We present two strategies for microspotting arrays of double-stranded DNAs (dsDNAs) onto a gold-coated glass slide for high-throughput studies of protein–DNA interactions by surface plasmon resonance (SPR) microscopy. Both methods use streptavidin (SA) as a linker layer between a biotin-containing mixed self-assembled monolayer (SAM) and biotinylated dsDNAs to produce arrays with high packing density. The primary mixed SAM is produced from biotin- and oligo(ethylene glycol)-terminated thiols bonded as thiolates onto the gold surface. In the first method, a robotic microspotter is used to deliver nanoliter droplets of dsDNA solution onto a uniform layer of this SA (\(\sim 2 \times 10^{12} \text{SA/cm}^2\)). SPR microscopy shows a density of \((5–6) \times 10^{11} \text{dsDNA/cm}^2\) (0.2–0.3 dsDNA/SA) in the array elements. The second method uses instead a microspotted array of this SA linker layer, onto which the micropots of dsDNA are added with spatial registry. SPR microscopy before addition of the dsDNA shows a SA coverage of \(2 \times 10^{12} \text{SA/cm}^2\) within the spots and a dsDNA density of \(8.5 \pm 3.5 \times 10^{11} \text{dsDNA/cm}^2\) (0.3–0.7 dsDNA/SA, depending on the length of dsDNA) after dsDNA spotting. We demonstrate the ability to simultaneously monitor protein binding with the SPR microscope in many 200-µm spots with 1-s time resolution and sensitivity to less than 1 pg of protein.

The advantages provided by microarray analysis of interactions between biomolecules are many. Parallel, high-throughput monitoring of binding between biomolecules has important implications in medical research and diagnostics,

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We present two strategies for microspotting 10 × 12 arrays of double-stranded DNAs (dsDNAs) onto a gold-coated glass slide for high-throughput studies of protein–DNA interactions by surface plasmon resonance (SPR) microscopy. Both methods use streptavidin (SA) as a linker layer between a biotin-containing mixed self-assembled monolayer (SAM) and biotinylated dsDNAs to produce arrays with high packing density. The primary mixed SAM is produced from biotin- and oligo(ethylene glycol)-terminated thiols bonded as thiolates onto the gold surface. In the first method, a robotic microspotter is used to deliver nanoliter droplets of dsDNA solution onto a uniform layer of this SA (\(\sim 2 \times 10^{12} \text{SA/cm}^2\)). SPR microscopy shows a density of \((5–6) \times 10^{11} \text{dsDNA/cm}^2\) (0.2–0.3 dsDNA/SA) in the array elements. The second method uses instead a microspotted array of this SA linker layer, onto which the micropots of dsDNA are added with spatial registry. SPR microscopy before addition of the dsDNA shows a SA coverage of \(2 \times 10^{12} \text{SA/cm}^2\) within the spots and a dsDNA density of \(8.5 \pm 3.5 \times 10^{11} \text{dsDNA/cm}^2\) (0.3–0.7 dsDNA/SA, depending on the length of dsDNA) after dsDNA spotting. We demonstrate the ability to simultaneously monitor protein binding with the SPR microscope in many 200-µm spots with 1-s time resolution and sensitivity to less than 1 pg of protein.

The advantages provided by microarray analysis of interactions between biomolecules are many. Parallel, high-throughput monitoring of binding between biomolecules has important implications in medical research and diagnostics,1–6 drug discovery,7–9 fundamental molecular and cellular biology investigations,10–12 and analytical sensor development.13–17

Surface plasmon resonance (SPR) microscopy is a promising tool for quantitative, real-time monitoring of biomolecule interactions taking place over a relatively large area of a sensing surface without the need for labeling.18–20 Recently, SPR microscopy (also called “imaging SPR”) has been used to study biomolecule interactions in a parallel fashion by creating an array of binding sites on a sensor surface and monitoring adsorption of biomolecules to those sites.21,22 Real-time quantitative measurement
of adsorption and desorption events using an SPR microscope is possible when reflected light intensity is monitored at a high-contrast angle near the SPR minimum.21,22,23 Changes in reflected light intensity monitored at such a high-contrast angle can be converted to real-time changes in effective refractive index, effective adlayer thicknesses, and absolute surface coverages of molecules using methods described in ref 33. These methods are based on the extension of formulas developed for quantitation of SPR spectroscopy data (SPR angle or wavelength shifts) and rely on a simple calibration of the SPR microscope’s response to changes in bulk solutions’ refractive index.24,25 Such quantitative SPR microscopy measurements are important whenever interactions between biomolecules are being studied.

Protein interactions with double-stranded DNAs (dsDNAs) are of fundamental importance in biology and medicine and play a vital role in cellular processes including transcription regulation, genetic recombination, DNA repair, and the restriction and modification of DNA.26 To use SPR microscopy for protein–dsDNA array analysis, a method must be developed to pattern dsDNAs on an SPR-active surface, typically a glass slide coated with a thin gold film (~50 nm). A few methods have been used to fabricate dsDNA arrays on gold. Brockman et al. used amine-terminated alkythiolates and hybridization between complementary single-stranded DNAs (ssDNAs) to create an array of dsDNAs for measuring protein–DNA interactions.30,31 They observed some nonspecific binding of a ssDNA-binding protein to array elements that should contain only dsDNAs.32 Smith et al. used this same technique to create an array of dsDNAs.33 Incomplete hybridization for a 38-base pair ssDNA was explained by possible accessibility problems due to nonspecific interactions of the immobilized ssDNA with the surface or formation of secondary structure.34

O’Brien et al. prepared an array of dsDNAs on a gold surface for atomic force microscopy studies of protein–dsDNA interactions35,36 by depositing disulfide-modified dsDNAs onto bare gold regions created by photopatterning. One drawback of this method is the potential for nonspecific interactions of dsDNA with bare gold, which may be why they observed smaller than expected heights for some array elements.37 Since alkythiolates often self-segregate in mixed monolayers on Au, this method could give a very nonuniform distribution of dsDNAs on the surface.38

