Centimeter-Scale Surface Interactions Using Hydrodynamic Flow Confinements

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Supporting Information

ABSTRACT: We present a device and method for selective chemical interactions with immersed substrates at the centimeter-scale. Our implementations enable both, sequential and simultaneous delivery of multiple reagents to a substrate, as well as the creation of gradients of reagents on surfaces. The method is based on localizing submicroliter volumes of liquids on an immersed surface with a microfluidic probe (MFP) using a principle termed hydrodynamic flow confinement (HFC). We here show spatially defined, multiplexed surface interactions while benefiting from the probe capabilities such as non-contact scanning operation and convection-enhanced reaction kinetics. Three-layer glass-Si-glass probes were developed to implement slit-aperture and aperture-array designs. Analytical and numerical analysis helped to establish probe designs and operating parameters. Using these probes, we performed immunohistochemical analysis on individual cores of a human breast-cancer tissue microarray. We applied α-p53 antibodies on a 2 mm diameter core within 2.5 min using a slit-aperture probe (HFC dimension: 0.3 mm × 1.2 mm). Further, multiplexed treatment of a tissue core with α-p53 and α-β-actin antibodies was performed using four adjacent HFCs created with an aperture-array probe (HFC dimension: 4 × 0.3 mm × 0.25 mm). The ability of these devices and methods to perform multiplexed assays, present sequentially different liquids on surfaces, and interact with surfaces at the centimeter-scale will likely spur new and efficient surface assays.

INTRODUCTION

Compartmentalization is central to studying the effect of various (bio)chemical microenvironments on biological entities. Such testing and analysis of multiple parameters are useful in (bio)chemical screening, analysis, synthesis, and characterization with applications, for instance, in drug discovery, studies of cell-to-cell communication, and tumor marker detection. Microtiter plates are currently one of the most common substrates for compartmentalized assays in both research and diagnostics. To increase the analytical throughput, the trend has been to reduce the footprint and volume of each well of the microtiter plates, with the current footprint of standard wells measuring 1.5 mm × 1.5 mm (1536 well plates). Further scaling of the microtiter plates is hindered by constraints in fabrication together with requirements for liquid and mechanical interfacing and imaging. These limitations have triggered a drive towards substrate-based assays. Such surface formats, called microarrays, use lithographic methods, inkjet printing and pin spotting to produce high-density patterns of specimens and reagents on surfaces. These surface assays have the potential to enable high-throughput analytical testing while simplifying read-out and detection. However, their lack of physical compartmentalization hinders multiplexing of liquid reagents and indicates the need for a new set of tools to enable targeted interaction with biological samples such as DNA/protein microarrays, tissue sections, and cell monolayers. Such a tool should ideally be able to (i) interact with the substrate on spatially distinct areas at the mm- to cm-scale; (ii) deliver different liquids to a surface in both a parallel and sequential manner; (iii) enable interaction with the surface without physical contact between the tool and the surface, and (iv) operate in a wet environment to avoid drying artifacts. Several techniques have been developed that allow local processing of immersed substrates and fulfill subsets of the above criteria. However, a versatile method to efficiently interact with immersed, cm-scale substrates in a localized manner remains elusive.

Pin spotters and inkjet systems are established technologies primarily used for patterning reagents on a dry surface and are not suitable to implement biological assays on surfaces. Aqueous two-phase systems implemented on immersed substrates by means of an inkjet-like nozzle have been used for patterning mammalian cells and bacteria, but the spatial resolution and the limitations imposed by diffusion between the two phases are not favorable for confining molecular reagents. Rapp et al. demonstrated cm-scale patterning of antibodies using selective UV irradiation enabled by a digital micromirror device. This method is limited to photoinitiated reactions and does not allow a selective change of the liquid environment on a surface. Local processing was also demonstrated by Kim et al. by conformably sealing microchannels on surfaces but without the ability to stain specific regions of interest. In addition, mechanical contact can introduce cross-contamination and adverse mechanical stress on the biological sample, which is...
also the case for another contact-based microfluidic device, namely the chemistode. Atomic force microscope (AFM)-based methods and their derivatives, such as the FluidFM and dip-pen lithography, enable local interaction with biological substrates with high resolution, but their narrow range of operation (which is \( \sim 150 \text{ nm} \times 150 \text{ nm} \times 20 \text{ nm} \)) is not compatible with cm-scale substrates. Other methods for high-precision interfacing with biological substrates are scanning ion conductance microscopy (SIM) and scanning electrochemical microscopy (SECM), both requiring implementation of reference electrodes and maintenance of specific homogeneous buffer conditions. This complicates the application of different processing liquids in (bio)chemical surface-based assays.

