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Controlling Oligonucleotide Surface Density in Light-Directed DNA Array Fabrication

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Abstract

Over the last two decades high-density DNA arrays have developed into a central technology for nucleic acid analyses. Important application areas include whole-genome gene expression studies, high throughput analyses of single nucleotide polymorphisms, and most recently, the determination of binding site specificities for transcription factors and other critical elements involved in gene regulation. A key parameter in the performance of DNA arrays is the density of the surface-bound oligonucleotides, which strongly affects both thermodynamic and kinetic aspects of DNA hybridization. In this report, we describe an approach for the control of oligonucleotide density in photolithographically fabricated DNA arrays, based upon a controlled UV light deprotection procedure. Modulation of the UV exposure permits a desired degree of deprotection of surface synthesis sites; a subsequent capping reaction to inactivate the exposed sites leaves only a desired fraction of active sites remaining for synthesis, corresponding to a lower oligonucleotide density. It is shown that the procedure is reasonably general, in that it is readily transferable to alternative substrate materials, yielding similar results.

INTRODUCTION

Planar arrays of oligonucleotide probes have brought fundamental changes in nucleic acid analysis, including genotyping,^{1, 2} resequencing,^{3, 4} genome-scale expression analysis,^{5, 6} single nucleotide polymorphism (SNP) detection,^{7–9} and sequence capture and enrichment.^{10, 11} These studies are based on either hybridization of labeled nucleic acids to arrays or enzymatic reactions on array surfaces. Recent years have also seen emergence of the application of DNA arrays to molecular recognition studies. Sequence-recognition properties of DNA-binding proteins and small molecules have been determined on DNA arrays in a high-throughput fashion.^{12–15}

Surface oligonucleotide density is crucial for a wide variety of applications of DNA arrays. The hybridization of complementary strands of DNA, which is the basis of all array-based techniques for nucleic acid analysis, is strongly dependent on surface oligonucleotide density both thermodynamically and kinetically.^{16–19} The thermodynamic stability of double-stranded DNA is not just a function of the nucleic acid sequences, but is also affected by nearest-neighbor interactions or molecular crowding as determined in part by oligonucleotide density on the surface.^{20, 21} Moreover, by affecting the kinetics of target/probe hybridization, surface

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oligonucleotide density plays an important role in the efficiency of duplex formation, as thermodynamic equilibrium may in some cases require excessively long incubation times.¹⁶ Many groups have also reported the effects of surface oligonucleotide density on signal intensity, equilibration time, and binding affinity for protein-DNA recognition studies on DNA arrays.^{22–24}

For DNA arrays fabricated by deposition and immobilization of pre-synthesized DNA sequences on functionalized substrates such as gold and glass slides, surface oligonucleotide density can be controlled by varying immobilization conditions, including pre-synthesized DNA strand concentration, solution ionic strength, interfacial electrostatic potential, whether duplex or single stranded oligonucleotides are used, and reaction time.^{16, 22, 23, 25–27} During the immobilization, surface oligonucleotide density is mainly determined by the electrostatic attraction/repulsion between oligonucleotides and the surface, and between oligonucleotides themselves as they become increasingly densely packed on the surface. Because of differences in the properties of various substrate materials, protocols for the control of surface oligonucleotide density are not generally transferable between different surfaces even when the same attachment chemistry is employed. Even for the same substrate material, reproducibility can be compromised by surface heterogeneity and other factors. It has also been demonstrated that non-electrostatic effects, such as DNA conformation, flexibility, or length, can also play a role in determining the density of the immobilized oligonucleotide probes.^{16, 28}

The situation is different, however, when arrays are prepared using *in situ* methods.²⁹ Light-directed oligonucleotide synthesis was the first such approach, and remains the most widely used. It provides an efficient and versatile method for fabricating arrays with hundreds of thousands of different oligonucleotide sequences/cm².^{30–33} Oligonucleotide building blocks with photolabile 5'-protecting groups such as 2-(2-nitrophenyl)-propoxycarbonyl (NPPOC),³⁴ ((methyl-2-nitropiperonyl)-oxy)carbonyl (MeNPOC),³³ or dimethoxybenzincarbonate (DMBOC)³⁵ have been used for synthesis. After the initial coupling of a 5'-protected monomer to an appropriately modified surface, the 5'-protecting group is removed by irradiation with light, exposing a hydroxyl group for the reaction with a subsequent monomer. The use of such photolabile protecting groups allows the synthesis to be performed in a spatially addressable format.