To measure protein–dsDNA interactions on a planar surface, the dsDNAs must be accessible (not too tightly packed) so the protein or protein complex is able to recognize its DNA binding site and have space to bind. Ideally, this spacing would be optimized without significantly decreasing binding signals. One major challenge in using the dsDNA immobilization strategies mentioned above is the difficulty in controlling the minimum spacing between dsDNAs. Each of those methods could result in closely packed DNA-rich regions displaying no or weak, nonspecific binding of proteins, complicating array analysis. Trying to overcome this problem, we recently used a streptavidin (SA) linker layer to immobilize biotinylated dsDNAs on a planar gold surface with high density (1–3) × 1012 DNA/cm², depending on the dsDNA length) and adequate spacing (~4 nm closest possible DNA–DNA separation at the surface) for the binding of proteins.39 This method is shown schematically in Figure 1. The SA monolayer, which offers ~5 × 1012 biotin sites/cm², was prepared first by adsorbing SA onto a mixed self-assembled monolayer (SAM) on gold, which contained biotin- and oligo(ethylene glycol)-terminated alkythiolates (BAT and OEG, respectively). Figure 2 shows the chemical structures of BAT and OEG. Oligo(ethylene glycol) is used as a headgroup on the OEG and as a spacer in the BAT because it resists nonspecific protein adsorption22,41–46 and nonspecific DNA adsorption to the surface. For the proper thiol mixture, the biotin headgroups allow the specific immobilization of SA to the SAM surface, reaching a packing density within ~20% of that for a crystalline SAM monolayer grown at the air–water interface.

Figure 1. Idealized cartoon of biotinylated double-stranded DNA immobilized on a SA monolayer. This protein layer is adsorbed on a mixed BAT/OEG binary alkythiolate monolayer. The biotin moiety of the biotinylated surface thiolate inserts ~14 Å into the SA binding pocket leading to the formation of a nearly close-packed monolayer of SA on the surface. Similarly, the biotinylated dsDNA then binds via its 5’-biotin modification on this SA layer. Note that the thiol molecules are not expected to display such high order and rigid orientation; a more accurate description of the composition, orientation, and order within these mixed monolayers is presented elsewhere.46 No attempt is made to show the known tilting of the alkyl chains. Not drawn to scale.
interface. The immobilized SA provides a very stable monolayer with an estimated half-life in excess of 100 days even with excess biotin in solution. One strand of the dsDNA is functionalized on its 5′ end with the biotin linker shown in Figure 2. The spacer between the DNA and the biotin provides flexibility and spacing so that the biotin tail group can fit into its binding pocket in the SA. The dsDNA-functionalized surface was shown to resist nonspecific protein adsorption and was used to measure the specific binding of a dsDNA-binding protein to its binding site immobilized on the surface.

In this paper, we describe an application of this SA linker layer to immobilize dsDNAs by microspotting them in an array. We also describe a method for microspotting the SA itself to form this linker layer and its application for microspotting dsDNA arrays. Successful application of the resulting SA and dsDNA arrays for simultaneously studying protein–DNA interactions with different sequences of dsDNA by SPR microscopy is described elsewhere.

Others groups have fabricated a SA array on a gold surface. Zizlsperger and Knoll patterned a mixed SAM containing biotin- and OH-terminated alkanethiols onto a bare gold surface using an ink jet printing technique. One potential problem with this method is the ability to form a high-quality SAM that will resist nonspecific DNA and protein adsorption. The adsorption time (~2 h) for the thiols is short compared to that generally used for SAM formation (12–24 h). Longer adsorption times are usually necessary to form a well-ordered, close-packed SAM. Zizlsperger and Knoll did not present results characterizing the nonspecific binding of protein or DNA to their surfaces.

Riepl et al. also prepared a SA array on a surface prefuctionalized with a SAM of biotin- and OEG-terminated thiociles. The SAM was formed on a commercial chip (XNA on Gold) that contained a pattern of bare gold spots surrounded by Teflon. SA was adsorbed from solution to these spots, and then biotinylated ssDNA was spotted onto the SA spots (diameter, 1.5 mm). The maximum SA packing density on their mixed SAM was approximately half that we typically achieve (see below). Riepl et al. attributed the low SA packing density to a decreased accessibility of the biotin headgroup to the SA due to the formation of a hydrogen-bonding network between neighboring amide groups. The decreased packing density means there are fewer binding sites per square centimeter available for immobilization of biomolecules such as DNA, which could result in a lower signal during related analyses.

Here we show how dsDNA can be immobilized in a 10 × 12 array of 200-µm spots with a high packing density (0.5–1.2 × 10^12 dsDNA/cm^2) on gold using a SA linker layer where ~0.2–0.7 dsDNA bind per SA, depending on the method. The ratios are consistent with that measured for similar length dsDNA adsorbed from solution to the same SA monolayer. The microspotting process and SA linker layer described here potentially could be used to immobilize a variety of other biotinylated molecules on the same surface for flexibility in array-based studies with SPR microscopy.

**EXPERIMENTAL SECTION**

**Substrate Preparation.** SPR microscope substrates (25 × 35 × 3 mm SF14 glass slide, Schott Glass Technology, Durea, PA) were cleaned and coated with 20 Å of chromium and 475 Å of gold. Immediately before use, the gold-coated substrates were cleaned as previously described by immersing them in basic peroxide solution at 65 °C for ~40 s. The slides were rinsed copiously with Nanopure water (18 MΩ resistivity), rinsed with absolute ethanol, and dried with nitrogen gas. Oligo(ethylene glycol)-terminated alkanethiol (OEG) was synthesized by Dr. Esmaeel Naemii and provided by Prof. Buddy Ratner (University of Washington). Biotin-terminated alkanethiol (BAT) was synthesized by Dr. M. M. Ribi, H. O.; Ringsdorf, H.; Kornberg, R. D. Biophys. J. 1991, 59, 387–396.


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![Bond-line structures of the thiols used in the mixed SAM and the biotin/linker functionality that is covalently attached to the 5′ end of one strand of the dsDNA for immobilization via a streptavidin linker layer.](Image 148x609 to 464x752)
removed from solution, rinsed liberally with absolute ethanol to
remove loosely bound thiols, and dried with nitrogen. Slides that
were not used immediately were placed in a covered Petri dish
filled with nitrogen and sealed with Parafilm. The Petri dish was
placed in a larger jar purged with nitrogen and sealed with
Parafilm for long-term storage.

