# Analyte capture in an array of functionalized droplets for a regenerable biosensor

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We describe in this work an advanced microfluidic chip for the capture of bioanalyte on the surface of droplets arranged in a dense array. We show the procedure for generating, functionalizing and arranging the droplets inside the device for capturing a specific bio-analyte. Then, we demonstrate the capacity of the array to capture analyte from a cross-flowing liquid, using a biotin/streptavidin model. The paper also proposes to use the droplets array, after integration with acoustic detection, as a regenerable detection interface for bioanalyte sensing. We model the arrangement of droplet in dense array and show that they present a larger effective capture surface and shorter capture distance than standard flat surface biosensor of the same footprint. As the droplets can be easily evacuated and replaced inside the device analysis chamber, the proposed biosensor would allow biointerface regeneration and chain measurement without dismounting the device.

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# I. INTRODUCTION

Since its inception in the 1990s, the research on microfluidic systems has allowed creating devices for biological detection with regular improvement in compactness, functionality integration and quantity of biological sample, leading to the concept of lab-on-chip. These developments have resulted in dramatic changes in the biomedical field, opening the possibility to perform multi-omics analysis (genomic, proteomic...), to prepare patient targeted drugs (ATMP), and to study cellular interactions or even organs, using organ-on-chip approach. An ultimate reduction in sample volume has been reached by using droplet as reaction chamber. This approach also offers an increase in speed of analysis by allowing large scale multiplexing, particularly interesting for drug research.



FIG. 1. Principle of operation of a biosensor with regenerable functionalized planar surface.

A particularly vibrant research direction in microfluidics focuses on the development of label-free biosensors for helping point-of-care diagnosis. These sensors are aimed at protein quantification or antibody detection

without the need for attaching fluorescent or magnetic labels<sup>3</sup>. Their principle of operation may be split in 5 elementary functions as shown in Fig. 1. Firstly, they require the preparation of a surface covered with bioreceptor targeting the bioanalyte of interest. We will call this operation the functionalization of the surface. Then we need the bioanalyte carrying fluid to circulate close to the functionalized surface for increasing the probability of capture. Actually, the third function is the capture of the bioanalyte with the bioreceptors on the surface. Usually captures with high specificity are required, and selective interactions have to be targeted, like antibody/antigen or ligand/protein bindings. Finally, to complete the sensor, we need a mechanism that could be used to measure the presence (or even the quantity) of bound bioanalyte on the surface. This can be accomplished using optical<sup>21,23,36</sup>, electric<sup>10</sup>, electrochemical<sup>2</sup> or acoustic transducers<sup>14,20</sup>, that should ultimately deliver an electrical signal proportional to the quantity of bound bio-analyte on the surface.

The main weakness of theses sensors lies in their need to replace the biodetection interface for performing a new measurement, which is obtained with a partially or totally disposable detection chamber. Then, cost issue does not allow to integrate with the chamber some of the most advanced detection scheme, like acoustic probing. To cicumvent this issue, some groups have recently tried to regenerate the bioreceptor layer on the detection interface using different chemical treatments<sup>5</sup>. Alternatively, it has been proposed to use functionalized magnetic microparticles that are immobilized close to the transducer using a strong magnetic field<sup>9</sup>. After capture on the particle surface and detection, the magnetic flux is removed, and the particles are flushed away. In this approach the integration with an an acaoustic transducer is difficult, and the electrochemical sensing used presents relatively low sensitivity. Moreover, the small magnetic particles

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are mixed with the biological material, which may pose regulatory or safety issue.

We propose to develop a new concept of biosensor where the functionalized surface is no more planar but is composed by the surface of an array of functionalized droplets. This architecture has an obvious interest in simplifying the reusability issue raised above: it is sufficient to flush away the functionalized array and to replace it with a new array of droplet, potentially with a different functionalization, to reuse the biosensor. Moreover, we will show that this architecture increases both the effective surface and the efficiency of capture of the bioanalyte in the tested sample. We also suggest that this architecture should use acoustic transduction for allowing to probe the droplet surface dynamically and measure with high sensitivity the quantity of bioanalyte captured in the  $\operatorname{array}^{6,29,32,37}$ . A summary of the different functions that would need to be integrated for such biosensor are illustrated in Figure 2. In this paper we only investi-



FIG. 2. Functions required for a droplet based biosensor with bioanalyte capture and regeneration.

gate the microfluidics aspects of the biosensor, detailing the strategy to generate functionalized droplets and arrange them in a regular array, then capture bioanalyte in the array and finally flush the droplet array for restarting from an empty chamber (the functions 1, 2, 3, 4 and 6 in Figure 2). The acoustic detection (function 5) is still an ongoing work.

