Fluid dynamical analysis of the distribution of ink jet printed biomolecules in microarray substrates for genotyping applications

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Oligonucleotide microarrays are tools used to analyze samples for the presence of specific DNA sequences. In the system as presented here, specific DNA sequences are first amplified by a polymerase chain reaction (PCR) during which process they are labeled with fluorophores. The amplicons are subsequently hybridized onto an oligonucleotide microarray, which in our case is a porous nylon membrane with microscopic spots. Each spot on the membrane contains oligonucleotides with a sequence complementary to part of one specific target sequence. The solution containing the amplicons flows by external agitation many times up and down through the porous substrate, thereby reducing the time delaying effect of diffusion. By excitation of the fluorophores the emitted pattern of fluorophores can be detected by a charge-coupled device camera. The recorded pattern is a characteristic of the composition of the sample. The oligonucleotide capture probes have been deposited on the substrate by using noncontact piezo ink jet printing, which is the focus of our study. The objective of this study is to understand the mechanisms that determine the distribution of the ink jet printed capture probes inside the membrane. The membrane is a porous medium: the droplets placed on the membrane penetrate in the microstructure of it. The three-dimensional (3D) distribution of the capture probes inside the membrane determines the distribution of the hybridized fluorescent PCR products inside the membrane and thus the emission of light when exposed to the light source. As the 3D distribution of the capture probes inside the membrane eventually determines the detection efficiency, this parameter can be controlled for optimization of the sensitivity of the assay. The main issues addressed here are how are the capture probes distributed inside the membrane and how does this distribution depend on the printing parameters. We will use two model systems to study the influences of different parameters: a single nozzle print head jetting large droplets at a low frequency and a multinozzle print head emitting small droplets at a high frequency. In particular, we have investigated the effects when we change from usage of the first system to the second system. Furthermore, we will go into detail how we can obtain smaller spot sizes in order to increase the spot density without having overlapping spots, leading eventually to lower manufacturing costs of microarrays. By controlling the main print parameters influencing the 3D distribution inside the porous medium, the overall batch-to-batch variations can possibly be reduced. © 2008 American Institute of Physics.

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I. INTRODUCTION

A. Microarrays

Microarrays¹ are used in life science and diagnostic applications to detect specific targets in a sample. Such a microarray is a solid support with microscopic spots that contain capture molecules that can bind specifically to a target molecule. Detection of the fluorescently labeled targets or probes is a common strategy to analyze the samples. The system we discuss here makes use of a porous membrane as the solid support. The support contains oligonucleotide capture probes located in individual spots, each of which is able to hybridize to one specific target DNA sequence. The targets have been amplified in a precedent step using a conventional end-point polymerase chain reaction (PCR).² The solution with amplified DNA sequences is forced to flow many times up and down through this porous membrane. During incubation of the sample with the array, the labeled DNA strands hybridize to the spot that contains the complementary capture probes. An optical setup consisting of LED illumination means, filters and a charge-coupled device (CCD) camera records the fluorescent pattern of the microarray. The recorded pattern is a characteristic of the composition of the sample.

B. Microfluidic aspects of microarray manufacturing

The common technology for depositing the capture probes onto the substrate in a precisely controlled manner is noncontact piezo ink jet printing. This technology allows for the patterned deposition of a large number of different fluids containing the different capture probes. Deposition of the whole amount of fluid in a single droplet on the substrate generally leads to larger spot sizes. In order to reduce the spot size to obtain higher density arrays, we apply a burst of smaller droplets that are placed after each other at the desired position.³

Upon hitting the surface of the membrane, a droplet spreads and the fluid starts to penetrate into the membrane. The time it takes for a single droplet of fluid to penetrate into the membrane depends, among other things, on the droplet number in a burst (except for a spot consisting out of a single droplet). The penetration time of the first droplet is short compared to the penetration time of the second and third, and so on. In this respect, our study differs from other investigations on the spreading and permeation of fluid in a spot on a porous substrate,^{4,5} as we include the dynamics during spreading upon impaction of multiple droplets.

The jetting frequency influences the permeation characteristics of the fluid into the membrane. At a low frequency, the total volume of the deposited droplets can penetrate before the next droplet lands. Shifting towards higher frequencies leads to a situation that at a certain moment in time there is still fluid present on the membrane surface when the next droplet impacts, enlarging the amount of fluid to be spread. The surface energy of the substrate is of crucial importance,⁶⁻⁸ as well as the surface tension of the fluid. Usually the biomolecules are dissolved in aqueous solutions, with different additives to optimize the stability of the droplet and spot formation processes.

The capture probes have to be in the very close neighborhood of the inner surface of the membrane in order to be caught by Van der Waals or electrostatic interaction forces between capture probes and functional groups present on surface of the inner structure of the membrane. The mechanism that causes transport of probes from the main stream of the fluid towards the wall is supposed to be (self) diffusion. This mechanism together with convection will determine how deep the capture probes are present in the membrane.

The objective of this study is to understand the mechanisms that determine the distribution of the ink jet printed capture probes inside the membrane. The main issues we want to resolve are as follows:

- (1) What happens when we go from low frequency, large droplet emission (50–150 Hz, 100–150 pl) to high frequency small droplet emission (up to 6 kHz, 10 pl)?
- (2) How can we control the printing process such in such a way that we end up with smaller sized dots? This is needed to increase the overall microarray density in order to be able to increase the degree of multiplexing using the same microarray substrate size.

The 3D distribution of the capture probes inside the membrane eventually determines the distribution of the hybridized fluorescent PCR products inside the membrane and thus the emission of light when exposed to the light source. Knowledge on these issues is of importance to optimize the sensitivity of the assay.

II. PRELIMINARY REMARKS

A. Process configuration of ink jet printing of oligonucleotides

The final distribution of capture probes inside the membrane is dependent on a number of different processes, such as the following.

Spreading dynamics. Spreading is a highly dynamic phenomenon involving the exchange of the kinetic energy into surface energy and heat due to viscous dissipation.⁷

Permeation. At the very moment the fluid hits the surface of the membrane, it starts to penetrate into its porous structure. The geometrics of the membrane structure and its wetting properties,^{6,8} and the extent the structure is already filled with fluid from the previously landed droplets determine the time it takes to fill the membrane. In this paper, we confine ourselves to one-dimensional permeation according to the Washburn equation.⁸

Convection. The mechanism that transports the capture probes in the membrane is convection associated with the main stream of fluid.⁹

Diffusion. The mechanism that transports the capture probes in the main stream towards the inner surface of the membrane is diffusion.⁹

Evaporation. After depositing all the droplets, the fluid evaporates out of the membrane leaving the capture probes inside the porous structure.⁹ During evaporation, convection of the solvent towards the surface transports the nonbound and still free-floating probes to the upper surface. It may happen that the permeation goes on until the fluid reaches the bottom surface of the membrane. In that case, we have to take into account evaporation from the bottom with induced flow from there.

