Supplementary Material (ESI) for Lab on a Chip

Managing evaporation for more robust microscale assays. Part 2: Characterization of convection and diffusion for cell biology – Supplementary Information.

Erwin Berthier, Jay Warrick, Hongmei Hu and David J. Beebe*

APPENDIX 1
Mass saturation concentration of water

The water vapor mass concentration C_0 at the interface of the drop is equal to the saturation concentration and can be estimated as a function of the temperature T using the empirical fit for temperature under 40 degrees [24]:

$$C_{sat} = 5.018 + 0.323T + 8.185.10^{-3}T^2 + 3.124.10^{-4}T^3 \quad (1)$$

Equation (1) states the mass saturation concentration for pure water. For a liquid of different osmolarity, the saturation concentration is given by the Raoult law, where x is the molar fraction of solute:

$$C_{sat}[x] = (1-x)C_{sat}$$

APPENDIX 2
Passive pumping flow

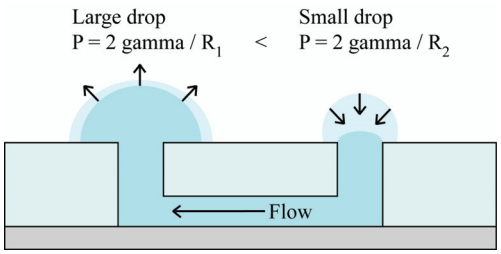

Figure 1 Schematic of pressures and flow during passive pumping.

Traditional passive pumping relies on a pressure differential created by surface tension. The pressure generated is inversely proportional to the radius, as stated by the Laplace law, with P the pressure in the drop, γ, the surface tension, and R, the radius of the drop.:

$$P = \frac{2\gamma}{R(t)} \quad (2)$$

Therefore a large drop will experience a small pressure, and a smaller drop placed on the opposite end of a channel will experience higher pressure. This will cause fluid flow from the smaller drop to the larger drop until pressure is equilibrated (fig.1).

APPENDIX 3
Evaporation induced flow

A. Introduction

When the system is stabilized, during a long duration experiment such as cell culture, evaporation causes a variation of volume on both sides of the channel. This variation in radius, thus in pressure, will be larger on the small drop, than on the large drop. An unbalance of pressure is created, and flow is generated to counter balance this pressure differential (fig.2). The direction of the flow is opposite to that of the passive pumping flow used to insert the particles of interest in the channel.

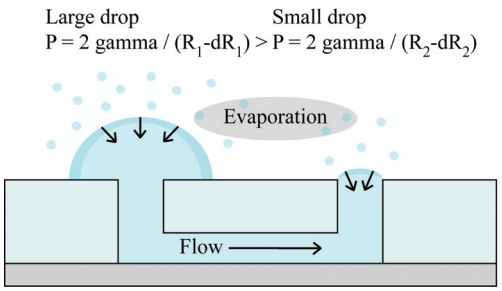

Figure 2 Schematic of pressures and flow during the exposition of a passive pumping system to evaporation.

B. Flow rate

Evaporation occurs on both sides of the channel. On the large drop evaporation is more intense and will cause the major variation of volume in the system. For a spherical cap of height H, provided the height is not too different from the radius of the drop, R, the evaporation rate is written:

$$E = \frac{2\pi D}{\rho}\Delta C_{sat-i}R = \lambda R \quad (3)$$

Evaporation occurring on the small spherical cap will be responsible for the flow. The air-liquid interface takes the geometry of a disk of radius R_p, and the flow rate can be written:

$$Q = \frac{2D_w}{\rho}\Delta C_{sat-i}R_w = \lambda R_w \quad (4)$$

C. Humidity in a container

The expression of the evaporation rate (4) requires the knowledge of the concentration drop from the interface of the liquid to the environment surrounding it, ie ΔC_{sat-i}. If the container is open, this drop will be easily measured using a hygrometer in the ambient air of the room. In a closed container however, this value can prove less straightforward to probe. Using a mass balance argument, it is clear that the total amount of vapor leaving the container is equal to the total amount of vapor produced through evaporation. The humidity leak has been calculated by defining a characteristic length of a container, termed the diffusive admittance, ζ, representing the tightness of the seal. The total evaporation is merely the addition of the evaporation at each surface given by (3). Thus, ΔC_{sat-i} can be determined using a value of the diffusive admittance of a container (table.1):

$$\Delta C_{sat-i} = \frac{\varsigma}{2\pi}\frac{\Delta C_{sat-e}}{\sum R} \quad (5)$$

D. Flow rate measurements

Verification of the analytical expression for the flow rate has been done experimentally. A passive pumping channel was filled and a 10µL drop was placed on one end. A petridish was placed over it such that only the evaporation port was exposed to atmosphere. To reduce as much as possible evaporation inside the pestridish, sacrificial droplets were placed and mineral oil was used to seal it. Time for total fluid loss was measured and the flow rate was deduced by assuming a constant flow rate over that time (fig.3).