A promising technology for local interaction with immersed substrates is the microfluidic probe and its variants (MFP). The MFP operates in a noncontact scanning mode and localizes a liquid on a surface by creating a hydrodynamic flow confinement (HFC; Figure 1a). Using the MFP, interactions with surfaces on length scales ranging from single \( \mu \text{m} \) to several hundred \( \mu \text{m} \) have been demonstrated. The MFP and its variants have been applied for multiplexed immunohistochemical analysis of tissue sections, biopatterning, and pharmacology on a single-cell level. In the configurations of the probes used thus far, the contact area of the processing liquid with the substrate is smaller than \( 200 \text{ \mu m} \times 200 \text{ \mu m} \).

Processing an area of, for instance, \( 1 \text{ cm}^2 \) would require scanning the area of interest for extended periods of time, when sufficient local incubation times are taken into account. Therefore, despite the favorable attributes listed above, excessively long processing times as well as varying processing conditions due to scanning render the microfluidic probe technology in its current form unsuitable for cm-scale surface assays.

In this paper, we present a new family of microfluidic probe devices along with a methodology for selective delivery of microliter volumes of processing liquids to immersed substrates on the cm and mm length-scales. This enables new strategies for multiplexed surface-based assays: uniform, localized exposure to a single reagent (Figure 1b, left), multiplexed exposure to several reagents (Figure 1b, right), and gradients of several reagents (Figure 1b, center). We present two families of probes, one with slit-aperture designs for interacting with a surface using a single reagent and creating concentration gradients, and the second one with aperture-array designs for multiplexed interaction and for creating concentration gradients. We demonstrate the efficacy and applicability of our devised designs in the context of surface assays by performing uniform and multiplexed immunohistochemistry on tissue sections and by patterning proteins on a surface (Figure S1).

## MATERIALS AND METHODS

### Microfluidic Probe Platform

The platform comprised a scanning unit, a holder for the sample, a holder for the probe and a flow control unit (Figure S2). The scanning unit held two stages for X-Y positioning of the sample relative to the probe, and another stage for Z-positioning of the probe over the sample (Zaber Technologies Inc., Canada). The entire scanning unit was placed on top of an inverted microscope (Eclipse TI-E, Nikon, Japan). The probe itself was mounted on a holder that allowed tilt adjustment of the probe relative to the sample. The flow control unit comprised reservoirs connected to the probe with 1/16 PEEK tubing (IDEX H&S, USA) and fluidic connectors (Dolomite microfluidics, UK). Vacuum or pressure was applied to the reservoirs using a pressure control device (Fluigent, France) (see Figure S3 for details on the simultaneous injection of multiple reagents). Flow rates were measured in real-time (Fluigent, France), allowing the system to operate in a closed-loop feedback mode with constant flow rates. A detailed description of the platform, alignment procedure and operation can be found elsewhere.

### Probe Fabrication

The probe is a glass-Si-glass device with microchannels defined by photolithography and etched to a depth of \( \sim 50 \text{ \mu m} \) using deep reactive-ion etching (DRIE) on both sides of a double-sided polished Si wafer. The microchannels were sealed by anodic bonding (1.3 kV, 475 °C) of the Si wafer with BF3 glass wafers. Details of the fabrication process for a two-layer Si/glass probes are described elsewhere. To fabricate the three-layer probes used here, each side of the processed Si substrate was bonded with glass sequentially.

