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Photoelectrophoretic Transport and Hybridization of DNA Oligonucleotides on Unpatterned Silicon Substrates

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Abstract: We describe a platform for photoelectrophoretic transport and electronic hybridization of fluorescence-labeled DNA oligonucleotides in a low conductivity electrolyte. At the core of the platform is a chemically stabilized semiconductor photodiode or photoconductor surface coated with a streptavidin–agarose permeation layer. Micro-illumination of this surface generates photoelectrochemical currents that are used to electro-phoretically transport and attach biotinylated DNA capture strands to arbitrarily selected locations. The same process is then used to transport and electronically hybridize fluorescence-labeled DNA target strands to the previously attached capture strands. Signal detection is accomplished either by a fluorescence scanner or a CCD camera. This represents a flexible electronic DNA assay platform that does not rely on pre-patterned microelectronic arrays.

Introduction

A growing number of micro-array based DNA oligonucleotide analysis systems are finding their way into commercial and academic laboratories.^{1,2} While all are designed to facilitate multiplex DNA analysis, a variety of approaches (both passive and active) have been taken to accomplish this. Passive micro-array systems use microspotting,^{3–5} or photolithography^{6,7} to anchor or synthesize oligonucleotides on an inert substrate. Active micro-array systems, however, use these techniques not only for fabrication but also to implement microcircuits or

simple electrodes for the manipulation or detection of DNA.^{8–12} A distinct advantage of certain active micro-arrays lies in the ability to manipulate DNA oligonucleotides, that is, to electro-phoretically transport, concentrate, and electronically hybridize them. We have reported previously on a number of active micro-array systems on which we demonstrated single nucleotide polymorphism (SNP) detection,^{10–12} short tandem repeat discrimination (STR),¹³ and even multiplex anchored amplification (SDA).¹⁴ These systems consist of microelectrode arrays (with or without CMOS driving circuitry) that are coated with an

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agarose permeation layer for DNA oligonucleotide attachment (biotin/streptavidin). In contrast, this work explores the use of unpatterned, photoactive silicon substrates as a simplified platform for electrophoretic or rather photoelectrophoretic transport and electronic hybridization of DNA oligonucleotides. Thus, a light beam, instead of discrete individual microelectrodes, is used to generate electrochemical currents at specific locations.

Inorganic and organic semiconductors are the materials of choice for the generation of photocurrents or photoelectrochemical currents. Although there are a rich variety of semiconductors suitable for solid state devices (solar cells, photodiodes, photoconductors, etc.), only a limited number of these are adequate for photoelectrochemical current formation. In addition, there is no single low band-gap semiconductor or compound semiconductor material known that can withstand the corrosive environment present during photooxidation of water.^{15–18} Photooxidation of water, however, is the dominant process at the positive electrode (anode) during electrophoresis of negatively charged DNA in aqueous electrolytes. To overcome this instability, a number of research groups involved in the field of photovoltaic technology have reported on the stabilization of inorganic semiconductor photoanodes against corrosion in water. Variable levels of stabilization were achieved by deposition of protective surface layers such as noble metals,^{19,20} metal oxides,^{21–25} or conducting polymers.²⁶ One report by Kainthla et al.²⁴ demonstrated excellent long-term stability of n-type silicon surfaces under conditions of water oxidation. The stabilizing layer in this case was Mn₂O₃ that was applied to the silicon surface by a simple solution-phase deposition process.

Thus, our approach was to integrate (and modify) this process for surface stabilization of photoactive silicon substrates with a previously developed streptavidin–agarose permeation layer¹² and to demonstrate photoelectrophoretic transport and electronic hybridization of DNA oligonucleotides.