Oligonucleotide densities on DNA arrays prepared using *in situ* methods are mainly determined by the properties of the substrates themselves and the chemistries employed for surface functionalization.³⁶ There have been very few reports on the control of oligonucleotide density for *in situ* synthesized DNA arrays.²⁰ We describe here an approach for the control of oligonucleotide density in the light-directed synthesis of DNA arrays. We first study the UV light exposure dose dependence of photo-deprotection of NPPOC-protected oligonucleotide building blocks on glass. By controlling the UV light exposure dose on the surface and capping exposed hydroxyl groups with non-photolabile groups, we are able to control the density of reactive sites for further phosphoramidite condensation with good reproducibility. Similar results are obtained on three different substrate materials where different attachment chemistries are employed, namely silane functionalized glass, UV photochemically functionalized amorphous carbon,³⁷ and glassy carbon,³⁸ indicating that the approach is generally applicable.

MATERIALS AND METHODS

Surface Preparation

Glass—Superclean glass slides (TeleChem International, Sunnyvale, CA) were immersed for 4 hours at room temperature in a stirred solution of 2% (v/v) N-(3-triethoxysilylpropyl)-4-

hydroxy-butylamide (Gelest, Morrisville, PA) in 0.1% acetic acid in 95% ethanol. The slides were then rinsed in 95% ethanol for 15 minutes. After being rinsed three times in diethyl ether, slides were transferred to a pre-heated (120°C) oven for a minimum of 2 hours, after which time they were cured under vacuum overnight. Slides were stored in a desiccator until ready for use.

Glassy carbon—Glassy carbon plates (2.5 cm × 4.0 cm) (Werkstoffe GmbH, Thierhaupten, Germany) were heated to ~900°C under vacuum and treated with a 13.56 MHz inductively coupled H-plasma for 20 minutes at 50 Torr to generate a hydrogen terminated surface.³⁹ Following hydrogen termination, 40 µL of 9-decene-1-ol (Aldrich, Milwaukee, WI) was placed directly onto the newly hydrogen-terminated surface and covered with a quartz coverslip. The surfaces were irradiated under N₂ purge with a low-pressure mercury vapor quartz grid lamp ($\lambda = 254$ nm, 0.35 mW/cm²) for 12 hours. After the photoreaction, they were rinsed with copious amounts of ethanol and deionized water, dried under nitrogen, and stored in a desiccator until ready for use.

Amorphous carbon—The amorphous carbon substrates were prepared on standard glass slides (VWR, PA). Prior to use, glass substrates were rinsed with copious amounts of deionized water and methanol and dried under a nitrogen stream. A 2.0 nm chromium layer followed by a 50 nm gold layer was applied to the substrates using an Angstrom Engineering Åmod metal evaporator (Cambridge, ON). After metal deposition, amorphous carbon thin films were applied to the surfaces by DC magnetron sputtering (Denton Vacuum, Moorestown, NJ). 40 µL of 9-decene-1-ol was placed directly onto the surfaces coated with amorphous carbon and covered with a quartz coverslip. The surfaces were irradiated under N₂ purge with a low-pressure mercury vapor quartz grid lamp ($\lambda = 254$ nm, 0.35 mW/cm²) for 12 hours. After the photoreaction, the surfaces were rinsed with copious amounts of ethanol and deionized water, dried under nitrogen, and stored in a desiccator until ready for use.

