Electronic Supplementary Information

Characterization of Sensitivity and Specificity in Leaky Droplet-based Assays

Yunhan Chen,^a Adi Wijaya Gani,^b and Sindy K. Y. Tang^{a,*}

^a Department of Mechanical Engineering, Stanford University, CA 94305
 ^b Department of Electrical Engineering, Stanford University, CA 94305
 *Corresponding author: <u>sindy@stanford.edu</u>

Supplementary movie 1 ("Intensity profiles.avi"). Spatial and temporal evolution of fluorescence intensity from the drops at $k_{perm} = 0$ (no leakage) and $k_{perm} = 10^{-8}$ m/s respectively. The other values used in the simulation to generate the movie are listed in Table 1.

ESI note 1. Derivation of mass flux across water-oil interface.

Following the descriptions in "Fundamentals of Transport Phenomena" Fahien R.W., McGraw-Hill 1983 (p.143-144, 154-157),¹ and "Analysis of Transport Phenomena" Deen, W.M. Oxford Press, 1998,² we elaborate on how we arrived at our boundary conditions for flux *J* across the water-oil interface:

$$J = D_{i,drop} \nabla C_{i,drop} = k_{perm} \left(C_{i,drop} - \frac{C_{i,oil}}{K_{part}} \right)$$
(Eq. 4 in text):

Molecules can have different solubilities in oil vs. in water droplets. The partition coefficient relates the equilibrium concentration of species *i* in the oil phase ($C_{i,oil}^{eq}$) to the equilibrium concentration of species *i* in the water (drop) phase ($C_{i,drop}^{eq}$):

$$K_{part} \equiv \frac{C_{i,oil}^{eq}}{C_{i,drop}^{eq}}$$

 $K_{part} < 1$ means species *i* is more soluble in water than in oil. $K_{part} = 1$ means species *i* is equally soluble in water and in oil. $K_{part} > 1$ means species *i* is more soluble in oil than in water.

As such, when $K_{part} \neq 1$, equilibrium does not mean equal concentrations of species *i* in the oil phase and in the water phase. At equilibrium, there is still a concentration discontinuity across the water-oil interface. In other words, $(C_{i,drop} - C_{i,oil})$ is non-zero at equilibrium. The direct use of $(C_{i,drop} - C_{i,oil})$ to calculate the driving force for net flux across the interface will result in a non-zero flux even at equilibrium which is incorrect. The expression for the driving force must account for the differences in the solubilities of the species in the different phases.

The correct driving force should be the difference in chemical potentials (or activities if the standard states are the same).¹ At equilibrium, the chemical potential of species *i* in the water phase equals the chemical potential of species *i* in the oil phase at the interface. Thus, flux *J* of species *i* across the water-oil interface with overall permeability or mass transfer coefficient *k* can be written as Eq. S1. Since we refer to species *i* only, we will drop the subscript *i* for the derivations below.

$$J = k \left(a_{drop} - a_{oil} \right)$$
 (Eq. S1)

with a_{drop} and a_{oil} being the chemical activities of species *i* in the water and oil phases respectively; γ_{drop} and γ_{oil} being the activity coefficients of species *i* in the water and oil phases respectively.

$$a_{drop} \equiv C_{drop} \gamma_{drop}$$
$$a_{oil} \equiv C_{oil} \gamma_{oil}$$

Eq. S1 can be written as:

$$J = k \left(C_{drop} \gamma_{drop} - C_{oil} \gamma_{oil} \right)$$
$$J = k \gamma_{drop} \left(C_{drop} - C_{oil} \frac{\gamma_{oil}}{\gamma_{drop}} \right)$$
$$J = k_{perm} \left(C_{drop} - \frac{C_{oil}}{\kappa_{part}} \right) \quad (Eq. S2)$$

where $K_{part} \equiv \frac{\gamma_{drop}}{\gamma_{oil}}$ (see "Derivation for partition coefficient" on the next page) and $k_{perm} \equiv k\gamma_{drop}$. K_{part} , γ_{drop} , γ_{oil} are usually assumed to be independent of concentration if the range of concentration considered is small.¹

Alternatively, one can follow the two-film model as first put forward by Lewis and Whitman³, also discussed in Fahien¹ (p.154-156) to derive Eq. S2:



Figure S1. Scheme showing two-film model.

