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SPR Imaging Measurements of 1-D and 2-D DNA Microarrays Created from Microfluidic Channels on Gold Thin Films

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Microfluidic channels fabricated from poly(dimethylsiloxane) (PDMS) are employed in surface plasmon resonance imaging experiments for the detection of DNA and RNA adsorption onto chemically modified gold surfaces. The PDMS microchannels are used to (i) fabricate "1-D" single-stranded DNA (ssDNA) line arrays that are used in SPR imaging experiments of oligonucleotide hybridization adsorption and (ii) create "2-D" DNA hybridization arrays in which a second set of PDMS microchannels are placed perpendicular to a 1-D line array in order to deliver target oligonucleotide solutions. In the 1-D line array experiments, the total sample volume is 500 μL; in the 2-D DNA array experiments, this volume is reduced to 1 μL. As a demonstration of the utility of these microfluidic arrays, a 2-D DNA array is used to detect a 20-fmol sample of in vitro transcribed RNA from the *uidA* gene of a transgenic *Arabidopsis thaliana* plant. It is also shown that this array fabrication method can be used for fluorescence measurements on chemically modified gold surfaces.

Surface plasmon resonance (SPR) imaging is a surface-sensitive optical technique that detects the affinity binding of unlabeled biological molecules onto arrays of molecules attached to chemically modified gold surfaces. We have recently demonstrated that SPR imaging measurements of DNA microarrays fabricated on gold surfaces can be used to monitor DNA−DNA, RNA−DNA, and protein−DNA interactions down to nanomolar concentrations.[1−3] In those initial sets of measurements, the DNA microarrays consisted of ∼60 500 μm × 500 μm squares with a total sample volume of 500 μL. For biological applications, such as gene expression and the detection of microbial species, it would be preferable to use a significantly smaller sample volume. One way to achieve this is to employ microfluidic networks.

Microfluidic devices provide a convenient means for manipulating very small amounts of sample and have been utilized in a variety of bioanalytical applications such as genetic analysis,[4,5] clinical analysis,[6,7] and immunoassays.[8] Microfluidic devices can be fabricated in a wide variety of materials such as glass, silica, and polymers, by the patterning techniques of either photolithography,[9] wet chemical etching,[10] or soft lithography.[11,12] Recently, microchip devices formed in poly(dimethylsiloxane) (PDMS) and then attached to either glass or gold surfaces have received an increased amount of interest as a simple, rapid, and low-cost fabrication methodology.[4,11,13−21] Microfluidic channels created in PDMS have been used in conjunction with a number of different detection methods such as fluorescence microscopy,[4,8,21] laser-induced fluorescence,[18] mass spectroscopy,[20] electrochemical detection,[16] and SPR-mass spectroscopy,[22] but not SPR imaging. We have found that microfluidic channels fabricated in PDMS can be easily applied to both the creation of DNA arrays on gold thin film surfaces and the detection of bioaffinity interactions in microliter sample volumes with SPR imaging.

In this paper, we describe (i) a general method for fabricating 1-D DNA line arrays using parallel microfluidic channels on chemically modified gold and silicon surfaces and (ii) a microliter

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detection volume methodology that uses 2-D DNA hybridization microarrays formed by employing the 1-D DNA line arrays in conjunction with a second set of parallel microfluidic channels for solution delivery. These arrays have been used to detect the specific adsorption of DNA and RNA with the techniques of SPR imaging and fluorescence microscopy. As a further demonstration of this method, the SPR imaging measurements of microfluidic channels were used to detect a 20-fmol sample of in vitro transcribed RNA from a partial clone of the *uidA* gene from a transgenic *Arabidopsis thaliana* plant (denoted as *GUS* gene RNA).

EXPERIMENTAL CONSIDERATIONS

Materials and Gold Thin Films. 11-Mercaptoundecylamine (MUAM; Dojindo), *N*-hydroxysuccinimidyl ester of methoxypoly(ethylene glycol)propionic acid MW 2000 (PEG-NHS; Shearwater Polymers Inc.), and sulfosuccinimidyl 4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate (SSMCC; Pierce) were all used as received. Absolute ethanol and Millipore-filtered water were used for rinsing and for making solutions. For the SPR imaging measurements, thin gold films (45 nm) were vapor deposited onto SF-10 glass slides (18 × 18 mm) as described previously.[23,24] A thin (<1 nm) chromium underlayer was used to enhance the adhesion of gold to the SF-10 slides. The fluorescence imaging experiments employed thicker gold films (200 nm) on silica substrates obtained from Evaporated Metal Films (Ithaca, NY).

