New Approach to Oligonucleotide Microarrays Using Zirconium Phosphonate-Modified Surfaces

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Abstract: A new approach to oligonucleotide arrays is demonstrated that utilizes zirconium phosphonate-derivatized glass slides. The active slides are prepared by binding Zr^{4+} to surfaces terminated with organophosphonate groups previously deposited using either Langmuir−Blodgett or self-assembled monolayer methods. Oligonucleotide probes modified with a terminal phosphate bind strongly to the active zirconium phosphonate monolayer, and arrays for detecting fluorescent targets have been prepared using commercial spotting and scanning instruments. Preferred binding to the surface of the terminal phosphate of the modified probes instead of the internal phosphate diester groups is demonstrated and shown to yield increased fluorescence intensity after hybridization with labeled targets. A significant decrease in background signal is achieved by treating the slides with bovine serum albumin after spotting and before hybridization. A further increase in fluorescence after hybridization is observed when using a poly-guanine spacer between the probe oligomer and the terminal phosphate. Combining these modifications, an intensity ratio of nearly 1000 is achieved when comparing 5′-phosphate-modified 33-mer probes with unmodified probes upon hybridization with fluorescent targets.

Introduction

DNA arrays have emerged as a convenient tool in molecular biological research, for rapid and accurate gene mapping, DNA sequencing, mRNA expression analysis, and diagnosis of genetic diseases.[1−4] Typical sensors consist either of double-stranded products (PCR) or single-stranded oligonucleotides of different sequences, called probes, bound to a surface and amenable to subsequent hybridization by targets. Two general processes have established themselves for producing arrays.[5−16] In-situ syn-

thesis,[9−13] such as the Affymetrix photolithographic on-chip synthesis,[14−16] can lead to very high density oligonucleotide arrays. On the other hand, flexibility to customize arrays is an important factor, and "spotting" techniques that use automated robots to array oligonucleotides previously synthesized by chemical or enzymatic methods is the other widely used strategy.[8,17−28]

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For spotting systems, glass substrates are preferred, and since oligonucleotides bind poorly to glass, many types of surface-activated glass slides have been developed. The simplest binding mechanism is electrostatic adsorption, for example, onto poly-lysine-coated or aminosilane-coated slides.[17,18] The potential drawback is that electrostatic binding encourages the probes to lie on the surface, reducing hybridization efficiency. Another approach is to create surface-bound functional groups that can form covalent bonds with reactive function introduced at the end, usually the 5′-end, of the oligonucleotide probe. Some combinations of surface/olignucleotide function that have been demonstrated include thiol/acrylamide,[19] activated carboxylic acid/amine,[20,21] amine/aldehyde,[22–24] epoxide/amine,[25] and biotin/streptavidin.[26–28] With these specific linkages, the population of probes is attached in a homogeneous manner with good surface coverage and the probes retain full activity, provided that no nonspecific attachment occurs through the nucleobases.

The use of oligonucleotide arrays is expanding beyond their high-profile role in sequencing the genome to other fundamental applications, like RNA profiling, and clinical uses, such as disease diagnostics.[8,29] The different and sometimes specialized uses will put varied demands on the oligonucleotide arrays, requiring alternative approaches for preparing arrays that fulfill changing requirements. In contrast to organic covalent linkages, the use of "organic–inorganic" interactions to immobilize oligonucleotide probes into arrays on a surface has largely remained unexplored. The principal exception is the use of thiol-derivatized oligonucleotides to attach to metallic gold via a sulfur–gold linkage.[30–33]

In this paper, we demonstrate a new approach to oligonucleotide arrays that utilizes active zirconium phosphonate-derivatized glass slides.[34–39] Oligonucleotide probes with a free phosphate group bind strongly to glass substrates coated with a zirconium phosphonate monolayer, and the methodology can be used to form efficient oligonucleotide arrays. The specificity of binding through the terminal phosphate and the sensitivity of the arrays for detecting targets have been evaluated. Using the metal phosphonate substrates with 5′-modified probes and a polyguanine spacer between the anchoring phosphate and the probe sequence, a sensitivity enhancement of 1000 relative to the case of unmodified oligonucleotides has been observed.