At the interface, the mass flux of species *i* in the water phase equals the mass flux of species *i* in the oil phase:

 $J_{drop} = J_{oil} = J$ at the water-oil interface (Eq. S3)

We can write the mass flux for the droplet phase and oil phase respectively in Eq. S4, where k_{drop} and k_{oil} are the permeability or mass transfer coefficients of species *i* in each phase:

$$J \equiv \begin{cases} k_{drop} (C_{drop} - C_{drop,film}^*), & \text{droplet phase} \\ k_{oil} (C_{oil,film}^* - C_{oil}), & \text{oil phase} \end{cases}$$
(Eq. S4)

Chemical activities are defined as follows with γ_{drop} and γ_{oil} being the activity coefficients for the water and oil phases respectively.

$$a_{drop} \equiv C_{drop} \gamma_{drop}$$

 $a_{oil} \equiv C_{oil} \gamma_{oil}$

$$a^{*}_{drop,film} \equiv C^{*}_{drop,film} \gamma_{drop}$$
$$a^{*}_{oil,film} \equiv C^{*}_{oil,film} \gamma_{oil}$$

Thus, Eq. S4 can be rewritten as:

$$J \equiv \begin{cases} k'_{drop} (C_{drop} \gamma_{drop} - C^*_{drop, film} \gamma_{drop}), & k'_{drop} = \frac{k_{drop}}{\gamma_{drop}} \\ k'_{oil} (C^*_{oil, film} \gamma_{oil} - C_{oil} \gamma_{oil}), & k'_{oil} = \frac{k_{oil}}{\gamma_{oil}} \end{cases}$$
(Eq. S5)

Two-film theory assumes equilibrium exists at the interface, i.e., that the chemical potentials or activities (not the concentrations) of species *i* at the interface are equal.

$$a_{drop}^* = a_{oil}^*$$
 (Eq. S6a)
 $C_{drop,film}^* \gamma_{drop} = C_{oil,film}^* \gamma_{oil}$ (Eq. S6a)

Incorporate Eq. S6 into Eq. S5, and add the mass flux in water phase to the mass flux in oil phase:

$$(k'_{oil}+k'_{drop})J = k'_{oil}k'_{drop}(C_{drop}\gamma_{drop} - C_{oil}\gamma_{oil})$$

$$(k'_{oil}+k'_{drop})J = k'_{oil}k'_{drop}\gamma_{drop}\left(C_{drop} - C_{oil}\frac{\gamma_{oil}}{\gamma_{drop}}\right)$$

$$J = \frac{k'_{oil}k'_{drop}\gamma_{drop}}{(k'_{oil}+k'_{drop})}\left(C_{drop} - C_{oil}\frac{\gamma_{oil}}{\gamma_{drop}}\right)$$

$$J = k_{perm}\left(C_{drop} - \frac{C_{oil}}{K_{part}}\right)$$
same as Eq. S2
where $K_{part} \equiv \frac{\gamma_{drop}}{\gamma_{oil}}$ and $k_{perm} \equiv \frac{k'_{oil}k'_{drop}\gamma_{drop}}{(k'_{oil}+k'_{drop})}$

Derivation for partition coefficient (adapted from p.143-144 of Fahien¹)

At equilibrium, the chemical potentials of species *i* at the water-oil interface are equal (again we omit the subscript *i*):

$$\bar{G}_{drop} = \bar{G}_{oil}$$
, at interface

where \bar{G}_{drop} = chemical potential of species *i* in water or droplet phase at interface \bar{G}_{oil} = chemical potential of species *i* in oil phase at interface

From thermodynamics, \bar{G}_{drop} and \bar{G}_{oil} can be related to \bar{G}°_{drop} and \bar{G}°_{oil} , their respective chemical potentials at standard states:

$$\bar{G}_{drop} = \bar{G}^{\circ}_{drop} + RTln(a_{drop})$$
$$\bar{G}_{oil} = \bar{G}^{\circ}_{oil} + RTln(a_{oil})$$

where $a_{drop} \equiv C_{drop} \gamma_{drop}$ is the activity of species *i* in droplets, and $a_{oil} \equiv C_{oil} \gamma_{oil}$ is the activity of species *i* in oil. γ_{drop} and γ_{oil} are the activity coefficients of species *i* in droplet and oil respectively.