To measure the flow at higher humidity levels, a container was fabricated incorporating a limited number of evaporative dishes, such that natural equilibrium occurs at a set humidity. The large container was modified to fit a humidity logger measuring humidity for the whole duration of the experiment verifying that the assumption of constant flow rate is correct.

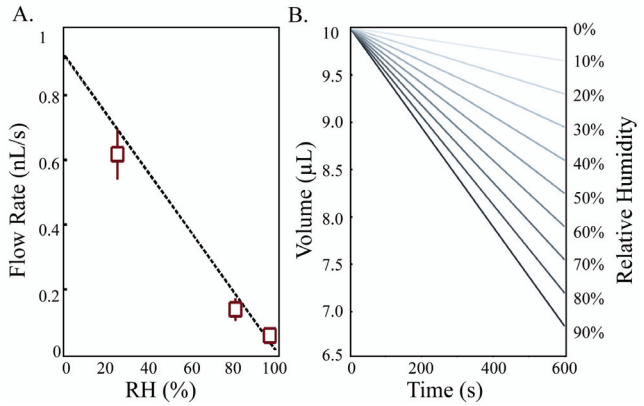

Figure 3 A. Flow rate in a passive pumping channel in function of the relative humidity and experimental measures (crosses). B. Variation of volume of the large drop during evaporation for relative humidity (RH) varying from 10% to 100% (shades of grey). At 100% no loss of volume or flow occurs.

APPENDIX 4

Experimental observations of convection/diffusion

Experimental verification has been performed to emphasis the necessity of quantifying evaporative flow. A bolus of dyes of various diffusion coefficients have been injected in a PDMS channel to observe the displacements of the fronts. Ability for the dye to diffuse "upstream" gave evidence of diffusion

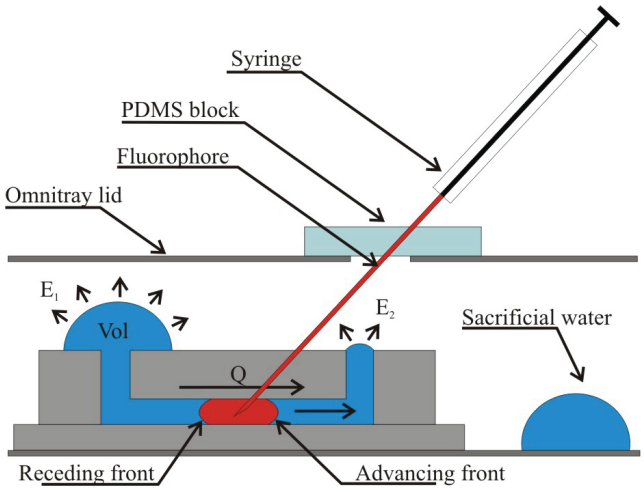

Figure 4 (Color Online) Experimental setup for diffusion/convection measurements. Evaporation is controlled by leaving or removing the lid.

Table 1 Characteristic diffusive admittance ζ for different platforms with standard deviation determined from experimental results.

Container	Perimeter	Diffusive admittance
Tissue culture flask (T50)	N/A	ζ = 0.6 +/- 0.02 10^{-3}m
Petridish 1''	79 mm	ζ = 2.5 +/- 0.1 10^{-3}m
Petridish 2''	160 mm	ζ = 3.3 +/- 0.3 10^{-3}m
Petridish 4''	315 mm	ζ = 4.9 +/- 0.7 10^{-3}m
Omnitray closed	430 mm	ζ = 6.5 +/- 0.3 10^{-3}m
Omnitray sealed	430 mm	ζ = 1.7 +/- 0.2 10^{-3}m

Table 2 Convection of diffusion for various dyes at different humidity. Experimental observation of the dominant condition is reported in the last column.