PDMS Microfluidic Channels. Microfluidic channels were fabricated in poly(dimethylsiloxane) (PDMS) polymer as described previously.[18,20] Briefly, PDMS microchannels were created by replication from 3-D silicon wafer masters that were created photolithographically from a 2-D chrome mask pattern. The chrome masks were created using e-beam photolithography by the group of Professor Paul Nealey of the University of Wisconsin—Madison and the Center for Nanotechnology. These masks contained a 2-D pattern of parallel channels (width 75 μm or 300 μm and length 1.4 cm) featuring circular reservoirs (d = 1.4 mm) at both ends of each channel. The 3-D patterns on a Si wafer were made with a negative photoresist (SU-8 50, Microlithography Chemical Corp., Newton, MA) that was spin-coated at 5000 rpm for 20 s and then exposed to 365-nm UV light. This 3-D Si master was then developed in propylene glycol methyl ether acetate (Microlithography Chemical Corp.) and then silanized by storing it in a desiccator under vacuum for 2 h with a vial containing a few drops of tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (United Chemical Technologies, Bristol, PA). This last step was necessary to ensure the easy removal of the PDMS replicas from the Si master. Replicas were formed from a 1:10 mixture of PDMS curing agent and prepolymer (Sylgard 184, Dow Corning, Midland, MI) that was degassed under vacuum and then poured onto the master to create a layer with a thickness of about 0.5−1 mm. The PDMS was then cured for at least 1 h at 70 °C before it was removed from the Si wafer. Reservoirs were created by cutting out the circular ends of each channel from the PDMS with a hole punch.

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Table 1. Oligonucleotide Sequences for the Surface-Bound DNA Probes

surface-bound probe DNA	DNA sequences (5′ →3′)	starting position
A	CCC AAC CTT TCG GTA TAA	1724[a]
B	CCT TCA CCC GGT TGC C	908[a]
C	CGT TAT CGA ATC CTT TGC	726[a]
D	CCA GTC CAG CGT TTT T	221[a]
E	GTC ATT GCG ACT AGT G	n/a[b]
F	GTG TTA GCC TCA AGT G	n/a

[a] Probe DNA is modified with a 5′-thiol modifier C6 and a 15-T spacer is appended on the 5′ end of the probe DNA. Sequences of probes selected for the detection of this RNA are designed to efficiently bind the *GUS* gene RNA. Sequences of probe DNA **E** and **F** were used with fluorescence microscopy experiments and were also designed to maximize discrimination to each other's complements. [b] n/a, not applicable.

SPR Imaging Apparatus. An SPR imaging apparatus using near-infrared excitation from an incoherent white light source as described previously[25] was used for the detection of 1-D DNA line arrays. For SPR imaging measurements of 2-D DNA hybridization arrays, this apparatus was modified with an additional collection lens to create a 4× magnification objective. This magnification was required in order to increase the number of CCD pixels for each DNA array element.

DNA Preparation. Two types of DNA were used: DNA "probe" molecules that were chemically attached to the gold surface and DNA "complement" molecules that were used to study hybridization to the DNA probes. The DNA probe molecules were C6 thiol-modified oligonucleotides synthesized on an ABI DNA synthesizer at the University of Wisconsin Biotechnology Center as described previously. [26] A 15-base-long thymine spacer was used before the specific sequence. Deprotection and purification of oligonucleotides were preformed as described previously.[1,2,26] The concentration of all probe DNA molecules used in this work was 1 mM. The sequences used in this paper are listed in Table 1, and denoted as **A**−**F**. The sequences for probes **A**−**D** were selected for the RNA hybridization experiments, and probes **E** and **F** were chosen as a pair of 16-mers that are 6-base mismatches. All DNA hybridization experiments were conducted at 27 °C in a buffer (pH 7.7) of 20 mM phosphate, 300 mM NaCl, and 1 mM EDTA.