**DNA Preparation.** Biotinylated DNAs containing 77 base
pairs (77-mer) and 100 base pairs (100-mer) were prepared from
single-stranded oligonucleotides (ss-oligos) synthesized by Inte-
grated DNA Technologies (Coralville, IA). For two complementary
ss-oligos, one strand contained no modification and the other
single-stranded oligonucleotides (ss-oligos) synthesized by Inte-
grated DNA Technologies (Coralville, IA) for microarray fabrication. The pin has a sample
holder. ChipWriter Pro software is used to control all aspects of
cleaning, a pin blotter station, and a 384-well microtiter plate
includes a water bath, a sonicator, a vacuum manifold for pin
arraying system (Virtek Biotech, Waterloo, Canada). The system
for microarray fabrication are part of a ChipWriter Pro robotic
robot and software used to deliver samples to the sensor surface
for microarray fabrication are part of a ChipWriter Pro.

**Microarray Fabrication. (1) Robotic Arraying System.** The
robot and software used to deliver samples to the sensor surface
for microarray fabrication are part of a ChipWriter Pro robotic
arraying system (Virtek Biotech, Waterloo, Canada). The system
includes a water bath, a sonicator, a vacuum manifold for pin
cleaning, a pin blotter station, and a 384-well microtiter plate
holder. ChipWriter Pro software is used to control all aspects of
robot motion. The robot controls the motion of a Stealth printhead
that holds ArrayIt microspotting pins from TeleChem, Interna-
tional, Inc. (Sunnyvale, CA) in order to deliver a droplet of sample
from each pin to the substrate surface in preselected sites on the
surface. Due to the constraints of the spacing between pins in
the printhead, software limitations, and a desire to minimize
sample consumption, we used a single pin (SM P7, TeleChem
International, Inc.) for microarray fabrication. The pin has a single
uptake volume of 250 nL and an estimated delivery volume of 1.7
nL, designed to create a spot diameter of 200–220 μm.52

**2 Streptavidin Immobilization in a Uniform Layer.**
Streptavidin was purchased from Calbiochem (San Diego, CA)
and used without further purification. Unless otherwise indicated,
SA solutions were prepared at a concentration of 0.05 mg/mL in
150 mM PBS buffer at pH 7.4. SA monolayers for microarray
fabrication were prepared by placing 500 μL of SA solution on
the substrate prefunctionalized with a BAT/OEG SAM. The
protein was allowed to adsorb for at least 30 min in a high-humidity
chamber. Then, the substrate was rinsed gently with a large
volume of 150 mM PBS buffer at pH 7.4. A final rinse with water
was used to remove buffer salts from the surface. A clean squirt
bottle was filled with humid air from the chamber, and this air
was squirted across the surface to gently remove bulk water. The
substrate was used immediately for microspotting. Exposure to
dry laboratory air was minimized during SA monolayer prepara-
tion.

**3 Microspotting Process.** Prior to microspotting, 8–10 μL
of SA solution or dsDNA solution was transferred to the appropri-
ate wells in a 384-well microtiter plate (Uniplate, 7701-5101,
Whatman, Clifton, NJ). The concentrations of these solutions for
each specific microarray are included in the Results and Discus-
sion section. The plate was covered with aluminum foil tape (1450,
3M Corp.) and a roller was used to ensure a good seal to minimize
evaporation. The plate was placed in a centrifuge (Centrifuge
5810R, Eppendorf, Westbury, NY) and spun for 1 min at 2000
rotations per minute in order to ensure the solutions were at the
bottom of the wells. After spinning, the tape was removed from
the top of the plate before it was placed in the microtiter plate
holder on the platform in the humidity-controlled chamber.

Humidity control is essential for fabricating reproducible
microarrays. The humidity level determines the time allowed
for adsorption of material to the functionalized surface. A relative
humidity of 70% at a temperature of 24.5 °C was found to keep
the surface wet for at least 2 h. When humidity dropped below
70% at this temperature, the solution deposited on the surface
evaporated more quickly. For example, when the relative humidity
was at 60% the surface dried in less than 1 min. The relative
humidity was maintained at 70–75% at temperatures that usually
ranged from 24.5 to 25.5 °C.

Initially, the pin was cleaned by a series of immersions in the
sonicator and the water bath. After each immersion, the pin was
dried in the vacuum manifold. The robot then dipped the dry pin
into the appropriate well of the microtiter plate to withdraw sample
trough capillary action. The pin moved to the blotter and tapped
lightly a specified number of times on a clean, glass slide. After
blotting, the robot moved the pin filled with sample to a position
above the substrate on the platform. The robot brought the pin
close to the substrate so the solution layer made contact with the
functionalized gold-coated surface. Ideally, at this point there is
no direct contact between the pin and the surface. This requires
careful alignment of the arrayer platform and pin robot before
use. The robot then moved the pin away from the surface,
depositing a droplet of solution on the surface. Usually the robot
was programmed to deposit multiple sample droplets in a row on
a single substrate or on multiple substrates after each sample
solution uptake. This minimizes sample volume requirements
and shortens the microarray fabrication time. The center-to-center
spot spacing is 450 μm. For the arrays described in this paper, each
row was made by filling the pin with a new solution from a well
in the microtiter plate and the elements in a row are formed from
replicate depositions from the pin after a single solution uptake.
There is no reason, however, that every spot could not be
functionalized with a different chemical species, but this would
require longer time and a greater variety of chemicals. After
depositing solutions for a row, the pin is cleaned using the process
described above before filling with a new sample solution.

After spotting, the substrates were allowed to sit in the high-
humidity chamber for at least 1 h to allow time for adsorption
while the spot maintains a thin water film. Longer times showed
no increase in sample adsorption. Then the substrate surfaces
were rinsed with copious amounts of 150 mM PBS buffer at pH
7.4 and stored in that same PBS buffer at 4 °C until analysis.

When SA arrays were made, the protein was allowed to adsorb
onto the mixed SAM from the spotted SA solution for at least 30
min before dsDNA was spotted onto the SA spots on the substrate.
In such a process, the robot's ability to align the DNA spots with
the SA spots was excellent (~1 μm). The slides were then
incubated for an additional hour before rinsing with copious

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amounts of PBS buffer stored as previously described. Rinsing took place in the humidity chamber, and exposure of the microarrays to dry room air was minimized.

