Supplemental information

Precise pooling and dispensing of microfluidic droplets

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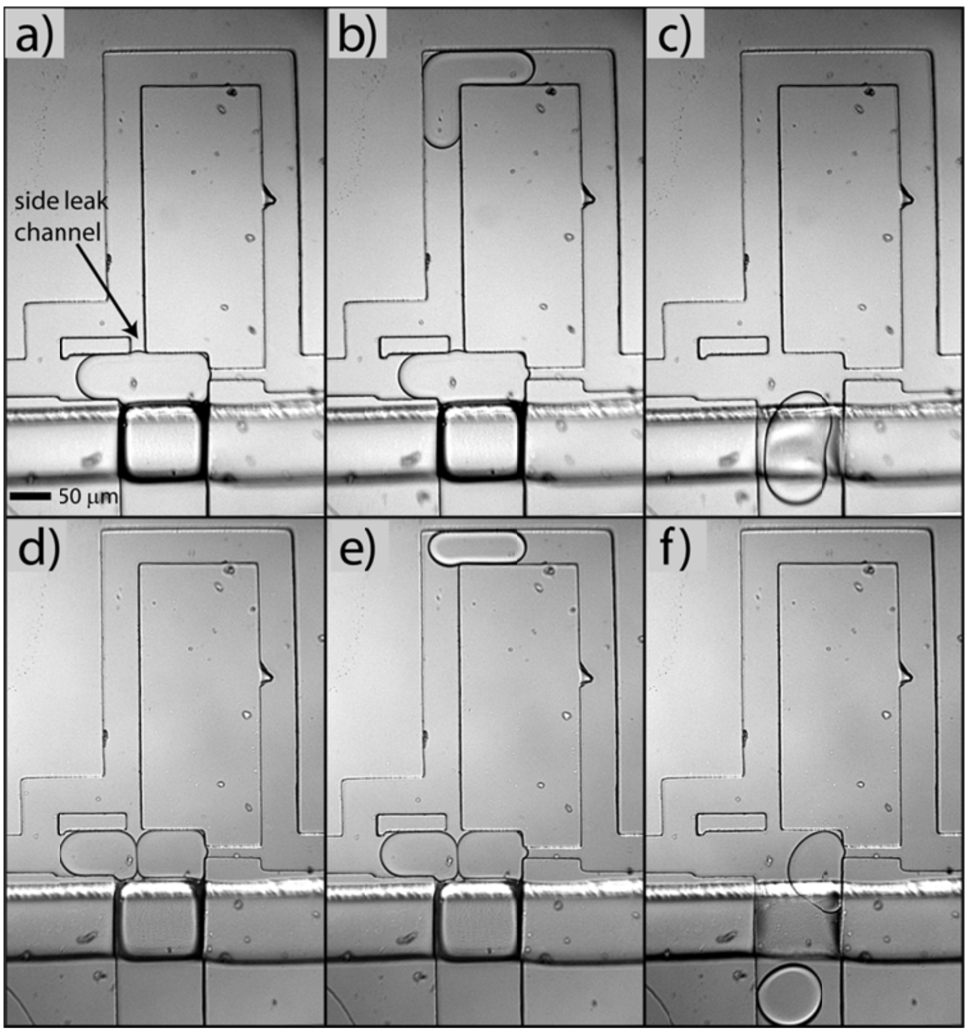
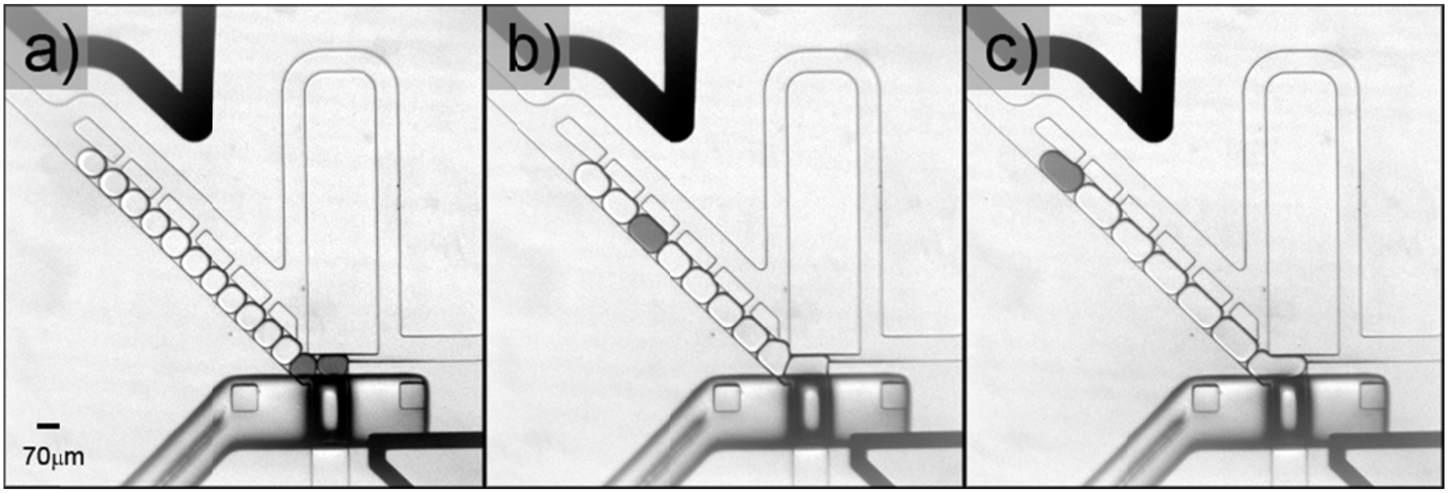
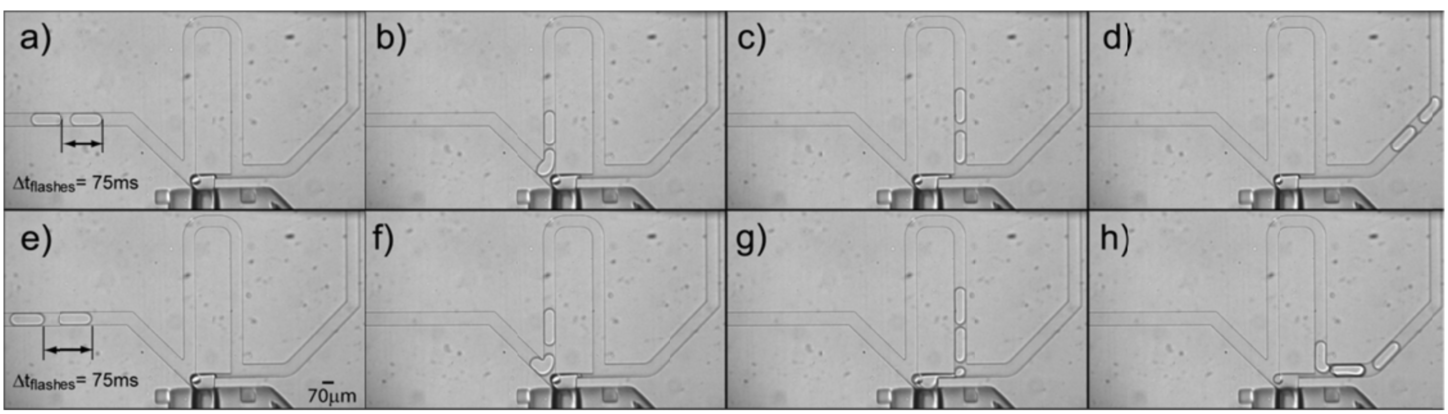
Supp Fig. 1 Expanding the design to trap larger or several droplets. a-c) We added a side leak channel to avoid splitting of large droplets at the bifurcation. d-f) The configuration also allows for trapping several droplets. The volumes of the trapping chamber and the droplets are respectively 1.75 nL and 1.44 nL (a-c) and 0.8 nL (d-f)..



