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Interaction of biomolecules sequentially deposited at the same location using a microcantilever-based spotter

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Abstract A microspotting tool, consisting of an array of micromachined silicon cantilevers with integrated microfluidic channels is introduced. This spotter, called Bioplume, is able to address on active surfaces and in a time-contact controlled manner picoliter of liquid solutions, leading to arrays of 5 to 20-μm diameter spots. In this paper, this device is used for the successive addressing of liquid solutions at the same location. Prior to exploit this principle in a biological context, it is demonstrated that: (1) a simple wash in water of the microcantilevers is enough to reduce by >96% the cross-contamination between the successive spotted solutions, and (2) the spatial resolution of the Bioplume spotter is high enough to deposit biomolecules at the same location. The methodology is validated through the immobilization of a 35mer oligonucleotide probe on an activated glass slide, showing specific hybridization only with the complementary strand spotted on top of the probe using the same microcantilevers. Similarly, this methodology is also used for the interaction of a protein with its antibody. Finally, a specifically developed external microfluidics cartridge is utilized to allow parallel deposition of three different biomolecules in a single run.

Keywords Microcantilevers · Microarray · Biomolecules · Hybridization · Protein interaction

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1 Introduction

The field of biotechnology instruments is evolving toward miniaturization especially thanks to the rapid progress of microfabrication techniques. Scaling laws predict faster mass and heat transport for smaller dimensions. Moreover, size reduction means quicker and cheaper analyses with much less biological samples, thus opening the way to mass fabrication, parallelization and automation. This trend encompasses drug discovery (Dittrich and Manz 2006), analytical chemistry (Auroux et al. 2002), chemical synthesis (Jähnisch et al. 2004), clinical diagnostics (McGlennen 2001), single molecule manipulation (Bockelmann 2004), as well as laboratory techniques including sampling, mixing, separation and analysis of biomolecules. A representative example in the field of micro total analysis systems (μTAS) is the integration of DNA extraction (Chung et al. 2004), amplification (Ali et al. 2004; Liu et al. 2004), electrophoresis (Effenhauser et al. 1994; Easley et al. 2006), and real time PCR (Lee et al. 2006) in a "pocket size" microfluidic device. With respect to screening for molecular interactions, many micro electromechanical systems (MEMS) devices have been proposed, for which biorecognition can be detected via a shift of resonance frequency of oscillating systems like microcantilevers (Fritz et al. 2000; Wu et al. 2001; Mukhopadhyay et al. 2005; Bergaud and Nicu 2000),

micromembranes (Guirardel et al. 2004, Nicu et al. 2005) or other microsensors with innovative design (Nicu and Bergaud 2004). However, the reduction in size of biosensors raises major issues concerning the proper coating of the sensing surface with bioactive layers. Direct immersion in a liquid solution is inappropriate due to the fragility of the microcircuits, and selective coating of active areas is a complicated task. Thus, microspotting is an interesting technology to overcome these challenges.

In the 1990s, the success of microarray analysis has contributed to the development of devices designed to address droplets at the micrometer scale. Today, many commercial companies propose arrayers able to deliver nanoliter droplets on glass slides, based on two main technologies: ink-jet printing (Okamoto et al. 2000) and contact deposition (Schena et al. 1995). Ink-jet printers work by propelling liquid onto the surface. The liquid is forced out of the nozzle using an increase of cartridge temperature (thermal ink-jet) or pressure (piezoelectric ink-jet). Concerning contact deposition devices, the principle is similar to fountain pen writing. Spotting pins, made of stainless steel or tungsten, are plunged into the solution that flows up into the channels by capillarity. When the pins come into contact with the surface, droplets are deposited. Whatever the technique, the size of the spots produced by these types of commercial devices ranges between 50 and 500 μm in diameter, depending on the type and the surface chemistry of the substrate (nylon membranes, glass slides, silicon, etc.). An alternative method for the parallel deposition of molecules on functionalized surface is the microcontact printing (μCP), which uses polydimethylsiloxane (PDMS) stamps exhibiting micrometer to nanometer size engraved patterns over large areas (Renault et al. 2003; Thibault et al. 2005). For higher size reduction, dip-pen lithography or nanodispensing techniques (NADIS) are today the most suitable techniques to reach nanometer scale dimensions. Both techniques rely on the use of an atomic force microscope (AFM) tip to deliver molecules onto a surface via a solvent meniscus. Dip-pen nanolithography has been used to directly fabricate protein arrays with 100 to 350 nm features (Lee et al. 2002), as well as to pattern 15 nm wide lines of a linker that mediates the adhesion of virus particles (Cheung et al. 2003). Using NADIS, droplet size can be controlled by the aperture size of the probe or by the surface energy of both tip outer wall and sample surface (Meister et al. 2004; Fang et al. 2007).