Capture probe–substrate interaction. The capture probes bind to the inner surface of the membrane by either Van der Waals or electrostatic forces.¹⁰

Coupling of the capture probes and washing of the nonbound capture probes. With different surface chemistries, the capture probes are coupled to the membrane surface. A subsequent washing step will remove the nonbound capture probes away. We assume homogenous immobilization and washing processes, so no change in distribution of bound capture probes will occur.

In this paper, we confine ourselves to the spreading and permeation dynamics and associated convection and diffusion phenomena.

B. Materials and methods

We use two different model systems in our study. The first model system is a Microdrop Autodrop pipette AD-K-501 (Microdrop, Norderstedt, Germany¹¹). This single nozzle glass pipette print head emits droplets of 100–150 pl that we use at a frequency of 50–150 Hz. The second model system is a Dimatix DMP-200 11610 cartridge (Fuji Film Dimatix, Santa Clara, CA¹²). This 16-nozzle print head dispenses 10 pl droplets that we run up to 6 kHz.

Both model systems will be used to study the three-dimensional (3D) distribution inside a membrane using a total of 1000 pl (1 nl) dispensed spot volume. For the pipette this means 7 droplets of 143 pl jetted at 100 Hz. The Dimatix print head emits 100 droplets of 10 pl at 4000 Hz out of one preselected nozzle of a 16 nozzle print head.

Throughout the paper we consider a Nytran SPC membrane (from Whatman, GE Healthcare¹³) having a thickness of 150 μm and an average pore diameter of 0.5 μm . From additional measurements, we learned that the membrane has a porosity of about 0.5. Furthermore, we assume an equilibrium contact angle $\theta_E=40^\circ$ on and in the membrane against water/air. This number needs some explanation. A porous membrane with submicron features does influence the

TABLE I. Data on droplet emission for a 1000 pl spot.

Droplet volume V (pl)	Droplet radius R (μm)	Droplet velocity v (m/s)	Change of velocity over 1 mm flight path (m/s)	Jetting frequency f (Hz)	Total number of droplets in a burst N	Total process time of printing (ms)
10	13.37	6	−0.86	4000	100	25
143	32.44	3	−0.15	100	7	70

wetting properties of its surface.^{6,8} As the fibers of the membrane are round and smooth we expect that Wenzel's theory holds true (no pinning at the surface structure, so the membrane structure will be filled directly) and the actual contact angle is given by

$$\cos \theta_E^w = r \cos \theta_E, \quad (1)$$

where r is the ratio of the actual area of the membrane surface and the projected area. The droplet size is much larger than the feature sizes of the membrane structure, the value r can be considered to be a constant, not that much different from unity. Therefore, we take the Wenzel contact angle to be characteristic for the equilibrium contact angle. The capture probes are generally dissolved in nM—mM concentrations in aqueous solvents. Some additives are used to optimize jetting performance. The density and viscosity of the fluid used are $\rho = 1000 \text{ kg/m}^3$ and $\eta = 0.001 \text{ Pa s}$, respectively. The surface tension γ equals 0.05 N/m .

The characteristics of the two typical cases are listed in Table I. The total number of droplets in a burst is calculated by dividing the total spot volume by the volume of a single droplet. The last column gives the total process time of printing the burst of N droplets with a frequency f and equals N/f .

When traveling from the nozzle to the substrate, the droplet feels an opposing force due to air friction. Following⁹ the force due to air friction is given by the modified Stokes' law

$$F_k = f_{\text{Stokes}} \pi R^2 \frac{1}{2} \rho_{\text{air}} v^2,$$

$$f_{\text{Stokes}} = \left(\sqrt{\frac{24}{\text{Re}}} + 0.5407 \right)^2 \quad \text{for } \text{Re} < 6000,$$

$$\text{Re} = \frac{2 \rho_{\text{air}} R v}{\eta_{\text{air}}}. \quad (2)$$

A droplet flying at speed v experiences a deceleration given by

$$\dot{v} = -\frac{F_k}{\rho V} = -f_{\text{Stokes}} \frac{3}{8} \frac{\rho_{\text{air}} v^2}{\rho R}, \quad (3)$$

with $\eta_{\text{air}} = 1.85 \times 10^{-5} \text{ Pa s}$ and $\rho_{\text{air}} = 1.2 \text{ kg/m}^3$ for the standard cases the decelerations are given in Table I. Note that we have calculated the deceleration of a droplet in terms of a decrease in velocity over a flight distance between nozzle and substrate of 1 mm.

In the following we will use as droplet speed the value at the moment of landing. For small droplets this means that they have to be jetted at a somewhat higher velocity.

TABLE II. Dimensionless numbers We, Oh, Re, and K .

Volume V (pl)	Radius R (μm)	Velocity v (m/s)	Droplet frequency f (Hz)	V_{os}^a (m/s)	We	Oh	Re	K^a
10	13.37	6	4000	6	19	0.03	160	81
143	32.44	3	100	1.5	12	0.02	195	59

^aReferences 7 and 14.

III. DYNAMICS OF DROPLET IMPACT ON SOLID SUBSTRATES

A. General remarks on droplet impact

In this chapter, we will treat two cases: (i) the impact of a droplet on a dry substrate and (ii) the impact of a droplet on a wet substrate. The latter case occurs when the droplet frequency f is so high that permeation cannot cope anymore and that there is fluid on the surface of the membrane at the instant the next droplet lands. Following Yarin,⁷ the We, Oh, Re, and K numbers, define the characteristic features of droplet impact (all material and geometric data used relate to the droplet):

$$\text{We} = \frac{2\rho R v^2}{\gamma}, \quad \text{Oh} = \frac{\eta}{\sqrt{2R\gamma\rho}}, \quad \text{Re} = \frac{2\rho v R}{\eta}, \quad K = \text{We Oh}^{-2/5}. \quad (4)$$

The Weber number We is a measure for the influence of the kinetic energy relative to the surface energy. The Ohnesorge number Oh relates the viscous forces to the surface tension forces, and the Reynolds number Re inertia forces to viscous forces. The composite number K is an important number defining the transition from spreading to splashing on dry substrates. For low impact velocities, the spreading is defined as a continuous process like the spreading of a pancake with a well-defined and clearly visible outer rim. When the impact velocity increases beyond a certain value, the spreading ends up into a crown consisting of a thin layer of liquid emerging almost perpendicular from the surface of the substrate, that immediately disintegrates in a large number of small droplets flying all over the place. Yarin⁷ refers to this effect as splashing.