Fluorophore	RH	D (m^2s^{-1})	Pe	Observation
1µm beads		5.10^{-12}	37	Convection
Dextran10kDa	50%	5.10^{-11}	3.7	Convection
Alexa488		2.10^{-10}	0.9	Both
1µm beads		5.10^{-12}	2.3	Convection
Dextran10kDa	95%	5.10^{-11}	0.2	Both
Alexa488		2.10^{-10}	0.06	Diffusion
1µm beads		5.10^{-12}	0	Diffusion
Dextran10kDa	100%	5.10^{-11}	0	Diffusion
Alexa488		2.10^{-10}	0	Diffusion

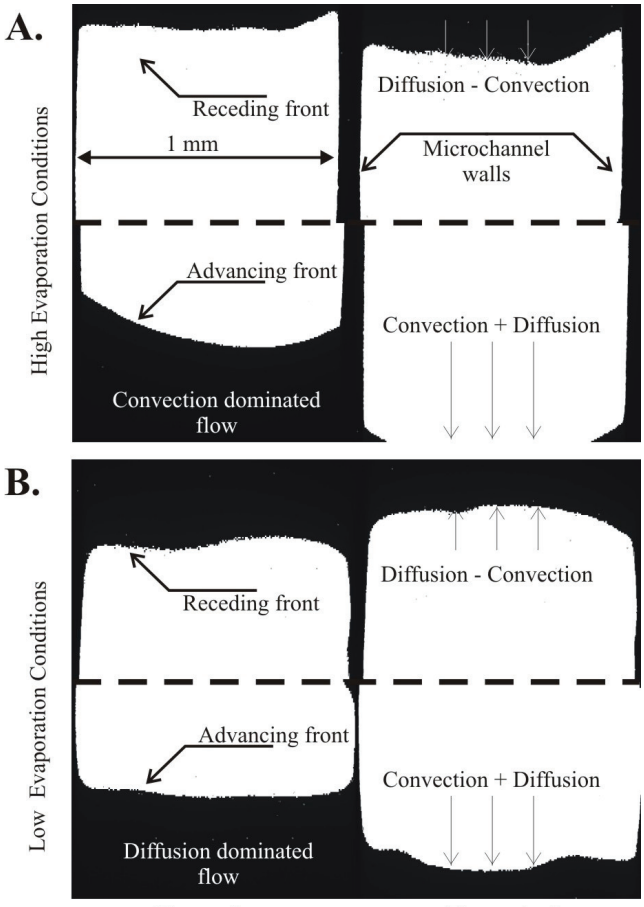

Figure 5 (Color Online) Fluorescence images of a plug of Alexa488 + Dextran10000 under different evaporation conditions at 25 degrees. A. High evaporation. B. No evaporation.

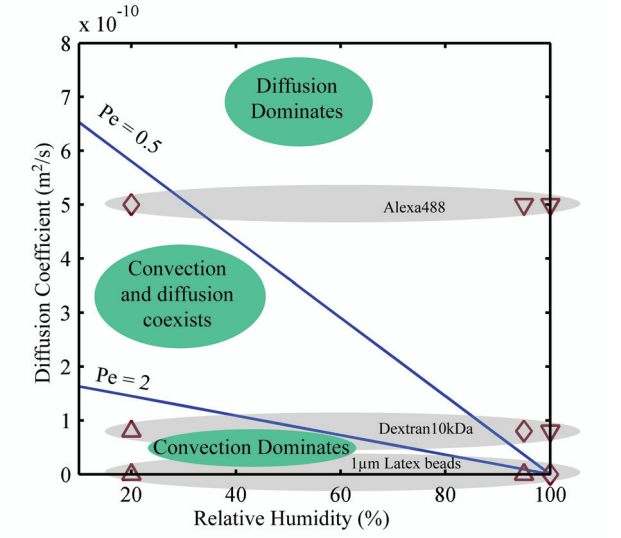

Figure 6 Effect of evaporation for different humidity on compounds depending on their diffusion coefficient, calculated using the Peclet number (6). In upward triangles, experimentally determined points in which convection was dominant; in downwards triangles diffusion was dominant; and in diamonds both were significant.

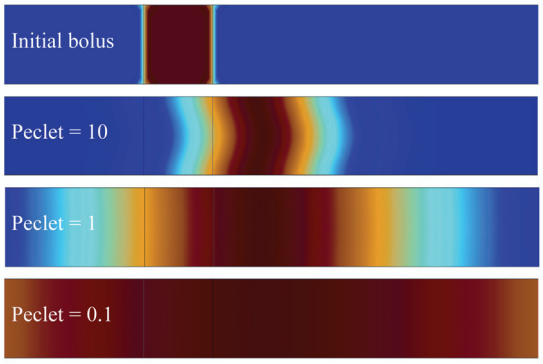

Figure 7 (Color Online) Experimental setup for diffusion/convection measurements. Evaporation is controlled by leaving or removing the lid.

dominating conditions, whereas general docility to the flow proved convection dominated conditions (fig.4 and 5).

Microchannels were fabricated using soft lithography techniques and PolyDiMethylSiloxane (Sylgaard 184, Dow Corning). The molds were created by coating SU-8 100 (Microchem) photoresist on a silicon wafer. The process is described in more detail elsewhere. A transparency film, a thin rubber sheet and a 7 kg iron weight are placed on top of the mold and uncured PDMS to allow the SU-8 ports to pierce through the PDMS layer. The stack is baked on a hot plate at 85 degrees for 1 hour 30 minutes. The PDMS is then peeled off the mold and laid on an Omnitray (NUNC, Rochester, NY). The channels are filled using a 2-20 µL pipettor.