In situ oligonucleotide synthesis

Oligonucleotide synthesis reagents activator (0.25 M 5-benzylthio-1H-tetrazole in anhydrous acetonitrile), dry wash (acetonitrile), amidite diluent (acetonitrile), and deblocking mix (3% dichloroacetic acid in dichloromethane (3% DCA/DCM)) were purchased from Glen Research (Sterling, VA); oxidizer solution was purchased from EMD Chemicals (Gibbstown, NJ); exposure solvent was purchased from Roche NimbleGen (Madison, WI). All anhydrous reagents were kept over molecular sieves (AldraSORB™ water trapping packets, Aldrich, Milwaukee, WI). All NPPOC (3'-nitrophenylpropyloxycarbonyl) protected phosphoramidites [5'-NPPOC-dAdenosine(tac) 3'-β-cyanoethylphosphoramidite (NPPOC-dA), 5'-NPPOC-dThymidine 3'-β-cyanoethylphosphoramidite (NPPOC-dT), 5'-NPPOC-dCytidine(ib) 3'-β-cyanoethylphosphoramidite (NPPOC-dC), 5'-NPPOC-dGuanosine(ipac) 3'-β-cyanoethylphosphoramidite (NPPOC-dG)] were manufactured by Proligo Biochemie GmbH (Hamburg, Germany) and purchased from Roche Nimblegen. NPPOC-Phosphoramidites were diluted (1 g in 60 mL) with amidite diluent. Cy3 phosphoramidite and 5'-dimethoxytrityl (DMT) -dThymidine 3'-β-cyanoethylphosphoramidite (DMT-dT) were purchased from Glen Research.

Light-directed photolithographic synthesis was performed as described in detail elsewhere.^{30, 31, 36} Briefly, a digital micromirror-based Maskless Array Synthesizer (MAS) was connected to a Perspective Biosystems Expedite Nucleic Acid Synthesis System (Framingham, MA). Oligonucleotide synthesis was carried out in a base-by-base manner using a modified DNA synthesis procedure, where the removal of the protecting group – photolabile NPPOC group was achieved by irradiation with 5.0 J/cm² of 365 nm light from a 350 watt mercury arc lamp (Newport, Stratford, CT), the power of which was fixed at 100 mW/cm² at 365 nm.

Addition of NPPOC-protected phosphoramidites proceeded as follows: (a) after condensation of the previous NPPOC-protected base to the growing DNA strand, the synthesis flow cell was flushed with 500 μL of exposure solvent; (b) a digital image on a Digital Micromirror Device (DMD) (Texas Instruments, TX) representing the locations for the next base addition illuminated the surface with 5.0 J/cm^2 . Exposure solvent was kept flowing through the flow cell during illumination, sufficiently maintaining the basic conditions needed to drive the photo-catalyzed elimination reaction; (c) following irradiation, the array was washed with acetonitrile ($\sim 500 \mu\text{L}$) to remove residual exposure solvent and dry wash ($\sim 300 \mu\text{L}$) to remove trace water; (d) coupling of the next base began with filling the flow cell with activator solution ($\sim 100 \mu\text{L}$) followed by a 1:1 solution of the desired phosphoramidite and activator. All 5'-NPPOC-protected amidites underwent a 60 second coupling step; (e) after amidite coupling, the array was washed with acetonitrile ($\sim 100 \mu\text{L}$) and either oxidized by flushing the cell with oxidizer solution ($\sim 500 \mu\text{L}$) or subjected to the next addition. The oxidation of the backbone phosphine groups was performed after every 4th coupling step and at the end of the synthesis, rather than at every coupling step; (f) after synthesis was complete, the nucleoside bases were deprotected in 1:1 ethylenediamine:ethanol solution (EDA/EtOH) at room temperature for 2 hours.

Surface oligonucleotide density control

Photodeprotection dose—Scheme 1 illustrates a typical strategy for controlling the photodeprotection ratio of the NPPOC-protected surface. Fifteen Ts were first synthesized on the functionalized glass slides using the standard light-directed synthesis protocol described above. A digital mask as shown in Scheme 1B was employed during synthesis. The black area in the image indicates light that is blocked by the DMD, while the white areas show the regions of illumination. The removal of the NPPOC group for the first fourteen Ts was achieved by 5.0 J/cm^2 irradiation from a 350 watt mercury arc lamp (50 seconds, 100 mW/cm^2).

For the deprotection of the fifteen Ts, a set of 15 digital masks was displayed in a predetermined order and for a predetermined duration as shown in Scheme 1A. This process generated an array of stripes with a gradient of increasing/decreasing exposure dose ranging from 0.2 J/cm^2 to 7.5 J/cm^2 (increasing in the first and third rows, decreasing in the second and fourth rows), and therefore increasing/decreasing the extent of deprotection. After this series of deprotection steps, the final pattern as shown in Scheme 1B was “stained” by coupling the Cy3 phosphoramidite or “capped” by coupling the DMT-dT phosphoramidite (Figure 1).