RNA Preparation. The 2-D arrays were used to detect the hybridization adsorption of a larger RNA fragment to the gold surfaces. This ssRNA molecule was 1731 base pairs in length and was created by the in vitro transcription from a partial clone of the *uidA* gene from a transgenic *A. thaliana* plant provided by Prof. Goodman's group in the department of Plant Pathology in the University of Wisconsin—Madison. The *uidA* gene, which encodes the enzyme β-glucuronidase (GUS), is commonly used as a reporter gene in eukaryotic organisms. To prepare *GUS* gene RNA, a partial uidA gene (bp 81−1812) from a transgenic *A. thaliana* plant was cloned;[27] the partial uidA gene was PCR

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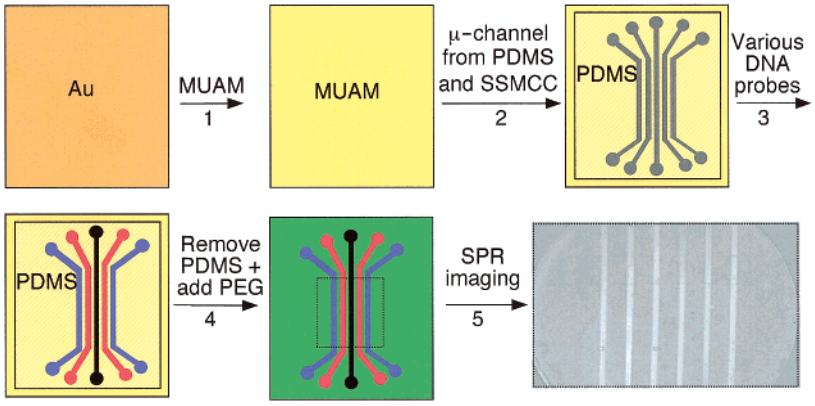

Figure 1. Schematic representation of the fabrication methodology used for creating 1-D DNA line and 2-D DNA hybridization microarrays. The region enclosed by the dotted box in step 4 represents a SPR difference image obtained for the entire central region ($\sim 8 \times 10^{-4}$ m^2) of the 1-D DNA line array shown in step 5. Six different DNA probes were used. The dimension of the microchannels used were 300 μm wide and 35 μm deep with a spacing of 1400 μm between channels. The full names of all the chemicals used for fabricating 1-D DNA line arrays are described under Experimental Considerations.

amplified from *Arabidopsis* genomic DNA using the primers GUS-F (5′-CTG TGG AAT TGA TCA GCG TTG GTG) and GUS-R (5′-TTC ACC GAA GTT CAT GCC AGT CC), and then the 1731-bp PCR product was cloned into the pGEM-T cloning vector (Promega). The orientation of the partial *uidA* gene within the pGEM-T vector was determined by restriction enzyme digestion. The partial *uidA* gene clone was then used as template in an in vitro transcription reaction (Ribomax, Promega), using the SP6 polymerase to generate the 1731-bp *uidA* RNA. The DNA template was removed with DNAse, and RNA size and concentration were determined by agarose gel electrophoresis. To denature the RNA before hybridization to the surface, samples were heated to ~100 °C for 10 min and then injected into the SPR imaging apparatus and left to hybridize for 1 h.

RESULTS AND DISCUSSION

Fabrication of 1-D DNA Line Arrays Using Microchannels. In a series of recent papers, we have demonstrated how SPR imaging measurements can be used to monitor the adsorption of biopolymers onto single-stranded DNA (ssDNA) microarrays that are attached to chemically modified gold thin film surfaces.[1−3] The fabrication of the DNA microarrays requires the creation of an amine-terminated alkanethiol self-assembled monolayer on the gold surface, followed by a series of attachment, photopatterning, and reversible protection/deprotection steps.[3] In this section, we describe an alternate version of this fabrication methodology for the creation of "1-D DNA line arrays" in which some of the surface modification reactions are performed in the parallel PDMS microfluidic channels attached to the gold surface. Figure 1 shows a schematic diagram of the methodology used for creating the 1-D DNA line arrays through the use of microfluidic channels in PDMS. The process required four steps:

(1) A gold thin film surface was reacted with MUAM for 2 h in order to form a self-assembled monolayer on the gold surface.