Experimental Section

Silicon Substrate Preparation. N-type single-crystal silicon wafers of (100) and (111) orientation and resistivities ranging between 0.1 and 0.9 Ω-cm and 1–4 Ω-cm were received from various commercial sources. The wafers were provided with an ohmic back contact by deposition of either a 300 nm aluminum layer (thermal evaporation) or a 50 nm platinum (sputter deposition) layer followed by thermal annealing. Annealing steps were performed under nitrogen at 300 °C for 1 h for aluminum layers and at 500 °C for 45 min for platinum layers. Amorphous silicon surfaces were obtained by sputter deposition

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of 50 nm silicon onto oxide stripped (buffered HF) n-type single-crystal wafers with ohmic back contacts.

The following procedure for deposition of Mn₂O₃ layers was modified from the original procedure published by Kainthla et al.: Individual samples of single crystal silicon or single crystal silicon coated with amorphous silicon with dimensions of about 1 cm² were cut from the respective wafers and sonicated in acetone followed by rinsing with 2-propanol and water. (Alternatively, larger samples can be pre-scribed with a diamond scribe and broken into individual pieces after deposition of the Mn₂O₃ layer.) After drying, the samples were placed in plastic Petri dishes²⁷ and treated with buffered HF (2 min) to strip the native oxide layer. Immediately after thorough rinsing with deionized water the sample surfaces were sensitized by exposure (2 min) to an aqueous solution containing 1 wt % SnCl₂ (Aldrich) and 4 vol % HCl. This step was followed by rinsing with 4 vol % HCl and deionized water. The sensitized surfaces were then decorated with Pd islands by immersion (2 min) in an aqueous solution containing 1 vol % HCl and 0.05 wt % PdCl₂ (Aldrich). Traces of Sn⁴⁺ were removed by soaking in 5% HCl for 5 min followed by rinsing with deionized water. The deposition of the Mn(OH)₂ layer was performed by adding a freshly prepared aqueous solution containing 0.25 M NH₄Cl, 0.1 M NH₄OH, and 0.03 M MnCl₂ to the samples in the Petri dish. Upon addition of the solution the Petri dishes were placed on a shaker table for 10 min to allow vigorous stirring. A light brownish precipitate was observed to form within 30–60 s. After completion of the deposition reaction, the samples were rinsed thoroughly with deionized water and dried in air. At this point, the sample surfaces have a slightly structured, brown-grayish appearance. The thermal conversion of Mn(OH)₂ into Mn₂O₃ was accomplished by heating the samples on a heating block in high vacuum (10⁻⁵ to 10⁻⁶ Torr) to 250 °C for 15 min. The samples were left overnight to cool to room temperature.

Permeation Layer and DNA Oligonucleotide Synthesis. Preparation and spin deposition of the streptavidin-loaded agarose permeation layer, as well as the synthesis of biotinylated and fluorescence-labeled DNA oligonucleotides have been described previously.¹²

Experimental Setup (Figure 1). A simple electrochemical cell was constructed by first attaching a stripe of conducting Cu tape to a glass slide. The Mn₂O₃- and agarose-coated silicon sample was then mounted onto the Cu tape using conducting silver paint (Ted Pella). The active electrode area was defined by attaching a 2 mm thin piece of Teflon sheet with a circular opening to the sample. The electrode area exposed to the light measured about 0.25 cm². The electrochemical cell was completed by placing a circular Pt auxiliary electrode and a Ag quasi-reference electrode above the sample surface. Sample illumination for photoelectrophoretic transport and hybridization was accomplished by a single-mode optical fiber (Thorlabs, 4 μm core, 125 μm cladding) that was mounted on a motorized micromanipulator stage (Eppendorf 5171) and coupled to a 8 mW 630 nm HeNe laser (Research Electro Optics) that was attenuated by a filter wheel (New Focus). Photoelectrochemical stability measurements were performed by illuminating the whole sample area with a halogen light source (Bausch & Lomb).

Instrumentation. Electrochemical experiments were performed either with a Pine AFRDE5 or an Autolab PGSTAT10 potentiostat system. Fluorescence signals were measured either on a high-resolution fluorescence scanner (Avalanche, Molecular Dynamics) or on a cooled CCD camera (Princeton Instruments). Surface morphologies and film thickness were recorded with a commercial atomic force microscope (Nanoscope III, Digital Instruments).