## **II. GENERATION OF FUNCTIONALIZED DROPLETS**

The generation of monodisperse droplets in microfluidics is obtained by simultaneously injecting two immiscible fluids inside different microchannel structures. For this sensor, the bionalyte being generally carried in water solution, we will need a continuous phase of water with droplets of oil. Hydrodynamics based generation uses two main configurations of channels: the T-junction<sup>35</sup> and the co-flowing junction<sup>15</sup>. In these cases, the drag forces appearing in the zone where the two liquids mix, induce the breakup of the liquid stream and the generation of droplets. For helping the generation and stabilizing the generated emulsion, surfactants are generally mixed with the fluids before injection in the microchannels junction.

For the fabrication, we are using a well controlled micromachining process based on silicon and glass wafer assembly, as shown in Figure 3. We are first pattern-



FIG. 3. (left) Flowchart for fabrication using silicon/glass process (right) Zoomed view of a microfluidic chip with generation of 80 µm biotinylated soja oil droplets in DI water with a T-junction in dripping regime.

ing with S1813 photoresist (Figure 3-1) and etching with a SPTS Rapier DRIE system the channels in a silicon wafer front side (Figure 3-2). Then, we pattern with thick AZ9260 photoresist (Figure 3-3) and etch from the silicon wafer backside the through holes for liquids access (Figure 3-4). The channels on the silicon wafer are then closed by anodic bonding with a BF33 1 mm thick glass plate (Figure 3-5). For increasing wetting of water with the silicon channels without changing wetting of oil, RCA1 solution (H<sub>2</sub>O:NH<sub>4</sub>OH:H<sub>2</sub>O<sub>2</sub> 2:1:1) is used to fill the channels by capillarity, and rinsed 3 times with DI water in the same manner. This process yields channels with controllable surface energy and low roughness, allowing to use the T-junction efficiently for the generation of oil droplet in water with diameter ranging between  $5\mu m$  and  $150\mu m^{33,34}$ .

However, if the generation of oil droplets is mastered, the ability to obtain functionalized oil droplets remains challenging. Actually, the standard techniques used for the functionalization of solid surfaces often makes the water/oil emulsion unstable, inducing coalescence of the droplets. In the literature we find only a handful of work dealing with droplets functionalization, with two main strategies:

- either the droplet is generated using functionalized surfactant that will place itself at the oil/water interface. This can be obtained by using biotiny-lated phospholipids<sup>7,17,25</sup> or using modified surfactants with different chemical termination (amine, carboxylic acid, biotin...)<sup>22</sup>,
- or the surfactant at the droplet surface is functionalized after the droplet is formed. This is obtained

directly after activation of fatty acid<sup>13</sup> or condensation of silanes at the droplet surface<sup>1</sup>. Alternatively, an indirect strategy has also been demonstrated, which consists in decorating the droplet with gold nanoparticles<sup>27</sup> allowing to later use standard thiol chemistry for the functionalization.

In order to demonstrate the capacity of the array of droplet to capture bioanalyte, we will be using the standard and very robust biotin/streptavidin interaction<sup>11</sup>. Accordingly, we have tested both strategies to decorate the oil droplets with biotin. The second strategy is more versatile, but our trial, inspired by a protocol proposed by Fattaccioli group<sup>13</sup>, did not yield droplets stable enough for forming droplet array. Thus, we based the generation of droplets on the first strategy, using biotinylated soy oil and DI water with Tween20 at 5 v/v% as shown in Figure 3-right. The droplets generated in the T-junction could have any diameter between 58 µm and 84 µm by controling oil and water flow rate as shown in Table I. This range of flow rate allows to remain in the dripping regime of generation, yielding high generation rate of small droplets with low diameter variation.

Parameter	$\operatorname{Symbol}$	Value
Oil channel width	$W_d$	20 µm
Water channel width	$W_c$	$100 \ \mu m$
Channel depth	h	$40~\mu{\rm m}$
Oil flow rate	$Q_d$	$3-4 \ \mu L/min$
Water flow rate	$Q_c$	5-30 $\mu L/min$

TABLE I. Parameter for biotinylated soja oil droplet generation.