(2) A 1-mm-thick PDMS polymer film containing parallel microchannels was then physically attached to the MUAM-modified gold surface. A surface pattern was created by flowing the heterobifunctional linker SSMCC through the PDMS microchannels over the gold surface. The SSMCC reacted with the MUAM to create a maleimide-terminated alkanethiol monolayer. To overcome insufficient flow by capillary action, a simple differential pressure pumping system was used to deliver the reaction solutions.

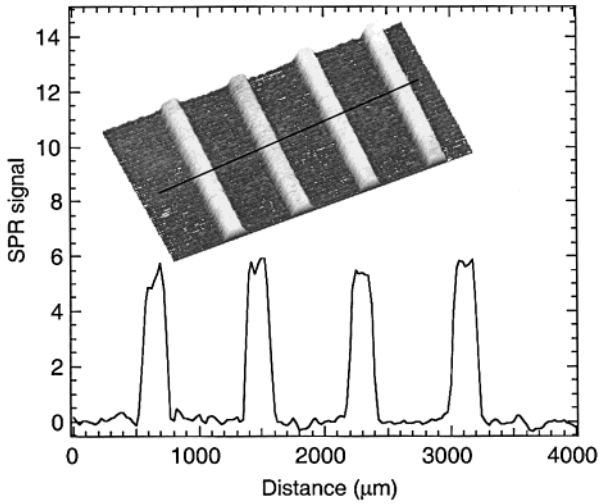

Figure 2. SPR difference images showing the hybridization of target DNA onto an array of surface-bound probe DNA. The 1-D DNA line array was composed of one probe DNA sequence (denoted as probe **A**) in all the channels. Hybridization onto the probe DNA array was indicated by a change in the percent reflectivity. The 1-D DNA line arrays were created as described in Figure 1. The dimension of the microchannels used were 300 μm wide and 35 μm deep with a spacing of 1400 μm between channels. Before each measurement, the hybridized array was denatured using 8 M urea. The concentration of all complementary DNA samples used in this experiment was 1 μM.

(3) A variety of 5′-thiol-modified ssDNA probes were each flowed into a separate PDMS microchannel and left to react with the maleimide-terminated gold surface for at least 4 h. This created a 1-D DNA line array of different oligonucleotides on the surface. The sequences of the DNA probes used in this paper are listed in Table 1.

(4) After the microchannels were cleaned with water, the PDMS was removed from the surface and the gold slide was

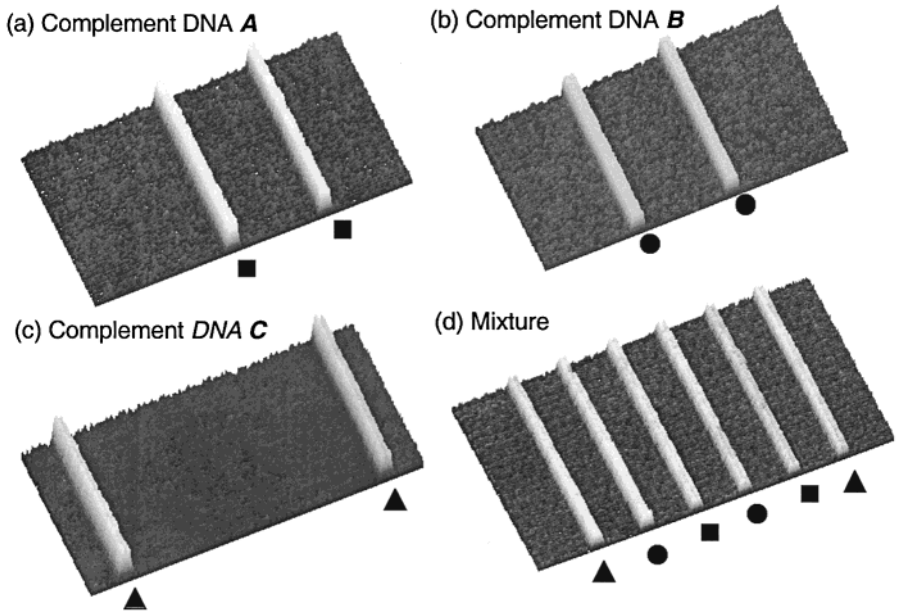

Figure 3. SPR difference images showing the hybridization of target DNA onto an array of surface-bound probe DNA. Hybridization onto the probe DNA array was indicated by a change in the percent reflectivity. The 1-D DNA line arrays were created as described in Figure 1. The dimension of the microchannels used were 300 μm wide and 35 μm deep with a spacing of 1400 μm between channels. The 1-D DNA line array consisted of multiple probes **A**, **B**, and **C**, and their sequences are listed in Table 1. Before each measurement, the hybridized array was denatured using 8 M urea. The concentration of all complementary DNA samples used in this experiment was 1 μM.