### Finite-Element Modeling

We performed steady-state 3D simulations with COMSOL Multiphysics (version 4.2). Nonslip boundary conditions were defined on all surfaces and a Neumann boundary condition for flows across the virtual interface between the immersion liquid and the liquid underneath the apex of the probe. At each aperture, a Dirichlet boundary condition defined the flow. All fluids were set to be water (incompressible Newtonian fluid with a density of 998 \( \text{kg/m}^3 \) and a dynamic viscosity of 0.001 \( \text{N s/m}^2 \)).

### Immunohistochemistry Protocol

Human breast-cancer tissue microarrays (TMA) (Novus Biologicals LLC, USA) were dried at 60 °C for 45 min, followed by removal of paraffin and gradual

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**Figure 1.** Strategies of interacting with immersed substrates on the cm-scale using hydrodynamic flow confinement. (a) The probe is positioned \( \sim 5 \) to \( 50 \text{ \mu m} \) above a substrate. Inset: an HFC between the probe and the surface is created by setting the ratio of injection flow rate \( (Q_i) \) to aspiration flow rate \( (Q_o) \) to \( \sim1:3 \). (b) Two families of probes, slit-aperture probes and aperture-array probes, enable several strategies of interfacing with surfaces: sequential exposure (left), multiplexed exposure to different reagents on spatially separated regions (right), and creation of gradients (center). Inset: schematic of antibodies patterned on a substrate immersed in physiological buffer.
rehydration. A hydrogen peroxide block was applied prior to the heat-induced epitope retrieval (target retrieval solution pH 9.0, Dako A/S, Denmark). The TMA was cooled gradually to room temperature in the target retrieval solution, followed by a protein block. With the probe, local areas of the tissue section were exposed to the primary antibody (Ab) solution. The primary Ab solutions used were α-p53 and α-β-actin, both produced in mouse (both Sigma-Aldrich, USA), and were diluted to 25 μg/mL in PBS. Rhodamine B was added to all antibody solutions at a concentration of 10 μM for flow visualization.

For staining, we used enzymatically amplified staining with the chromogen 3,3′-diaminobenzidine (DAB) for bright-field visualization (mouse-specific HRP/DAB IHC Kit, Abcam plc, UK).

Biopatterning and IgG–Anti-IgG Assay. A Si stencil (mask) was used to generate the protein patterns on the surface. The surface of the stencil was cleaned with air plasma (200 W, 2 min), and then the stencil was placed on a sheet of polydimethylsiloxane (PDMS; 0.25 mm, HT-6240, Rogers Corp., USA) supported by a microscope glass slide. The channels of the stencil were filled with a solution containing 50 μg/mL IgG from rabbit serum (Sigma-Aldrich, USA), followed by 30 min incubation. After rinsing with BSA (1% in PBS), the stencil was removed. The PDMS sheet was then incubated with BSA (1% in PBS) for 15 min prior to treatment with the MFP. The liquid applied with the probe as processing liquid contained fluorescently labeled α-rabbit IgG at 25 μg/mL and BSA (1% in PBS).

## RESULTS AND DISCUSSIONS

Probe Design and Operating Conditions for mm-Scale HFCs. Two-layer Si/glass probes allow the formation of a μm-scale, drop-shaped HFC by simultaneously injecting and aspirating a processing liquid between adjacent apertures at the apex (Figure 2a). Extending the HFC in one dimension can potentially enable processing on the cm-scale by scanning in the direction perpendicular to the direction of extension. Intuitively, one would design probes with increased spacing between the two adjacent apertures, resulting in an HFC that is elongated along its main axis. This however is not a viable approach for the following reasons: (i) the resulting HFC would be broad in the proximity of the injection aperture and rather narrow in the proximity of the aspiration aperture (a “stretched” drop shape). This change in width would result in nonuniform incubation times at different positions of the HFC when scanning the substrate in the direction perpendicular to the direction of extension (main axis of the HFC, Figure 2a). (ii) To realize HFC at reasonable flow rates (Q/Q0 ≥ 1/5), the distance between adjacent apertures may not be larger than the shortest distance between an aperture and the edge of the apex. The maximum spacing between the adjacent apertures is therefore limited to the thickness of the glass and Si substrates.