Recently, an alternative contact deposition tool able to directly pattern 1 to 100 μm diameter spots was proposed as a solution to fill in the gap between the performances offered by commercial devices and dip-pen nanolithography in terms of spot size (Belaubre et al. 2003). The scale addressed by this microspotting tool, called Bioplume, corresponds to MEMS typical surface areas (100–10,000 μm^2). Bioplume consists of an array of micromachined silicon cantilevers integrating a fluidic channel fixed to a five-degree-of-freedom motion control system. To control the contact force with the surface during deposition, displacement sensors are incorporated into the array by means of piezoresistors integrated into the silicon cantilevers: this is a major asset in the perspective of depositing solutions on fragile active surfaces, e.g. the one of some microbiosensors. Bioplume has already been used to deposit proteins, DNA, and nanoparticles on functionalized surfaces (Leïchlé et al 2005), and it has been recently adapted for the electrodeposition of metals and polymers (Leïchlé et al. 2006a, b).

In this paper, we explore the capability of the Bioplume microspotting tool to perform sequential depositions of biomolecules at the same location on an active surface. This intermixed deposition principle is described in Fig. 1. As a proof-of-concept, successful hybridization of two complementary short DNA sequences and interaction of a protein with its antibody are reported. To complete the study, an external loading fluidic chip is used to load the cantilever array with three different biological solutions. The chip, specifically fabricated for that purpose, enables the addressing of different biological solutions in a single run, thus allowing the possible parallelization of the Bioplume technology.

2 Methods

2.1 Chemical, biochemical reagents and solutions

Epoxide slides "Nexterion slide E" were purchased from Schott, and aldehyde-slides were fabricated as previously described (Trévisiol et al., NJC 2003). The fluorophore Alexa 647 carboxylic acid succinimidyl ester (Alexa 647-NHS) was purchased from Molecular Probes. Nanosep® 30 K devices were from Pall Corporation. Oligonucleotide probes were synthesized by standard phosphoramidite methods by Eurogentec (Seraing, Belgium) or Operon (Germany) and the probe oligonucleotides were flanked at the 5′ terminus by an amine group via a 6-carbon alkyl spacer. Sequences used were: "Cy5-35mer-NH_2" 5′-Cy5-TTTAGCGCATTTTGGCATATTTGGGCGGACAACTT-NH_2-3′; 35mer probe sequence "35mer-NH_2" 5′-NH_2-GTGATCGTTGTATCGAGGAATACTCCGATACCATT-3′; a non-complementary sequence "26mer-NC" 5′-NH_2-CATTTAGTTGGACGTTGTCACATTTA-3′; and two fluorescently labeled sequences: "15mer-Cy5" 5′-Cy5-AATGGTATCGGAGTA-3′ complementary to "35mer-NH_2" and "16mer-Cy3" 5′-Cy3-CATATTCGACGGCATC-3′.

Gluthation-S-Transferase (GST), monoclonal IgG anti-GST, BSA, sodium dodecyl sulphate (SDS), glycerol, and

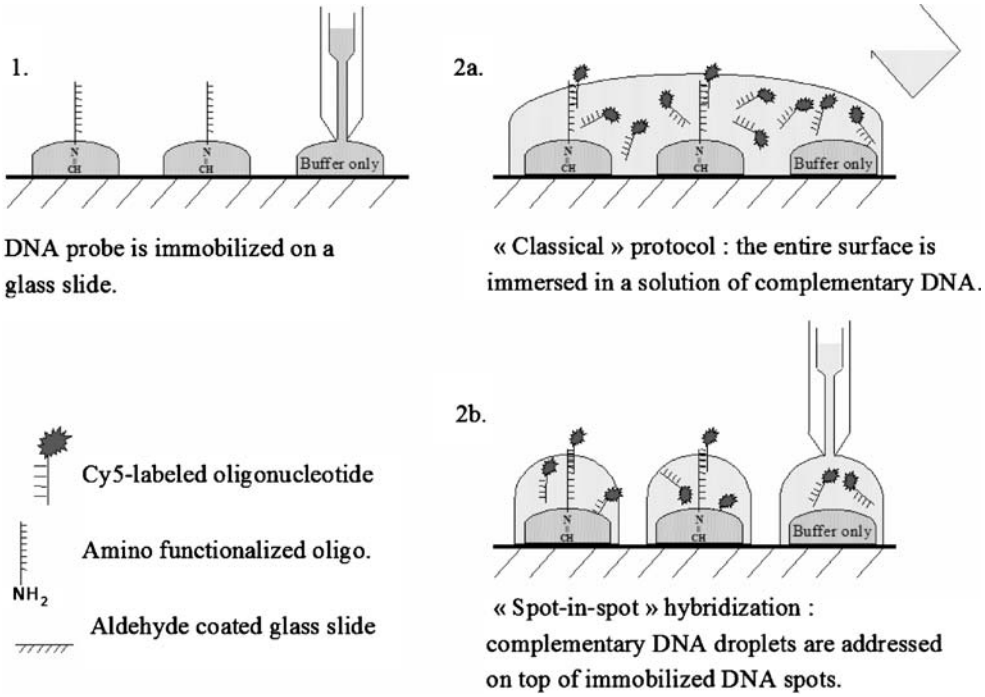

Fig. 1 In-spot localized hybridization principle

sodium citrate buffer (SSC 20×) were purchased from Sigma-Aldrich (France).