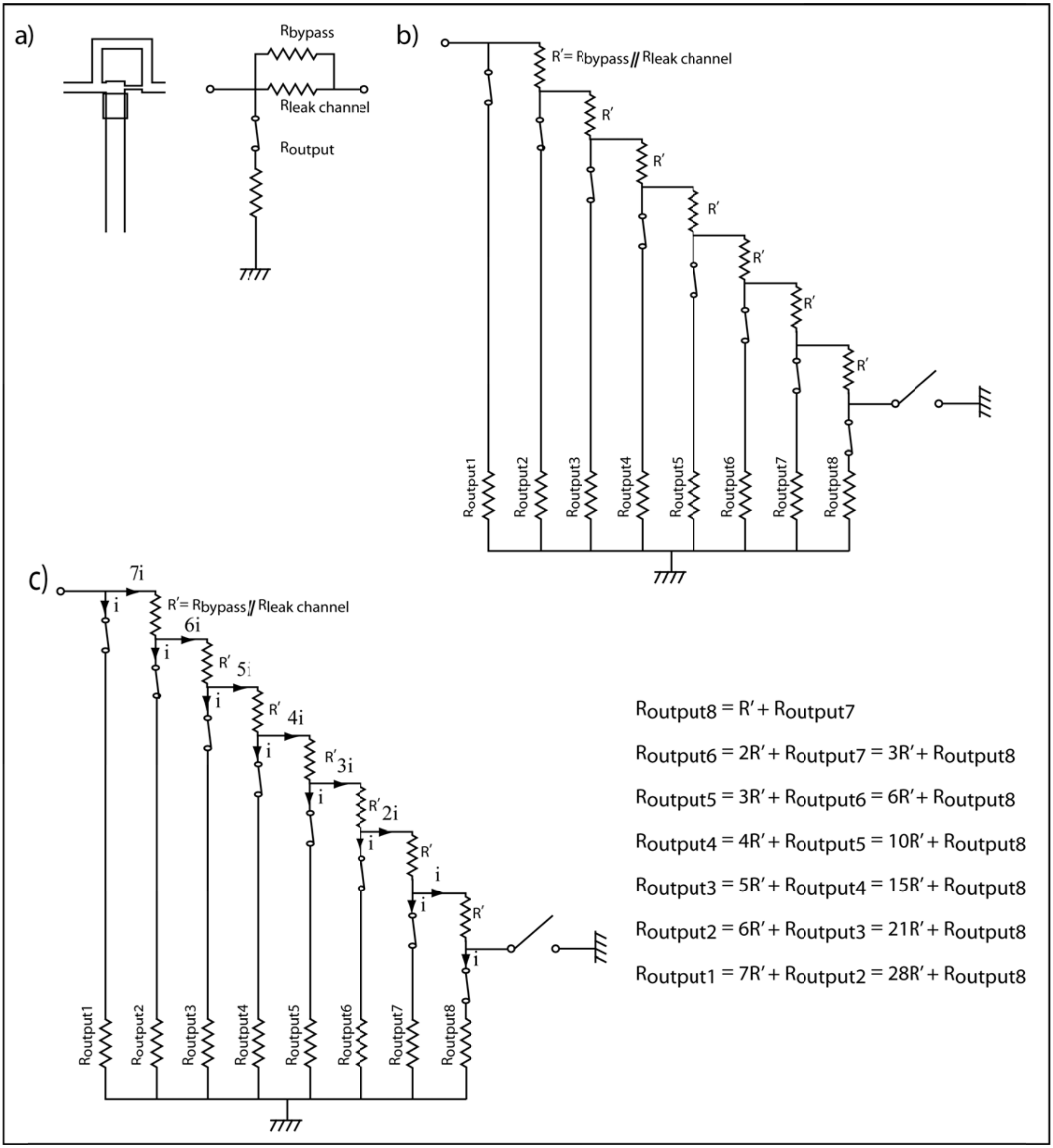
Supp Fig. 2 To demonstrate the flexibility of the droplet trapping design described in Fig.2, we used it to trap either: a) 13 droplets (Fig. 2); b) 11 droplets; or c) 7 droplets. The precise number of droplets that can be pooled is purely determined by ratio of the volume of the trapping chamber and droplet volume.