Assay Procedure. A solution containing 50 nM of a DNA oligonucleotide in 50 mM of L-histidine (electrolyte) was pipetted into the electrochemical cell. The single mode optical fiber (not illuminated) was then placed above the sample surface and a constant electrochemical potential was applied to the cell (typically 1.5–2 V vs Ag/Ag⁺). Transport and/or hybridization of the DNA oligonucleotides were induced by illuminating the sample surface for 5–60 s with a light power of 4–80 μW. Between runs with different DNA oligonucleotides, the cell was rinsed three times with 50 mM L-histidine. At the end of hybridization experiments, an additional rinse with 0.1 M phosphate

(27) Samples with aluminum back contacts were attached to the Petri dishes with double-sided tape to prevent corrosion of the aluminum.

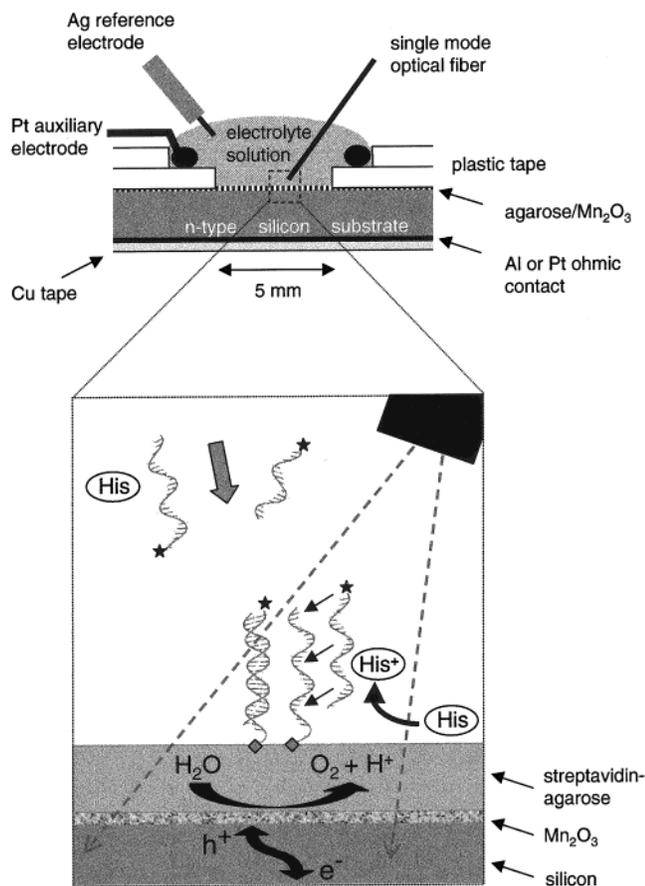


Figure 1. Schematic cross-section of the electrochemical setup for photoelectrophoresis experiments. *Insert:* The insert outlines the mechanism of photoelectrochemical transport and hybridization of DNA oligonucleotides. Illumination of the silicon surface creates electron (e^-) and hole (h^+) pairs. In presence of an applied potential, these electron hole pairs generate a photoelectrochemical current that causes electrophoretic transport of DNA oligonucleotides to the illuminated area. The oligonucleotides are then bound to the agarose layer through a biotin/streptavidin interaction or through hybridization to a previously deposited capture strand. The Mn_2O_3 layer serves to protect the silicon surface from oxidation. (His = histidine as a neutral zwitterion, His^+ = protonated histidine having a net positive charge, diamonds indicate biotin/streptavidin attachment, stars indicate fluorescent dye).

buffer pH 7.4 containing 1% sodium dodecyl sulfonate was performed to reduce non-specific background.

Bead transport experiments were performed with thoroughly washed $1\ \mu\text{m}$ diameter carboxylated polystyrene beads (Bangs) in 50 mM L-histidine.

