acceleration of 500 rpm/sec.
4. Pipette 25 μL of filter onto the chip. Make sure the liquid does not spill out of the sensor (light-colored) area. Basically, make sure the liquid does not wet anywhere outside the sensor area. Be careful not to touch the chip surface with the pipette tip. Use 2 hands to steady the pipette.
5. Run the spin-coater right after dropping the dye filter onto CMOS.
6. Soft-bake the chip using a hotplate set at 110° C for 2 min.
7. Hard-bake the chip using a hotplate set at 220° C for 2 min.
8. Allow the chip to cool for 2 min on a tabletop or metal slab.
9. Repeat steps 4-8 to achieve the desired number of coated filter layers.

III) Spin-coating PDMS layer onto CMOS chips and bonding microchannel

Fabricating PDMS microchannel

PDMS channels were fabricated using standard soft lithographic methods. To facilitate bonding of channels to the CMOS sensor, the size of the PDMS stamp was smaller than the sensor area of CMOS chips to avoid contact with the wire-bonding on the chip. A minimum spacing of 0.5 mm between the edge of the PDMS stamp and the channels was used to avoid leakage of fluids from the channels.

IV) Preparing PDMS solution and spin-coating PDMS layer onto CMOS chip

1. Prepare 20 mL of 25% (w/w) solution of pre-mixed PDMS (10:1 base to curing agent ratio) in hexane (ACS grade, Aldrich).
2. Mix the solution thoroughly.
3. Blow-dry the CMOS chip sensor surface to make sure it is clean of particles.
4. Pipette 50 µL of the 25% (w/w) PDMS-hexane solution onto the CMOS sensor surface.
5. Spin-coat the CMOS chip at: i) 300 rpm for 10 s with an acceleration of 100 rpm/s; ii) 3000 rpm for 2 min with an acceleration of 1000 rpm/s. According to Lange *et al*., *Anal. Chem.* **2009,** *81,* 10089–10096, this should yield a final PDMS film thickness of ~0.93 µm, assuming that the thickness h of PDMS approximately scales with spin rate ω as: $h \sim \omega^{-1}$.
6. Put CMOS chip into the oven to evaporate hexane for 24 hours at 65 °C.

V) Bonding PDMS microchannel to CMOS chip

1. Expose the surfaces of PDMS microchannel and the PDMS-coated CMOS chip to oxygen plasma. Gently place the microchannel into the CMOS chip surface for bonding.
2. Incubate the bonded device into a 65 °C oven for 6+ hours.
3. Treat the microfluidic channel with Aquapel to make the channel hydrophobic. At this point, the device is ready for experiments.

Figure S1. (a) Transmitted intensity with and without 15 layers of green filter measured with a spectrometer. A white LED was used as the light source, which determined the transmission spectrum without filter (red line). (b) Optical density of 15 layers of green filter. The thickness of 15 layers of the green filter was about 6 μm measured using a profilometer.