On wet substrates, the transition from spreading to splashing depends on the experimentally determined value of a threshold velocity defined by^{7,14} (SI units),

$$V_{os} = 18 \left(\frac{\gamma}{\rho} \right)^{1/4} \left(\frac{\eta}{\rho} \right)^{1/8} f^{3/8}. \quad (5)$$

On dry substrates, the value for the composite dimensionless group K defining the transition from spreading to splashing is about 650.⁷ Table II gives the relevant data for our two cases.

First of all, we see that for both cases kinetic effects dominate over influences associated with surface tension and viscosity because both the We and Re numbers are large compared to unity. As the Oh number is very small, damping due to viscous dissipation plays a minor role. It is the exchange between kinetic and surface energy that matters. Based on the results given in Table II we conclude that for our two cases on dry surfaces no splashing will occur. On wet surfaces, however, for the large droplet and low frequency case we may expect splashing, because the droplet velocity is larger than V_{os} . In order to end up with well-defined spots, splashing must be avoided. Therefore, the state of the surface, dry or wet, is of concern at the instant the droplet impacts.

B. Single droplet case

Consider a droplet with radius R , density ρ , and surface tension γ approaching the surface of a porous medium with velocity v (see Fig. 1). This surface is characterized by its surface tension in air γ_{SO} and its surface tension in the liquid of the droplet γ_{SL} . Based on experimental and

FIG. 1. Geometric details of a droplet before and after impacting on a solid surface. The radius of the free flying droplet is given by R , the radius and thickness of the dot on the surface containing the volume of the droplet by R_s and h_s , respectively.

computational observations with nonsplashing droplets,^{7,14–22} impacting droplets spread like pancakes. For our consideration, we follow the terminology and symbols used by de Gennes *et al.*⁸ In static equilibrium (velocity of contact line zero), the difference between the surface energies of the substrate against air and liquid follows from Young's relation:

$$\gamma \cos \theta_E = \gamma_{SO} - \gamma_{SL}. \quad (6)$$

θ_E is the equilibrium contact angle of the fluid on the solid surface.

For the moving contact line problem, we have to distinguish between:

- i. The advancing contact line case: with advancing (dynamic) contact angle θ_D . The fluid wets the solid surface and
- ii. The receding contact line case: with receding contact angle θ_R . The fluid de-wets the surface.

For the advancing contact angle we use the Jiang, Soo-Gun, and Slattery formula,^{23,24}

$$\frac{\cos(\theta_E) - \cos(\theta_D)}{\cos(\theta_E) + 1} = \tanh(4.96 \text{Ca}^{0.702}), \quad (7)$$

with θ_E the static contact angle, θ_D the dynamic advancing contact angle, and Ca the capillary number defined by $\text{Ca} = \eta U / \gamma$ (η , dynamic viscosity; U , velocity of moving contact line; and γ , surface tension). The receding contact angle θ_R is usually zero because often a very thin layer of fluid stays on the surface. This results into a pinned contact line. To investigate the spreading dynamics we proceed as follows: First, we estimate the contact line velocity. We show that the contact line velocity is so high that significant tumbling of the contact angle will take place. Next, by solving the equation of motion of the spreading droplet taking into account the tumbling effect, we calculate the extent and timing of the spreading.

Based on experimental considerations,^{7,15,17–21} initially the spherical droplet upon impacting changes into a domed cylinder with the same radius as the droplet, then into a hatlike-shaped dot, and later into a pancake shape (see Fig. 2).

FIG. 2. Transformation of a droplet landing on a flat surface into an expanding flat pancakelike cylinder. Depicted here are four stages: the situation just before impact, the situation that the radius of the domed cylinder in contact with the substrate is equal to the droplet radius (the height of the domed cylinder follows from conservation of mass), the situation that the droplet starts to spread beyond the radius of the droplet, and finally the situation that the dot expands like a pancake.

Directly after first contact the inertia of the droplet flattens the contact region in short time to a cylindrical shape, as visualized in Fig. 2. Assuming that the velocity of the mass of fluid of the droplet while changing into a cylinder stays equal or almost equal to v , the mean contact line velocity during this time interval is of the order of magnitude

$$U \approx \frac{\text{change in location of contact line}}{\text{time}} = \frac{R}{\left(\frac{2R - \frac{5}{3}R}{v}\right)} = 3v. \quad (8)$$

From this particular moment on the cylinder expands via a hat-shaped dot into a pancake following the dynamics already explained. Meanwhile, the no-slip boundary condition at the substrate surface is established and a shear wave starts to penetrate into the expanding pancake of fluid. The reach (penetration depth) δ of the shear wave is given by^{9,25}

$$\delta = 4 \sqrt{\frac{\eta}{\rho}}. \quad (9)$$

For the large droplet the penetration depth of the shear wave at the end of the initial stage of spreading as shown in Fig. 2 equals $7.6 \mu\text{m}$; for the small droplet we find $3.5 \mu\text{m}$. These values indicate that almost immediately the shear wave penetrates over a considerable distance into the expanding dot. Following the spreading event, we observe that the capillary pressure of the cylindrical part is half of the capillary pressure of the spherical dome. So, the expanding fluid more easily breaks through capillary barrier of the cylindrical part, while the dome of fluid still moves downward with the droplet velocity. At that moment in time, the contact line velocity is given by

$$2\pi R^2_3 RU = \pi R^2 v \rightarrow U \approx \frac{3}{4}v. \quad (10)$$

Using the contact line velocity given by Eq. (10) we can calculate the Ca number and the tumbling of the contact line, by using Eq. (7). The results are given in Table III.

Initially, there is strong contact angle tumbling beyond 90° . At the end of the spreading where the contact line velocity approaches zero, the equilibrium value of the contact angle is valid again. In our analysis we use for the whole spreading event the value calculated above. This means that in order to wet the surface, energy has to be supplied (contact angle larger than 90°), this will limit the final spreading.

We will compare the dynamic spreading data with the static spreading of a nonevaporating droplet on a surface with contact angle θ_E . See Fig. 3 below for geometric details.