soaked in a PEG−NHS solution in order to modify the MUAM background. This PEG-coated background was necessary in order to resist the nonspecific adsorption of ssDNA during the hybridization experiments.

Surfaces created by this four-step process were used in subsequent SPR imaging or fluorescence measurements of ssDNA and ssRNA hybridization adsorption. As an example, Figure 1 shows a SPR image of a ssDNA pattern of six 300-μm lines formed on a gold thin film. We have fabricated these 1-D DNA line arrays using microchannels as narrow as 50 μm. To maintain the integrity of the PDMS seal during array fabrication, the ratio of the distance between lines to the line width must be equal to or greater than 1.5. For 50-μm lines, this leads to a line density of 80 lines/cm, which results in a maximum of 144 different ssDNA lines on the 1.8-cm gold thin films employed in the SPR imaging measurements.

These 1-D line arrays were used in a series of SPR imaging experiments to detect and identify DNA molecules by hybridization adsorption onto the gold surface. These experiments employed the same 100-μL sample cell and total sample volume of 500 μL that were used in our previous studies.[1−3] The results of these SPR imaging experiments are shown in Figures 2 and 3. In Figure 2, a 1-D ssDNA line array in which all of the lines were the same 18-base sequence (probe **A**) was exposed to a 1 μM solution of a ssDNA 18-mer that was the complementary sequence (denoted as "complement A"). In a previous paper,[1] we showed that the change in percent reflectivity in the SPR image upon adsorption can be related to the solution concentration of the target ssDNA molecules. Thus, these ssDNA line arrays can be used as a quantitative measurement of solution DNA concentration in situ. A "line profile" taken from this SPR differential reflectance image is shown in Figure 2 and demonstrates the uniformity of DNA hybridization onto the different line array elements. No nonspecific adsorption to the background was observed in this experiment, and a high S/N ratio could be obtained by creating a line profile that was integrated over a large section of the line array. SPR imaging of the line arrays could detect the hybridization adsorption from ssDNA solutions as low as 10 nM.

In a second experiment, three different ssDNA probes (probes **A**, **B**, and **C**) were used to create a line array. As seen in Figure 3, when this array was exposed to each of the individual ssDNA complements, only the correct array elements were visible in the SPR difference image. Exposure of the surface to a mixture of all three complements resulted in a SPR difference image that contained all of the array elements. These hybridization experiments were all performed on the same surface; after each hybridization adsorption step, the DNA complements were removed from the surface by exposure to 8 M urea. These surfaces were stable for up to as many as 30 hybridization/denaturation cycles. From these experiments, it is clear that this method for creating 1-D DNA line arrays via microfluidics provides a rapid and facile route for the creation of surface arrays when only a small number (<100) of different ssDNA sequences is required.

Fabrication of 2-D DNA Hybridization Arrays Using Microchannels. In a second set of experiments, we used microfluidic channels for the delivery of target molecule solutions in conjunction with the 1-D DNA line arrays described above in order to create 2-D hybridization arrays for SPR imaging studies. In these experiments, a 1-D line array of ssDNA probe molecules was first created on the gold surface. Then, a second set of PDMS microchannels was attached to the surface perpendicular to the 1-D DNA line array, as shown in Figure 4, to deliver target molecules to the surface. The intersection of the line arrays with the microchannels created a "2-D hybridization array" of dsDNA probe spots. The elements of these 2-D arrays were either 300 × 300 μm for SPR imaging measurements, or 75 × 75 μm for fluorescence microscopy experiments. The microfluidic channels are typically 35 μm deep, so that a 300-μm-width channel has a