Results and Discussion

I. Silicon Surface Preparation. The original procedure for surface protection of n-type single-crystal silicon surfaces with Mn_2O_3 layers published by Kainthla et al.²⁴ involved a stain etch pretreatment ($HF:HNO_3:HCl$) followed by electrochemical deposition of a Pd monolayer and deposition of a $Mn(OH)_2$ precursor film from solution. The $Mn(OH)_2$ film was then converted into Mn_2O_3 by heating in high vacuum. Kainthla et al. developed the Mn_2O_3 system for photovoltaic applications where long-term stability and high current densities are desired. However, we were interested in a platform with good short-term stability that supports high current densities and provides good lateral resolution. The latter point raised concerns that insufficient control over the thickness of the Pd seed layer would produce excessive lateral conductivity and, consequently, poor

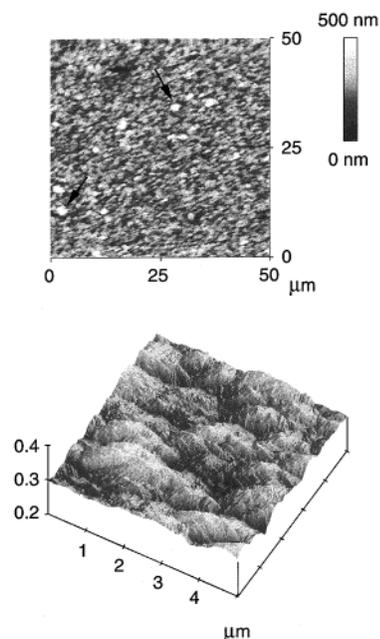


Figure 2. Tapping mode AFM images of a Mn_2O_3 film deposited onto single crystal n-type silicon. The film thickness of this sample is approximately 270 nm with a medium roughness of 50 nm. (a) Low-resolution image showing the oriented granular structure of the Mn_2O_3 film. Arrows indicate larger precipitates on the surface. (b) High-resolution surface plot of the same sample.

resolution. We also found that the electrodeposition of Pd seed layers on silicon substrates was problematic in general, thus leading to large variations in surface stabilization by Mn_2O_3 films. For this reason, we decided to deposit the Pd seed layers chemically, rather than electrochemically. Procedures to accomplish this are well-known and have been used for many years to catalyze electroless plating of a variety of metals.²⁸

In short, the surfaces to be plated are sensitized in a first step by adsorption of Sn^{2+} ions. In a second step, the Sn^{2+} ions are used to reduce Pd^{2+} to metallic Pd, thereby producing a submonolayer of Pd. This change in deposition of the Pd monolayer allowed us also to omit the recommended but undesirable stain-etch pretreatment that was part of the original procedure by Kainthla et al.

A further modification involved the actual $Mn(OH)_2$ deposition step. It was observed that the use of 1.4 M NH_4OH leads to immediate precipitation of $Mn(OH)_2$ and not to a gradual formation of a light brown precipitate as described in the original paper. This rapid precipitation was found to introduce further irreproducible behavior that we were able to avoid by decreasing the NH_4OH concentration to 0.1 M. The resulting surfaces still displayed a certain degree of visual inhomogeneity but had very reproducible photoelectrochemical characteristics.

Figure 2 shows two tapping mode atomic force images of a typical Mn_2O_3 surface at $50\ \mu\text{m}$ and $5\ \mu\text{m}$ full scale. The surface has a granular structure with an average grain size of approximately $2\ \mu\text{m}$ and a mean roughness of 50 nm (with a small number of larger precipitates). The grains exhibit a preferred orientation probably caused by fluid flow during solution deposition of the $Mn(OH)_2$ precursor film. Film thicknesses obtained from step height measurements ranged from 250 to 350 nm, which is at least a factor of 10 more than the thickness reported by Kainthla et al. The difference reflects the increased