## **III. ARRANGEMENT OF DROPLETS IN ARRAY**

Arrangement of soap bubbles in large and closely packed arrays at the free surface of an open reservoir has been demonstrated in the past, most famously in 1947 by Sir L. Bragg for studying crystals<sup>8</sup>. For droplets generated in close channels, the need to drain the interstitial liquid for forming a closely packed array poses new problems. In fact the generation of droplet by hydrodynamic forces in channel junctions<sup>16,35</sup> requires a certain ratio of flow rate  $\phi = Q_d/Q_c$  between the dispersed and the continuous phases that is not necessarily compatible with the production of a closely packed array. Accordingly, in the literature, we find essentially two strategies to overcome this issue: either use a large phase fraction  $\phi^{19,24}$  or drain the continuous phase from the array after generation.

The second strategy has the advantage to be mostly independent of the droplet generation mechanism and the  $\phi$  ratio. It is based on the the use of narrow lateral channels for draining the continuous phase interstitial liquid after generation, while at the same time retain the droplets in the main channel or chamber<sup>18,26,28,34,38</sup>. We propose to use this strategy in our sensor for obtaining a closely packed array of monodisperse droplets, and we will thus combine a T-junction droplet generator with a chamber equipped with sieves and drain, as shown in the Figure 4. The input port 3 and 4 are used for generating



FIG. 4. Layout of the chip with 8 standard ports (port 2 is unused) for generation of oil droplets and their organization in closely packed array.

the droplets, while the other ports are mostly used as purging channels at this step (the port 1 and 6 will be used for flowing the bioanalyte across the droplets array in the next section).

A computerized control system allows to propose a systematic method for filling the chamber with densely packed droplets, as shown in Figure 5. In a first step we stabilize the generation of droplet by using only the port 3 (Oil), 4 (Water) and 5 (dump) as in the previous section. Then we start filling the chamber using only the central channel dump (port 7). In a third step we open the drains (ports 6 and 8) for densifying the droplet array by removing interstitial water. At the end of this step, the droplet get compressed and deformed because of the continuous flow coming from the droplet generator. In a last step we stop the droplet get their round shape, and finally fully filling the chamber.

The flow behavior during this process has been partially simulated using COMSOL Multiphysics FEM simulation software and is presented in Figure 6. In this chamber much more wide (3 mm) than deep (40 µm), the behavior is close to Hele-Shaw flow and a 2D analysis is sufficient. Moreover, the chamber is symmetric and we simulated only half the chamber with one drain to decrease the computing time. We clearly see here that when the drains are closed (step 2) the liquid bypass the chamber by the side, slowing down in the central section which helps densifying the droplet array. However the liquid is re-injected at the end of the chamber, finally lifting the droplet from the chamber side and disorganizing the droplet array as we observed previously. When we open the drain dump in step 3, we remove this issue and slow down the liquid until the end of the chamber allowing the droplets to form the closely packed array.



FIG. 5. Chip ports control sequence for organizing droplets in a densely packed array in the detection chamber.

We had to introduce sieves between the chamber and the drain for preventing the droplets from flowing out of the chamber. The shape of the sieves is crucial for preventing the droplets from deforming and squeezing out of the chamber, while at the same time keep an acceptable fluidic resistance. We observed that 500 µm-long and 20 µm-wide channels<sup>34</sup> did not work well, and we studied the possibility to use 10 µm and 5 µm-wide channels instead. However, they suffer from a dramatic increase in hydraulic resistance (even taking into account that more could be packed by unit length) respectively by 500% and 2500%, as obtained from simulation from COMSOL Multiphysics<sup>™</sup>. Instead of using uniform narrower channels for the sieve, we choose to design new forked channels with narrow but short branches at their end (5 µm wide



FIG. 6. 2D simulation of flow rate in cm/s in the (half) chamber during step 2 and step 3 of Fig. 5 (water flow is from left to right) inset: SEM view of the forked sieves end.

branch over 25  $\mu$ m), as shown in the inset in Fig. 6. This architecture showed an increase of the fluidic resistance by only 57% but proved to be much more efficient at preventing the droplet from escaping the chamber than the original uniform 20  $\mu$ m-wide channels.