**SPR Microscopy Measurements.** (1) SPR Microscopy Image Capture and Analysis. The microarrays were characterized using our home-built SPR microscope described and characterized in more detail elsewhere.\(^2,3\) Briefly, a stabilized 632.8-nm HeNe laser serves as the excitation source for the system. The laser beam is p-polarized, expanded, and collimated before traveling through an SF14 glass hemicylindrical prism and substrate to illuminate an ~24-mm\(^2\) area of a gold-coated sensor surface. The detection optics are connected to an aluminum rail that is attached to a motorized rotation stage. This stage is mounted under an identical stage that holds the prism. The centers of rotation for the two stages are aligned. A computer connected to a stage controller/driver is used to separately and equally vary the angle of incidence and the angle of detection so the CCD detector stays at the specular angle for each angle of incidence. The reflected light is focused and directed by a lens directly onto the CCD detector of a video camera, creating an image that is automatically digitized by a framegrabber card (DT3155, Data Translation, Marlboro, MA) and stored using image acquisition software (KSA400, k-Space Associates, Inc., Ann Arbor, MI). This software controls the CCD camera exposure time, frame averaging, and conversion of measured light intensity values to gray scale levels. A neutral density filter decreases the laser power to prevent flooding of the CCD camera. The entire system is mounted on a laser table and covered by a black box to minimize stray light, dust, and air flow effects. The fluidics system is mounted on a laser table and covered by a black box to eliminate the contribution of the laser beam profile.

**SPR Microscopy Data Quantitation.** The reflected intensity changes monitored by the SPR microscope were converted into surface coverages using a method for quantitation of SPR microscopy data.\(^3\) This new method is an extension of the formalism described previously.\(^3\) This method involves a very simple calibration of the instrumental sensitivity (s) based on the sensor response to changes in bulk index of refraction, an exponential probe depth estimated from Fresnel equations, and the known index of refraction for the adsorbate.\(^3\) The index of refraction for SA is 1.57\(^4\) and that for DNA is 1.75.\(^5\) A sensitivity (s) of 300% reflected intensity RIU for the SPR microscope was measured by doing a calibration of the system’s response to bulk changes in refractive index.\(^3,4\) For clarity, RIU is used to indicate “refractive index unit,” a unitless quantity. A decay length of 234 nm was calculated using previously described methods.\(^4\) Specific volumes of 0.77\(^6\), 0.85\(^7\), and 0.61 cm\(^2\)/g\(^8\) were used for SA and DNA, respectively.

**RESULTS AND DISCUSSION**

Macroarray Fabrication. Microarrays were fabricated using two different strategies (method 1 and method 2) that are shown schematically in Figure 3. Both methods begin with a gold surface prefunctionalized with the mixed BAT/OEG SAM and use SA as
to the surrounding surface because of the higher reflected
in Figure 4. At this angle, the DNA spots appear bright compared
created by directly spotting dsDNA solution on the SA array. Then a dsDNA array is
form a dsDNA array. The spots are labeled (S1, S2, etc.) to show
is spotted onto the surface using a robotic microspotting system to
SA to form a SA monolayer on this mixed SAM. Then dsDNA solution
OEG monolayer. The next step in method 1 involves adsorption of
begin with a gold-coated glass slide that is functionalized with a BAT/
spotted onto the mixed SAM. Then the robotic microspotting system is
is to form a SA monolayer by protein adsorption from solution
SA on a BAT/OEG SAM-functionalized gold-coated substrate. Then a SA monolayer is created using both of these methods.

a linker layer to immobilize dsDNAs. For method 1, the next step is to form a SA monolayer by protein adsorption from solution onto the mixed SAM. Then the robotic microspotting system is used to deliver nanoliter droplets of biotinylated dsDNAs to preselected areas of the SA monolayer to create the dsDNA microarray elements.

Instead of using a preformed SA monolayer, method 2 uses the BAT/OEG SAM as the foundation for first creating a SA microarray, SA in solution is deposited by the microspotting pin on the BAT/OEG SAM to create the SA microarray. Then, a dsDNA array is created on the SA microarray by spotting biotinylated dsDNAs on top of the SA array elements. We present below the SPR microscopy characterization of microarrays fabricated using both of these methods.

(1) dsDNA Microarray Spotted on a Uniform Streptavidin Monolayer. A dsDNA microarray was prepared by preadsorbing SA on a BAT/OEG SAM-functionalized gold-coated substrate. Then dsDNA (0.2 mg/mL) in 400 mM PBS buffer, pH 7.4, was spotted in a 12 x 8 array on this uniform SA monolayer (method 1). This array contains only two different dsDNA species, which were spotted in complete rows: a 100-mer (rows 1, 2, 5, and 6, starting from the top) and a 77-mer (rows 3, 4, 7, and 8). Figure 4 shows representative SPR reflectivity versus angle curves collected under buffer solution on and off the dsDNA spots. The SPR resonance shifts to higher angles where the DNA is present, as expected since DNA has a higher refractive index than the buffer.

Figure 5 shows a representative SPR microscope image of this dsDNA array, acquired at the high-contrast angle (54.44°) shown in Figure 4. At this angle, the DNA spots appear bright compared to the surrounding surface because of the higher reflected intensity in these areas (Figure 4). While it is possible to see the 200 μm array elements in the image, the spot boundaries are ill-defined due to the very small difference in effective refractive index caused by the addition of the DNA. A profile of the line in the image is shown in Figure 5B. The reflected intensity data (dotted line) and the data smoothed by averaging a data point at the smoothing location with the two closest neighboring data points on each side (solid line) are shown in the profile.

The amount of dsDNA in the array spots was quantitated using the intensity integration analysis method provided by the KSA software. The percent reflected intensity was integrated over a 0.02-mm² area in the center of each element of the array and in the same size area of the SA layer located just below each array element. The difference in percent reflected intensity was calculated by subtracting the intensity for the surrounding SA layer from that for the array element. These percent reflected intensity differences were averaged for each row and used to calculate the dsDNA effective thickness and packing density following the procedures outlined above. The values are shown in Table 1. The effective DNA film thickness for the 100-mer spots is systematically about one-third thicker than the 77-mer spots, consistent with their length difference. This results in much more similar dsDNA packing densities, which range from 5 x 10¹¹ to 6 x 10¹¹ dsDNA/cm². The spot-to-spot standard deviations in reflected light intensity in Table 1 are much larger than the standard deviation with time within a given spot (~0.1% at one measurement per second; see below). The spot-to-spot deviation is due to spatial noise in the scattered light image, which arises from imperfections in the optics. It does not limit the instrument’s sensitivity for detecting adsorption in real time within a given spot (see below).