2.2 Bioplume spotter description

The spotting system, called Bioplume, consists of a silicon-based microcantilever array fixed on a five-stage automated spotter (Fig. 2). The chip array includes ten depositing cantilevers, fabricated using conventional microfabrication techniques. Each cantilever (shown in Fig. 2) is 1,500 μm long, 120 μm wide and 5 μm thick. A fluidic channel is incorporated in the cantilever tip for liquid loading and deposition. Droplet deposition is obtained by contact of the cantilever tip with the surface to pattern. To control the contact force and time during deposition, displacement sensors are incorporated into the array by means of piezoresistors integrated into the silicon cantilevers (Leïchlé et al. 2006c). The homogeneity of the array of the printed droplets is guaranteed by using two piezoresistive displacement sensing cantilevers added on the side of the array to control the trim of the array regarding the deposition surface. A dedicated electronics relying on a modified

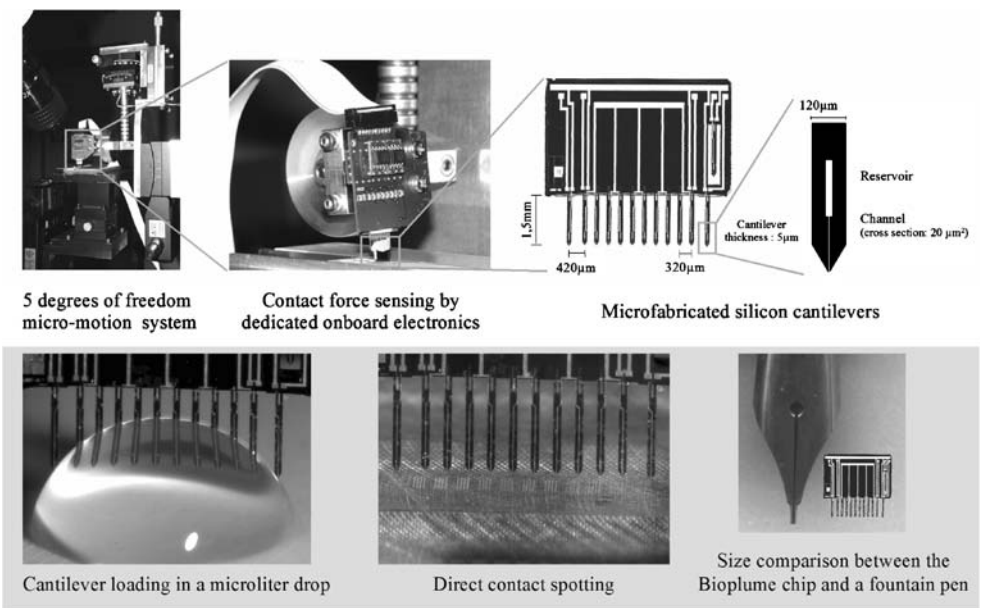

Fig. 2 Cantilever-based contact deposition device Bioplume

Wheatstone bridge is used to read-out the piezoresistor change, hereby providing means to measure the bending of the cantilevers.

2.3 Mode of deposition and washing procedure of the microcantilevers

The microcantilevers were filled by immersion in the liquid solution thanks to capillary forces. Contact of the microcantilevers on the slide surface led to the formation of spots exhibiting time dependent diameters. In the presented experiments, a contact time of 300 ms was set, resulting in the production of spot of 20 μm in diameter. Matrices of drops were carried out by combining the deposition step with X and Y translations of the substrate stage, without the need for refilling the cantilevers. Washing the microcantilevers was achieved by dipping the microcantilevers into water. After a short agitation, the cantilevers were removed from the reservoir drop, thus allowing water evaporation to occur within a few seconds. The aminated oligonucleotides and proteins were diluted in a sodium phosphate buffer (Na_2HPO_4 100 mM, pH 9.0) or in a sodium carbonate buffer ($NaHCO_3$ 100 mM, pH 8.4), respectively. Both buffers contained 25% of glycerol to prevent the evaporation of the spotted drops. After deposition, oligonucleotide and protein droplets were incubated on the surface respectively for 30 min or for 3 h at room temperature, thus allowing the covalent immobilization of the biomolecules.

2.4 Slide blocking

After DNA deposition, the aldehyde-functionalized slides were immersed for 30 min in a sodium borohydride solution (3.5 mg/ml), rinsed two times with water during 10 min and then dried under a stream of nitrogen. In the case of protein deposition, the slides were blocked by incubation in a 20 mg/ml bovine serum albumin (BSA) in PBS ×1 (10 mM Na_2HPO_4, 2.7 mM KCl, 140 mM NaCl, pH 7.4, 140 μl). To avoid any substrate movement, the slide was fixed on the robotic table and all blocking and washing steps were carried out directly on the surface. Solutions were deposited with a micropipet without touching the slide surface, liquid was circulated by bidirectional pipetting, and the last washing solution was drawn up.