TABLE III. Dynamic contact angles ($\theta_E=40^\circ$).

Volume (pl)	Radius (μm)	Velocity at impact (m/s)	Mean contact line velocity, Eq. (10) (m/s)	Ca	θ_D ($^\circ$)
10	13.37	6	4.5	0.09	121
143	32.44	3	2.25	0.045	98

Given the volume V and the equilibrium contact angle θ_E , the radius R_d (radius of curvature of dome), the radius of the wetted area R_s and the height h of the dome can be calculated with²⁶

$$\cos \theta_E = \frac{R_d - h}{R_d}, \quad \sin \theta_E = \frac{R_s}{R_d}, \quad R_d = \left(\frac{3V}{\pi(2 - 3 \cos \theta_E + \cos^3 \theta_E)} \right)^{1/3}. \quad (11)$$

To calculate the spreading dynamics we use Lagrange's method^{27,28} to derive the equation of motion.

The droplet spreads like a pancake, with $h_s(t)$ and $R_s(t)$ the momentary thickness and spreading radius. We define as generalized coordinates the position of the center of gravity $Z_{\text{center}}(t)$ and the velocity of the center of gravity $\dot{Z}_{\text{center}}(t)$. The idea is visualized in Fig. 4. As the shear wave penetrates fast into the expanding dot Eq. (9), we assume that the velocity profile in the spreading dot is parabolic, with zero velocity at the fluid-solid interface and zero derivative at the fluid air interface (zero stress at surface). A radial velocity profile that obeys the no-slip condition at the fluid-substrate interface and the zero-stress condition at the fluid-air interface is given by

$$v_r = -f(r)(z^2 - 2h_s z),$$

$$z = 0, \quad v_r = 0,$$

FIG. 3. Static equilibrium shape of a droplet on a flat surface.

FIG. 4. Pancake model of an expanding dot.

$$z = h_s, \quad \frac{\partial v_r}{\partial z} = -f(r)(2z - 2h_s) = 0. \quad (12)$$

Using the equation of continuity, we obtain an expression for v_z

$$\begin{aligned} \frac{1}{r} \frac{\partial}{\partial r}(rv_r) + \frac{\partial v_z}{\partial z} &= 0, \\ \frac{\partial v_z}{\partial z} &= (z^2 - 2h_s z) \frac{1}{r} \frac{\partial}{\partial r}\{rf(r)\}, \\ v_z &= \left(\frac{1}{3}z^3 - h_s z^2\right) \frac{1}{r} \frac{\partial}{\partial r}\{rf(r)\}. \end{aligned} \quad (13)$$

Going from the second to the third line, use has made of the fact that for $z=0$: $v_z=0$. For the pancake model planes with constant z move parallel to the plane $z=0$, so v_z is not a function of r . Consequently [with $f(r)=0$ for $r=0$],

$$\begin{aligned} v_r &= -\frac{1}{2}C_1 r(z^2 - 2h_s z), \\ v_z &= C_1 \left(\frac{1}{3}z^3 - h_s z^2\right), \\ C_1 &= -\frac{24}{5} \frac{1}{h_s^3} \dot{Z}_{\text{center}}. \end{aligned} \quad (14)$$

The kinetic energy in Lagrange's equation expressed in terms of the generalized coordinates can now be evaluated,

$$T = \int_0^{h_s} \int_0^{R_s} \frac{1}{2} \rho 2\pi r dr dz (v_r^2 + v_z^2) = \frac{1}{2} \rho V \dot{Z}_{\text{center}}^2 \left(0.256 \frac{R^3}{Z_{\text{center}}^3} + 2.413714 \right). \quad (15)$$

The potential energy equals the excess spreading energy

$$U = \gamma[(1 - \cos \theta_D) \pi R_s^2 + 2 \pi R_s h_s - 4 \pi R^2] = \gamma \left[(1 - \cos \theta_D) \frac{V}{2Z_{\text{center}}} + 2 \sqrt{2 \pi V Z_{\text{center}}} - 4 \pi R^2 \right]. \quad (16)$$

Note that we have used θ_D rather than θ_E , in order to take into account the effect that due to the high contact line velocity and tumbling of the contact angle beyond 90° energy has to be supplied by the expanding dot to wet the surface of the membrane.

With the latent energy $L = T - U$ we obtain by evaluation of Lagrange's equation

$$\frac{d}{dt} \frac{\partial L}{\partial \dot{Z}_{\text{center}}} - \frac{\partial L}{\partial Z_{\text{center}}} = 0, \quad (17)$$

the equation of motion of the center of gravity of the spreading dot,

$$\ddot{Z}_{\text{center}} \left(0.512 \frac{R^3}{Z_{\text{center}}^3} + 4.827 \, 428 \right) - 0.768 R^3 \frac{\dot{Z}_{\text{center}}^2}{Z_{\text{center}}^4} + \frac{\gamma}{\rho Z_{\text{center}}^2} \left[- (1 - \cos \theta_D) + \sqrt{6 \frac{Z_{\text{center}}^3}{R^3}} \right] = 0. \quad (18)$$

To introduce the viscous drag into the equation of motion, we observe that the pancake model with a no-stress condition at the upper surface is half of the case for which a certain fixed amount of fluid is squeezed between two parallel plates. At some moment in time, the force needed to move the plates with respect to each other is given by (see Ref. 9, the formula adapted to our notation)

$$F(t) = - \frac{3}{80 \pi} \eta \frac{V^2}{Z_{\text{center}}^5} \dot{Z}_{\text{center}}. \quad (19)$$

This force acts throughout the whole pancake. So the equation of motion including the effect of viscosity reads

$$\begin{aligned} \ddot{Z}_{\text{center}} \left(0.512 \frac{R^3}{Z_{\text{center}}^3} + 4.827 \, 428 \right) - 0.768 R^3 \frac{\dot{Z}_{\text{center}}^2}{Z_{\text{center}}^4} \\ + \frac{\pi}{15 \rho} \eta R^3 \frac{\dot{Z}_{\text{center}}}{Z_{\text{center}}^5} + \frac{\gamma}{\rho Z_{\text{center}}^2} \left[- (1 - \cos \theta_D) + \sqrt{6 \frac{Z_{\text{center}}^3}{R^3}} \right] = 0. \end{aligned} \quad (20)$$

In order to solve this equation we need initial conditions. From Fig. 2 we learn that from the second situation (domed cylinder) on, the dot expands more or less as a pancake. This situation is taken as $t=0$, with $t=0: Z_{\text{center}} = \frac{2}{3}R$. We assume that at the very moment our solution of the equation of motion makes sense, the kinetic energy of the cylinder equals the kinetic energy of the impacting droplet. So for $t=0$ we have

$$\begin{aligned} \frac{1}{2} \rho V \dot{Z}_{\text{center}}^2 \left(0.256 \frac{R^3}{Z_{\text{center}}^3} + 2.413 \, 714 \right) = \frac{1}{2} \rho V v^2, \\ \dot{Z}_{\text{center}} = -0.55235 v. \end{aligned} \quad (21)$$

Starting at $t=0$ the equation of motion is solved by discrete time stepping. This process is repeated until the velocity changes sign. Figure 5 shows a typical example of the solution for a droplet of 143 pl.