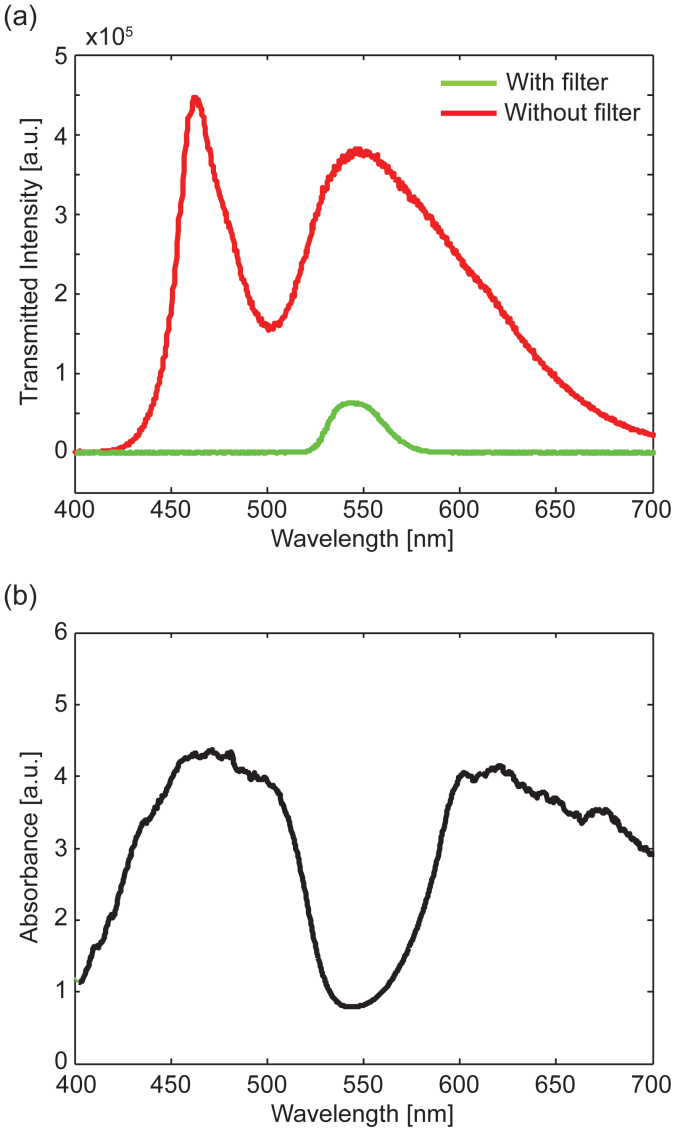


Table S1. Expected droplet interrogation rate as a function of drop size, assuming a monolayer of drops is imaged at a time and that the channel height is exactly one droplet diameter. We further assume that the drops advance by at most 1 drop diameter per frame and the volume fraction of drops in the emulsion is 85%.

Drop size [pL]	20	30	40	50	60
Maximum distance per frame [um/frame]	33.68	38.55	42.43	45.71	48.57
Maximum flow rate [mL/hr]	28.86	37.81	45.81	53.16	60.03
Droplet interrogation rate [drop/sec]	340,745	297,607	270,397	251,055	236,211

Figure S2. (a), (c), (e): Images of different channel geometries tried, and the corresponding droplet flow profiles in (b), (d) and (f) respectively. A concentrated emulsion with volume fraction of 85% was injected into the channels. A high speed camera was used to image the drops and to extract the velocity of the drops as a function of y-position in the channel.