2.5 Fluorescent labeling of antibody and protein

Alexa 647-NHS (6 μl in DMSO) was added to a solution of anti-GST antibody (100 μg) in carbonate buffer (0.1 M, pH 8.4, 120 μl). The solution was incubated for 1 h at room temperature in the dark, with gentle agitation. Reaction was stopped by incubation in Tris–HCl buffer (14 μl, 1 M, pH 8.8) 1 h at room temperature and then overnight at 4°C. The labeled antibody (140 μl) was purified by size exclusion using a Nanosep® 30 K column: the labeling solution was poured on top of the column and centrifugated at 5.9 g during 2 min 30. The Alexa 647 excess was then washed two times by PBS 1× at 7.5 g during 2 min 30. The purified fluorescent labeled antibody was collected on the top of the column (50 μl in PBS 1×). The labeling quality was checked by UV using the Nanodrop ND-1000 spectrophotometer.

2.6 Conditions for biological interaction

Hybridization of "35mer-NH_2" deposited on the slides with the complementary Cy5-labeled oligonucleotide target ("15mer-Cy5") was carried out for 30 min at room temperature, either in a SSC 3×/SDS 0.3% solution (sodium citrate 45 mM, pH 7.0; NaCl 450 mM; SDS 0.3% *w*/*v*) or in commercial "Pronto Short Oligo Hybridization" buffer (Corning), both containing glycerol (25% *w*/*v*). Slides were then washed 5 min in SSC 3×/SDS 0.3%, 2 min in SDS 0.1%, 2 min in SSC 1× and finally 1 min in SSC 0.1×.

Interaction of the covalently bound GST protein with Cy5-labeled anti-GST antibody was carried out at room temperature in the commercial "reaction buffer" containing 10% "ChipHybe" (Ventana Molecular Discovery Systems) and glycerol 25%, for 50 min. The slide was washed for 5 min with "high salt" solution (PBS 1× containing Tween 20, 0.1% *w*/*v* and 850 mM NaCl) and then another 5 min in the same buffer without salt.

2.7 Data acquisition

After hybridization and washing steps, the slides were scanned using the Axon 4100A (Axon Instruments) and the images were captured with the Genepix 6.0 software. Circle grids were aligned on each image. The fluorescence intensity of each spot was calculated as the average fluorescence signal (measured in pixels). Due to the low resolution of the scanner (5 μm) as compared to the size of the spots (20 μm), the fluorescence signal of the spots showed a standard variation of at least 25%.

3 Results and discussion

3.1 Accuracy of the Bioplume spotter for successive deposition at the same location

The aim of this work was to demonstrate the ability of the Bioplume spotter to deposit in a sequential manner and at the same location active biomolecules on functionalized surfaces. Prior to evaluate this feasibility in a biological

context, several controlling steps were validated. The first validation concerned the possibility to reuse the same microcantilevers for the successive deposition of different liquid solutions without any cross-contamination. To this aim, a simple washing procedure was implemented. The microcantilevers were filled in with either the buffer solution alone or with the Cy3-labeled oligonucleotide "16mer-Cy3" in the buffer solution. After deposition on a glass slide, they were dipped in a water drop for 5 min with gentle mechanical agitation and then reused for a new deposition run. The efficiency of this washing procedure, estimated as the percent of contamination, was obtained by the following equation:

$$\%\ contamination = \frac{Buff.fluor.with\ ''16mer\ Cy3''\ contaminated\ pins - Buff.self\ fluor.}{''16mer\ Cy3''\ fluor.} \quad (1)$$

Results obtained with 140 spots deposited with seven different cantilevers showed that 96% of the previously deposited solution was removed after a single rinse of the cantilevers for 5 min in water. A repetition of this experiment with a Cy5-labeled oligonucleotide confirmed this result. A reduction to less than 1% of cross-contamination could be obtained by dipping the cantilevers in harsher solutions such as sodium hydroxide 0.1 M and/or detergents (SDS 1%; data not shown). However, we did not consider using these solvents for the subsequent biological experiments because this method would require additional cleaning in water. Thus, this residual contamination was considered to be satisfactory for our application.

The second critical step toward sequential spotting consisted in confirming that the Bioplume spotter could address biological solutions several times at the same location, i.e. with sufficient spatial resolution. For this purpose, three grids (2×10 spots each) of Cy5-labeled oligonucleotide (so-called "15mer-Cy5") were deposited with three adjacent cantilevers (see Fig. 3). After washing the cantilevers, three grids of Cy3-labeled oligonucleotide "16mer-Cy3" were deposited in a second run. As indicated in the figure, spotting position was shifted downwards (with respect to the figure) so that half the spots should be superposed. The first five deposited rows of spots, seen at the bottom of the figure, exhibited a green fluorescence, thus proving the efficiency of the washing process. As expected, because the remaining ten spots were deposited on top of the previously spotted "15mer-Cy5", a yellow signal was obtained: this indicated a correct superposition of the two fluorescent oligonucleotides. This yellow color was due to mixing and not hybridization of the two labeled oligonucleotides since they were not complementary to each other. The spatial resolution for spot superposition was thus estimated to be within the scanner resolution, *i.e.* 5 μm.