Fluorescence labeling and imaging—The free hydroxyl groups produced on the glass surface after light-directed removal of the NPPOC groups were terminally labeled by coupling with a Cy3 phosphoramidite / DMT-dT phosphoramidite mixture (5 mM Cy3 phosphoramidite in amidite diluent containing 45 mM DMT-dT phosphoramidite). This dilution of the Cy3 phosphoramidite reduces fluorescence quenching due to dye-dye interactions on the surface.^{40, 41} The mixture was subjected to two 300 second coupling steps. The DMT and Cy3 monomethoxytrityl (MMT) protecting groups were then removed by pumping 3% DCA/DCM through the flow cell. The slides were then soaked in a solution of 1:1 EDA/EtOH solution at room temperature. After a 30 minute incubation period, the terminally Cy3 labeled slides were rinsed with ethanol and water and dried with nitrogen. The surface fluorescence was imaged in 1 $\times$ SSPE buffer (10 mM NaH_2PO_4 , 0.15 M NaCl, 1 mM EDTA, pH 7.4) using a Genomic Solutions GeneTAC UC 4 $\times$ 4 scanner (Ann Arbor, MI).

DMT-dT capping—The free hydroxyls generated by photoremoval of NPPOC groups were subjected to DMT-dT phosphoramidite (67 mM DMT-dT phosphoramidite in amidite diluent) capping. Two 60 second coupling steps were employed to eliminate non-capped hydroxyl groups on surfaces. Then a 5.0 J/cm^2 UV irradiation was used to remove remaining NPPOC

groups, exposing hydroxyl groups which provided a starting point for subsequent light-directed synthesis of the remaining 19 bases of oligonucleotide 1. DMT groups which remained on the surfaces were removed by 3% DCA/DCM at the end of the synthesis. The slides were then soaked in 1:1 EDA/EtOH solution at room temperature for 2 hours before hybridization.

DNA hybridization and imaging—Fluorescently-tagged oligonucleotide 2, complementary to oligonucleotide 1, was purchased from IDT (Coralville, IA; see Table 1 for all oligonucleotide sequences). All arrays were hybridized by placing 50 μ L of the fluorescently-tagged complement (1 μ M in 1 $\times$ SSPE buffer) on the surface, covering with a coverslip, and incubating for 30 minutes in a humid chamber. Nonspecifically bound DNA was removed by incubating the surface in 1 $\times$ SSPE buffer at 37°C for 10 minutes. Arrays hybridized with fluorescein-labeled complementary strands were imaged in 1 $\times$ SSPE buffer.

RESULTS AND DISCUSSION

Hydroxyl groups are the initiation sites for light-directed DNA synthesis on both glass-based and carbon-based substrates.^{36, 42} Accordingly, varying the density of surface hydroxyl groups controls the density of sites available for phosphoramidite coupling on surfaces. The central idea of the work presented here is to control surface oligonucleotide density by using the UV irradiation dose to manipulate the density of free surface hydroxyl groups.

Figure 1 shows a schematic diagram of the strategy employed. We generally utilize a dT₁₅ spacer for all surface oligonucleotides, as such a spacer is necessary for optimum hybridization efficiency on surfaces.^{43–45} Such a fifteen T spacer is first synthesized at all accessible sites on the substrate using standard NPPOC-amidite chemistry. A subset of the terminal NPPOC groups on the 15th T is then removed by UV exposure and capped with DMT-dT phosphoramidite. Those probes on the surface with NPPOC protecting groups are available for reaction in the next round of light-directed synthesis, while those probes capped with non-photolabile DMT groups are no longer available. In this manner, the density of probes synthesized on the surface (starting after the 15th T of the polyT spacer) may be controlled.