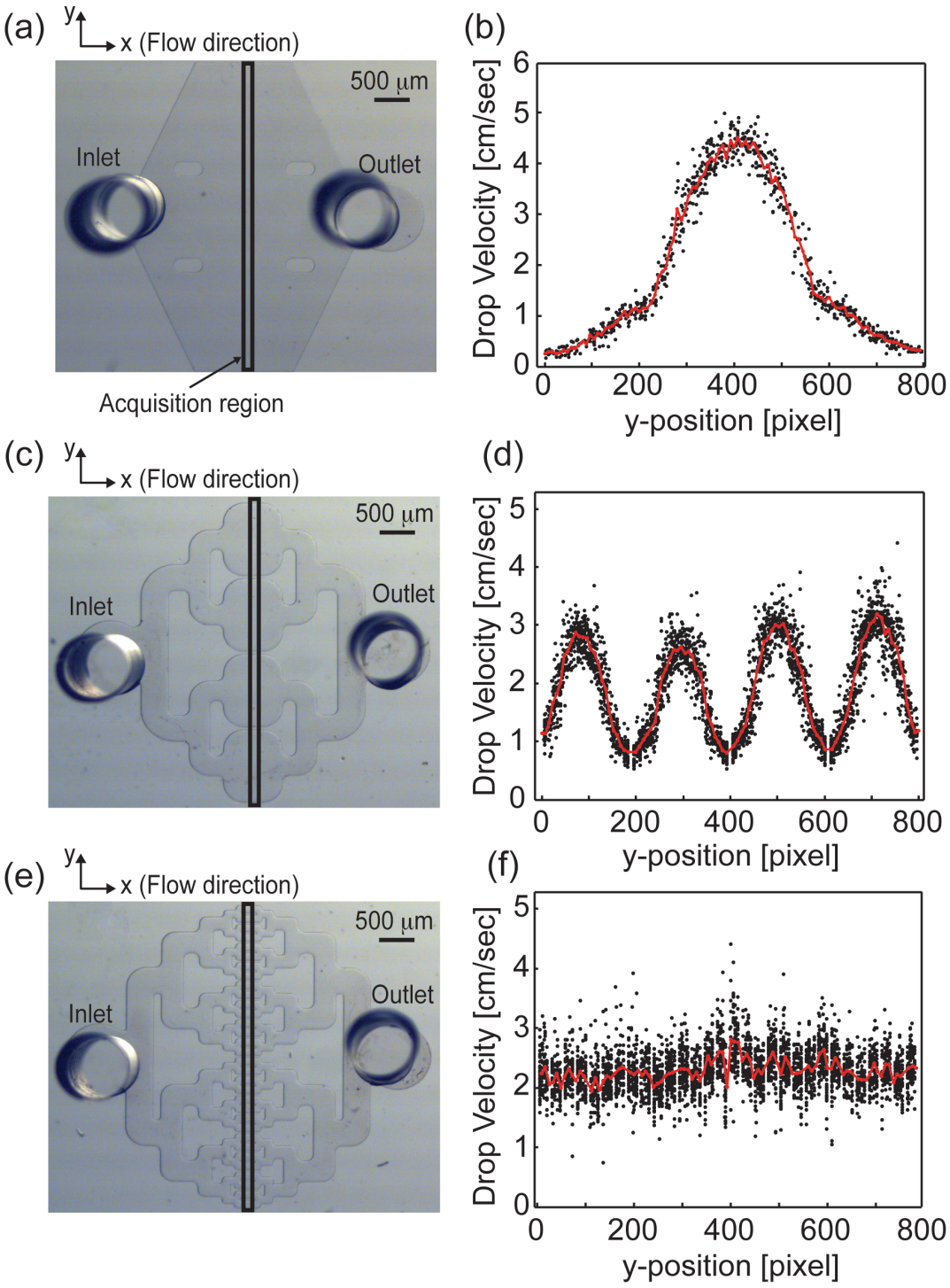


Table S2. Percent of the channel where the mean velocity of the drops (depicted by red lines in Figure S2) is less than half of the maximum velocity in the channel (i.e., $V < V_{max}/2$).

# of channels	1	4	16	32
Percent of Channel, $V < V_{max}/2$	64%	39.8%	4.255%	0%

Note S2. Calculation of expected droplet intensity profile.

To calculate the intensity profile of fluorescence emitted from a fluorescent drop as imaged by the CMOS chip, we use the inverse square law of light propagation to account for the intensity I change as a function of distance d between a point source and a pixel on the CMOS sensor:

$$I \sim \frac{1}{4\pi d^2} \qquad \text{Eq. (S1)}$$

We assume that a drop is cylindrical in shape, and is a collection of point sources. We also assume that fluorophores are distributed homogeneously inside the drop. We define a mesh over the volume of the drop such that each grid point on the mesh contains a single fluorophore or point source. As the geometry of the drop is known, the position of each grid point can be identified and distance d between each grid point and a pixel on CMOS sensor is obtained. The inverse square law of light propagation is then used to identify the intensity at each pixel where the intensity from all point sources is summed up.

Figure S3. (a) Calculated intensity profile (as imaged by the CMOS sensor) arising from two positive drops in direct contact using Eq. (S1). (b) Calculated intensity profile arising from two positive drops sandwiching a negative drop. In our calculation, the drops were separated from the sensor by 0.9 μm of PDMS and 6 μm of filter material.