Partition coefficient can be written as: $K_{part} \equiv \frac{C_{i,oil}^{eq}}{C_{i,drop}^{eq}} = \frac{\frac{1}{\gamma_{oil}} \exp\left(\frac{\overline{G}_{oil} - \overline{G}^{\circ}_{oil}}{RT}\right)}{\frac{1}{\gamma_{drop}} \exp\left(\frac{\overline{G}_{drop} - \overline{G}^{\circ}_{drop}}{RT}\right)}$

If $\bar{G}^{\circ}_{drop} = \bar{G}^{\circ}_{oil}$, and $\bar{G}_{drop} = \bar{G}_{oil}$ at interface at equilibrium:

$$K_{part} \equiv \frac{C_{i,oil}^{eq}}{C_{i,drop}^{eq}} = \frac{\gamma_{drop}}{\gamma_{oil}}$$

ESI note 2. Expression for fluorescence intensity.

Fluorescence intensity *I* can be expressed as:

$$I = \varphi I_{abs} = \varphi (I_0 - I_{tx}) = \varphi I_0 (1 - I_{tx}/I_0) = \varphi I_0 (1 - e^{-\varepsilon IC})$$

where I_0 , I_{tx} , I_{abs} are the incident intensity, transmitted intensity, and absorbed intensity respectively. φ is the ratio of fluorescence intensity to absorbed intensity, and is a property of the fluorophore. The ratio I_{tx}/I_0 is given by the Beer Lambert Law. ε , l, and C are the extinction coefficient, path length, and concentration of the fluorophore. If $\varepsilon l C$ is small, the equation can be approximated as:

$$I = \varphi I_0 (1 - e^{-\varepsilon lC}) \sim \varphi I_0 (\varepsilon l C) = \varepsilon' C$$

 ε ' is the molar fluorescence intensity used in the text.

ESI note 3. Estimation of partition coefficient from Courtois et al.

In the estimation of the partition coefficient of fluorescein in the work by Courtois et al.,⁴ we assume fluorescence intensity is linearly proportional to the concentration of fluorescein which is valid for dilute solutions of fluorescein. By mass conservation, the reduction in fluorescence intensity in the droplet must be due to the loss of fluorescein to the oil phase. The partition coefficient of fluorescein between the oil and water phase could be estimated from the ratio of fluorescence intensity lost from the drop, to the fluorescence intensity remaining in the drop.

We note that, however, the volumetric flow rates of oil and aqueous phase were 100 μ L h⁻¹ and 60 μ L h⁻¹ respectively in the reported experiment. Since there was no mention of ways to increase the volume fraction of droplets downstream after the drops were generated, we assume the volume of oil was 100/60 that of water in the observation chamber or reservoir. This higher volume of oil would lead to greater loss of intensity from the drop; the K_{part} calculated would be higher than expected. The actual K_{part} should be adjusted by the volume ratio between the oil phase and the water phase (or total droplet volume), i.e.,

$$K_{part} \equiv \frac{C_{oil}^{eq}}{C_{drop}^{eq}} \approx \frac{I_{drop}(t=0) - I_{drop}^{eq}}{I_{drop}^{eq}} \left(\frac{V_w}{V_{oil}}\right) = \frac{1 - 0.88}{0.88} \left(\frac{60}{100}\right) = 0.082$$