The spreading equals 2.31 and the time it takes to reach maximum spreading is about $3R/v$; the values agree well with experimental and theoretical data found in the literature.^{17,18,20} The results for the two cases are summarized in Table IV (surface tension 0.05 N/m, equilibrium contact angle 40° , density 1000 kg/m^3 , viscosity 0.001 Pa s).

FIG. 5. Time evolution of the spreading characteristics after impacting of a droplet of 143 pl. The arrows indicate the corresponding Y axes. Droplet velocity $v=3$ m/s, surface tension $\gamma=0.05$ N/m, equilibrium contact angle $\theta_E=40^\circ$, and viscosity $\eta=0.001$ Pa s.

Directly after maximal spreading the excess potential energy accelerates the fluid of the dot inwards. We have to distinguish two cases (no permeation in the substrate considered):

(i) The fluid de-wets the surface immediately and the potential energy is transferred into kinetic energy again and as viscous dissipation is small (the Oh number is small in both cases) the droplet leaves the surface with almost the same velocity as the velocity at impact.

(ii) During retraction, the receding contact angle is much smaller than the advancing contact angle or even zero in case a thin layer of fluid is left behind on the de-wetted surface. The contact line stays pinned and the potential energy available for transfer into kinetic energy is much less and the dot stays at the surface.

We pose that the last case applies to the spreading dynamics of a dot on a porous membrane. Namely, during spreading of the dot on the porous membrane, the fluid starts to penetrate into the porous structure of the membrane, so during retraction, the surface stays wetted and the contact angle θ_R becomes zero. The only change in surface energy comes from the rim of the pancake. The energy available at the end of the spreading event and at the start of the retraction equals

$$E_{\text{spreading}} = \gamma 2\pi R_s h_s. \quad (22)$$

The condition that the dot after maximal spreading changes again in a droplet leaving the surface is (available energy: $E_{\text{spreading}}$ minus surface energy droplet, has to be larger than zero)

$$\gamma(2\pi R_s h_s - 4\pi R^2) > 0,$$

TABLE IV. Summary of results on spreading and timing.

Volume (pl)	Radius (μm)	Velocity (m/s)	Static spreading [Eq. (11)]	R_s/R [Eqs. (20) and (21)]	Spreading time (μs)
10	13.37	6	1.91	2.36	7
143	32.44	3	1.91	2.31	32

FIG. 6. Droplet impacting on the wetted area of a previously deposited droplet. The droplet spreads over an already wetted area.

$$\frac{2R}{3R_s} - 1 > 0. \quad (23)$$

Referring to Table IV, the minimum value for $R_s/R \cong 2.3-2.4$ at the moment the retraction starts, so for our two cases this condition will never be fulfilled and after impact and spreading the dot stays on the surface. The remaining energy is viscously dissipated during fluid motions inside the dot.

C. Multidroplet case

In order to investigate what happens when more droplets land on the same place on top of each other we use the method used earlier. For the sake of argument, we assume no permeation of fluid in the substrate and no evaporation to the environment. Instead of landing on a dry substrate, the droplet lands on a wetted surface in the dot of the previously deposited droplets as depicted in Fig. 6.

The expression for the surface energy has to be adapted for the fact that the substrate is wetted. On top of the wetted area the equilibrium contact angle is zero, so the advancing contact angle on the already wetted area equals ($\theta_E=0$). On substitution $\theta_E=0$ in the Jiang, Soo-Gun, and Slattery formula^{23,24} [Eq. (7)] we get

$$\frac{\cos(0) - \cos(\theta_{DW})}{\cos(0) + 1} = \tanh(4.96 \text{Ca}^{0.702}),$$

$$\cos \theta_{DW} = 1 - 2 \tanh(4.96 \text{Ca}^{0.702}),$$

$$\text{Ca} = \frac{\eta U}{\gamma} = \frac{3}{4} \frac{\eta v}{\gamma}. \quad (24)$$

Using the same method as explained earlier, we find that the spreading radius is slightly larger. On a wetted surface, the spreading of a 10 pl droplet equals $2.39R$ (on a dry substrate we found $2.36R$). Likewise, on a wetted surface, the spreading of a 143 pl droplet equals $2.4R$ (on a dry substrate $2.31R$).

FIG. 7. Square unit cell of porous substrate. The fluid is applied from the top of the membrane and has penetrated over a distance x .

IV. ABSORPTION OF FLUID INTO THE POROUS STRUCTURE OF THE MEMBRANE

A. Definition of the porous structure model

For our analysis of the fluid dynamics of droplet impact on and permeation in the porous structure of the membrane, we model the membrane in a one-dimensional way as a stack of parallel equally sized tubes with radii R_{pore} at a square and regular pitch L .⁸ The thickness of the membrane is H . For the definition of the square unit cell, see Fig. 7.

The porosity P (filling ratio or spot density) is defined by

$$P = \pi \frac{R_{\text{pore}}^2}{L^2}. \quad (25)$$

For the calculation of the permeation depth as a function of time we use the Washburn equation (1921),⁸

$$x = \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} t}, \quad t = \frac{2\eta}{\gamma \cos \theta_E R_{\text{pore}}} \frac{x^2}{}, \quad (26)$$

with γ and η being the surface tension and viscosity of the fluid, and θ_E the contact angle between the fluid and the inside of the pore.

B. Single droplet case

For the single droplet impact and permeation we assume that the permeation takes place over the circular wetted area defined by R_s . With

$$\frac{1}{P} V = \frac{1}{P} \frac{4}{3} \pi R^3 = \pi R_s^2 \Delta x,$$

TABLE V. Permeation depths and times for 10 pl and 143 pl droplets.