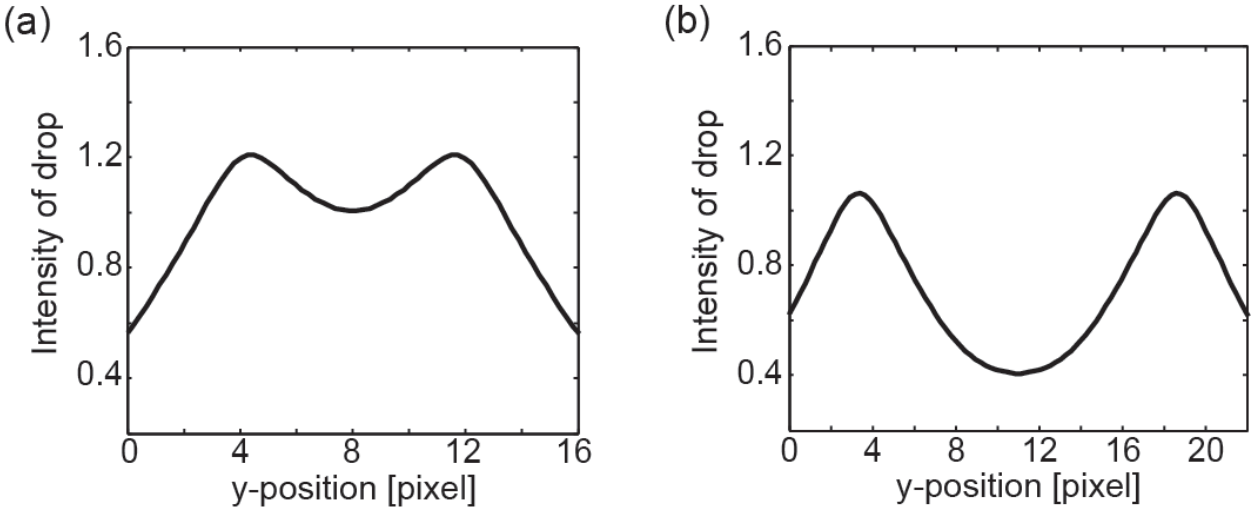


Figure S4. Histogram of size distribution of digitized blobs. The ratio of positive drops to the total number of drops was 1 to 1000.

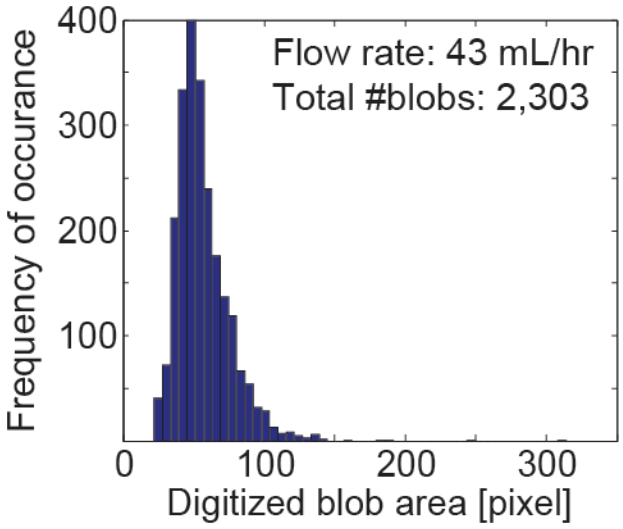
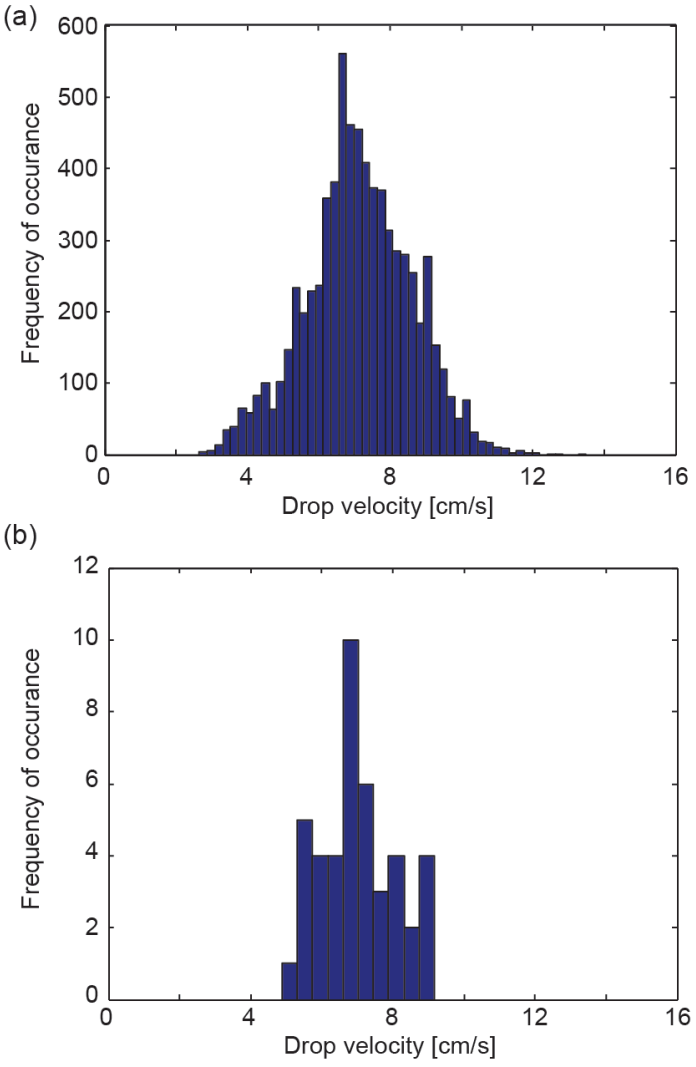
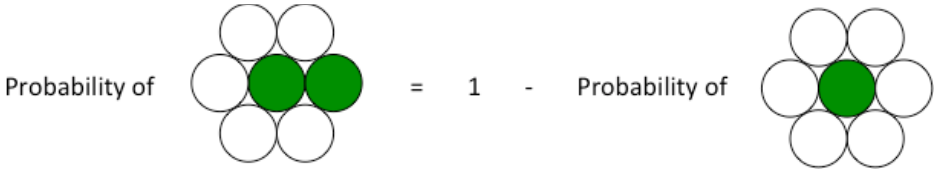