 $C_{0} = original \ concentration \ of \ dye \ in \ water \ (at \ time \ 0)$ $C_{oil}^{eq} = concentration \ of \ dye \ in \ oil \ at \ equilibrium$ $C_{drop}^{eq} = concentration \ of \ dye \ in \ water \ at \ equilibrium$ $N_{0} = initial \ total \ number \ of \ moles \ of \ dye \ in \ water \ (zero \ in \ oil)$ $N_{oil}^{eq} = total \ number \ of \ moles \ of \ dye \ in \ water \ at \ equilibrium$ $N_{drop}^{eq} = total \ number \ of \ moles \ of \ dye \ in \ water \ at \ equilibrium$ $V_{oil} = volume \ of \ oil$ $V_{w} = total \ volume \ of \ all \ water \ drops$ $N_{0}/V_{w} = C_{0}$ $N_{oil}^{eq} / V_{will} = C_{oil}^{eq}$ $N_{drop}^{eq} / V_{w} = C_{drop}^{eq}$ $N_{0} = N_{oil}^{eq} + N_{drop}^{eq} \ (mass \ conservation)$

Accordingly,

$$K_{part} \equiv \frac{C_{oil}^{eq}}{C_{drop}^{eq}} = \frac{\frac{N_{oil}^{eq}}{V_{oil}}}{\frac{N_{drop}^{eq}}{V_w}} = \frac{N_{oil}^{eq}}{N_{drop}^{eq}} \left(\frac{V_w}{V_{oil}}\right) = \frac{N_0 - N_{drop}^{eq}}{N_{drop}^{eq}} \left(\frac{V_w}{V_{oil}}\right) = \frac{\frac{N_0 - N_{drop}^{eq}}{V_w}}{\frac{N_{drop}^{eq}}{V_w}} \left(\frac{V_w}{V_{oil}}\right)$$
$$= \frac{C_0 - C_{drop}^{eq}}{C_{drop}^{eq}} \left(\frac{V_w}{V_{oil}}\right) \approx \frac{I_{drop}(t=0) - I_{drop}^{eq}}{I_{drop}^{eq}} \left(\frac{V_w}{V_{oil}}\right) \quad \text{(for dilute solutions)}$$

ESI note 4. Order-of-magnitude estimation of micelle size.

Here we seek an order-of-magnitude estimation of the hydrodynamic radius of micelles formed by surfactant Abil EM 90, to estimate the order of magnitude of micelle diffusivity. The structure of Abil EM 90, the surfactant used by Courtois et al.,⁴ is reported to have this form:⁵



where $PE=(C_2H_4O)_{12}$ —H, o=2, m=8, n=20, p=9, q=3. The PE group is hydrophilic. When in the form of a reverse-micelle in a hydrocarbon-based continuous phase, the two silicone tails face outwards. Assuming a bond length of ~ 0.1 nm, we estimated the head group to have a length of ~ 0.2 nm x (12 + q) = 3 nm. The tails have a length ~ 0.2 nm x n = 4 nm. The radius of the micelle is thus approximately on the order of ~ 7 nm.

Figure S2. Periodic vs. no-flux boundary condition

We compare the effect of periodic v.s. no-flux boundary condition at the edge of the droplet array in Figure 3. We simulated an array of 342 hexagonally packed drops, consisting of 155 positive drops (containing fluorescein only) and 187 negative drops. The locations of the positive drops were randomly chosen in Matlab. We simulated 1 set of positive drop distributions only. a) Positive drops are indicated in red, and negative drops are indicated in white. The number in a positive drop indicates the number of immediate neighboring drops that are negative. The number in a negative drop indicates the number of immediate neighboring drops that are positive. b) Normalized intensity from the array of drops at t = 6 hrs in the x-y plane at z=0. The intensity is normalized with respect to the maximum intensity. c) Change in fluorescence intensity from positive drops $(I_{c,+})$ as a function of time, when surrounded by different number of immediate neighboring drops that are negative. d) Change in fluorescence intensity from negative drops (I_{c}) as a function of time, when surrounded by different number of immediate neighboring drops that are positive. The solid lines represent the mean intensity values when the boundary of the array of drops is periodic, and the dashed lines represent mean intensity values when the boundary of the array is no-flux. The height of the error bars indicates one standard deviation of intensity values from the mean calculated using all drops within the array in (a) having a given number of immediate neighbors. $k_{perm} = 3.5 \times 10^{-10}$ m/s, $K_{part} = 0.082$, $D_{oil} = 10^{-11}$ m²/s, R = 22.5 µm, and C_s (t=0) = 10 µM. The values of other parameters used are marked with * in Table 1.