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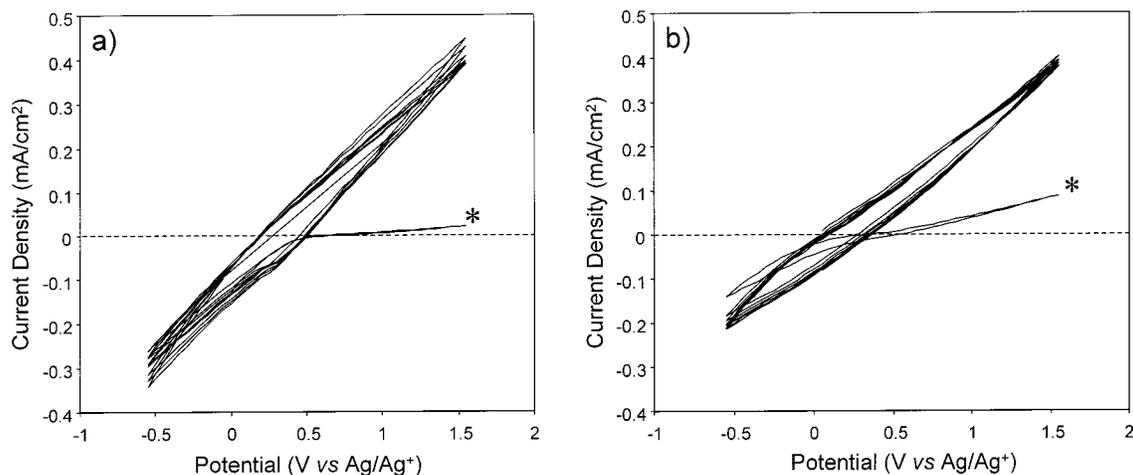


Figure 3. Current–voltage characteristics of Mn_2O_3 -coated n-type silicon electrodes measured in 50 mM L-histidine solutions. The 10 consecutive cyclic voltammograms acquired in the dark (*) and under illumination were measured (a) before and (b) after deposition of the streptavidin–agarose permeation layer. The scan rate was 100 mV/sec. (Electrochemical potentials were not compensated for ohmic potential drops across the low conductivity electrolyte.)

deposition rate of $\text{Mn}(\text{OH})_2$ that is caused by the lower concentration of NH_4OH used in our procedure.

II. Photoelectrochemical Behavior. Previous work on microelectrode arrays^{9,12} has shown that 50 mM L-histidine in water is an ideal electrolyte for DNA oligonucleotide transport and electronic hybridization. Its low conductivity ($<100 \mu\text{S}/\text{cm}$) allows for efficient electrophoretic transport of oligonucleotides at low current densities. More importantly, L-histidine actively promotes hybridization of oligonucleotides during electronic assays by buffering the acidic conditions present at the microelectrode and by reducing the repulsion between complementary DNA strands after acquiring a net positive charge.⁹ (Figure 1: insert) Consequently, all photoelectrochemical measurements were performed in solutions of 50 mM L-histidine.

Typical cyclic voltammograms of Mn_2O_3 -coated silicon electrodes before and after deposition of the agarose permeation layer are shown in Figure 3a and 3b, respectively. The two sequences of consecutive scans show that Mn_2O_3 coatings deposited with our modified procedure stabilize the silicon surface against photocorrosion. That is, after an initial decrease of about 5–20%, photocurrents are stable for hours of continuous operation (>12 h). The initial loss is probably due to rapid passivation of unprotected electrode areas such as pinholes. Dark current levels are typically 10–50 times smaller than photocurrents but tend to increase after deposition of the agarose permeation layer. Mn_2O_3 -coated thin film amorphous silicon electrodes displayed very similar cyclic voltammograms compared to single-crystal silicon electrodes. However, the amorphous silicon electrodes exhibited better performance in photoelectrophoresis experiments, as will be discussed below.

III. Photoelectrophoretic Transport and Hybridization of DNA Oligonucleotides. The ability to photoelectrophoretically transport DNA oligonucleotides to specific locations on an agarose-coated silicon substrate depends on the ratio between photocurrent and dark current as well as on the degree of lateral diffusion of the photocurrent. Thus, a large dark current leads to reduced contrast between illuminated and non-illuminated areas and eventually leads to a reduced signal-to-background ratio during fluorescence detection. Lateral diffusion of the photocurrent on the other hand, reduces the spatial resolution that is otherwise limited by the spot size of the illumination source.