Figure S5. (a) Histogram of droplet velocity distribution for all drops in the 16-channel design used in our main text. The mean was 7.14 cm/s and the standard deviation was 20.7%. (b) Histogram of droplet velocity distribution for drops at one fixed location in the channel.



Note S3. Derivation of equation 3 in the main text.

The channel in our experiment had a height of less than one droplet diameter, and drops flowed in the channel as a 2D monolayer. Projections of drops on the 2D monolayer plane were approximated as uniform circles. All concentrated emulsions used in our experiment had a volume fraction φ = 85.6 ± 3.1%. At this volume fraction, drops in the channel were assumed to arrange themselves in hexagonal packing configuration, as shown in figure below.



For a given fluorescent ("positive") drop, the probability of six non-fluorescent ("negative") neighbor drops is given by Eq.(1):

$$P_1 = (C_-)^6 = (1 - C_+)^6 \qquad \text{Eq. (1)}$$

where C_+ is the concentration of positive drops among all drops in the emulsion ($C_+ = 1$ if the entire emulsion is made of positive drops). The probability of two or more drops in direct contact is given by Eq.(2).

$$P_{multi} = 1 - (1 - C_+)^6 \qquad \text{Eq. (2)}$$

We assume the amount of three or more positive drops in direct contact is negligible since these cases are very rare at low concentrations of positive drops. Refer to Note S4 for the probability of two positive drops in contact and that of three or more positive drops in contact. Here, we focus on two positive drops that are in direct contact and form a positive pair, and assume that the probability of multiple drops in contact is approximately equal to the probability of two drops in contact (see Note S4 for justification):

$$P_{multi} \approx P_{pair} \qquad \text{Eq. (S4)}$$

The number of pairs of two positive drops can be calculated by Eq. (S5):

$$N_{pair} \approx \frac{1}{2} N_+ P_{multi} = \frac{1}{2} N_{tot} C_+ [1 - (1 - C_+)^6] \qquad \text{Eq. (S5)}$$

where N_+ is the total number of positive drops, and N_{tot} is the total number of drops in emulsion.