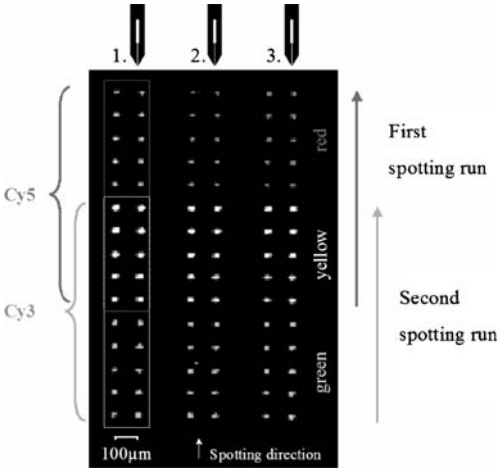

Fig. 3 Spatial resolution of the intermixed deposition with microcantilevers. Three grids of "15mer-Cy5"oligonucleotide 10 μM in 25% glycerol were spotted using Bioplume. Each grid, consisting of two rows of ten spots was deposited by a single cantilever (number 1, 2 and 3 on the figure). The cantilevers were then washed for 15 min in water, and reused to produce a second grid of "15mer-Cy3" oligonucleotide 1 μM in 25% glycerol spots, so that half of them was addressed exactly on top of the previously spotted spots

3.2 Hybridization of DNA molecules

Prior to testing the in-spot localised hybridization between complementary DNA strands, we checked that the buffer condition in combination with the scale reduction of the spot diameter (20 μm in the present case compared with 100 μm with classical microarrayers) did not alter the interaction process. This was carried out by spotting several times the "35mer-NH_2" oligonucleotide prepared in the buffer solution containing 25% of glycerol using Bioplume on an aldehyde-functionalized glass slide. Buffer was also spotted as a negative control. After chemical blocking of the spotted oligonucleotides during 30 min with sodium borohydride ($NaBH_4$) and washing off the excess of chemicals, the entire glass surface was covered with the hybridization buffer containing "15mer-Cy5" in the presence of 25% glycerol. After 30 min hybridization, the slide was washed to remove non-hybridized oligonucleotides. Fluorescent signals 100-fold above the background signal were measured only on spots bearing the "35mer-NH_2" probes. This indicated that scale reduction and presence of high concentration of glycerol in the spotting and hybridization buffer did not impair DNA hybridization.

In-spot localized DNA hybridization was therefore investigated as depicted in Fig. 4. The "35mer-NH_2"oligonucleotide (lines d and e in Fig. 4) and buffer alone (lines a, b) were spotted several times on an aldehyde-activated glass slide using Bioplume. After blocking and washing the