Another approach for enlarging the HFC is to increase the dimensions of the apertures perpendicularly to the main axis of the HFC. In a two-layer probe, this can be done by increasing the etch depth d, which is as well limited by the thickness of the Si substrate. We note that fabrication techniques with different materials, e.g., plastics, might provide additional options for scaling of the apertures. However, in the context of biological assays, probes in Si and glass offers several advantages, such as (i) high-resolution features and geometries, (ii) chemical inertness, (iii) robust thermo-mechanical properties, (iv) good optical properties, (v) controllable surface properties, and (vi) robust and established protocols for fabrication and quality control. To enable scaling of the apertures while leveraging the advantages of Si/glass probes, we present a three-layer probe design, which allows the main axis of the HFC to be oriented perpendicularly to the Si and glass layers (Figure 2b). In such a design configuration, the HFC can be extended homogeneously in one dimension by scaling w. Also, w can be varied independently for each aperture, and scaling is independent of the thicknesses of the Si and glass substrates. This allows the fabrication of probes with adjacent slit apertures (Figure 2b) or arrays of adjacent apertures (Figure 2c).

Slit-Aperture Probes. We performed numerical simulations of liquid flow in the gap between the probe and the substrate to study and optimize the flow patterns generated by slit-aperture probes. We fixed the distance between the probe and the substrate at 30 μm and Q/Q0 = 1/5. Figure 3a is a false-color image of the relative amplitude of the flow velocity in the center plane between the probe and the substrate (XY-center plane) for a design with identical slit lengths. The operating condition Q/Q0 = 1/5 is reflected by high velocity amplitudes in the vicinity of the aspiration aperture. Figure 3c (top) highlights the flow path of the processing liquid in the YZ-center plane for slit apertures of identical length and the operating conditions mentioned. The simulation results suggest that under such operating conditions, a sheath flow of immersion liquid prevents the confined processing liquid from contacting the substrate. The sheath flow results from the fact that more liquid is aspirated from the region of the injection aperture, than the injection aperture supplies. Therefore, flow from the injection aperture is complemented with a flow of immersion liquid. To further understand this failure mode, we developed a simplified analytical model in which we neglect the effects at the edges of the apertures and only consider flow in the Y-direction. In three-layer Si/glass probes with slit apertures, the distance between the aspiration aperture and the closer edge of the apex in the Y-direction...
Figure 3. Optimization of slit-aperture probe designs and operating parameters. (a) Numerical simulation of relative amplitude of flow velocity (zero flow velocity in black regions, high flow velocity in bright regions) and flow pattern of injected processing liquid (white streamlines) in the horizontal middle plane between probe and substrate for a probe design with slit apertures of identical length. (b) A probe design with an aspiration aperture that is 4× longer than the injection aperture. (c) Relative amplitude of flow velocity in vertical planes along the lines A and B indicated in (a) and (b). For slit apertures of same length (top), a sheath flow of immersion liquid screens the confined processing liquid from contacting the surface (see sketch on the right. Read arrows: flow of processing liquid, blue arrows: flow of immersion liquid). In a slit-aperture probe (bottom) with the design illustrated in (b), no sheath flow of immersion liquid is formed. (d) Working regime of $Q_I/Q_A$ for different values of the aperture length ratio $L_I/L_A$.

Insets: photographs of HFCs of a processing liquid containing fluorescein for an aperture length ratio of 1:4 at different values of $Q_I/Q_A$.

(lower edge of the apex) is 500 μm, whereas the distance to the farther edge (upper edge of the apex) is 775 μm. We assumed that the pressure difference between the aspiration aperture and both, the lower and the upper edge, is identical. We further assumed that the hydrodynamic resistance between the aspiration aperture and the edges scales linearly with distance. Therefore, the relative aspiration flow supplied from the upper edge of the apex can be calculated as $100% \times (1 - \frac{775}{500 + 775}) \approx 40\%$. If the injection flow is lower than this 40% fraction of the aspiration flow, as is the case for $Q_I/Q_A = \frac{1}{5}$, additional liquid flow is required from the upper edge of the apex. For slit apertures of the same length, this results in a sheath flow of immersion liquid under the flow of processing liquid, thereby hindering interaction of the processing liquid with the substrate. Contact between the processing liquid and the substrate can be enforced by providing an injection flow that exceeds the aspiration flow in the vicinity of the injection aperture. The fraction of the aspiration flow drawn in from the region of the injection aperture would nonetheless remain unchanged and not all of the injected liquid would be recollected by the aspiration aperture. In such a case, the processing liquid would not be confined. Slit apertures of identical length are therefore unsuitable for interacting with a substrate using HFCs, as there is no operating range between the two failure modes of (i) no contact between the processing liquid and the substrate and (ii) the loss of confinement of the processing liquid.