Volume (pl)	Radius (μm)	Velocity (m/s)	R_s/R [Eq. (20)]	Absorption depth Δx (μm) [Eq. (27)]	$\Delta t_{\text{penetration}}$ (μsec) [Eq. (27)]
10	13.37	6	2.36	6.4	8.55
143	32.44	3	2.31	16.2	54.8

$$\Delta x = \frac{1}{P} \frac{4}{3} R \frac{R^2}{R_s^2},$$

$$\Delta t_{\text{penetration}} = \frac{2\eta}{\gamma \cos \theta_E} \frac{\Delta x^2}{R_{\text{pore}}}, \quad (27)$$

we make Table V ($P=0.5$, $\eta=0.001$ Pa s, $R_{\text{pore}}=0.25 \times 10^{-6}$ m = $0.25 \mu\text{m}$, $\gamma=0.05$ N/m, and $\theta_E=40^\circ$).

For large droplets deposited at a frequency of 100 Hz the time between the droplets is 10 ms, so the second droplet will land on a dry surface because the penetration time is much shorter. Small droplets are deposited at 4000 Hz; the time between subsequent droplets is 0.25 ms: for this case surely the second droplet lands on a dry surface as well.

C. Diffusion time constants for the single droplet case

Upon comparing the results of Tables IV and V, we observe that the penetration times and the spreading times are comparable in magnitude. We compare these numbers with the time it takes for a molecule to diffuse from the middle of the porous structure towards the wall by self-diffusion. We assume that when the molecule reaches the surface of the pore, it is caught by van der Waal's or electrostatic forces¹⁰ and will not be detached by the flow.

As the concentration of biomolecules in solution generally is low we take the self-diffusion constant equal to the diffusion constant. There are three cases to consider.²⁹

(i) $t_{\text{diffusion}} \ll \Delta t_{\text{penetration}}$: this implies that the molecules are being caught in the upper part of the membrane and do not flow along with the fluid that further penetrates into the membrane.

(ii) $t_{\text{diffusion}} \sim \Delta t_{\text{penetration}}$: for this case the 3D distribution of the capture probes is the same as the 3D distribution of the fluid.

(iii) $t_{\text{diffusion}} \gg \Delta t_{\text{penetration}}$: the capture probes go along with the solvent molecules into the membrane. Now the time scale $t_{\text{evaporation}}$ of the evaporation of the solvent through the upper part of the membrane becomes important. Although not part of this paper, we will briefly mention two cases. When $t_{\text{evaporation}} \gg t_{\text{diffusion}}$, the capture probes evenly distributes throughout the membrane. The three-dimensional distribution of the capture probes will be uniform in the space occupied by the fluid in the membrane. In case the evaporation time $t_{\text{evaporation}}$ is shorter than the diffusion time, the capture probes will be collected at the surface of the membrane.

Diffusion of proteins and oligonucleotides has been studied extensively.^{30–36} For oligonucleotides it is found that $D_{AB}(\text{oligo/water}) \cong 10^{-10}$ m²/s. The diffusion coefficient of small noninteracting spherical particles in a fluid (dilute solution) is given by the Stokes–Einstein formula⁹

$$D_{AB(\text{oligo/solvent})} = \frac{kT}{6\pi\eta_B R_A}, \quad (28)$$

with species A denoting the capture probes and species B the solvent, k is the Boltzmann's constant, T the absolute temperature, η_B the viscosity of the solvent, and R_A the radius of the particles.

FIG. 8. Stacking of fluid portions per droplet in the membrane.

The diffusion coefficient calculated by means of the Stokes–Einstein formula is based on the notion of the random walk. That means that the statistical value of the change in position of the molecule from its initial position does not depend on the direction. In other words, the particle moves over a certain distance in course of time but any direction is equally probable. Therefore, it can travel right away to the wall over the straight pore, but it is equally well probable that it moves along the center line of the pore. Now we have to remember that the porous medium is not a simple stack of parallel straight channels but a random structure with a pore size of R_{pore} . In order to estimate the diffusion time, it is reasonable to use the following formula:

$$t_{\text{diffusion}} = \frac{R_{\text{pore}}^2}{D_{AB(\text{oligo/solvent})}}, \quad (29)$$

in which R_{pore} is the radius of the pore. Given the radius of the pore to be equal to $0.25 \mu\text{m}$, and a diffusion coefficient of $10^{-10} \text{ m}^2/\text{s}$, we find an estimate for diffusion time $t_{\text{diffusion}}$ of 0.625 ms . We see that the diffusion time is much longer than the spreading and permeation times calculated for the two single drop cases. This means that for the single droplet case the particles flow along with the fluid and that the distribution of the probes is uniform in the part of the membrane occupied by the fluid.

D. Multidroplet case

In order to make a spot that consists of a volume larger than a single droplet, more droplets are placed on top of each other at the same position of the membrane. In the following, it is assumed that the print head is stationary with respect to the membrane. Let us suppose for the time being that the timing of placement is such that the volume of the already placed dots has just completely penetrated the membrane so that the next droplet lands on a dry substrate and will end up with the same spreading. The penetration will add up with the number of droplets (see Fig. 8).

When jetting at constant frequency the largest penetration time will determine this constant jetting frequency such that all droplets land on a dry surface. With N the number of droplets needed to make a dot, the time it takes for the last droplet to penetrate into the membrane, based on formula (27), is given by

$$\begin{aligned}\Delta t &= \frac{2\eta}{\gamma \cos \theta_E} \frac{x_N^2 - x_{N-1}^2}{R_{\text{pore}}} = \frac{2\eta}{\gamma \cos \theta_E} \frac{(x_N + x_{N-1})(x_N - x_{N-1})}{R_{\text{pore}}} = \frac{2\eta}{\gamma \cos \theta_E} \frac{(x_N + x_{N-1})\Delta x}{R_{\text{pore}}} \\ &= \frac{2\eta}{\gamma \cos \theta_E} \frac{(2N-1)\Delta x^2}{R_{\text{pore}}} = (2N-1)\Delta t_{\text{penetration}}.\end{aligned}\quad (30)$$

The highest constant frequency that ends up with stacking of N fluid portions inside the membrane is given by

$$f_{\text{max}} = \frac{1}{\Delta t} = \frac{\gamma \cos \theta_E}{2\eta} \frac{R_{\text{pore}}}{(2N-1)\Delta x^2} = \frac{1}{(2N-1)\Delta t_{\text{penetration}}}.\quad (31)$$