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Experimental Section

Materials. Glass substrates were purchased from Gold Seal Products (cat no. 3010, $3 \times 1''$, thickness 0.93–1.05 mm). Oligonucleotides were purchased from MWG Biotech with the following structures (C = cytosine, G = guanine, A = adenine, T = thymine): 5′-$(H_2O_3PO)_{x}$–$(B)_n$–GACCCCAGAGGTATACATACGTTGCAGT-CAGGA-3′, **O33(X)** ($x = n = 0$), **5′H$_2$O$_3$PO–O33(X)** ($x = 1$ and $n = 0$), **5′H$_2$O$_3$PO–(G)$_n$–O33(X)** ($x = 1$, $n = 1, 3, 5, 7, 9, 11$ and B = G); 5′-$(H_2O_3PO)_{x}$–$(B)_n$-TGCACTCGAGATTCAGATCCTC-TTCTGAGATGA-3′, **O33(Y)** ($x = n = 0$), **5′H$_2$O$_3$PO–O33(Y)** ($x = 1$ and $n = 0$), **5′H$_2$O$_3$PO–(G)$_{11}$–O33(Y),** ($x = 1$, $n = 11$ and B = G); 5′-$(H_2O_3PO)_{x}$–$(B)_n$-CCGCCGGTAACCGGAGGTTAAGAT-CGAGATCCA-3′, **O33(Z)** ($x = n = 0$), **5′H$_2$O$_3$PO–O33(Z),** ($x = 1$ and $n = 0$), **5′H$_2$O$_3$PO–(G)$_{11}$–O33(Z),** ($x = 1$, $n = 11$ and B = G); 5′-(Cy3)-AGGACTGACGTTGCATACATATGGAGACCCCAG-OPO$_3$H$_2$-3′, **5′Cy3-O33(W)–OPO$_3$H$_2$**; 5′-(Cy3)-TCCTGACTGCAACG-TATGTATACCTCTGGGGTC, **comp-5′Cy3–O33(X)**; 5′-(Cy3)-TCATCTCAGAAGAGGATCTGAATCTCGAGTGCA, **comp-5′Cy3–O33(Y),** and 5′-(Cy3)-TGGATCTCGATCTTAACCTCCGGTTACC-GGCGG, **comp-5′Cy3–O33(Z)**. Reagents were of analytical grade and used as received from commercial sources, unless indicated. Zirconated octadecyl phosphonic acid (ODPA-Zr) Langmuir–Blodgett templates were prepared as described previously.[39] The procedure of Katz et al.[40] was followed to prepare slides derivatized with covalently linked phosphonic acids.

Typical Spotting Conditions. The slides were spotted with a quill-type pin microarrayer (SDDC2, Virtek) at a 250 μm spacing, using 16 steel tips and 5–50 μM oligonucleotide solutions in 1× SSC (saline sodium citrate, adjusted to pH 6 by addition of HCl). The spotted slides were placed overnight in a sealed slide box at room temperature and then rinsed successively with 2× SSC containing 0.1% SDS (sodium dodecyl sulfate, 2 min), 1× SSC (2 min), 0.2× SSC (2 times 2 min), and spun dry. Slides treated with BSA (bovine serum albumin) to passivate unspotted areas were treated after spotting with a solution of 1% BSA, 3.5× SSC, and 0.3% SDS at 42 °C for 1 h and then rinsed 5 times using ultrapure water and spun dry (centrifugal force = 40g). Hybridizations were performed in a mixture of 0.3% SDS, 3.5× SSC, 50% formamide, 5× Denhart's, and 10% TE (Tris-EDTA buffer, pH 7.8) containing the Cy3-labeled complementary oligonucleotide corresponding to a final 0.002 OD units/μL (concentration ≈ 5 μM), except where noted. The mixture was applied to the surface within marked boundaries, and a cover slip was placed over the solution. The arrays were sealed in a chamber with an underlayer of 1× SSC to provide humidification, after which they sat at 42 °C overnight. The arrays were immersed in 2× SSC containing 0.1% SDS for 2 min to remove the target solution and the cover slip, and they were allowed to rock gently in 1× SSC (2 min) and 0.2× SSC (2 times 2 min) and spun dry. The slides were scanned on a ScanArray 4000 scanner (GSI Lumonics, Packard). The fluorescence intensities in color images of the slides are color-coded, varying from blue (low) to green, yellow, red, and then white (saturation). The location of each analyte spot on the array was outlined using the mapping software GenePix (Axon Laboratories, Palo Alto, CA) and ScanAnalyze.[41] The background, calculated as the median of pixel intensities from the local area around each spot, was subtracted from the average pixel intensity within each spot.