Supp Fig. 3 Method to measure trapping efficiency of different designs. A first droplet is trapped before flowing a second droplet at different velocities and monitoring its effect on trapping stability. We use a stroboscopic system to image the same droplets after a specific time interval on a single picture. Here the time interval is 75 ms, and the series (a-d) shows the case in which the second droplet does not destabilize the trapping (c-d). On contrary, the velocity of the second droplet in the series (e-h) is sufficient to destabilize the trapping and release the first droplet (g-h).



Supp Fig. 4 Calculations of relative hydrodynamic resistances for multiplexed droplet delivery. a) Equivalent electrical circuit of a single trapping module. b) Equivalent electrical circuit of the 8-plex droplet delivery system. c) Computation of the electrical circuit to assure the same current in each delivery channel. Calculations show that the differential in resistances required by such a configuration increases dramatically with the number of trapping-delivery modules. Practically, it is not possible to design such a system and we decided to use a series of pairs of trapping-delivery modules instead.



Supp movies

Supp. Movie 1 (see Fig. 1): Single droplet loading and delivery that demonstrates the basic module – First sequence: 1 cycle in real-time to describe each cycle step by step; Second sequence: 12 cycles (accelerated 5.5 fold) to demonstrate robustness of the basic module.

Supp. Movie 2 (see Fig. 2): Pooling, merging and delivering a precise number of droplets (13 droplets, real-time).

Supp. Movie 3 (see Fig. 6): Multiplex delivery of 8 droplets with stage moving with droplets - First sequence: 1 cycle in real-time to describe each cycle step by step; Second sequence: 9 cycles (accelerated 6.3 fold) to demonstrate robustness of the multiplex module.

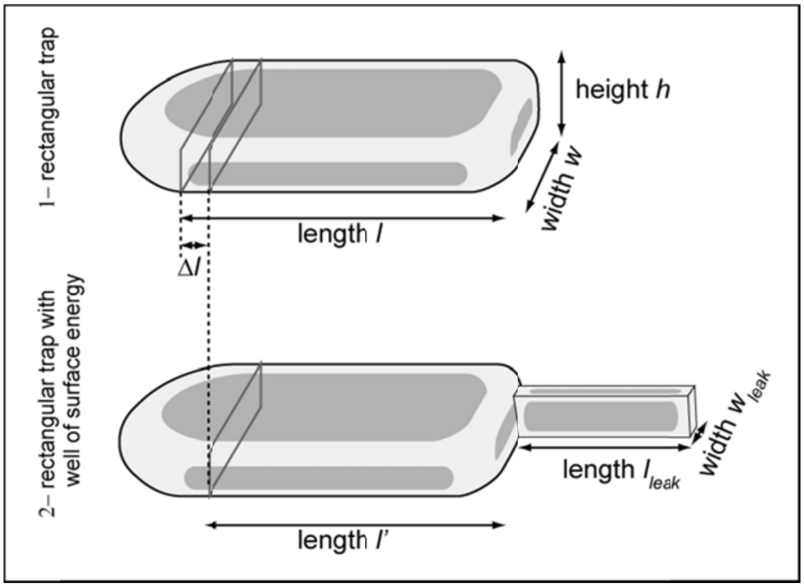
Supp. Movie 4 (see Fig. 6): Wide-field movie of multiplex delivery of 8 droplets – (25 fps, real-time, 267 seconds).

Supp. Movie 5 (see Fig. 7): Auto correction mechanism of droplet trapping**-** First phase accelerated 3-fold, second phase is real-time.

Supp. Movie 6: Movie showing failure modes of the droplet delivery system including a case where droplets are not spaced properly, a case where a small droplet blocks a trapping chamber, and a situation where the air used to deliver droplets pushes a droplet out of the trapping chamber (4 fps, 21 seconds). 1) “near misses”: 9.7sec, 4 fps; 2) “small droplet”: 2.1sec, 4fps; 3) “too many droplets”: 3.1sec, 4fps

Supplemental calculations.

Calculations to estimate the trapping energy due to the leak channel



Our goal is to evaluate the trapping energy added by using a leak channel. The calculations are based on the facts that the trapping energy is proportional to the surface tension and the change in surface area induced by the presence of the microfluidic anchor:

$$E = \gamma \Delta A \qquad (1)$$

And that the droplet volume remains constant across the two situations:

$$\Delta V = 0 \qquad (2)$$

In our configuration, we estimate that the bulk of the droplet remains the same except for its length that changes from *l* to *l'*, such that the change in surface area in eq. (1) is the sum of the area of a band of length Δ_l= *l* -*l'* and the surface of the leak channel:

$$\Delta A = -A_{\Delta_l} + A_{leak} \qquad (3)$$

In addition, from eq. (2) we can infer that the volume of the band of length Δ_l is equal to the volume of the cap:

$$V_{\Delta_l} = V_{leak} \qquad (4)$$

In our configuration,

$$A_{\Delta_l} = 2\,\Delta l(w + h) \qquad (5)$$

and

$$V_{\Delta_l} = wh\Delta l \qquad (6)$$

hence

$$A_{\Delta_l} = 2(w + h)\frac{V_{leak}}{wh} \qquad (7)$$

and

$$\Delta A = -2\frac{w+h}{wh}V_{leak} + A_{leak} \tag{8}$$

$$\Delta A = 2hl_{leak}\left(1 - \frac{w_{leak}}{w}\right) \tag{9}$$

$$\mathrm{E} = 2\gamma hl_{leak}\left(1 - \frac{w_{leak}}{w}\right) \tag{10}$$

Calculations to estimate the trapping energy due to the microfluidic anchor

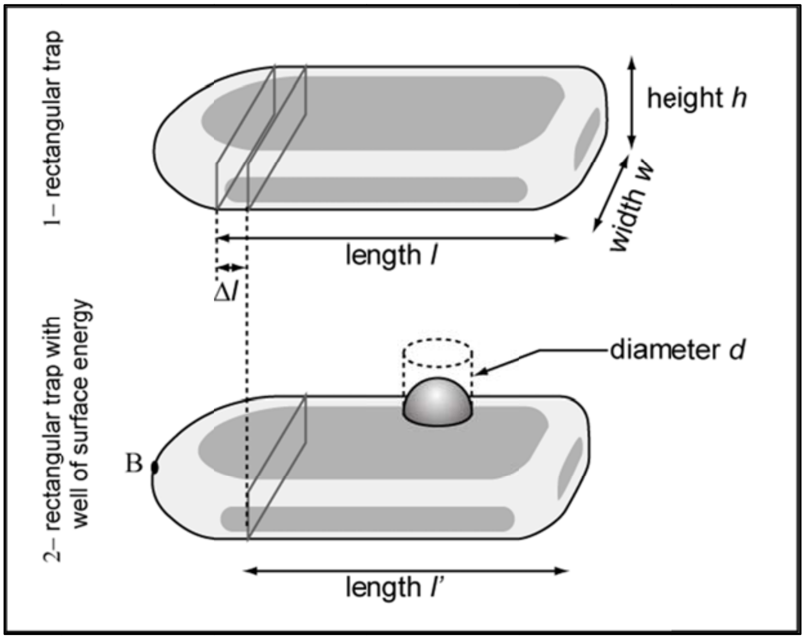


Figure. Definition of the parameters for estimating the added trapping energy allowed by using a well of surface energy. Panel 1 represents a droplet in a rectangular trap alone; Panel 2 represents a droplet in a rectangular trap that a comprises a well of surface energy. The main differences are the creation of a cap that enters the well, as well as a reducing of the droplet length in the rectangular trap. The added energy is proportional to the surface tension and the surface changes due to the creation of the cap and the loss of a band of width Δl because of the change in the droplet length. Droplet volume remains constant.

Our goal is to evaluate the trapping energy added by using a well of surface energy. The location of this well has no consequence on this estimation, and we moved the well of surface energy in the figure for clarity purpose. The calculations are based on the facts that the trapping energy is proportional to the surface tension and the change in surface area induced by the presence of the microfluidic anchor:

$$\mathrm{E} = \gamma \Delta A \qquad (11)$$

And that the droplet volume remains constant across the two situations:

$$\Delta V = 0 \qquad (12)$$

In our configuration, we estimate that the bulk of the droplet remains the same except for its length that changes from *l* to *l'*, such that the change in surface area in eq. (1) is the sum of the area of a band of length $\Delta_l = l - l'$ and the surface of the cap created by the presence of the microfluidic anchor:

$$\Delta A = -A_{\Delta_l} + A_{cap} \qquad (13)$$

In addition, from eq. (2) we can infer that the volume of the band of length Δ_l is equal to the volume of the cap:

$$V_{\Delta_l} = V_{cap} \qquad (14)$$

In our configuration,

$$A_{\Delta_l} = 2\,\Delta l(w + h) \qquad (15)$$

and

$$V_{\Delta_l} = wh\Delta l \qquad (16)$$

hence

$$A_{\Delta_l} = 2(w + h)\frac{V_{cap}}{wh} \qquad (17)$$

and

$$\Delta A = -2\frac{w+h}{wh}V_{cap} + A_{cap} \qquad (18)$$

To calculate V_{cap} and S_{cap} we consider the shape of the cap to be a spherical cap defined by its radius R_{cap} or curvature C_{cap} and the radius of the base of the cap d/2. To estimate C we consider that the droplet is at equilibrium without external forces and that the Laplace pressure is constant across the droplet, such that the curvature of the cap should be the same as the curvature of the free surface at the entrance of the trap (point B in Fig.). In point B we assume that the curvature is dictated by the geometry such that the local curvatures are h/2 and w/2, hence:

$$C_{cap} = \frac{1}{2}\left(\frac{2}{h} + \frac{2}{w}\right) = \frac{w+h}{wh} \qquad (19)$$

$$R_{cap} = \frac{wh}{w+h} \qquad (20)$$

The surface area of a spherical cap is:

$$A = \pi(a^2 + h^2) \qquad (21)$$

where *a* is the radius of the base of the cap, and *h* is the height of the cap. The relationship between *h*, *R*, and *a* is the following:

$$h = R - \sqrt{R^2 - a^2} \qquad (22)$$

hence,

$$A = 2\pi R^2\left(1 - \sqrt{1 - \frac{a^2}{R^2}}\right) \qquad (23)$$

The volume of the spherical cap derives from:

$$V = \frac{\pi h^2}{3}(3R - h) \qquad (24)$$

$$V = \frac{2\pi}{3}R^3\left(2 + \sqrt{1 - \frac{a^2}{R^2}}\right)\left(1 - \sqrt{1 - \frac{a^2}{R^2}} - \frac{a^2}{2R^2}\right) \qquad (25)$$

In our case $a = \frac{d}{2}$, and $R_{cap} = \frac{wh}{w+h}$, by defining b as $b = \frac{a}{R}$ we obtain:

$$A = 2\pi R^2\left(1 - \sqrt{1 - b^2}\right) \qquad (26)$$

and

$$V = \frac{2\pi}{3}R^3\left(2 + \sqrt{1 - b^2}\right)\left(1 - \sqrt{1 - b^2} - \frac{b^2}{2}\right) \qquad (27)$$

now

$$\Delta A = -2\frac{w+h}{wh}V_{cap} + A_{cap} \qquad (28)$$

$$\Delta A = -\frac{2}{R}V_{cap} + A_{cap} \qquad (29)$$

$$\Delta A = -\frac{2}{R}\frac{2\pi}{3}R^3\left(2 + \sqrt{1 - b^2}\right)\left(1 - \sqrt{1 - b^2} - \frac{b^2}{2}\right) + 2\pi R^2\left(1 - \sqrt{1 - b^2}\right) \qquad (30)$$

$$\Delta A = 2\pi R^2\left(1-\sqrt{1-b^2}\right)\left[1-\frac{2}{3}\left(2+\sqrt{1-b^2}\right)\left(1-\frac{b^2}{2\left(1-\sqrt{1-b^2}\right)}\right)\right] \quad (31)$$

$$\Delta A = 2\pi R^2 S(b) \quad (32)$$

where

$$S(b) = \left(1-\sqrt{1-b^2}\right)\left[1-\frac{2}{3}\left(2+\sqrt{1-b^2}\right)\left(1-\frac{b^2}{2\left(1-\sqrt{1-b^2}\right)}\right)\right] \quad (33)$$

and

$$R = R_{cap} = \frac{wh}{w+h} \quad (34)$$

<u>Simple calculation to estimate the upper limit of the trapping energy</u>

We can easily estimate the upper limit of the effect of the microfluidic anchor by considering that the most efficient trapping would occur if the value of the cap diameter is equal to the diameter of the well of energy or D_{cap}=d. In this case, we can still use:

$$\Delta A = -2\frac{w+h}{wh}V_{cap} + A_{cap} \quad (35)$$

with $V_{cap} = \frac{\pi}{12}d^3$, and $A_{cap} = \frac{1}{2}\pi d^2$, hence

$$\Delta A \leq -2\frac{w+h}{wh}\frac{\pi}{12}d^3 + \frac{1}{2}\pi d^2 \quad (36)$$

$$\Delta A \leq -\frac{\pi}{6}\frac{w+h}{wh}d^3 + \frac{1}{2}\pi d^2 \quad (37)$$

$$\Delta A \leq \pi d^2\left(\frac{1}{2} - \frac{w+h}{6wh}\,d\right) \quad (38)$$

<u>Numerical applications</u>:

With d = 50 μm, h = 35 μm, w = 110 μm, w_{leak} = 20 μm and l_{leak} = 110 μm:

	ΔA
Leak channel (eq. 10)	6300
Microfluidic anchor (eq. 32)	1420
Microfluidic anchor upper limit (eq. 38)	1460

Numerical application in our configurations shows that:

$$E_{leak\ channel} \sim 6.3 * 10^3 \gamma \quad (1)$$

$$E_{microfluidic\ anchor} \sim 1.4 * 10^3 \gamma \quad (3)$$