From the results shown in step 4 of Fig. 5, it is clear that the strategy we developed allows to fill the chamber with a densely packed array of droplets, even if we could observe some limited coalescence of droplets.

We have also verified that we could empty the chamber completely, by flushing water through port 4 while port 7 was left open, and the other ports closed. The chamber was then refilled with droplet array using the same strategy, and this process could be repeated multiple times, without observing any degradation in the formed droplet array. This is one of the advantages of our architecture, where the regeneration of the capture surface (function 5 in Fig. 1) is easily obtained for a fully reusable sensor.

#### IV. FLOW THROUGH THE DROPLET ARRAY

For studying the flow through the droplet array, we injected water with fluorescein (1 mg/mL) in the chip (Fig. 7) through port 1 and left port 7 open, while all the other ports were closed (see Fig. 4).

A sequence of image was recorded during the injection of fluid at low flowrate (0.5  $\mu$ L/min). In each image we took the intensity along the two lines shown in Fig. 7 and stacked the lines progressively atop each others in a diagram with the time as vertical axis. This kinogram is showing the fluorescence front progression as a function of time and the slope of the black/white frontier gives access to the front speed in the different zones of the chip.

The flow in the sieves and in the dense droplet array has similarities with flow in a porous medium and the



FIG. 7. Flow of fluorescent liquid into the droplet array (Zones 1 and 5 correspond to the two drains, zones 2 and 4 to the two sieves and zone 3 to the droplet array).



FIG. 8. Kinograms of the flow of the fluorescent liquid in the chip along (top) the central line and (bottom) the left line shown in Fig. 7 (numbered zones correspond to the zone shown in the figure).

total flow rate is then described by Darcy's equation :

$$Q = \frac{kA}{\eta L} \overrightarrow{\nabla} P$$

with k the permeability (linked with the pore characteristics) and A the medium section. We may use this equation to obtain the velocity of the fluid in the pores, by noticing that  $v = Q/\varphi A$ , where we have introduced  $\varphi$ the porosity, to account for the fact that in porous media only a fraction of the cross-section area allows liquid circulation. We have :

$$v=\frac{k}{\eta\varphi L}\nabla P$$

This equation may be used to describe the flow in the zones 2, 3 and 4 of the chamber.

We note that the difference of fluid velocity that is typically observed in the two sieves (zones 2 and 4) that have the same geometry, and hence the same porosity and permeability, indicates that the fluid path is not uniform. In fact, the fluid concentrates in some part of the exit sieve which is traced back to defects in the droplet array as seen in Figure 8: a bigger droplet (coalescence) in the chamber right is blocking the flow through a few pores of the sieve and a few missing droplets in the left of the array lower the fluidic resistance in this region. These combined effects concentrate the flow in the central and left part of the chamber. In fact, we see that the flow velocity increases by about 50% between the input sieve (zone 2) and the output sieve (zone 4), meaning that only about 2/3 of the pore in the exit sieve participates to the flow.

The porosity of the sieves may be directly estimated from the geometry (a channel 20 µm wide every 40 µm) and is  $\varphi_s = 0.5$ . Accordingly, considering that the flow rate Q and the section A (that is, the depth and width of the chamber) are approximately kept constant between the sieve and chamber zones, we may write:

$$Q = v_{\rm s}\varphi_{\rm s}A = v_{\rm d}\varphi_{\rm d}A$$

where  $\varphi_{d}$  is the porosity of the array of droplet in the chamber. Thus, by using velocity in the top sieve, we obtain the porosity of the droplet array as:

$$\varphi_{\rm d} = \frac{v_{\rm s}}{v_{\rm d}} \varphi_{\rm s} \approx 0.18$$

In the droplet array the pores (i.e. the space between the oil droplets) are fully wet and the array porosity is in fact equal to the water fraction  $\varphi_{\rm w} = \varphi_{\rm d}$ , meaning that water makes only  $\varphi_{\rm w} = 18\%$  of the volume and oil is making 82%.

When the droplets are constrained by the chamber top and bottom surfaces they do not assume a spherical shape. We still refer to their radius (R), by using the apparent radius of their projected shape seen from the top, as they remain circular from that direction when they don't touch the side walls. In fact, if the droplet top diameter is larger than the depth of the chamber (2R > h), the droplet assumes normally a barrel-like shape as shown in the top-right inset in Figure 9. It spreads (and may wet) the top and the bottom of the chamber and shows an apparent contact angle  $(\theta)$ , whose magnitude depends on the fluids interfacial tension and on the droplet volume. By varying the apparent contact angle between  $\pi/2$  and  $\pi$  for droplets with constant top diameter we may model different barrel droplet shapes, as shown in the insets in Figure 9.