These failure modes however can be avoided by designing the aspiration aperture to be longer than the injection aperture. We performed numerical analysis of the flow conditions for a probe with apertures with a length ratio of $L_I/L_A = 1/4$. With the ratio of flow rates remaining at $Q_I/Q_A = 1/5$, significantly less liquid is aspirated from the direction of the injection aperture (Figure 3b) and the injection flow is not smaller than the aspiration flow in the vicinity of the injection aperture. This results in the formation of a stagnation zone between the edge of the apex and the injection aperture, indicating that a screening sheath flow of immersion liquid is not formed and that thus the confined processing liquid gets in contact with the surface (Figure 3c, bottom).

A longer aspiration aperture also ensures a stable confinement of the processing liquid over a broader range of $Q_I/Q_A$ values, because on both sides of the injection aperture there is still flow of immersion liquid from the upper edge of the apex. This results in a shielding flow of immersion liquid around the HFC. Before the confinement of the processing liquid fails and the processing liquid streams into the immersion liquid from the upper edge of the apex, the injection flow would have to exceed this shielding flow of immersion liquid. Therefore, in a specific range of $Q_I/Q_A$, the fraction of the injection flow that exceeds the aspiration requirement in vicinity of the injection aperture is still recollected by the aspiration aperture and, in part, compensates for the flow that would be aspirated from the lower edge of the apex.

As per the model, the minimum $Q_I/Q_A$ ratio required for the processing liquid to contact the substrate scales with $L_I/L_A$:

$$\frac{Q_I}{Q_A} = 0.4 \times \frac{L_I}{L_A}$$

(1)

Leakage of the processing liquid from the upper edge of the apex occurs when the injection flow saturates the fraction of the aspiration aperture that corresponds to the length of the injection aperture (see Figure 3c, hatched area). An upper threshold for $Q_I/Q_A$ can be therefore defined by

$$\frac{Q_I}{Q_A} = \frac{L_I}{L_A}$$

(2)

Figure 3d illustrates the operating range to be expected based on the above estimations. For choosing the appropriate ratio of lengths of apertures, two general trends have to be considered: a high ratio of $L_I/L_A$ results in a higher ratio of $Q_I/Q_A$ required to ensure contact between the processing liquid and the substrate. This makes the HFC more prone to perturbations.
caused by scanning movements of the probe. A low ratio of $L_i/L_A$ in turn enables the generation of a more stable HFC but offers a smaller working range for the ratio of $Q_i/Q_A$. We experimentally verified the validity of the discussed solution using a design with $L_i/L_A = 1/4$, as this ratio of lengths offers a comfortable working range, while providing good stability during scanning movements. For this design we also analyzed the confinement conditions at different values of $Q_i/Q_A$ with a processing liquid containing fluorescein (Figure 3d, inset). The model predicts a loss of confinement for $Q_i/Q_A > 1/4$. We verified experimentally that at this condition the HFC starts to bend around the edges of the injection aperture and is susceptible to leakage of the processing liquid, in particular during scanning mode of operation (Figure 3d, inset II).