This formula holds true as long as the fluid has not reached the other side of the membrane. This is certainly true for depositing seven large droplets. For the small 10 pL droplets the bottom side is reached after 24 droplets. The last issue we have to resolve is about what happens when either the penetration of liquid in the membrane cannot cope with the volumetric rate of flow associated with the stream of droplets and/or the fluid has reached the bottom side of the membrane. Suppose droplets are jetted at a higher frequency than the maximum constant jetting frequency for minimal spreading. The first droplets can penetrate fast enough, but at a certain moment in time, the time between two successive droplets becomes equal to the penetration time needed for a droplet to be completely absorbed by the porous structure of the membrane. With N^* the number of droplets after which the membrane cannot keep track anymore with absorbing the volume rate of flow associated with the stream of droplets and f the jetting frequency, we have

$$\begin{aligned}\frac{1}{f} &= (2N^* - 1)\Delta t_{\text{penetration}}, \\ N^* &= \text{trunc} \left[\frac{1}{2} \left(\frac{1}{f\Delta t_{\text{penetration}}} + 1 \right) \right] + 1.\end{aligned}\quad (32)$$

During the period of time that the droplets permeate fast enough (with n the number of droplets jetted so far and $n \leq N^*$) we have to deal with two time scales: one real time t that follows the jetting frequency and another t_0 given by the penetration of the fluid in the membrane. This last time scale is governed by the Washburn equation. For $n = N^*$ we have

$$\begin{aligned}t &= \frac{N^*}{f}, \\ x_0 = N^* \Delta x &= \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} t_0}, \\ t_0 &= \frac{2\eta}{\gamma \cos \theta_E} \frac{(x_0)^2}{R_{\text{pore}}}.\end{aligned}\quad (33)$$

The $N^* + 1^{\text{st}}$ droplet lands on a wet surface and spreads further than the previously landed droplets according to the theory developed in Chap. III C. The absorption takes place along two paths as depicted in Fig. 9.

In case the core has not reached the bottom side of the membrane, the core of the liquid with radius R_s moves with the speed given by the penetration depth x_0 . As the droplet $N^* + 1$ has landed on a wet surface, the spreading is larger and given by the radius R_w . The penetration length is given by x_1 . As $x_1 \ll x_0$ the velocity at which the fluid is absorbed in annular region around the core is much faster (note that this is not necessarily the case for the respective volume rates of flow).

FIG. 9. Telescopic penetration of fluid in the membrane.

We refer to this pattern of filling of the membrane as to telescopic penetration. So, for the $n = N^* + 1$ droplet and where V_{excess} is the volume in the dot on the surface of the membrane just before impact of the following droplet, we have

$$\Delta x_0 = \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} \left(t_0 + \frac{1}{f} \right)} - \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} t_0},$$

$$x_0 = \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} t_0} + \Delta x_0,$$

$$x_1 = \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} \frac{1}{f}},$$

$$V_{\text{excess}} = V_d - P[\pi R_s^2 \Delta x_0 + \pi(R_w^2 - R_s^2)x_1]. \quad (34)$$

As long as neither x_0 and/or x_1 have reached the bottom of the membrane, for the n th droplet ($n > N^*$) we have (V_{excess} the volume in the dot just before impact of the following droplet):

$$\Delta x_0 = \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} \left(t_0 + \frac{n - N^*}{f} \right)} - \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} t_0},$$

$$x_0 = \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} t_0} + \Delta x_0,$$

$$x_1 = \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} \frac{n - N^*}{f}},$$

FIG. 10. Telescopic penetration of fluid into the membrane via a core and two annular regions.

$$V_{\text{excess}} = (n - N^*)V_d - P[\pi R_s^2 \Delta x_0 + \pi(R_w^2 - R_s^2)x_1]. \quad (35)$$

At a certain moment a situation occurs for which the static spreading after impacting of the next droplet is larger than the dynamic spreading on a wetted surface (spreading radius R_w). This case is characterized by the following condition (droplet number N^{**}):

$$R_d \sin \theta_E = \left(\frac{3(V_{\text{excess}} + V_d)}{\pi(2 - 3 \cos \theta_E + \cos^3 \theta_E)} \right)^{1/3} \sin \theta_E > R_w. \quad (36)$$

A new annular region becomes available for draining fluid from the volume collected in the dot on the substrate to the inside of the membrane. This is visualized in Fig. 10.

For $n = N^{**}$ we have three time scales, t_0 , t_1 , and t_2 , and three penetration depths, x_0 , x_1 , and x_2 . The excess volume in the dot just before impacting of the next droplet is given by (as long as x_0 and/or x_1 and/or x_2 have reached the bottom of the membrane),

$$\Delta x_0 = \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} \left(t_0 + \frac{N^{**} - N^*}{f} \right)} - \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} t_0},$$

$$x_0 = \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} t_0} + \Delta x_0,$$

$$x_1 = \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} \frac{N^{**} - N^*}{f}},$$

$$x_2 = \sqrt{\frac{1}{2} \frac{\gamma \cos \theta_E}{\eta} R_{\text{pore}} \frac{1}{f}}$$

TABLE VI. Overview of permeation characteristics for the multidroplet cases.

Volume (pl)	Radius (μm)	Jetting frequency (Hz)	Droplet speed (m/s)	f_{max} (Hz)	Permeation depth (μm)	Spreading factor	Spreading radius (μm)
10	13.37	4000	6	2548	150	4.65	62
143	32.44	100	3	1404	113	2.31	75

$$V_{\text{excess}} = (N^{**} - N^*)V_d - P[\pi R_s^2 \Delta x_0 + \pi(R_w^2 - R_s^2)x_1 + \pi\{(R_d \sin \theta_E)^2 - R_w^2\}x_2]. \quad (37)$$

In order to go on, we check for each next droplet whether the spreading after impacting is larger than the previous value. When this is the case, a new annular region is defined with inner radius equal to the value belonging to the previous droplet and the outer radius equal to the newly calculated value. Accordingly, we also define a new penetration depth and associated time scale. In the mean time, we check whether x_0 , x_1 , x_2 , and so on have reached the bottom of the membrane. When this is the case, the suction in that particular region stops and no more fluid can be drained into that area. Over the course of time, the total volume of all droplets on the substrate is absorbed into the membrane. In case for all regions defined the bottom side of the membrane is reached, suction stops and solvent of the remaining fluid evaporates, leaving the biomolecules on the surface of the membrane. Table VI summarizes the results for multidroplet printing.