From the geometry of the cross-section of the constrained droplet in the chamber shown in Figure 9, the volume of the droplet<sup>4</sup> may be expressed with the parameters R, h and  $\theta$  only and it is given by:

$$V_{\rm d} = 2\pi \left( \frac{h}{2} (R^2 - 2rR + 2r^2) - \frac{1}{3} (h/2)^3 + (R - r)r^2 \left[ \theta - \frac{\pi}{2} - \frac{\sin(2\theta)}{2} \right] \right) \quad (1)$$

where the tore radius  $r = -h/2\cos\theta$  has been left in the equation to make it easier to read.

Considering the dense arrangement of droplet, that defines a hexagonal symmetry as shown in Figure 9, we



FIG. 9. Evolution of droplet array porosity as a function of the normalized droplet diameter 2R/h and for different values of the apparent contact angle  $\theta = \pi/2, 125^{\circ}, 3\pi/4, \pi$ . We show in the figure sketch of typical droplets for different values of normalized diameter and contact angle (top-right inset: sketch of the oil droplets arrangement and cross-section of a droplet inside the chamber when the droplet diameter is larger than the chamber depth (2R > h)).

compute the water fraction as:

$$\varphi_{\rm w} = \frac{\sqrt{3}R^2h - V_{\rm d}/2}{\sqrt{3}R^2h}$$

where we used the difference between the volume of the circumscribed isosceles prism  $(\sqrt{3}R^2h)$ , grayed out in the inset of Figure 9, with the inscribed volume of the oil droplet  $(V_d/2)$  to find the volume of water. We find that this dimensionless porosity depends only on 2R/h and  $\theta$ . We plotted the function in Figure 9 and we see that it varies between 9.3% and 39.5% for  $1 < 2R/h < \infty$  and  $\pi/2 < \theta < \pi$  corresponding to the range of meaningful values for the droplet array model.

For the droplets of radius  $R = 40 \ \mu\text{m}$  in a chamber of depth  $h = 40 \ \mu\text{m}$  (that is 2R/h = 2), corresponding to the case presented in Figure 7, the porosity may vary between 9,3% and 26.5%, when the contact angle varies between 90° and 180°. By solving numerically the equation we find that in the droplet array considered ( $\phi_d = 0.18$ ) we have  $\theta \approx 125^\circ$  (this point is indicated with a cross in Figure 9). This figure is compatible with measurements of contact angle using sessile drop experiment made separately, and with simple geometry measurements (by measuring the droplet radius R and the contact radius a) although these measurements are not very precise.

The droplet array is now characterized in detail (the determination of the permeability coefficient k of the ar-

ray is not possible in this experiment as we can not measure the pressure inside the chip) and we will now model and demonstrate its capability in capturing bioanalytes.

# V. CAPTURE OF BIOANALYTE ON DROPLET SURFACE

The dense arrangement of functionalized droplets for bioanalyte capture present multiple advantages compared to the functionalization of a chamber flat surface:

- it makes regeneration of functionalized surface straightforward,
- it increases the bionalyte capture surface for the same chamber footprint,
- it provides shorter distance for complete bioanalyte capture.

If the first point has already been established, the two other points require more investigation.

The surface of the oil droplet in contact with circulating water (thus excluding the two discs at top and bottom) is obtained by computing the surface integral using the geometry shown in Figure 9 and we obtained after verification with Maple<sup>TM</sup>:

$$S_d = -\frac{2\pi h}{\cos(\theta)} \left[ \left( R + \frac{h}{2\cos(\theta)} \right) \left( \theta - \frac{\pi}{2} \right) + \frac{h}{2} \right]$$

In the special case where 2R = h the droplet shape goes from a cylinder to a sphere of same radius when  $\theta$ goes from  $\pi/2$  to  $\pi$ . We verify easily that the side surface  $S_d = 2\pi h^2/2 = 4\pi h^2/4 = \pi h^2$  is the same in both cases, as Archimedes already noticed in 225 BC.