The density of surface hydroxyl groups was monitored by reacting the dT₁₅-modified surface with the phosphoramidite derivative of the fluorescent dye Cy3 as described in the Materials and Methods section. The Cy3 phosphoramidite is diluted 1:10 with a DMT-dT phosphoramidite to reduce surface quenching effects.^{40, 41} Figure 2A shows an example of the results obtained when the UV exposure time is systematically varied from 0 to 75 seconds. As shown in Figure 2B, the resultant fluorescence intensities are described well by a first-order exponential with a rate constant k of 0.091 s⁻¹ (half-life of 7.6 seconds), as expected for a simple first-order deprotection reaction. The range of exposure values below approximately 40 seconds (4.0 J/cm²) is the region within which it is possible to control the density of surface hydroxyl groups by varying exposure time. A similar value for the exposure dose needed for complete deprotection was obtained in previous studies.³⁶ Figure 2B shows that the density of surface sites is readily controlled over approximately an order of magnitude by variation of the exposure dose. For example, 80%, 60%, 40%, and 20% relative densities are obtained with exposure doses of 0.4 J/cm², 0.7 J/cm², 1.0 J/cm², and 1.7 J/cm², respectively. If the exposure dose is increased to 2.2 J/cm², a surface density of only 10% of the original full coverage is obtained.

In addition to the above characterization of the number of reactive surface hydroxyls present on the surface after various exposure times, we also wished to determine the relationship between the exposure dose and the eventual density of hybridizable oligonucleotides (“hybridization density”) fabricated on the surface. This measurement was done by hybridization of a fluorescently labeled complement to the DNA array, followed by

fluorescence imaging. A dT₁₅ spacer was synthesized at full density, and variable exposure doses for the 15th dT were employed to vary the density of surface hydroxyl groups. DMT-dT phosphoramidite was then used to cap the newly exposed hydroxyl groups. Subsequent synthesis occurred only on the non-capped (NPPOC-protected) oligonucleotides. The remaining 19 bases of oligonucleotide 1 were then synthesized. After synthesis of oligonucleotide 1 at different densities was complete, the slides were hybridized with fluorescein-tagged complementary strands (oligonucleotide 2) and scanned under buffer. The results are shown as hybridization fluorescence images in Figure 3A, and in graphical form in Figure 3B. These measured hybridization fluorescence intensities were averaged and normalized as described for the terminal Cy3 labeling experiments. As seen in Figure 3B, the plot of normalized hybridization fluorescence intensities (inverted triangles ▼) matches very well with the relative probe densities derived from the terminal fluorescence labeling experiments (squares ■), indicating that the hybridization efficiency was approximately constant for all probe densities. This result is not surprising, as previous work from our laboratory has shown the density of hybridizable sites on the glass surfaces employed for these experiments to be about 2.7×10^{12} molecules/cm²,³⁶ and it has been shown that at surface densities in that range essentially 100% of probes can be hybridized.^{24, 46}

In contrast to previously described strategies for the control of surface oligonucleotide density, which generally depend upon the immobilization or synthesis conditions employed, surface oligonucleotide density in the approach presented here is determined solely by the NPPOC deprotection efficiency, which in turn is determined by the UV irradiation dose. This suggested that the same approach would work equally well for the control of oligonucleotide density on a variety of surfaces other than the glass substrates employed for the experiments presented above. Accordingly, we evaluated two other surfaces, glassy carbon, and amorphous carbon deposited as a thin film on a gold sublayer.⁴² Unlike glass substrates, which are modified using reactive silane reagents dissolved in organic solvents, carbon substrates may be functionalized by UV light-mediated reactions with alkenes, yielding carbon-carbon bonds between the surface and the molecule of interest.³⁷ Biomolecular arrays prepared on such carbon-based substrates offer superior stability compared to their glass counterparts under a variety of conditions.^{36, 42}

Figure 4 shows the results of hybridization fluorescence intensity studies identical to those of Figure 3B, but on the two carbon-based substrates. The hybridization observed for the glassy carbon substrate is indistinguishable from that obtained on glass. The results obtained from the amorphous carbon substrate were also quite similar, but deviated somewhat at higher surface coverages. The small difference observed in hybridization intensity as a function of exposure time for the amorphous carbon substrate compared with the glass and glassy carbon substrates is likely due to two factors: first, the probe density is higher on amorphous carbon than on the two other substrates (data not shown); second, the hybridization efficiency is lower at higher probe density – that is, a smaller fraction of sites are occupied by complements in a given time period. These two factors cause a more gradual decrease in the hybridization intensities for the amorphous carbon surfaces.