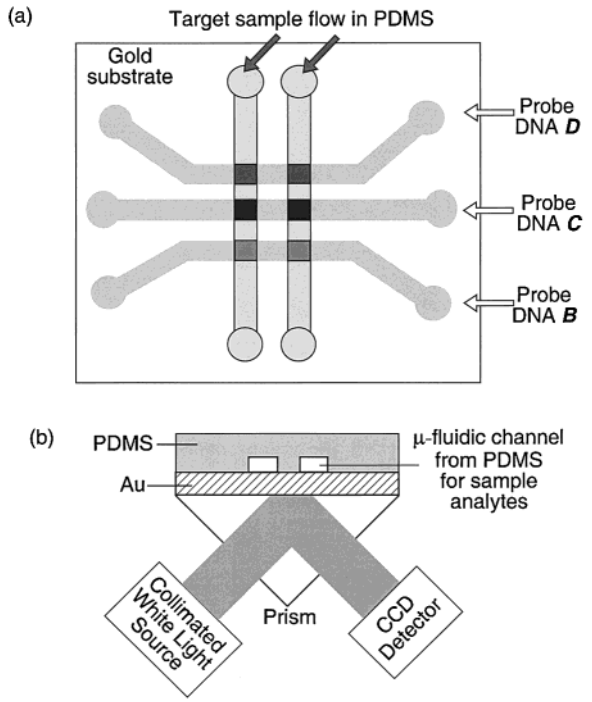

Figure 4. (a) Schematic representation of 2-D DNA hybridization microarray elements used for SPR measurements. One set of PDMS microchannels was used to create 1-D DNA line arrays as described in Figure 1. A second set of PDMS microchannels was attached to the surface perpendicular to the 1-D DNA line arrays to deliver DNA or RNA samples. Hybridization/adsorption events occur at the intersections of a 2-D DNA hybridization array and can be detected either with SPR imaging or fluorescence microscopy. (b) Schematic representation of the SPR imaging experimental setup incorporating microfluidics. Collimated white light passes first through a narrow band-pass filter (800 nm), a polarizer, and a glass prism. A glass slide with a thin (45-nm) coating of gold is optically coupled to the prism. For 2-D array experiments, the conventional SPR imaging lens was replaced with a 4× magnification objective and attached to the CCD camera in order to increase the number of CCD pixels for each array element. The CCD camera was used to detect changes in the percent reflectivity.

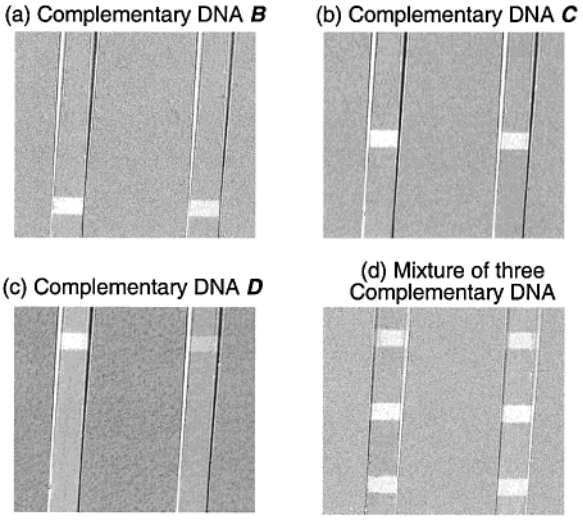

Figure 5. SPR difference images showing hybridization of target DNA onto a 2-D DNA hybridization array of surface-bound probe DNA molecules. The 2-D DNA hybridization array was created as described in Figure 4a. The dimensions of the microchannels used were 300 μm wide and 35 μm deep with a spacing of 1400 μm between channels. The area of detection for hybridization/adsorption events between complementary target DNA and the 2-D hybridization array of surface-bound probes was defined by the intersection area of the 1-D DNA line array and the channels used for solution delivery. Hybridization onto the probe DNA array was indicated by a change in the percent reflectivity. The probe DNA molecules attached on the surface were probes **B**, **C**, and **D**, and their sequences are listed in Table 1. Before each measurement, the hybridized array was denatured using 8 M urea. The concentration of all complementary DNA samples used in this experiment was 100 nM.

typical interaction volume of 100 pL. The total volume of the sample used in these experiments was typically 1 μL or less, which is 500 times smaller than in our previous SPR imaging experiments. With a previously determined detection limit for 18-mer oligonucleotides of 10 nM,[1] this means that we should be able to detect 10 fmol of ssDNA. This number is even smaller for larger protein, DNA, or RNA molecules.