The deposition of the 143 pl droplets goes so slow that the volume of each droplet is completely absorbed before the next droplet lands. As the fluid has not reached the bottom side of the membrane, this means that the final spot diameter is equal to the diameter of the first dot. This is not the case for the deposition of the small 10 pl droplets at a high frequency of 4000 Hz. For the development of the spreading and permeation see Fig. 11.

FIG. 11. Evolution of dynamic spreading R_s/R , static spreading R_{static}/R , and excess volume as functions of time after the start of the printing process. The data used for the calculations are as follows: droplet volume $V=10$ pl, droplet speed $v=6$ m/s, droplet frequency $f=4000$ Hz, density fluid $\rho=1000$ kg/m³, viscosity $\eta=1$ mPas, $\gamma=0.05$ N/m, $\theta_E=40^\circ$, $P=0.5$, $R_{\text{pore}}=0.25$ μm , membrane thickness $H_{\text{membrane}}=150$ μm .

Figure 11 contains all the features we have discussed so far. For the first 19 droplets the suction power of the membrane can cope with the volume rate of flow associated with the stream of droplets. Then starting at $N=22$ the spreading radius is increased to R_w . From that moment on, the excess volume increases. At the impact of droplet 25 the spreading radius increases beyond R_w . From that moment on, the spreading radius increases steadily. At the end of the droplet emission, the excess volume on the membrane upper surface contains almost 136 pl, the volume of about 14 droplets. As not all the telescopic regions are filled draining goes on after the jetting has stopped. After filling all the telescopic regions fluid still remains on top of the membrane (88.6 pl which is around nine droplets).

E. Diffusion time constants for the multidroplet case

As discussed above for the low frequency printing of large droplets the fluid portions belonging to the different droplets are stacked on top of each other. The diffusion time of the biomolecules from the center line of the pores towards the wall of the pores is estimated to be of the order of magnitude of 0.62 ms. The time between successive droplets is 10 ms (100 Hz). All probes have found a binding site before the next droplet lands and the fluid is cleared from free-floating capture probes. The fluid of this next droplet displaces this cleared fluid deeper into the membrane. The time it takes for the fluid of the next droplet to penetrate into the porous membrane structure is longer than for the previous droplet. For the single droplet case the diffusion time is much longer than the permeation time; this difference will decrease as more and more droplets land on the same spot of the membrane. Using Eq. (30) we find for the permeation time of the last droplet ($N=7$) $\Delta t=0.6$ ms. Even for the last droplet, the permeation time is of equal magnitude compared with the diffusion time. This means that all capture probes are caught in the area occupied by the fluid of the first droplet (penetration depth equal to $16.2 \mu\text{m}$). This statement holds true as long as all capture probes can find a binding site. When all sites are occupied, the volume occupied by the fluid of the first droplet and displaced deeper into the membrane by the fluid of the second droplet, and so on, will transport the still free-floating probes. For the case discussed here the fluid will not reach the bottom side of the membrane.

The situation of depositing the 1 nl of fluid by jetting 100 small 10 pl droplets at a high frequency is more complicated. The diffusion time (0.625 ms) is slightly larger than the time in between successive droplets (0.25 ms). Therefore, the deposition time of the first, let us say five, droplets is again about equal to the diffusion time scale, which leads to the conclusion that capture probes penetrate at least over a distance of about $30 \mu\text{m}$. It depends on the availability of binding sites whether the capture probes will go deeper into the structure of the membrane. At the very moment the suction power of the porous membrane cannot cope any longer with the flow rate associated with the stream of droplets, the spreading increases. The telescopic areas are filled in over time. Looking to Fig. 11 after spreading beyond the initial spreading radius there is always sufficient fluid available for permeation into the telescopic regions. This means that the penetration of capture probes will be deeper there (up to about $75 \mu\text{m}$). This conclusion follows from the one-dimensional nature of the Washburn model and needs either experimental or theoretical verification (by using a two-dimensional permeation model), which is not part of this paper. The fluid has reached the bottom side of the membrane.

V. CONCLUSIONS

We have investigated the different processes determining the 3D distribution of capture probes inside a porous membrane. These capture probes are deposited on well-defined spots on a substrate, in our case a membrane, by ink jet printing. We have calculated the different characteristic times of spreading, permeation, and diffusion. By doing so we are able to predict in detail the distribution of the probes inside the membrane. We summarize here our results for the two cases discussed.

(1) *Low frequency jetting of 143 pl droplets.* 143 pl droplets spread considerably. Penetration takes place over quite a large surface area. Because of the low jetting frequency (100 Hz) all the

fluid has been absorbed before the next droplet lands. The penetration manifests itself like a stack of equally sized cylinders. Capture probes are uniformly distributed in the area occupied by the fluid of the first droplet. It depends on the availability of binding sites whether capture probes will be found deeper in the membrane structure.

(2) *High frequency jetting of 10 pl droplets.* 10 pl droplets spread over a much smaller surface area. Initially the penetration is so fast that the spreading cannot take place completely. After a certain number of droplets landed, an excess volume collects in a fluid dome on the upper surface of the membrane because of the high jetting frequency. This excess volume can be so large that the spreading increases. According to the one-dimensional Washburn model the membrane structure fills in a telescopic manner, as a core surrounded by annular regions. For the case considered, the permeation is deeper compared to the large droplet case and the fluid can reach the other side of the membrane. In case this happens for either the core region or one or more annular regions, the draining in these regions stops. It might happen that the excess volume cannot be drained away and drying of the spot on the upper side of the membrane causes a higher density of capture probes at that location compared to the density of probes inside the membrane. Above a certain concentration, the probes cannot find binding sites anymore and will be washed away. The penetration of capture probes is deeper compared to the large droplet low frequency case. According to our one-dimensional analysis the penetration in the telescopic regions is deeper than in the core region.

Smaller-sized dots can be obtained by depositing smaller amounts of fluid.

(i) For low frequency deposition of 143 pl droplets, the only possibility is to decrease the droplet speed.

(ii) The use of a print head that jets small-sized droplets at high frequency opens all kinds of ways of controlling the dot size: number of droplets, droplet speed, and droplet frequency. By reducing the droplet speed, the initial spreading radius is reduced. By lowering the droplet frequency, the time for absorbing the volume of the impacting droplets increases. Another method is to jet just a few droplets more than the membrane can absorb; the excess volume stays on the upper side of the membrane increasing the density of the capture probes near and on the surface.

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