A packing density for the SA monolayer was estimated by measuring with the SPR microscope the amount of additional SA that could be adsorbed from solution onto the areas around these dsDNA elements. These areas should contain the mixed thiol monolayer and, ideally, a saturation coverage of SA. SA (0.08 mg/mL) in 150 mM PBS buffer was allowed to adsorb to the surface, and the resulting changes in reflected intensity were measured using the SPR microscope. The average change in reflected light intensity measured for six spatially separated areas of the surface

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was 1.6 ± 0.1% reflected intensity. This intensity change converts to a SA effective thickness of 0.20 nm and a surface coverage of 2.6 × 10^{11} \text{SA/cm}^2. This is 10% of the typical SA saturation coverage of 2.4 × 10^{12} \text{SA/cm}^2 measured on these monolayers. Adsorption of this additional SA on the surface is most likely due to the binding of SA to available biotin sites left vacant due to loss of SA from the surface caused by drying of the saturated SA monolayer prior to dsDNA spotting.

If we assume the SA coverage was 2.4 × 10^{12} \text{SA/cm}^2 prior to drying (i.e., the saturation coverage) and subtract the amount lost after drying (10% as measured above), the estimated SA packing density at the time of dsDNA immobilization was 2.14 × 10^{12} \text{SA/cm}^2. The binding ratios for each of the dsDNA spots were estimated using this SA packing density and are shown in Table 1. The dsDNA/SA binding ratio of 0.24 ± 0.02 is consistent for each row of the array and is somewhat below the saturation binding ratio (0.44 dsDNA/SA) measured for a similar length dsDNA adsorbed from solution onto a SA monolayer immobilized on the same composition BAT/OEG SAM. Since each SA exposes two biotin binding sites to the solution, only ~12% of these sites are bound with the biotinylated DNA. This value of ~20% observed previously was explained as due to repulsive interactions between the DNAs, preventing them from occupying nearest-neighbor biotin sites. Since DNAs of this length are still much like rigid rods, these repulsive interactions must be electrostatic and not just due to the excluded volume of the polymer molecule.

(2) Streptavidin Microarray on a BAT/OEG Monolayer.

The dsDNA spotting strategy discussed above (method 1) results in low contrast in the SPR microscopy images of the dsDNA microarray, making it difficult to choose the DNA areas of the image for real-time monitoring of binding events (for example, of proteins subsequently binding to these DNAs). Therefore, we next discuss the fabrication of a dsDNA array on a SA array prespotted onto the BAT/OEG SAM, method 2 in Figure 3.

The first step in this process is to microspot a SA array onto the mixed BAT/OEG SAM. An SPR microscope image of such a SA microarray acquired at a high-contrast angle is shown in Figure 6A. Each spot in the 10 × 12 array is made by replicate depositions of SA solutions (0.14 mg/mL in 150 mM PBS, pH 7.4). The high contrast in the image makes it easy to see the 200-µm-diameter SA spots. The rectangular spot shapes may be a consequence of the rectangular shape of the slit in the microspotting pin tip.

The line profile in Figure 6B shows the good signal-to-noise ratio compared to the line profile for the dsDNA fabricated on the SA monolayer shown in Figure 5B. The line profile was used to quantitate the surface coverage of SA in the spots. The difference in reflected intensity for the 10 spots is 12.5 ± 0.5% and corresponds to an effective SA thickness of 1.6 nm. This thickness converts to a surface density of 2.1 × 10^{12} \text{SA/cm}^2. This is 88% of a typical saturation coverage measured by SPR for a SA monolayer on these BAT/OEG SAMs.

The ability to fabricate a SA array with uniform coverage of SA in the individual spots is shown by the data in Table 2. The percent reflected intensity was integrated over a 0.018-mm^2 area in the center of each element of the array and in the same size area of a region just below each array element. The difference in percent reflected intensity was calculated by subtracting the intensity for the surrounding BAT/OEG monolayer from that for the SA array element. These percent reflected intensity differences were averaged for the spots in each row and used to calculate the SA effective thickness and packing density. The resulting values are shown in the table. A homogeneous SA surface coverage in all of the spots is shown by the low deviations for the average reflected intensity values in each row and the good agreement in the intensity values for all of the rows ((2.1–2.4) × 10^{12} \text{SA/cm}^2).

To verify the binding capacity of the BAT/OEG SAM and check whether SA coverage in the spots was at saturation, we adsorbed SA from solution to the microarray surface. Real-time SA adsorption and desorption curves measured for multiple regions of the microarray from the image in Figure 6A are shown in Figure 7. The changes in percent reflected intensity after SA was introduced to the array surface were measured by simultaneously integrating the reflected intensity at a high-contrast angle for eight spatially separated preselected 0.018-mm^2 areas of the

After establishing a baseline with 150 mM PBS buffer, pH 7.4, a sample of SA (0.08 mg/mL) in buffer was introduced into the flow cell. After adsorption reached saturation, the surface was rinsed with buffer. The region of the curves highlighted by the box in Figure 7A is expanded and plotted in Figure 7B. These curves demonstrate the ability to use the SPR microscopy area integration method to measure adsorption and desorption events simultaneously at many preselected regions of the surface with high sensitivity and fast time resolution (1 s). The sensitivity can be estimated from the small peak-to-peak noise on the curves:

\[ <3 \times 10^{10} \text{SA/cm}^2 \] or \( \sim 5 \times 10^6 \) SA molecules (~0.5 pg of protein) in one of the spots. The curves are similar and verify the optimization of the fluids system for rapid, simultaneous delivery of sample to all areas of the sensing surface.

An image of the surface after SA adsorption (data not shown) shows that the SA spots have disappeared after the SA adsorbed to the surrounding BAT/OEG SAM and filled in the areas between the microarray elements. The adsorption curves show a much larger increase in overall reflected intensity for the SAM than for the SA spots. The average increase in reflected intensity was 14.8 (1.1% for 12 different areas on the bare thiolate monolayer between the spots and 2.9 (0.7% on the 12 SA spots. These changes in percent reflected intensity correspond to effective SA thicknesses of 1.9 and 0.4 nm for the bare SAM and the SA spots, respectively. The thickness of 1.9 nm for the SA on the SAM corresponds to a surface coverage of 2.14 \( \times 10^{12} \) SA/cm\(^2\). This is within 4% of a typical saturation coverage for SA on the BAT/OEG SAM.\(^{41,46,59}\)

The thickness of 0.4 nm for the additional adsorption of SA on the SA spots corresponds to a coverage of 5.3 \( \times 10^{11} \) SA/cm\(^2\). This surface coverage is \( \sim 20\% \) of a typical full SA monolayer. When this additional SA coverage is added to the initial average spot coverage measured using the line profile, the final coverage is \( \sim 2.6 \times 10^{12} \) SAs/cm\(^2\), again very close to typical saturation coverage, as expected.