SUMMARY

We present here a study using UV irradiation on NPPOC-protected oligonucleotide building blocks to control surface oligonucleotide densities on light-directed synthesized DNA arrays. Unlike most of the other density control strategies which involves time-consuming immobilization or synthesis condition adjustment and optimization, in this approach surface oligonucleotide density could be controlled over an order of magnitude with good reproducibility by simply adjusting the UV irradiation dose. This approach is readily applicable to other light-directed synthesis-compatible substrates.

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REFERENCE

1. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shaperro MH, Carson AR, Chen WW, Cho EK, Dallaire S, Freeman JL, Gonzalez JR, Gratacos M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang FT, Zhang JJ, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME. *Nature* 2006;444:444–454. [PubMed: 17122850]
2. Lucito R, Healy J, Alexander J, Reiner A, Esposito D, Chi MY, Rodgers L, Brady A, Sebat J, Troge J, West JA, Rostan S, Nguyen KCQ, Powers S, Ye KQ, Olshen A, Venkatraman E, Norton L, Wigler M. *Genome Res* 2003;13:2291–2305. [PubMed: 12975311]
3. Herring CD, Palsson BO. *Bmc Genomics* 2007;8. [PubMed: 17210083]
4. Herring CD, Raghunathan A, Honisch C, Patel T, Applebee MK, Joyce AR, Albert TJ, Blattner FR, van den Boom D, Cantor CR, Palsson BO. *Nat. Genet* 2006;38:1406–1412. [PubMed: 17086184]
5. Nuwaysir EF, Huang W, Albert TJ, Singh J, Nuwaysir K, Pitas A, Richmond T, Gorski T, Berg JP, Ballin J, McCormick M, Norton J, Pollock T, Sumwalt T, Butcher L, Porter D, Molla M, Hall C, Blattner F, Sussman MR, Wallace RL, Cerrina F, Green RD. *Genome Res* 2002;12:1749–1755. [PubMed: 12421762]
6. Griffith M, Tang MJ, Griffith OL, Morin RD, Chan SY, Asano JK, Zeng T, Flibotte S, Ally A, Baross A, Hirst M, Jones SJM, Morin GB, Tai IT, Marra MA. *Nat. Methods* 2008;5:118–118
7. Wang L, Luhm R, Lei M. *Adv. Exp. Med. Biol* 2007;593:105–116. [PubMed: 17265721]
8. Chen Y, Shortreed MR, Olivier M, Smith LM. *Anal. Chem* 2005;77:2400–2405. [PubMed: 15828773]
9. Chen Y, Shortreed MR, Peelen D, Lu MC, Smith LM. *J. Am. Chem. Soc* 2004;126:3016–3017. [PubMed: 15012108]
10. Okou DT, Steinberg KM, Middle C, Cutler DJ, Albert TJ, Zwick ME. *Nat. Methods* 2007;4:907–909. [PubMed: 17934469]
11. Olson M. *Nat. Methods* 2007;4:891–892. [PubMed: 17971778]
12. Bulyk ML. *Curr. Opin. Biotechnol* 2006;17:422–430. [PubMed: 16839757]
13. Bulyk ML, Gentalen E, Lockhart DJ, Church GM. *Nat. Biotechnol* 1999;17:573–577. [PubMed: 10385322]
14. Warren CL, Kratochvil NCS, Hauschild KE, Foister S, Brezinski ML, Dervan PB, Phillips GN Jr, Ansari AZ. *Proc. Natl. Acad. Sci. U. S. A* 2006;103:867–872. [PubMed: 16418267]
15. Navani NK, Li YF. *Curr. Opin. Chem. Biol* 2006;10:272–281. [PubMed: 16678470]
16. Peterson AW, Heaton RJ, Georgiadis RM. *Nucleic Acids Res* 2001;29:5163–5168. [PubMed: 11812850]
17. Watterson JH, Piuino PAE, Wust CC, Krull UJ. *Langmuir* 2000;16:4984–4992.
18. Gong P, Harbers GM, Grainger DW. *Anal. Chem* 2006;78:2342–2351. [PubMed: 16579618]
19. Gong P, Lee CY, Gamble LJ, Castner DG, Grainger DW. *Anal. Chem* 2006;78:3326–3334. [PubMed: 16689533]
20. Song F, Krull UJ. In *Optical Waveguide Sensing and Imaging* 2008:195–210.
21. Wong KY, Pettitt BM. *Theor. Chem. Acc* 2001;106:233–235.
22. White RJ, Phares N, Lubin AA, Xiao Y, Plaxco KW. *Langmuir* 2008;24:10513–10518. [PubMed: 18690727]
23. Ricci F, Lai RY, Heeger AJ, Plaxco KW, Sumner JJ. *Langmuir* 2007;23:6827–6834. [PubMed: 17488132]
24. Yu F, Yao DF, Knoll W. *Nucleic Acids Res* 2004;32:e75. [PubMed: 15155822]
25. Vainrub A, Pettitt BM. *Biopolymers* 2003;68:265–270. [PubMed: 12548628]