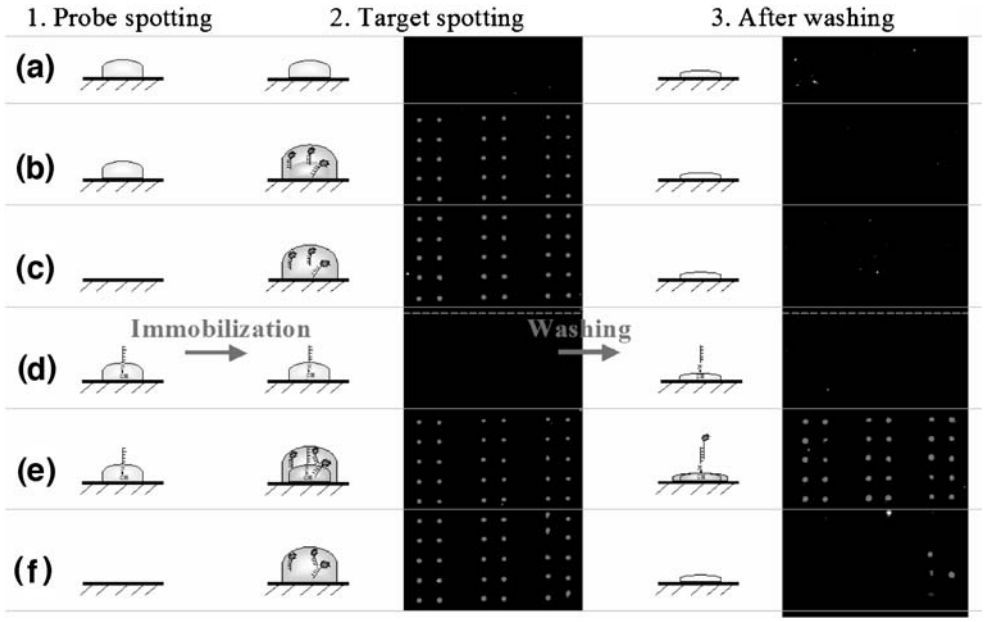

Fig. 4 In-spot localized hybridization. A 50 μM 35mer-NH_2 amino-oligonucleotide in buffer Na_2HPO_4 0.1 M, pH 9 containing glycerol 25% was deposited several times on a glass slide using Bioplume microcantilevers (*lines d* and *e*). Buffer alone was also spotted as negative control in *lines a*, and *b*. After leaving the slide for 30 min, it was treated with a solution of $NaBH_4$ 3.5 mg/ml for 30 min and then washed with water. Then, complementary Cy5-labeled 15mer oligonucleotide at 500 nM in commercial "Pronto Short Oligo hybridization buffer" was addressed at the same location as the previous spotting. After 30 min hybridization at 25°C, the slide was successively washed with SSC 3×/SDS 0.3%; SSC 1×/0.1% SDS, SSC 1×, and finally SSC 0.1×. Fluorescent signals were captured at 635 nm with the laser scanner Axon 4100A setting the photomultiplier at 500 before washing and at 800 after slide washes

slides with caution to avoid any movement, the "15mer-Cy5" oligonucleotide complementary to the "35mer-NH_2" probe (lines b, c, and e) was addressed using the same microcantilevers, previously washed. After 30 min of hybridization at 25°C followed by the washing steps, the slides were scanned with the Genepix 4100A instrument. As indicated in Fig. 4, a red signal was captured only in spots containing both immobilized "35mer-NH_2" probes and "15-mer Cy5" complementary targets (line e). Moreover, the washing steps enabled to remove all non-hybridized oligonucleotides. As a conclusion, this in-spot localized hybridization is as efficient as the classical protocol using microarrays bearing 100–200 μm spots (data not shown) with the notable advantage to reduce the volume of the biological solution by a factor 100 to 1,000.

3.3 Protein interaction

The same experimental approach was used to study the interaction between the glutathione-S-transferase (GST) and its specific anti-GST antibody. GST and BSA (the latter used as a negative control) were addressed on an aldehyde-functionalized glass slide using Bioplume. After blocking the slide with a BSA solution, the Alexa 647-labeled anti-GST antibody was addressed with the same washed cantilevers on top of each spot. The interaction between the spotted GST and the addressed anti-GST took place during 60 min at 25°C. After washing with a buffer solution to remove unbound labeled antibodies, fluorescent signals were captured with Genepix 4100A instrument. As shown in Fig. 5, only the spots bearing GST and labeled anti-GST resulted in a positive fluorescent signal. As a conclusion, this intermixed deposition principle can also be employed for protein-protein interaction, with the advantage of size and volume reductions as compared to the current protein chip technology (Templin et al. 2003).

3.4 Deposition of different molecules using a loading fluidic chip

The approach of using several cantilevers is relevant only if this technology can be multiplexed, i.e. it can deliver different biological materials on a same surface at the same time. As a preliminary step toward this objective, we fabricated and used an external loading fluidic chip associated to the cantilever array. Such delivery apparatuses have already been proposed to simultaneously load an array of dip-pen (Banarjee et al. 2003; Ryu et al. 2004). This chip enables to load the microcantilevers by reducing from millimeter size fluidic inputs to micrometer size outputs. For this purpose, the fluidic chip includes three input reservoirs and ten dispensing holes, each reservoir being linked to three or four holes

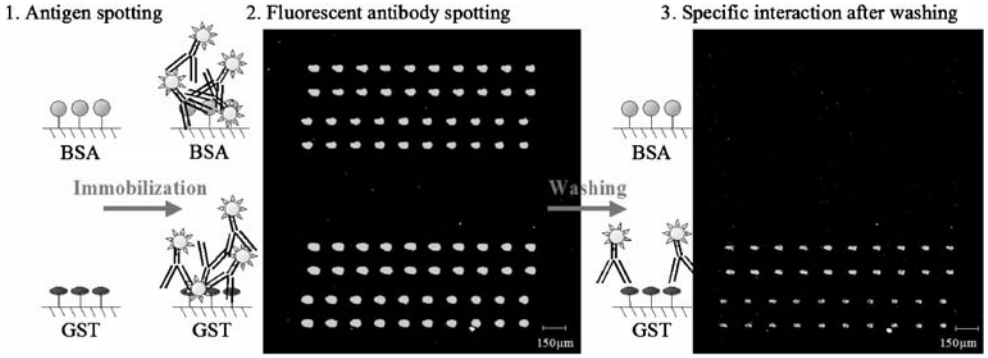

Fig. 5 In-spot localized protein interaction. The GST and BSA proteins, at a concentration of 0.1 mg/ml in $NaHCO_3$ pH 8.5 buffer, glycerol 25% were deposited on a glass slide. After 3 h immobilization, a blocking solution of BSA at 20 mg/ml in PBS 1× was poured onto the spots, followed 30 min later by a PBS 1× wash. Alexa 647-labeled anti-GST antibody at a concentration of 0.1 mg/ml in commercial buffer containing 25% glycerol was addressed on top of previously spotted proteins. After 60 min incubation at room temperature, the slide was washed 5 min with PBS 1× containing Tween 20, 0.1% *w/v* and 850 mM NaCl and then another 5 min in the same buffer without salt. Fluorescent signals were captured at 635 nm with the laser scanner Axon 4100A setting the photomultiplier at 500