Figure 5 shows the SPR difference image for hybridization adsorption of the three complementary DNA probes onto the line arrays via the microfluidic channels. The complementary DNA solution concentrations were all 100 nM, which with a total solution volume of 1 μL corresponds to a total of 100 fmol of each ssDNA sample. As in the case of the 1-D line arrays, the gold surface was reacted with NHS−PEG in order to inhibit nonspecific adsorption prior to the attachment of the second set of PDMS microchannels. No hybridization to incorrect array elements was observed, and as in the previous measurements, a solution containing a mixture of the three ssDNA complements resulted in the SPR difference signal of all of the array elements. As with the 1-D line arrays, this series of hybridization experiments was performed on the same surface after removal of the previously hybridized target DNA by exposure to 8 M urea.

As an additional test and demonstration of these microfluidic 2-D DNA hybridization arrays, the three-probe surface used in Figure 5 was also exposed to a solution of ssRNA to monitor RNA−DNA hybridization. The results of the RNA hybridization experiment are shown in Figure 6. Specifically, 20 fmol of *GUS* gene ssRNA in a total solution volume of 1 μL was introduced into the PDMS microchannels to create a 2-D hybridization array by RNA−DNA hybridization adsorption. The ssDNA probes **B**, **C**, and **D** are in fact the three sequences that have been designed specifically for hybridization to the *GUS* gene ssRNA. The GUS gene sequence is 1731 base pairs, and the use of three complementary 16−18-mer probes ensures the unique identification of this RNA fragment. These probes were selected using a free-energy calculation procedure that is described in detail elsewhere.[28,29] Listed in Table 1 along with the probe sequences are the starting positions for where the probe molecules hybridize to the ssRNA. Different amounts of SPR signal are observed for the three different probes in Figure 6. These differences are attributed to differences in the binding efficiency of the *GUS* gene ssRNA to the different surface-bound ssDNA probes. We have observed similar differences in other ssRNA binding experiments; these differences are predicted by free-energy calculations.[28,29] The

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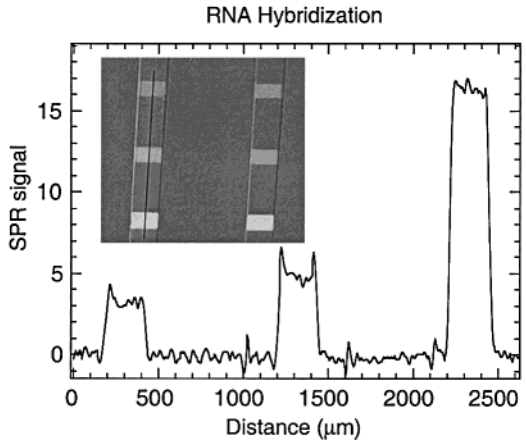

Figure 6. SPR difference images showing hybridization of the *GUS* gene RNA onto a 2-D hybridization array of surface-bound probe DNA. The 2-D DNA hybridization array fabricated in Figure 5 was used in this experiment after denaturing with 8 M urea. The surface-bound probe DNA molecules were probes **B**, **C**, and **D**. The specific sequences, the starting position, and the corresponding $\Delta G^{\circ}_{\text{binding}}$ of these probe molecules for hybridization of the *GUS* gene RNA molecules are summarized in Table 1. Hybridization of the target RNA onto the probe DNA array was indicated by a change in the percent reflectivity. Different signal strengths for the different probes were attributed to variations in binding efficiency of the *GUS* gene RNA to the surface-bound ssDNA probes.