26. Peterson AW, Wolf LK, Georgiadis RM. *J. Am. Chem. Soc* 2002;124:14601–14607. [PubMed: 12465970]
27. Cattaruzza F, Cricenti A, Flamini A, Girasole M, Longo G, Prosperi T, Andreano G, Cellai L, Chirivino E. *Nucleic Acids Res* 2006;34:e32. [PubMed: 16507670]
28. Steel AB, Levicky RL, Herne TM, Tarlov MJ. *Biophys. J* 2000;79:975–981. [PubMed: 10920027]
29. Gao XL, Gulari E, Zhou XC. *Biopolymers* 2004;73:579–596. [PubMed: 15048782]
30. Fodor SPA, Read JL, Pirrung MC, Stryer L, Lu AT, Solas D. *Science* 1991;251:767–773. [PubMed: 1990438]
31. Singh-Gasson S, Green RD, Yue Y, Nelson C, Blattner F, Sussman MR, Cerrina F. *Nat. Biotechnol* 1999;17:974–978. [PubMed: 10504697]
32. Lipshutz RJ, Fodor SPA, Gingeras TR, Lockhart DJ. *Nat. Genet* 1999;21:20–24. [PubMed: 9915496]
33. Pease AC, Solas D, Sullivan EJ, Cronin MT, Holmes CP, Fodor SPA. *Proc. Natl. Acad. Sci. U. S. A* 1994;91:5022–5026. [PubMed: 8197176]
34. Hasan A, Stengele KP, Giegrich H, Cornwell P, Isham KR, Sachleben RA, Pfeleiderer W, Foote RS. *Tetrahedron* 1997;53:4247–4264.
35. Pirrung MC, Fallon L, McGall G. *J. Org. Chem* 1998;63:241–246.
36. Phillips MF, Lockett MR, Rodesch MJ, Shortreed MR, Cerrina F, Smith LM. *Nucleic Acids Res* 2008;36:e7. [PubMed: 18084027]
37. Sun B, Colavita PE, Kim H, Lockett M, Marcus MS, Smith LM, Hamers RJ. *Langmuir* 2006;22:9598–9605. [PubMed: 17073485]
38. Fitzer E. *Angew. Chem., Int. Ed. Engl* 1980;19:375–385. [PubMed: 6773441]
39. Yang WS, Auciello O, Butler JE, Cai W, Carlisle JA, Gerbi J, Gruen DM, Knickerbocker T, Lasseter TL, Russell JN, Smith LM, Hamers RJ. *Nat. Mater* 2002;1:253–257. [PubMed: 12618788]
40. McGall GH, Barone AD, Diggelmann M, Fodor SPA, Gentalen E, Ngo N. *J. Am. Chem. Soc* 1997;119:5081–5090.
41. Beier M, Hoheisel JD. *Nucleic Acids Res* 2000;28:e11. [PubMed: 10648799]
42. Lockett MR, Weibel SC, Phillips MF, Shortreed MR, Sun B, Corn RM, Hamers RJ, Cerrina F, Smith LM. *J. Am. Chem. Soc* 2008;130:8611–8613. [PubMed: 18597426]
43. Guo Z, Guilfoyle RA, Thiel AJ, Wang RF, Smith LM. *Nucleic Acids Res* 1994;22:5456–5465. [PubMed: 7816638]
44. Southern E, Mir K, Shchepinov M. *Nat. Genet* 1999;21:5–9. [PubMed: 9915493]
45. Shchepinov MS, Case-Green SC, Southern EM. *Nucl. Acids Res* 1997;25:1155–1161. [PubMed: 9092624]
46. Vainrub A, Pettitt BM. *J. Am. Chem. Soc* 2003;125:7798–7799. [PubMed: 12822987]