by means of closed microchannels (see Fig. 6). The chip dimension is 20-mm long and 12-mm wide and dispensing holes are 200 µm long, 200 µm wide and 525 µm deep. The total volume for a single liquid reservoir is about 13 µl. The fabrication process is based on reactive ion etching of silicon to build the channels, the loading reservoirs and dispensing holes, and pyrex bonding to cover the channels. Liquid is supplied to the input reservoirs by pipetting, and fills up the microchannels and the dispensing holes by capillary forces. Loading of the microcantilever arrays is then achieved by dipping the cantilevers in the dispensing holes. To avoid the large deflection of the tilt monitoring cantilevers during the Bioplume loading, two dispensing holes free from liquid supply are added.

To verify that the delivery through the dispensing holes occurred without mixing, the reservoirs were filled up with distinct colored liquid solutions (see Fig. 6, bottom right picture). The picture in Fig. 2 (bottom left) shows how loading was previously achieved from a microdroplet reservoir, illustrating the advantage of using a fluidic chip in terms of reduction of the volume required for loading.

To investigate the efficiency of the loading chip for parallel deposition, three different experiments were carried out. In the first one, the three reservoirs of the fluidic cartridge were filled in with solutions of Cy5, Cy3 and Cy3+Cy5, respectively. The Bioplume microcantilevers were dipped in the dispensing holes for several seconds and a matrix of spots was deposited on an aldehyde-activated glass slide. The spots were then observed with a laser scanner. As shown in Fig. 7(a), a specific pattern of red, green and yellow spots was obtained in agreement with the labeled oligonucleotides contained in the reservoirs, indicating the correct working operation of the fluidic chip.

The second experiment concerned the use of this external fluidic chip for parallel hybridization. To this aim, matrices of spots were arrayed from reservoirs

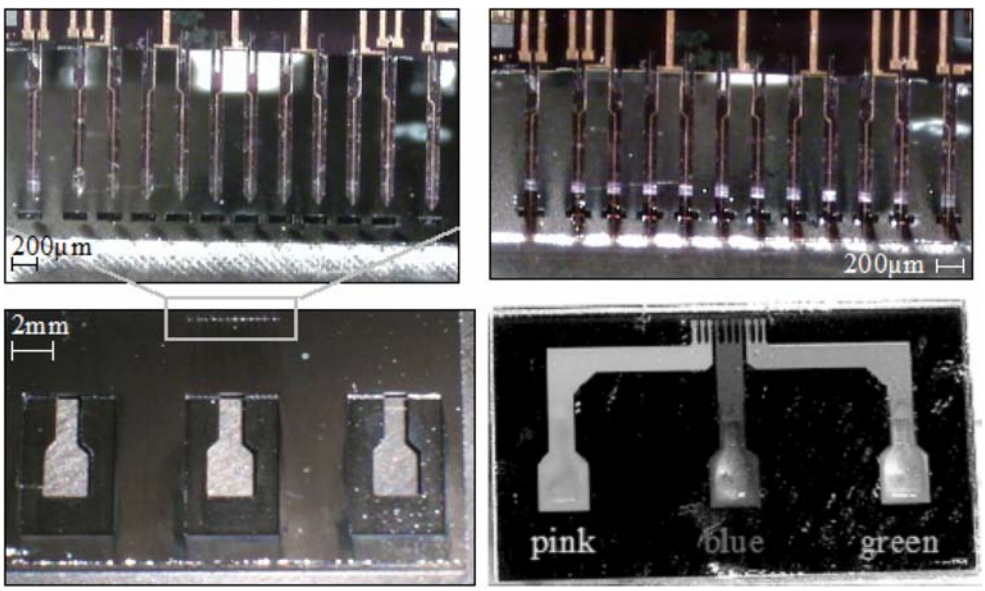

Fig. 6 External loading fluidic chip. *Top* Picture of the loading process before and after dipping the cantilevers in the dispensing holes. *Bottom* Picture of the external loading chip. *Left* Front side of the chip showing the reservoirs and the dispensing holes. *Right* Back side showing the reservoirs and the channels filled in with colored inks