For high evaporation rates, the tray is placed lidless on the microscope exposing the device to 50% air humidity. For low evaporation rates, 1mL of sacrificial water is placed in the tray with the lid on; the air humidity rises to 95% (fig.4). For minimum evaporation the tray is sealed with parafilm (SPI supplies, West Chester, USA). Holes are made in the lid of the Omnitray to add the inlet drops; PDMS blocks seal these holes (fig.4). All of these measurements have been made at room temperature (25 degrees), the shutter of the microscope being opened only during the measurements.

To measure the velocity and the diffusion in the channel, a bolus of fluorophore is injected into the channels using a syringe and needle (fig.4). 1 µm fluorescent beads (Fluorospheres, Invitrogen, Carlsbad, CA) are used for low diffusion rate dye, Alexa 488 bonded to Dextran10000 is used for a intermediate diffusion rate dye, and Alexa 488 alone is used for a high diffusion rate dye. Images of the advancing front and receding front of the plug of dye are recorded with an Olympus IX70 fluorescent microscope every 20 seconds for 4 minutes. The displacements of some fronts are displayed in (fig.5).

The experimental results, compiled in table 2, allow us to make several observations. The observation of the diffusion of fluorophores in a channel is coherent with the predictions given by the Peclet number (fig.5). We observe that in the case of beads, ($D=5.10^{-12}m^2s^{-1}$), diffusion is dominant only for no evaporation. For an average protein with a molecular weight of 10kDa ($D=5.10^{-11}m^2s^{-1}$) diffusion is dominant for no evaporation conditions, and convection becomes observable yet non dominant at 95% humidity (fig.6). Finally, for a rapidly diffusing molecule, usually smaller than a protein ($D=2.10^{-10}m^2s^{-1}$), diffusion seems to dominate even for high evaporation, although convection becomes non-negligible at very low humidity.

Fig.6 illustrates the "cutoff" level for the diffusion constant under which flow will cause the compound to be washed away. For this we consider a characteristic length L equal to an average distance between two "communicating" cells of 100µm. Two threshold Peclet numbers have been considered; Compounds with a Peclet number under 0.5 are considered diffusion driven and those with a Peclet above 2 are considered convection driven. Observations using fluorophores of known diffusion constant show good compatibility with the Peclet number as illustrated in fig.6.

COMSOL simulations of a bolus of particles with $D=10^{-10}$ after 2 minutes in the channel presents the displacement and smearing of the initial band (fig.7). This illustrates the results that were sought in the experimental setup. We can see that the value of the maximum concentration is displaced with the same value for all three Peclet numbers. Only the diffusion allows the particle to diffuse upstream at a Peclet of 0.1 or under. At Pe=1, both diffusion and convection are distinguishable, however it is clear that diffusion upstream will be hindered. For a Peclet above 10, diffusion upstream is prevented, but diffusion downstream cannot homogenize the channel and signaling can be impaired.

APPENDIX 5
Trigonometric relationships in a spherical cap

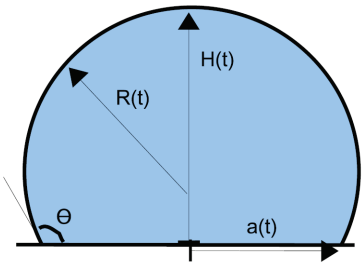

Figure 8 Spherical cap of Radius R and height H.

Lengths:
Following are the different relation ships in spherical caps linking geometrical parameters to each other. To express R in function of H and a:

$$R(t) = \frac{H(t)^2 + a^2}{2H(t)}$$

H in function of the contact angle and R:

$$H(t) = (1 - cos\theta)R(t)$$

Finally a in function of R and the contact angle:

$$a(t) = R(t)sin\theta$$

Areas:
The surface area of the spherical cap is:

$$S(t) = 2\pi R(t)H(t)$$

Volumes:
The volume V can be written in function of the wetted radius and the height:

$$V = \frac{\pi}{6}H(t)(3a^2 + H(t)^2)$$

Or in function of the contact angle and the height:

$$V = \frac{\pi(2 - 3cos\theta + cos^3\theta)}{3(1 - cos\theta)^3}H^3 = g(\theta)H^3$$

Or in function of the contact angle and the wetted radius:

$$V = \frac{\pi(2 - 3cos\theta + cos^3\theta)}{3}\frac{a^3}{sin^3\theta} = f(\theta)\frac{a^3}{sin^3\theta}$$