The ratio of the surface of the droplet  $(S_d)$  and the footprint of the array of droplet  $(S_p)$  has been estimated from geometric consideration as:

$$G_{\rm s} = \frac{S_{\rm d}}{S_{\rm p}} = \frac{\pi h}{\sqrt{3}\cos(\theta)R^2} \left[ \left( R + \frac{h}{2\cos(\theta)} \right) \left( \theta - \frac{\pi}{2} \right) + \frac{h}{2} \right]$$

where h is the depth of the chamber, R the radius of the droplets and  $\theta$  the contact angle of the droplet at the channel top and bottom surface. When the surface ratio  $G_s$  becomes larger than 1, it means that we have a larger capture surface than what could be obtained with a flat sensor of same footprint. Accordingly,  $G_s$  is considered as a gain of surface for droplet based bioanalyte capture, that could be even more important if droplets are stacked-up in multiple layers and not only in a single layer. We have plotted in Figure 10 this surface gain for different droplet diameter/height ratio as a function of the normalized droplet diameter, and we see that it is normally around 2 and may exceed 3.5.

For comparing the capacity to capture bioanalyte between the array of droplet and the flat surface, we start by establishing the length required for capturing all the



FIG. 10. Ratio of bioanalyte capture surface for a droplet sensor and a flat sensor of same occupancy as a function of the normalized droplet diameter 2R/h and for different values of the apparent contact angle  $\theta = \pi/2, 3\pi/4, \pi$ . The cross represent the typical case shown in Figure 7.

analyte in a channel. We follow a reasoning inspired by Squires<sup>31</sup> that is illustrated in Figure 11. We will only consider here the transport phenomenas (advection and diffusion) and assume that the capture process is the same in the two situations and do not limit the complete process (in microfluidics terms, it means that we assume we work with large Damkohler number<sup>31</sup>  $Da \gg 1$ ).

Inside the microfluidic chip, the fluid flows parallel to the channel surfaces while carrying the bionalyte (advective flow). At microscale, we have laminar flow without turbulences and the bioanalyte particles would normally flow parallel to the surface and would have no chance of being captured. Hopefully, the bioanalyte particles are small and they are subject to diffusion (diffusive transport) that will help drive them toward the surface of capture. We will estimate the typical distance L it takes for



FIG. 11. Advective and diffusive transport of bioanalyte for reaching the surface of capture from the channel top.

capturing all the bioanalyte particles present in the fluid by studying their motion under simultaneous advective (i.e. due to carrier liquid flow) and diffusive (i.e. due to diffusion) flows. The advection time it takes to move the bioanalyte by a distance l is given by:

$$t_{\rm a} = \frac{l}{\bar{v}} = \frac{lhu}{Q}$$

where Q is the volumetric flowrate of the liquid carrier and hw the area of the channel.

Because we want to estimate the distance for full capture we will consider the bioanalyte that is the hardest to capture. This bioanalyte enters the channel the farthest from the surface and has to diffuse by the full channel height h before it may be captured by the surface. The diffusion time needed for this bioanalyte to finally reach the surface is estimated by using typical diffusion length expression:

$$t_{\rm d} = \frac{h^2}{4D}$$

where D is the diffusion constant of the bioanalyte, that can be related to bioanalyte size, temperature and fluid properties using the Stokes-Einstein relationship<sup>12</sup>. Thus the maximum distance L required for capturing this farthest bioanalyte, and consequently all the bioanalyte in the channel, is obtained when  $t_a = t_d$ , giving:

$$L = \frac{Qh}{4Dw}$$

The application of this expression to the case of a chamber of width w and depth h with a plane functionalized surface is straightforward and we have:

$$L_{\rm p} = \frac{Qh}{4Dw}$$

If the chamber is filled with droplets, the flow may be approximated as if it went through an array of parallel channels in the interstices between the droplets. The depth of each of these equivalent channels is given by the distance between droplets  $h_{\rm a}$  (assumed here to be uniform), while their equivalent width is given by the chamber depth w and the flowrate in these equivalent interstitial channels is noted q, giving:

$$L_{\rm d} = \frac{qh_{\rm a}}{4Dh}$$

Again here for showing the interest of our structure we will compare the capture length between these two cases:

$$G_{\rm c} = \frac{L_{\rm p}}{L_{\rm d}} = \frac{h^2}{h_{\rm a}w} \frac{Q}{q}$$

If we impose the same volumetric flow rate for the chamber with plane functionalized surface and with functionalized droplets, it means that q = Q/n where n is the number of parallel channels in the droplet array. Accordingly, we find that:

$$G_{\rm c} = \frac{h^2}{h_{\rm a} d}$$

where we have used the fact that in the chamber the droplet are touching each others and thus  $w \approx nd$  with d the droplet diameter. For a typical case where the droplet diameter is 80µm,  $h_a \approx 1µm$  and the channel is 40µm deep, we find  $G_c = 20$ . It means that, for the same flow rate, the capture distance is 20 times shorter in the droplet array than in the planar configuration, ensuring complete capture in a more compact device. In practice the capture will even be more efficient as the path between the droplets is not straight but zigzagging, forcing the bioanalyte toward the functionalized surface.

For demonstrating the capability of the functionalized droplet array to capture bioanalyte, we will be using the streptavidin/biotin interaction. Actually, the streptavidin protein and the biotin coenzyme have a very high affinity and make strong bond, unaffected by solvents, detergent, heat, etc<sup>11</sup>. After injecting the streptavidin solution through the sieves in the biotinylated droplet array for 1 min, we have disrupted the array of droplets by sending a high flowrate through port 1 and 6 and observed the behavior of the droplets using a camera. We observed that the droplet instead of gliding on each other as it is usually observed, are actually sticking to fixed points. This behavior may be observed in Figure 12 on the sequence of images extracted with Fiji<sup>30</sup> from a video where the lower 'arm' appears to rotate. We also pushed the biotinylated droplets still present in the smaller stabilization chamber after the T-junction, and verified that these droplets, which hadn't seen the streptavidin, had retained their original behavior and did not stick to each others. The real behavior is dynamic and is better understood by directly comparing the video of biotinylated droplets after streptavidin injection (see Video 1 in supplementary material) with the video of biotinylated droplets without streptavidin (see Video 2 in supplementary material) in the supplementary material online.

The visible stickiness of the droplets when they move around is attributed to the bridging of droplets by streptavidin. Actually, streptavidin has 2 binding sites on each side of the molecule, which could produce biotin-streptavidin-biotin bridges between droplets. Such bridge (inset Figure 12) have already been observed by Fattaccioli et al.<sup>13</sup> using biotin decorated droplet with streptavidin.

A quantitative technique (our fluorescent set-up is unfortunately not sensitive enough) will be needed for quantifying precisely the capture efficiency of the functionalized droplets array. However, this experiment validates for the first time the capacity of such droplet array to capture bioanalyte.

# VI. CONCLUSION

We have designed and tested a microfluidic chip for capturing bioanalyte on the functionalized surface of droplets. We also proposed that this structure could be



FIG. 12. (top) Sequence of image extracted from a video showing biotinylated droplets sticking to each other (rotation of lower 'arm') (see Video 1 in supplementary material) (bottom) formation of streptavidin bridges between biotynilated droplets.

used for a new type of biosensor with regeneration capability after integration of acoustic probing.

In this paper we have focused on designing and demonstrating the microfluidic functions of the biosensor. We successfully demonstrated the generation of functionalized droplets, their arrangement in a dense array, the injection of bioanalyte through this array and finally the binding of the bioanalyte to the surface of the droplets. Moreover we have presented a model showing the advantage of our sensor architecture compared to a standard planar sensor: firstly we increase by a factor of 2 or more the capture surface and we decrease by a factor of 20 or more the capture length of the bioanalyte for the same flowrate.

We are currently developing an integration strategy, based on AlN deposition on SOI wafer, for placing piezoelectric excitation and sensing below the chamber. The test of this future chip will establish the sensitivity of our method and we will investigate its capabilities to fully capture bioanalyte of interest in small samples.

It should be noted that without any integrated detection, the architecture proposed here can already be used as a regenerable bioanalyte filter. In fact, the microfluidic functions described in this paper allow to collect targeted bioanalyte in a sample and extract them by flushing the droplets for further processing.

## SUPPLEMENTARY MATERIAL

See in the supplementary material a first video presenting the motion of biotinylated oil droplet in water after streptavidin injection (the center of the video corresponds to the sequence of images shown in Fig. 12), where the droplets seem to be sticking to each others because of streptavidin bridges. The second video is showing the motion of biotinylated oil droplets just after generation before exposure to streptavidin, where droplets can be seen to move freely and glide on each others.

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