### Table 1. Surface Coverage of dsDNAs in the Microarray in Figure 5\(^a\)

<table>
<thead>
<tr>
<th>array row number</th>
<th>dsDNA length (mer)</th>
<th>av diff in % reflected intensity</th>
<th>std dev (reflected intensity)</th>
<th>effective dsDNA layer thickness (nm)</th>
<th>surface density ((10^{11} ) dsDNAs/cm(^2))</th>
<th>binding ratio (dsDNAs/SA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>3.8</td>
<td>1.3</td>
<td>0.31</td>
<td>5.0</td>
<td>0.23</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>3.8</td>
<td>1.5</td>
<td>0.31</td>
<td>5.0</td>
<td>0.23</td>
</tr>
<tr>
<td>3</td>
<td>77</td>
<td>2.9</td>
<td>1.1</td>
<td>0.24</td>
<td>4.9</td>
<td>0.23</td>
</tr>
<tr>
<td>4</td>
<td>77</td>
<td>2.9</td>
<td>2.2</td>
<td>0.24</td>
<td>4.9</td>
<td>0.23</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>3.7</td>
<td>1.8</td>
<td>0.30</td>
<td>4.9</td>
<td>0.23</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>4.1</td>
<td>1.3</td>
<td>0.34</td>
<td>5.4</td>
<td>0.25</td>
</tr>
<tr>
<td>7</td>
<td>77</td>
<td>3.0</td>
<td>1.7</td>
<td>0.25</td>
<td>5.1</td>
<td>0.24</td>
</tr>
<tr>
<td>8</td>
<td>77</td>
<td>3.6</td>
<td>1.6</td>
<td>0.30</td>
<td>6.1</td>
<td>0.29</td>
</tr>
</tbody>
</table>

\(^a\) Values for percent reflected intensity were found by using Scan Mode to integrate the light intensity in a 0.02-mm\(^2\) region in each array element and a region below the array element. Changes in percent reflected intensity were found by subtracting the intensity for the surrounding region from the spot intensity. A SA packing density of 2.14 \( \times 10^{12} \) SAs/cm\(^2\) was used to estimate the binding ratios.

### Table 2. Surface Coverage of SA in Array in Figure 6\(^a\)

<table>
<thead>
<tr>
<th>array row no.</th>
<th>diff in % reflected intensity</th>
<th>std dev (% reflected intensity)</th>
<th>effective SA layer thickness (nm)</th>
<th>surface density ((10^{11} ) SA/cm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.7</td>
<td>1.2</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>12.9</td>
<td>1.7</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>3</td>
<td>13.4</td>
<td>1.4</td>
<td>1.7</td>
<td>2.2</td>
</tr>
<tr>
<td>4</td>
<td>12.9</td>
<td>1.5</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>5</td>
<td>12.7</td>
<td>1.4</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>6</td>
<td>12.6</td>
<td>1.3</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>7</td>
<td>14.2</td>
<td>0.8</td>
<td>1.8</td>
<td>2.4</td>
</tr>
<tr>
<td>8</td>
<td>12.9</td>
<td>1.3</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>9</td>
<td>13.4</td>
<td>0.8</td>
<td>1.7</td>
<td>2.2</td>
</tr>
<tr>
<td>10</td>
<td>13.0</td>
<td>1.1</td>
<td>1.7</td>
<td>2.2</td>
</tr>
</tbody>
</table>

\(^a\) Values for percent reflected intensity were found by using Scan Mode to integrate the light intensity in a 0.018-mm\(^2\) region in each array element and a region below the array element of the microarray. Changes in percent reflected intensity were found by subtracting the intensity for the surrounding region from the spot intensity. The reflected intensity for each row was found by averaging the values for the SA spots in that row.

Figure 6. High-contrast-angle SPR microscope image and line profile of a SA microarray fabricated on a BAT/OEG SAM under buffer solution. (A) Rows were made by replicate SA sample depositions after a new sample uptake. (B) The profile was found by averaging the line profiles for a 4-pixel-wide column (67 \( \mu \)m) located in the position shown by the line in (A).

SA array. After establishing a baseline with 150 mM PBS buffer, pH 7.4, a sample of SA (0.08 mg/mL) in buffer was introduced into the flow cell. After adsorption reached saturation, the surface was rinsed with buffer. The region of the curves highlighted by the box in Figure 7A is expanded and plotted in Figure 7B. These curves demonstrate the ability to use the SPR microscopy area integration method to measure adsorption and desorption events simultaneously at many preselected regions of the surface with high sensitivity and fast time resolution (1 s). The sensitivity can be estimated from the small peak-to-peak noise on the curves:
The ability of additional SA to adsorb on the array spots may be due to incomplete adsorption from the solution spotted on the surface during the microspotting process. Alternatively, the additional SA may be filling sites on the BAT/OEG SAM where SA was lost due to drying. As mentioned previously, drying of the surface was minimized, but not completely eliminated. There is always some drying of the surface prior to mounting the flow cell on the substrate/prism assembly. The extent of this drying is difficult to control because it depends in part on the relative humidity in the laboratory.

The loss of SA due to drying of the microarray surface also is corroborated by the fact that the SA coverage after microspotting was 20% less than the typical saturation coverage on these mixed monolayers. When the additional SA adsorbs, the final SA coverage in the spots is within 4% of the coverage measured for the surrounding monolayer and similar to what has previously been measured.

(3) Relationship between SA Solution Concentration and SA Surface Coverage. To maximize the SA coverage in the microarray spots for dsDNA immobilization, we investigated the relationship between the SA solution concentration used for microspotting and the protein coverage in the microarray. SA solutions ranging in concentration from 0.46 to 0.05 mg/mL were used to fabricate the SA microarray shown in the SPRM image in Figure 8A. We chose these concentrations based on the number of SA monolayers present in solution when we assume a drop delivery volume of 1.7 nL based on the specifications for our pin, a spot diameter of 200 μm, and a SA monolayer saturation coverage of 2.4 × 10^12 SA/cm². The estimated number of SA monolayers contained in each droplet of each SA solution concentration is shown in Table 3.