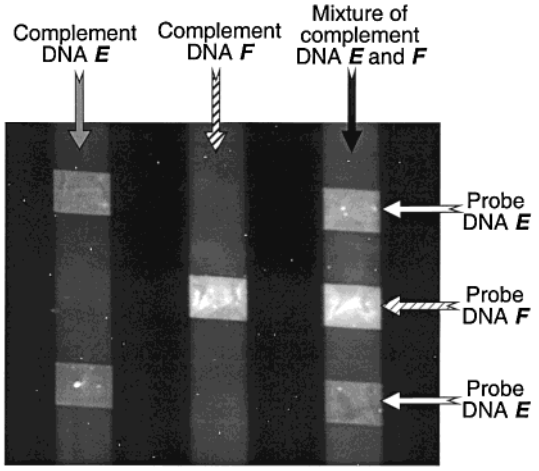

Figure 7. Fluorescence image showing hybridization of fluorescently labeled target DNA onto a 2-D hybridization array of surface-bound probe DNA. The 2-D DNA hybridization array was created as described in Figure 4a. The intersections of the 2-D DNA hybridization array are the fluorescence sensing regions where the hybridization/adsorption occurs between the fluorescently labeled target complementary DNA and the 2-D hybridization array of surface-bound probe DNA. The surface-bound probe DNA molecules were probes **E** and **F**, and their sequences are listed in Table 1. Before each measurement, the hybridized array was denatured using 8 M urea. The dimensions of the microchannels were 75 μm wide and 35 μm deep with a spacing of 100 μm between channels. The concentration of all complementary DNA samples used in this experiment was 2 μM.

ability to detect 20 fmol of ssRNA is sufficient for a number of biological applications, including the direct detection of messenger RNA (mRNA) from highly expressed genes, and the detection of ribsomal RNA (rRNA) from complex biological samples.[28,29] However, the general detection of gene expression via the detection of mRNA normally requires an amplification step prior to readout, as in the gene expression array work of Brown et al.[30]

In a final experiment, the 2-D hybridization arrays were used in conjunction with fluorescence microscopy to observe the hybridization adsorption of fluorescently tagged target ssDNA molecules. These experiments were performed in order to demonstrate that the microfluidic array fabrication methods described here are not only useful for SPR imaging but will be useful for all methods that require creation of attached DNA arrays on gold surfaces. Fluorescence measurements of DNA microarrays on gold surfaces have been demonstrated previously.[31−33] Fluorescence quenching by the gold surface reduces the signal as compared to silica surfaces but also reduces the background fluorescence. For this experiment, a 2-D ssDNA microarray of 75 μm × 75 μm square detection regions was created as described above. Figure 7 is a fluorescence microscope image showing two different sequences of complementary DNA that have been adsorbed onto the detection elements. Unlike the SPR imaging measurements, it was necessary to thoroughly rinse the target DNA out of the microchannels before imaging in order to avoid any contributions from the solution fluorescence. A total of 1 μL of a 2 μM fluorescently labeled complementary DNA was used in each channel in order to create the double-stranded DNA (dsDNA) arrays. As observed with the SPR imaging measurements, the specific hybridization of each ssDNA complement was obtained without observing any nonspecific binding onto the background. In principle, these PDMS microchannels can also be used for fluorescence measurements with higher density arrays that would create multiple adsorption elements in a single microchannel.

CONCLUSIONS

The use of microchannels described in this paper represents a new methodology for the creation of 1-D and 2-D DNA microarrays on gold surfaces for SPR imaging measurements of DNA−DNA, DNA−RNA, and DNA−protein interactions. The 1-D line arrays allow us to create surface patterns as narrow as 50 μm and are particularly useful whenever a surface array of only a small number (<100) of different ssDNA sequences is required. For systems where the number of different ssDNA sequences is greater than 100, the generalized photopatterned array fabrication methodology that we have described previously is more applicable.[3]

In conjunction with the 1-D line arrays, we have developed a "2-D" hybridization array configuration that uses a second set of PDMS microchannels for target solution delivery that allows us to use sample volumes as small as 1 μL; this is particularly relevant when dealing with small amounts of target biopolymers from complex biological samples. In fact, if smaller microwells are employed, the solution volume can be reduced from 1 μL to 200 pL. In addition, the 2-D array configuration allows for the simultaneous detection of multiple hybridization reactions during

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SPR experiments. We are currently exploring the use of these 2-D arrays in SPR imaging measurements for the direct detection of mRNA and rRNA from microbial samples. This microchannel fabrication methodology can also be implemented on other surfaces such as silicon substrates that have been modified with an amine-terminated monolayer.[22,34,35]

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