In general, dark current levels can be minimized by careful preparation of the semiconductor surface (avoiding large

Table 1. Four-Point Probe Resistance Data

sample	resistance (M Ω)	thickness (nm)	resistivity (Ω -cm)
glass/a-Si	0.4 ± 0.02	50 ± 5	9 ± 0.7
glass/a-Si/Pd	0.2 ± 0.03	50 ± 5	5 ± 0.8
glass/a-Si/Pd/ Mn_2O_3	0.5 ± 0.15	350 ± 55	(75 ± 22)

numbers of surface traps) and by working with a semiconductor with moderate to low conductivity.²⁰ Photocurrent diffusion depends on the minority carrier diffusion length within the semiconductor depletion zone and, specifically for this platform, on the lateral electrical conductivity of the Pd and Mn_2O_3 layers. Minority carrier or hole diffusion within the semiconductor substrate can be reduced to a few micrometers by using highly doped silicon,²⁹ however, this comes at the expense of higher dark currents. Minimal minority carrier diffusion lengths can also be achieved by using amorphous silicon (a-Si) coatings or substrates.

The contributions of a-Si, Pd, and Mn_2O_3 to the lateral conductivity were determined by four-point probe measurements performed on thin films that were deposited on glass slides (Table 1). The a-Si and Pd layer had a moderate dark conductivity (5–10 Ω -cm), whereas the Mn_2O_3 film was found to be essentially insulating. (The literature value for room-temperature resistivity of dry, crystalline Mn_2O_3 is 16–100 Ω -cm).³⁰ In light of the cyclic voltammograms recorded (Figure 3) it must be assumed that the Mn_2O_3 film is porous or exhibits increased conductivity in the presence of water.

The first substrate studied for DNA transport was Mn_2O_3 -protected single-crystal silicon (1–4 Ω -cm resistivity) coated with a layer of streptavidin–agarose. The DNA oligonucleotides (capture probe sequence C1, Table 2) were both biotinylated and fluorescence-labeled (BTR 493/503 nm). Illumination of the electrode surface with 40–80 μW for 15–120 s at an applied potential of 1.5 V resulted in easily detectable fluorescent spots. These spots did not wash off during rinsing as compared to non-biotinylated oligonucleotides, demonstrating that the biotinylated oligonucleotides were not only photoelectrophoretically transported to the illuminated locations but also bound to the streptavidin present in the agarose.

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Table 2. Sequences of DNA Oligonucleotides

name	sequence
C1	5'-Bio-GAGAGCACATGAG(*)C ^a
C2 (ATA5)	5'-GATGAGCAGTTCTACGTGG-Bio ^b
T1	5'-TGATACGGTG(493)TCTCGACTCATGTGCTCTC ^c
T2 (RCA5)	5'-(493)CCACGTAGAACTGCTATC

^a * = NH₂ or fluorescent dye. ^b bio = Biotin. ^c 493: fluorescent dye with 493 nm absorption and 503 nm emission.