Figure S3. Effect of Kpart

(a,b) Change in fluorescence intensity along line AC as a function of time, when a central positive drop containing a fluorophore (fluorescein) is surrounded by 6 negative neighboring drops that contain a non-fluorescent buffer. We set the border of the continuous phase to be no-flux. The partition coefficients are (a) $K_{part} = 0.082$, and (b) $K_{part} = 2$, respectively. (c) Distribution of drops used to generate S3d. Positive drops with fluorescein are indicated in red and green. The intensity of the 6 green drops are monitored to generated the curves in S3d. Negative drops are indicated in blue. The border of this array of drops is periodic. (d) Average fluorescence intensity (I_{c+}) from the 6 positive drops indicated in green as a function of time at different values of K_{part} . All these 6 drops have 4 negative immediate neighbors. The height of the error bar indicates one standard deviation of intensity values from the mean calculated using these 6 drops. Here, $k_{perm} = 3.5 \times 10^{-10}$ m/s, $D_{oil} = 10^{-11}$ m²/s, R = 22.5 µm and $C_s(t=0) = 10$ µM. The values of other parameters used are marked with * in Table 1.



Figure S4. Effect of the shape of the drop.

Comparison of leakage from drops with vertical sidewalls and drops with curved sidewalls. Here we consider leakage of a fluorophore (e.g., fluorescein) only without reaction. For cases 2-4, the contact angle between water and the wall of the chamber is 135°. We assume the curvature profile is an arc of a circle. For case 5, the drops are spherical. We performed the simulation in 3D. a) Scheme of the two drops we simulated in cases 2 to 5. b) Scheme in x-z plane. c) Fluorophore concentration in the negative drop as a function of time for the 5 cases. Table S1 lists the geometries used in the 5 cases. The values of other parameters used are marked with * in Table 1.



Table	S1 .
-------	-------------

Case	Droplet sidewall profile	R=h(µm)	s(µm)	s _{min} (nm)	Area exposed to oil (m ²)	Droplet Volume(m ³)	Area/Volume ratio (A/V) (m ⁻¹)	Note
Case 1	vertical	10	4.2	4200	6.28E- 10	3.14E-15	2.00E+05	
Case 2	curved	9.64	4.2	207.0	7.37E- 10	3.69E-15	2.00E+05	same A/V ratio as case 1
Case 3	curved	10	4.2	57.9	7.93E- 10	4.11E-15	1.93E+05	same R and h as case 1
Case 4	curved	9.14	4.2	414.1	6.63E- 10	3.14E-15	2.11E+05	same droplet volume as case 1
Case 5	sphere	R=9.09 h=2R	22.38	4200	1.04E-9	3.14E-15	3.30E+05	same droplet volume as case 1; spherical shape

Figure S5. Effect of spacing between drops.

Variation of SNR with time at various spacing *s* between the drops. The values of other parameters used are marked with * in Table 1.



References

- 1. R. W. Fahien, Fundamentals of Transport Phenomena, McGraw-Hill, New York, 1983.
- 2. W. M. Deen, Analysis of Transport Phenomena, Oxford University Press, New York, 1998.
- 3. W. K. Lewis and W. G. Whitman, *Industrial and Engineering Chemistry*, 1924, **16**, 1215-1220.
- 4. F. Courtois, L. F. Olguin, G. Whyte, A. B. Theberge, W. T. S. Huck, F. Hollfelder and C. Abell, *Analytical Chemistry*, 2009, **81**, 3008-3016.
- 5. N. Terren, M.-M. Roux and S. Favre, US Pat., 7083800, L' Oreal S.A., 2006.