Figure 1. Schematic of the Cy3 terminal labeling and density control on *in situ* synthesized DNA arrays. NPPOC groups are partially deprotected by UV irradiation after 15Ts were synthesized on the substrates. Cy3 phosphoramidite is coupled to the surface to monitor the percent of hydroxyl groups remaining after photodeprotection. DMT-dT phosphoramidite is used to cap newly exposed hydroxyl groups. Subsequent DNA synthesis is at reduced oligonucleotide densities as fewer NPPOC-protected sites are available. After the synthesis is complete, 3% DCA/DCM is used to remove DMT groups.

Figure 2.

(A) Fluorescence images of a glass substrate after partial NPPOC deprotection and Cy3 phosphoramidite coupling. The numbers on the image correspond to exposure times used for NPPOC deprotection for the stripes below. (B) Normalized fluorescence intensity as a function of UV irradiation dose. The background signal (region without UV irradiation) was subtracted from the measured fluorescent intensities and the results were normalized to a range from 0 (no UV irradiation) to 1 (75 sec or 7.5 J/cm²). The data points and error bars correspond to the averages and standard deviations obtained from 4 identical features, respectively. The UV irradiation dose is linearly proportional to exposure time, with a conversion factor of 0.1 J/cm²•sec. The data were fit to the first-order exponential shown in the figure (solid line). The rate constant of 0.091 s⁻¹ corresponds to a half life of 7.6 seconds.

Figure 3.

(A) Fluorescence images of a glass substrate after hybridization of fluorescein-tagged complementary strands to surface probes with different densities. The numbers on the image correspond to exposure times used for NPPOC deprotection for the stripes below. Longer initial exposure leaves fewer residual NPPOC-protected probes to serve as sites for further synthesis, and thus yields lower probe densities. (B) Normalized hybridization fluorescence intensity (▼) and percent of residual NPPOC groups (■) as a function of UV irradiation dose. The percent of residual NPPOC groups is replotted here from the data in Figure 2B, as $(1 - \text{normalized fluorescence intensity of Cy3 terminal labeling}) \times 100\%$, with the value of 75 seconds (7.5 J/cm^2) set to correspond to complete NPPOC deprotection (minimum fluorescence). The NPPOC percentage data were fit to the first-order exponential function shown in the figure (solid line).

Figure 4.
Comparison of normalized hybridization fluorescence intensities on different substrates.

Scheme 1.

Serial photodeprotection of NPPOC-protected surface. (A) a set of 15 digital masks were displayed serially for a predetermined duration. The first bar in the upper left corner was never illuminated in this deprotection series, the second bar in the first row from left was exposed for 2 seconds, the third bar for $2 + 2 = 4$ seconds, the fourth bar for $2 + 2 + 2 = 6$ seconds, ..., the fifteenth bar for $2 + 2 + 2 + \dots + 7 + 10 + 12 = 57$ seconds, and the sixteenth bar for $2 + 2 + 2 + \dots + 7 + 10 + 12 + 18 = 75$ seconds; (B) a digital mask employed for DNA synthesis. DNA synthesis only occurs in the white areas where UV light is reflected from the digital micromirror device. This is also the final deprotection pattern after the deprotection series. The numbers shown indicate the UV illumination time for each bar.

Table 1

Sequences used

	Sequence: 5' - 3'
Oligonucleotide 1	5'-ACTCTTGCAGGTCATCGGC(T) ₁₅ -3'
Oligonucleotide 2	5'-GCCGATGACCTGCAAGAGT-FAM-3'