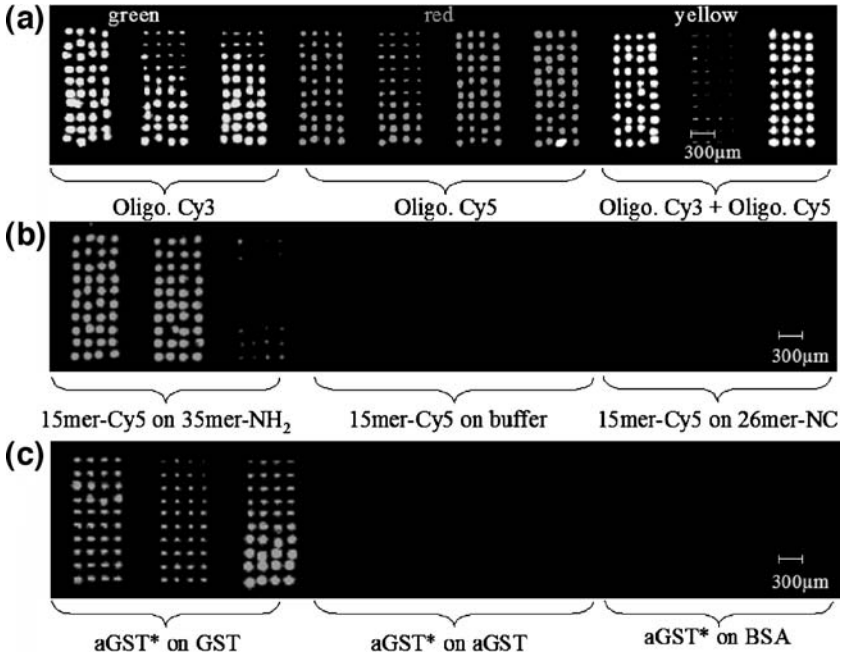

Fig. 7 Parallelization of the intermixed deposition at the micron scale. (**a**) Cy3, Cy5 and Cy3+Cy5 labeled oligonucleotides in Na_2HPO_4 0.1 M pH 9.0 buffer, glycerol 25% were spotted by an array of 10 microcantilevers from three independent reservoirs of the fluidic chip. (**b**) A solution of "15mer-Cy5"oligonucleotide was deposited on previously spotted "35mer-NH_2" probes, buffer alone and the non-complementary sequence "26mer-NC" (negative controls). (**c**) A solution of Alexa 647 anti-GST antibody at a concentration of 0.1 mg/ml in commercial buffer containing 25% glycerol was addressed to previously spotted and immobilized GST, anti-GST and BSA. Fluorescent signals were captured at 635 nm (*red*) and 532 nm (*green*) with the laser scanner Axon 4100A setting the photomultiplier at 600 (*red*) and 400 (*green*)

containing the "35mer-NH_2" oligonucleotide, the non complementary sequence "26mer-NC" and the buffer alone, according to the procedure aforementioned. Hybridization was carried out by depositing a solution of Cy5-labeled "15mer-Cy5" oligonucleotide complementary to the "35mer-NH_2" probes. As expected [Fig. 7(b)], a red fluorescent signal was captured only in spots where both "35mer-NH_2" and "15mer-Cy5" were present at the same location. It should be noticed that the fluorescence signal resulting from one of the matrix was much lower than from the other two. This difference could be explained by a poor loading efficiency of the third cantilever or by an insufficient contact onto the surface.

The last experiment consisted in applying this method to localised interactions using three different proteins at the same time. A matrix of spots bearing GST, anti-GST antibody and BSA were first deposited using the loading cell coupled to the Bioplume spotter. The labeled anti-GST antibody was addressed in a second step with the same cantilevers on each of these spots. As shown in Fig. 7(c), the interaction occurred only in the spots bearing the GST protein. Again, we observed a lack of homogeneity in the fluorescent intensity between the spots that was likely due to a lack of surface contact during deposition with Bioplume (that could be solved by using the piezoresistor response to monitor the deposition process in real time), together with a poor resolution of the scanner. To prevent these spotting defaults, the loading cell was designed so that each solution could be spotted by at least three independent cantilevers. Nonetheless, this considerable scale reduction enables to repeat the spots of the same solution without requiring much surface area.

4 Conclusion

In this work, we have demonstrated that a spotting tool called Bioplume, based on the use of micromachined cantilevers, is fully adapted for delivering in a sequential manner and at the same location various liquid solutions. This intermixed deposition methodology was validated for two basic biological applications, namely DNA hybridization and protein-protein interaction. The possibility of multiplexing this methodology was further evaluated by coupling the deposition microcantilever system to an external loading fluidic chip. Several advantages are foreseen using this methodology, as compared to the current microarray technology. A first and non negligible advantage with respect to scarcity of the biological material is a drastic volume reduction, which can be estimated to range between factors of 100 to 1,000, without reducing the quality of the results. A clearly dedicated application would

be the high throughput pharmaceutical screening of biomolecules interactions (i.e. inhibitors, drugs, etc.) on specific probes (proteins, peptides, etc.), since they are often prohibitive and available in very low quantities. A second advantage is the possibility to address chemical solutions with high spatial resolution on small and fragile surfaces, as for instance for the coating of the MEMS-based microsensors and for the deposition of highly sensitive biomolecules under conditions that would warrant their biological activity, which is likely not the case using ink-jet printing or other dispensing method that requires high temperature or high pressure.

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