The size and intensity of the array spots decrease for lower SA solution concentrations. The line profile in Figure 8B shows the decrease in the spot intensity for the column indicated by the line in the SPRM image. The SA coverage was calculated by measuring the difference in percent reflected intensity for the array elements using line profiles. The results were averaged for each row and are shown in Table 3. Figure 9 plots the resulting SA surface coverage within a spot versus the SA solution concentration used in spotting. Although there were several monolayers worth of SA per droplet in several of the spotting solutions according to our estimations, less than a monolayer of SA coverage was found for all of the spots. This may be due to a lower than estimated droplet volume delivered to the surface, incomplete SA adsorption from solution and its subsequent
Table 3. SA Surface Coverage for Different SA Solution Samples Used for Microspottinga

<table>
<thead>
<tr>
<th>SA solution concn (mg/mL)</th>
<th>est no. of SA monolayers in droplet</th>
<th>av diff in % reflected intensity</th>
<th>std dev (% reflected intensity)</th>
<th>effective SA layer thickness (nm)</th>
<th>surface density (10^12 SA/cm^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.46</td>
<td>5</td>
<td>12.4</td>
<td>1.6</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>0.28</td>
<td>3</td>
<td>10.4</td>
<td>2.0</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td>0.14</td>
<td>1.5</td>
<td>8.8</td>
<td>1.9</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>0.09</td>
<td>1</td>
<td>6.8</td>
<td>1.7</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>0.05</td>
<td>0.5</td>
<td>3.5</td>
<td>1.2</td>
<td>0.4</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*a The difference in average reflected intensity relative to the surrounding regions was measured using the SPR microscope line scans for the microarray in Figure 8 in contact with PBS buffer.

Figure 9. Relationship between the SA solution concentration for microspotting and the resulting density of SA measured in the microarray on a BAT/OEG SAM functionalized gold-coated substrate.

rinsing, or loss of SA due to drying/rinsing. This last effect could only be dominant for the highest SA concentration. The values for Table 3 and Figure 9 were obtained from line profiles rather than using intensity integration over the spot area due to the need to adjust the integration area for each spot as the spot size changed. However, to compare results obtained using both methods, we used area intensity integration for the top row of spots, which are the largest. As previously described, the reflected intensity values were found by integrating the intensity in a 0.02-mm^2 area centered over each spot and over an area of the BAT/OEG surface just below the spot. The difference in reflected intensity was found by subtracting the intensity value for the area below the spot from the value for the spot. The resulting intensity values were averaged for the top row of spots to give a difference in reflected intensity of 12.0 ± 1.3%. This value agrees well with that found using the line profiles (12.4 ± 1.6% reflected intensity), showing that both line profiles and area integration are valid methods for measuring reflected intensity values.

(4) dsDNA and SA Microarray on a BAT/OEG Monolayer.

Finally, we used a SA array as the basis for immobilizing dsDNA by spotting biotinylated dsDNA directly on the array of SA spots (method 2 of Figure 3). The resulting dsDNA microarray is shown in Figure 10A. The array was made by first spotting SA (0.14 mg/mL) onto a BAT/OEG mixed monolayer. After protein was allowed to adsorb in the high-humidity chamber for 30 min, dsDNA (0.2 mg/mL) in 400 mM PBS buffer, pH 7.4, was spotted on top of the SA spots. Again, only two types of dsDNA were used: 100- (rows 2, 3, 6, and 7) and 77-mers (rows 4, 5, 8, and 9).

The top and bottom rows were spotted only with SA solution, while the other rows contain both dsDNA and SA. The high contrast between the spots and the surrounding surface makes it easy to identify the spots and their boundaries. As expected, the reflected light intensity for the SA spots is slightly lower than that for the spots composed of SA and dsDNA. This is also shown by the line profile in Figure 10C. This is the profile for the line in Figure 10A. The amount of SA and dsDNA in the spots was quantified using line profiles. The differences in percent reflected intensity for the dsDNA spots were calculated by subtracting the average difference in percent reflectivity for the SA spots from the difference in percent reflected intensity for the spots containing dsDNA and SA. The values are presented in Table 4. The dsDNA surface density ranged from 5 × 10^11 to 12 × 10^11 dsDNA/cm^2. The average packing density for the SA spots was 1.7 × 10^12 SA/cm^2 or 70% of typical SA saturation coverage on these SAMs. This SA coverage was used to calculate the dsDNA/binding ratios shown in Table 4. The binding ratios for most of the rows are in very good agreement with those found for dsDNA immobilized on a uniform SA monolayer measured previously (~0.4 dsDNA/SA). However, the ratios for the second and eighth array rows are slightly higher (0.74 and 0.61 dsDNA/SA, respectively), and may be due to a higher SA coverage for those rows.

SA (0.08 mg/mL) in 150 mM PBS buffer, pH 7.4, was allowed to adsorb to this dsDNA microarray. The reflected intensity changes due to SA adsorption are seen as a decrease in contrast between the spots and the surrounding area in the SPR image of the array after SA adsorption, shown in Figure 10B. It is more difficult to identify the dsDNA spot boundaries, and as expected, the DNA-free SA spots disappear completely.

The line profile for the array after SA adsorption is shown in Figure 10D. The lower signal-to-noise ratio is similar to that obtained for the dsDNA fabricated on the preadsorbed SA monolayer shown in Figure 5. The two surfaces are essentially the same at this point. The advantage of using the SA array as the basis for the dsDNA monolayer is that the spot locations (i.e., the exact pixels on the video camera image) can be identified easily prior to filling in the rest of the surface with SA. This is necessary when measuring real-time adsorption and desorption using Scan Mode, since these areas must be preselected prior to kinetic measurements.

Elsewhere, we show that dsDNA arrays spotted by the methods outlined here can be used successfully to simultaneously monitor in real time with 1-s time resolution the binding of DNA-binding proteins to each element of the array with a sensitivity of ~0.5 pg of protein on the spot.

Loss of Streptavidin due to Drying. While fabricating microarrays, we found that the treatment of the microarray surface during preparation for microspotting and prior to analysis with the SPR microscope was important for obtaining consistent high-quality arrays. Initially, we dried the substrate modified with the SA layer with N2 prior to dsDNA microarray fabrication. A dry surface is necessary to prevent the droplets of DNA solutions from running together on the surface. This processing of the substrates failed to lead to reproducible dsDNA microarrays that could be observed with the SPR microscope. With SPR microscopy analysis, we measured a SA saturation coverage that was >25% lower than that typically measured on the BAT/OEG SAM after normal adsorption from solution. We also observed adsorption of additional SA on surfaces that should already have had a saturation...
coverage of SA. We suspected that this apparent loss of SA from the surface was due to drying of the surfaces during microarray fabrication. We investigated this further using SPR spectroscopy and atomic force microscopy. These experiments and results are presented in ref 60.