**Aperture Array Probes.** An array of HFCs enables multiplexed and simultaneous processing of a substrate with different reagents. Numerical analysis of the flow patterns in the XY-plane (gap distance 30 μm, $Q_i/Q_A = 1/4$) suggests that in such a probe design there can be cross-talk between the HFCs at the extremities of the array and their neighbors (Figure 4a, top). The aspiration apertures for the HFCs at the extremities of the array can aspirate liquid from the X-direction (Figure 4a, bottom). This is not the case for the other aspiration apertures in the array, as there are competing aspiration apertures on both sides in the X-direction, resulting in a stagnation of flow between neighboring apertures. The additional flow to the aspiration apertures at the edges of the array results in less liquid being aspirated from the direction of the respective injection apertures. A fraction of the processing liquids injected at the extremities of the array is therefore aspirated by the neighboring aspiration apertures, which alters the adjacent HFC, albeit to a lesser extent. One solution to this problem would be to inject only buffer from the outermost injection apertures and only use the HFCs at the center of the array for confining the actual processing liquids. A more general solution however is to create flow conditions for the HFCs at the extremities of the array that mimic the conditions within the array. This can be achieved by including additional aspiration-only apertures beyond the extremities of the array in the X-direction, which we term stabilization apertures. These stabilization apertures suppress flow from the X-direction to the aspiration apertures of the flanking HFCs resulting in stagnation of flow between the stabilization apertures and the aspiration apertures at the extremities of the array. Those aspiration apertures therefore aspirate mainly from the Y-direction, which leads to a stronger aspiration and therefore a better confinement of the respective processing liquids. The further the stabilization apertures are positioned apart from the array, the stronger aspiration to the stabilization apertures has to be to sufficiently stabilize the HFCs. The cross-talk between HFCs and also the efficacy of the stabilization apertures for averting cross-talk were experimentally verified and are in concordance with numerical simulations (Figure 4a,b, insets).

**Immunohistochemistry on Tissue Sections.** Using slit-aperture and aperture-array probes, we demonstrated the staining of individual cores of a tissue microarray (TMA). By this we show that the slit aperture and aperture array probes presented in this study (i) allow to generate stable confinement of processing liquids on the mm-scale suited for interaction with standard samples, (ii) short residence times observed in studies with μm-scale HFCs can be transferred to the mm- to cm-range, and (iii) the presented devices and methods enable unique strategies to process an immersed, cm-scale sample. Individual cores could be treated with different primary antibodies, or a single core could be exposed to distinct primary antibodies. Importantly, with these new families of probes, it is feasible to stain a core of interest or perform in-depth analysis of a selected core. For uniform mm-scale processing of a single core, we used a design with an injection aperture of 1 mm × 0.05 mm and an aspiration aperture of 4 mm × 0.05 mm (Figure 5a, inset).

This results in an HFC with a width of 1.2 mm, staining an area of about 2.4 mm² when scanned across a tissue core with a diameter of 2 mm. The unprocessed area on this core could be used, for example, to perform analysis with an additional antibody.28 We performed IHC on a breast-cancer-tissue microarray slide. Here, the primary antibody (Ab) ($\alpha$-p53, at a concentration of 25 μg/mL) was delivered with the MFP. We added rhodamine B at a concentration of 10 μM to visualize the HFC. Rhodamine B binds noncovalently to the tissue and is washed by the flow of immersion liquid during scanning of the probe over the tissue (Figure 5a). The HFC extends about 330 μm in the Y-direction, thus the local incubation time of the primary Ab with the tissue section at the scanning speed of 0.01 mm/s chosen was about 33 s. All other steps of IHC were performed for the entire TMA slide. In contrast to conventional...
methods, where the primary Ab incubation is on the order of hours, here the incubation is performed within seconds. This is due to the convective transport of the molecules to the substrate, resulting in an enhancement of reaction kinetics, and also to the application of higher concentrations of primary Abs. Using the set of parameters given above, we incubated a 2 mm TMA core with primary Abs in 2.5 min, consuming approximately 7.5 μL of processing liquid. The concentration of primary Abs used in conventional IHC (on the order of 1 μg/mL) typically is significantly lower compared to the concentrations used in the discussed experiments. We did not focus on minimizing the Ab consumption. However, consumption can be reduced by optimizing the flow rates, incubation times and the Ab concentration. The total time required for processing an entire TMA can be further optimized by using multiple probes, stacked probes, or by creating HFCs with a larger contact area with the substrate.