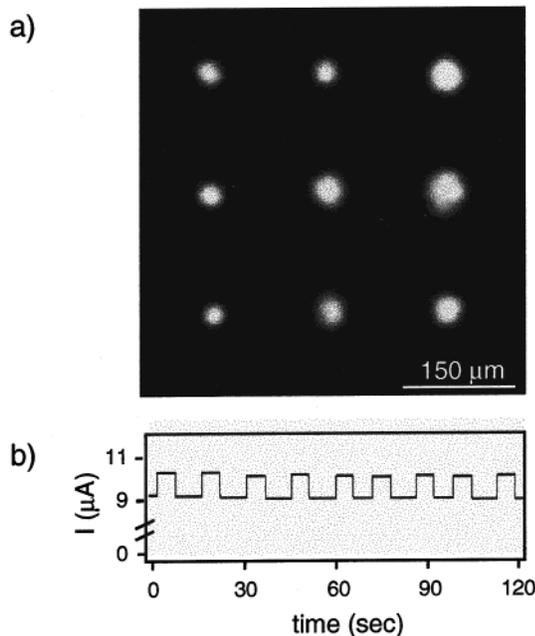


Figure 4. (a) Image of an array of fluorescent spots that was produced by photoelectrophoretic transport and biotin-streptavidin mediated immobilization of fluorescence-labeled DNA oligonucleotides on a streptavidin-agarose and Mn₂O₃-coated amorphous silicon electrode. Individual spots were illuminated for 5 s at a light intensity of 4 μW (630 nm) and an applied electrochemical potential of 1.5 V. (b) Photocurrent transient recorded during formation of the array.

However, large variations in spot size and fluorescence intensity were observed for experiments run under identical conditions, and in some cases, no fluorescent signal was detected at all even though substantial photocurrents were generated. Since there was no clear correlation between photocurrents and detected fluorescence levels, it was suspected that the surfaces exhibited variable degrees of photocurrent spreading, preventing the detection of localized fluorescence signals. For this reason, silicon substrates with lower resistance and substrates with amorphous silicon coatings were investigated.

While a decrease in resistance (0.1 and 0.9 Ω-cm) did not seem to enhance the reproducibility of DNA oligonucleotide binding, much improved results were obtained with amorphous silicon coated electrodes. On these surfaces, fluorescent spots were reproducibly generated at any location on a given chip with signal-to-background ratios greater than 100. For example, Figure 4a shows an array of fluorescent oligonucleotide spots that was produced by successive illumination of nine individual locations on a streptavidin-agarose and Mn₂O₃-coated amorphous silicon sample. Overall, there was still some degree of non-uniformity in spot size (25% SD fwhm) and intensity (40% SD), however, the mean spot size of 28 μm (fwhm) compared well with the area illuminated by the optical fiber of approximately 30 μm. (N.B. the laser light emerges as an uncollimated beam from the 4 μm diameter fiber optic core and expands rapidly with distance). Figure 4b shows the current

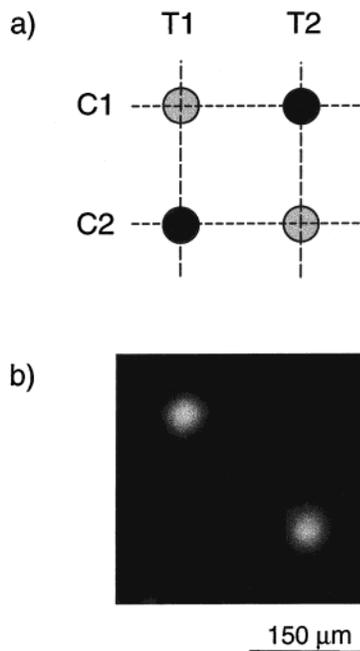


Figure 5. Electronic DNA hybridization. (a) Schematic illustration of the process used for photoelectrophoretic transport and electronic hybridization of fluorescence-labeled DNA oligonucleotides (C1, C2 = capture oligonucleotides; T1, T2 = target oligonucleotides). (b) Fluorescence image of two sets of two spots with capture strands C1 and C2 after electronic hybridization with fluorescence-labeled target strands T1 and T2. Fluorescence signals are only observed at the two locations with complementary capture and target strands. Oligonucleotides were deposited by illuminating individual spots with 4 μW at an applied potential of 1.5 V. Illumination times were 15 s for capture strands and 10 s for target strands.

transient recorded during formation of the array of the fluorescent oligonucleotide spots. As can be seen, stability and reproducibility of the photocurrents recorded at different locations is excellent. Furthermore, the relatively low light levels and short illumination times sufficient to immobilize fluorescence-labeled DNA oligonucleotides on amorphous silicon electrodes make it possible to use other fluorescent dyes with absorption maximums at higher wavelengths (e.g., BTR 588/616 or BTR 630/650). This was not possible under the conditions applied for single-crystal silicon electrodes where severe photobleaching was observed for these dyes. At this point, it is unclear if the superior performance of the amorphous silicon samples is due to a reduction of the minority carrier diffusion length or rather to an improvement in the interface between the Mn₂O₃ layer and the underlying substrate. The remaining non-uniformities may be attributed to inhomogeneity within the Mn₂O₃ layer that could affect the local transport or binding efficiency of DNA oligonucleotides.