Due to the importance of hydration to maintain a high-density and robust SA monolayer, we minimized drying of the surface between uniformly coating it with SA and microspotting biotinylated dsDNAs onto it. After adsorbing SA from solution to form a SA monolayer, the surface is rinsed with buffer and then pure water as described previously. However, rather than drying this surface with a rapid N2 flow as usual, the bulk water was instead gently blown off the surface by squirts of air from a clean squirt bottle filled with humid air from the microspotting chamber. Drying also is minimized after array fabrication by rinsing the surfaces in the high-humidity chamber and then immediately

---

**Table 4. Surface Coverages of SA and dsDNA in the Array Shown in Figure 10**

<table>
<thead>
<tr>
<th>row no.</th>
<th>spot compn</th>
<th>av diff in % reflected intensity</th>
<th>std dev (% reflected intensity)</th>
<th>diff in % reflected intensity due to dsDNAs</th>
<th>effective thickness (nm) of SA or dsDNA</th>
<th>surface density (10^11 molecules/cm²)</th>
<th>binding ratio (dsDNAs/SA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SA</td>
<td>10.8</td>
<td>1.7</td>
<td>1.38 (SA)</td>
<td>1.38 (SA)</td>
<td>18 (SA)</td>
<td>0.74</td>
</tr>
<tr>
<td>2</td>
<td>SA+100-mer DNA</td>
<td>19.4</td>
<td>2.2</td>
<td>9.4</td>
<td>0.77</td>
<td>12</td>
<td>0.74</td>
</tr>
<tr>
<td>3</td>
<td>SA+100-mer DNA</td>
<td>16.5</td>
<td>1.4</td>
<td>6.5</td>
<td>0.53</td>
<td>8.5</td>
<td>0.51</td>
</tr>
<tr>
<td>4</td>
<td>SA+77-mer DNA</td>
<td>13.8</td>
<td>1.8</td>
<td>3.8</td>
<td>0.31</td>
<td>6.5</td>
<td>0.39</td>
</tr>
<tr>
<td>5</td>
<td>SA+77-mer DNA</td>
<td>13.5</td>
<td>2.4</td>
<td>3.5</td>
<td>0.29</td>
<td>5.9</td>
<td>0.36</td>
</tr>
<tr>
<td>6</td>
<td>SA+100-mer DNA</td>
<td>13.7</td>
<td>1.5</td>
<td>3.7</td>
<td>0.30</td>
<td>4.8</td>
<td>0.29</td>
</tr>
<tr>
<td>7</td>
<td>SA+100-mer DNA</td>
<td>14.3</td>
<td>2.1</td>
<td>4.3</td>
<td>0.36</td>
<td>5.7</td>
<td>0.34</td>
</tr>
<tr>
<td>8</td>
<td>SA+77-mer DNA</td>
<td>16.0</td>
<td>2.3</td>
<td>6.0</td>
<td>0.49</td>
<td>10</td>
<td>0.61</td>
</tr>
<tr>
<td>9</td>
<td>SA+77-mer DNA</td>
<td>13.3</td>
<td>1.5</td>
<td>3.3</td>
<td>0.27</td>
<td>5.7</td>
<td>0.34</td>
</tr>
<tr>
<td>10</td>
<td>SA</td>
<td>9.2</td>
<td>1.0</td>
<td>1.17 (SA)</td>
<td>1.17 (SA)</td>
<td>15 (SA)</td>
<td></td>
</tr>
</tbody>
</table>

* Differences in reflected intensity were calculated from the unsmoothed line profiles. The average of the SA packing densities for the top and bottom rows was used to estimate the binding ratios.

---

**Figure 10.** SPRM images and line profiles for a dsDNA microarray fabricated on a SA array on a BAT/OEG SAM. All rows contain SA. The dsDNA rows are labeled with the specific dsDNA spotted in that row. The images were acquired at a high-contrast angle with the surface in contact with PBS buffer (A) before and (B) after SA adsorption from solution. (C) Profile for the line in (A). (D) Profile of the same area as after SA adsorption from solution. Both line profiles were found by averaging the line profiles for a 9-pixel-wide (250 μm) region centered on the lines shown in the images. The curves are a five-point smoothed version of the raw data. Array elements in the line profile are labeled by the row number starting from the top of the array.
placing the array-functionalized substrates in buffer for storage. Exposure of the substrates to the dry laboratory air is minimized prior to analysis of the microarrays with the SPR microscope. After the substrate is mounted on the prism, the flow cell is quickly fastened on top of the substrate and filled with buffer solution.

The drying effect also has relevance for storage of the slides. For the best results, the SA monolayer needs to be formed in the humid chamber just prior to microspotting. Substrates functionalized with microarrays are also stored under buffer until use. There were no obvious changes in the microarrays observed after storage under buffer for three weeks. Stability of the arrays during longer-term storage was not investigated. Elsewhere we discuss a soluble coating that allows drying the SA layers without loss of SA.60

CONCLUSIONS

We describe here two methods for microspotting of dsDNA arrays on a gold surface. Both are based on first creating a SA layer (either uniformly across the surface or as a microspotted array) on a BAT/OEG SAM. This nearly close-packed SA layer provides \( \sim 5 \times 10^{12} \) binding sites/cm\(^2\) for biotin. We showed the ability to immobilize dsDNAs on this layer so that the surface coverage of dsDNAs \((5\sim 12) \times 10^{11}\) dsDNA/cm\(^2\) should allow enough space between dsDNAs for recognition and binding of DNA-binding proteins when their recognition sites are immobilized on this surface. The fabrication of a SA array with uniform coverage, followed by deposition of a biotinylated dsDNA directly on the SA array, demonstrates the possibility of using such an SA array for the immobilization of a variety of biotinylated molecules for SPR microscopy array-based studies. We also demonstrated the ability to use the SPR microscope to measure real-time binding of protein to a patterned surface with good time resolution \((1 \text{ s})\) and sensitivity \((\sim 0.5 \text{ pg of protein in a spot})\). The combination of such real-time adsorption and desorption SPR microscopy measurements with the SA array fabrication on the gold SPR-active surface will allow for SPR microscopy array-based studies of interactions between a variety of biomolecules with high throughput.

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