In a kymograph of imaged emulsion, a pair of two positive drops appears as one bright spot ("blob"). We denote N_{blob} as the total number of blobs. N_{blob}, N_{pair}, and N_+ must satisfy Eq. (S6).

$$N_{blob} + N_{pair} = N_{tot} C_+ \qquad \text{Eq. (S6)}$$

Substitute Eq.(S5) for N_{pair} and rearrange, we have:

$$2\frac{N_{blob}}{N_{tot}} = C_+[1 + (1 - C_+)^6] \qquad \text{Eq. (S7)}$$

If C_+ (concentration of positive drops) is much smaller than 1, the term $(1 - C_+)^6$ on the right-hand side of Eq. (S7) can be expanded.

$$(1 - C_+)^6 = 1 - 6C_+ + 15{C_+}^2 + \cdots \qquad \text{Eq. (S8)}$$

Since $C_+ \ll 1$, we ignore higher order terms and leave only the first two terms in Eq. (6). By plugging Eq. (S8) into Eq. (S7) and rearrange, we end up with a quadratic equation.

$$3{C_+}^2 - C_+ + \frac{N_{blob}}{N_{tot}} = 0 \qquad \text{Eq. (S9)}$$

The two solutions of Eq. (S9) are straightforward, as shown in Eq.(S10).

$$C_+ = \frac{1}{6}\left(1 \pm \sqrt{1 - 12\frac{N_{blob}}{N_{tot}}}\right) \qquad \text{Eq. (S10)}$$

We should only take the solution with a negative sign in front of the square root because the other solution is not physically meaningful. The final solution of expected concentration of positive drops is given as:

$$C_+ = \frac{1}{6}\left(1 - \sqrt{1 - 12\frac{N_{blob}}{N_{tot}}}\right) \qquad \text{Eq. (3)}$$

Alternatively, one can directly solve Eq. (S7) for C_+.

Note S4. Probability of three or more drops in direct contact.

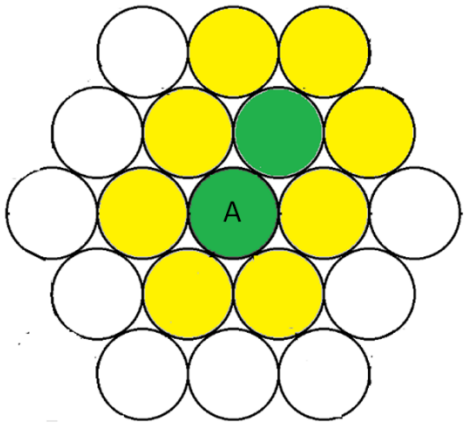
In Eq.(2), P_{multi} not only contains the probability of two drops in contact, but also contains the probability of three or more drops in contact:

$$P_{multi} = 1 - (1 - C_+)^6 \qquad \text{Eq. (2)}$$

In Note S3, we only considered the case of two positive drops in contact, assuming those cases of three or more positive drops in direct contact are increasing unlikely for low concentrations of positive drops. Here we justify this assumption.

While it's not straightforward to calculate probability of three or more positive drops in direct contact, we can first calculate P_{pair}, the probability of only two positive drops stuck together. By comparing P_{multi} and P_{pair}, we can check if it's reasonable to ignore those cases of three or more drops in contact.

Imagine we have a positive drop, A, in the center, as shown in the diagram below. The case of only 2 positive drops in contact should satisfy the two conditions:



1. One of the six drops that are next to A must be positive. In the diagram, the two positive drops in direct contact are green. The probability from this step is:

$$P1 = C_6^1 * C_+ = 6 * C_+ \qquad \text{Eq. (S11)}$$

2. The 8 drops (in yellow) that surround the two positive drops must be negative. The probability from this step is:

$$P2 = (1 - C_+)^8 \qquad \text{Eq. (S12)}$$

Therefore, the overall probability of only two positive drops in direct contact is:

$$P_{pair} = 6 * C_+ * (1 - C_+)^8 \qquad \text{Eq. (S13)}$$

For a given C_+, now we can compare P_{multi} in Eq. (2) and P_{pair} in Eq. (S13). The results are shown in Table S3.

Table S3. Probability that drops are in direct contact.

C_+ [ratio of positive drop to the total number of drops]	1/20	1/100	1/1,000	1/10,000	1/100,000	1/1,000,000
C_+ [ppm]	5 x 10^4	10^4	10^3	10^2	10	1
P_{multi}	0.2649	0.05852	0.005985	0.0006	6E-05	6E-06
P_{pair}	0.1990	0.05536	0.005922	5.995E-04	5.999E-5	5.999E-6

As the value of C_+ decreases below 10^4 ppm, the assumption that

$$P_{multi} \approx P_{pair} \qquad \text{Eq. (S4)}$$

is increasingly valid.