In addition to photoelectrophoretic transport, electronic hybridization was investigated. In electronic hybridization one set of unlabeled oligonucleotides (capture strands) are first targeted to specific locations and anchored.⁹ A second set of fluorescence-labeled oligonucleotides (target strands) is then targeted to the same locations and actively hybridized to the capture strands. In the example shown, two sets of biotinylated DNA capture probes (sequences C1 and C2, Table 2) were successively transported and anchored to four different locations on a streptavidin-agarose and Mn₂O₃-coated amorphous silicon substrate, as outlined in Figure 5a. Two fluorescence-labeled target strands (sequences T1 and T2) were then each transported

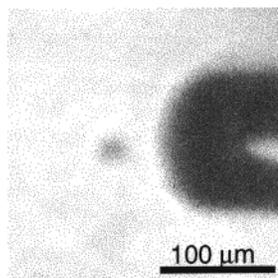


Figure 6. Microphotograph of photoelectrophoretic bead accumulation on a Mn_2O_3 -coated n-type single-crystal silicon electrode. The accumulated $1\ \mu\text{m}$ beads appear as a dark spot next to the tip of the single mode optical fiber in the right half of the picture. Accumulation was performed by illuminating with $80\ \mu\text{W}$ for 1 min and scanning the applied potential between 0 and 0.6 V (200 mV/sec).

to a location with complementary capture probes and a location with non-complementary capture probes and were electronically hybridized. This step produced two clearly detectable fluorescence signals at the two locations with matching sequences (Figure 5b). The average value (multiple experiments) for the ratio between signal and non-specific background was close to 10, however, the standard deviation was more than 60%. This high value is expected since it derives from the product of the standard deviations of both the capture and target strand deposition steps. Nevertheless, the signal to non-specific background ratios were sufficient to allow easy discrimination between the matching and non-matching DNA sequences.

Although additional optimization of the silicon substrate preparation and Mn_2O_3 film deposition is necessary to achieve the reproducibility and accuracy currently attained on prepatterned microchip arrays,^{10–12} the ability to pattern different capture probes in an arbitrary fashion with arbitrary spot sizes offers, in concept, alternatives in assay design as compared to those currently available and accommodates sample handling procedures and imaging requirements alternative to those now employed.

IV. Photoelectrophoretic Transport of Beads. As an expansion of the above methodology, we also performed photoelectrophoretic transport using negatively charged polystyrene beads on Mn_2O_3 -coated single-crystal silicon samples ($1\text{--}4\ \Omega\text{-cm}$). Figure 6 shows an example of localized bead aggregation induced by application of a scanned potential under illumination with the optical fiber. The diameter of the bead cluster is only slightly larger than the illuminated spot size of about $20\ \mu\text{m}$ (no agarose), indicating minimal photocurrent spreading on this sample. The applied potential was scanned rather than kept constant because it was observed that the beads adhered to the surface unless the potential was periodically lowered to 0 V. By slowly changing the position of the optical fiber, beads can also be transported across the surface. Potentially, this may be useful for micropositioning applications performed in aqueous or non-aqueous environments.³¹

Conclusions

We have shown that Mn_2O_3 -stabilized n-type silicon photoelectrodes coated with a streptavidin–agarose permeation layer constitute a simple platform for rapid manipulation of DNA oligonucleotides by photodirected electrophoretic transport. In addition, specific electronic hybridization between arbitrarily pre-positioned capture oligonucleotides and phototransported complementary oligonucleotides has been demonstrated. We have also shown that the same platform can be used for accumulation or transport of micrometer-sized objects, for example, polystyrene beads, a technique that might ultimately be employed in micropositioning or object sorting tasks.

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