We also performed multiplexed staining by confining two primary Ab solutions (α-p53 and α-β-actin, both at 25 μg/mL) in 4 parallel, independent HFCs using an aperture-array probe capable of creating 8 HFCs (Figure 5b, inset). The remaining 4 HFCs were run with PBS as processing liquid (processing liquids in the eight adjacent HFCs: PBS − PBS − (α-p53) − (α-β-actin) − PBS − (α-p53) − (α−β-actin) − PBS). The injection flow rate for each HFC was 0.5 μL/min and Q_I/Q_A = 1/4. The area processed with each of the two Ab solutions was about 0.5 mm², which sufficiently provides a measure of the antigen expression within the sample.28

CONCLUDING REMARKS

The devices and methods presented enable versatile and localized interaction of different processing liquids with immersed, centimeter-scale substrates. Surface (bio)chemical assays in general would benefit from the ability to sequentially and simultaneously apply different liquids onto a surface. These multiplexing techniques, in combination with the scanning capability of the probe, enable versatile spatiotemporal alterations of selected regions of a substrate. Slit-aperture probes and also aperture-array probes enable the creation of localized gradients on a surface. Using a slit-aperture design, gradients can be created by supplying different liquids to the injection aperture through independent channels. Probes with
aperture-array designs allow the creation of discrete gradients by confining different proportions of two liquids in adjacent HFCs. The width of single HFCs and also the spacing between neighboring HFCs in such an array could be scaled down significantly to provide a higher resolution for creating such gradients. To enable conformal processing of a substrate with parallel HFCs, aperture-array probes could also be scanned over a substrate at an inclined angle (rotation around Z-axis) to enable an overlap between the areas processed with separate HFCs.

We further believe that using the methods presented in this paper, in combination with sequencing different liquids within each injection line, would be helpful for implementing a range of (bio)chemical assays on a surface. This could be done by the controlled insertion and removal of immiscible spacers, thereby minimizing Taylor dispersion. Moreover, we believe it is conceivable to integrate the methods presented with techniques to perform local temperature alterations to assist in minimizing Taylor dispersion. Additionally, we believe it is conceivable to integrate the methods presented with techniques to perform local temperature alterations to assist in performing efficient chemical reactions.

A limitation of the devices and methods presented is the relatively high consumption of reagents compared with that of on-bench protocols. An implementation of the discussed designs in probes made of plastics might allow the distance between the apertures in a slit-aperture design to be increased to several millimeters. This would increase the surface area the processing liquid interacts with before being reaspirated, which would result in a more efficient usage of the injected reagents. Another approach toward efficient use of the processing liquids would be to create HFC with minimal dilution in combination with liquid recirculation.

In a research setting, the key use of the devices and methods presented for cm-scale interaction with a substrate lies in enabling multiplexing in all steps of an analysis which are performed in liquid environment. In a clinical environment, the short incubation times enabled by HFC of the processing liquids might allow e.g. IHC analysis to be done during surgery, while the ability to acquire more detailed information on selected areas of interest can potentially enable a more targeted and more efficient analysis of tissue sections.

We believe that the methods proposed here can enable novel, complex, and dynamic (bio)chemical processes to be implemented on a range of biological substrates and for many applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.6b02983.

Centimeter-scale patterning of Abs on an elastomeric surface; schematic of the experimental setup; details pertaining to interfacing with a 96-plate well for parallel injection of various liquids. (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge financial support by the European Research Council (ERC) Starting Grant, under the 7th Framework Program (Project No. 311122, “BioIProbe”). We thank Aditya Kashyap, Anna Fomitcheva, and Dr. Ali Oskooei for discussions and Ute Drechslers, Marcel Burge, and the model shop team for technical assistance. Prof. Philippe Renaud (EPFL), Dr. Emmanuel Delamarche, Dr. Bruno Michel, and Dr. Walter Riess are acknowledged for their continuous support.

ABBREVIATIONS

HFC, hydrodynamic flow confinement; MFP, microfluidic probe; AFM, atomic force microscope; PDMS, poly(dimethyl)siloxane; IgG, immunoglobulin G; IHC, immunohistochemistry; TMA, tissue microarray; Ab, antibody

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