Results and Discussion

Zirconium Phosphonate Surface and Spotting Conditions. Zirconium phosphonate-modified surfaces have previously been studied as starting points for depositing monolayer and multi-

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layer thin films of phosphonate-containing organic molecules[34−40,42−52] and for polyelectrolyte layer-by-layer assembly.[53,54] The starting surfaces have been prepared in several ways, including the direct adsorption of Zr^{4+} ions on silica,[51] covalent attachment of phosphorylated groups to silica[40,43−50] or Au,[40,42,43,45,52] and Langmuir−Blodgett deposition of an organophosphonic acid.[34−39] Our experience is that exceptionally smooth and uniform films that are structurally stable but chemically active can be generated on hydrophobic supports using Langmuir−Blodgett methods.[34−39]

The LB process begins with an octadecylphosphonic acid (ODPA) Langmuir monolayer that is deposited onto the hydrophobic solid support in such a way that the hydrophilic acid group (PO_3H_2) is directed away from the support.[34,35] The substrate is then removed from the LB trough and exposed to a solution of Zr^{4+} ions that bind to give a monolayer of the zirconated octadecylphosphonic acid (ODPA−Zr). In zirconium phosphonates, each Zr^{4+} ion coordinates to more than one phosphonate molecule and the phosphonates bind to more than one metal ion. Therefore, the extremely strong binding of the zirconium ions cross-links the original monolayer and provides a stable, well-defined interface of zirconium phosphonate sites.[34,35] The ODPA−Zr monolayer sticks strongly to the surface because it is no longer a traditional LB film of individual molecules physisorbed to the surface but rather a network or monolayer tape where adhesion comes from the sum of all molecules in a cross-linked array. The ODPA−Zr monolayers can be stored in water for months and retain activity with no evidence of desorption.

The metal ions now on the surface are active and react readily when exposed to other phosphonic acids or organophosphates to bind them to the surface (Figure 1). Extensive "self-assembled monolayer" chemistry based on binding organophosphonates to the zirconium phosphonate surface has been developed.[35−39] Monolayers prepared in this way are stable to a wide range of temperatures and aqueous and organic media. For example, in a recent study, we used these surfaces to bind monolayers of a phosphorylated manganese porphyrin oxidation catalyst and studied organic transformations at the monolayer with no desorption of the film.[39]

The coordination properties of free phosphate (OPO_3H_2) are very close to those of the phosphonic acid function, and phosphate species graft similarly to zirconium phosphonate surfaces.[45] As terminal phosphorylation of oligonucleotides can

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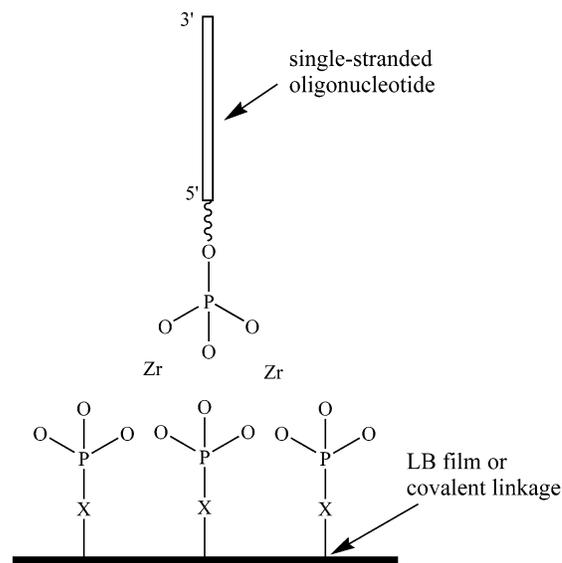

Figure 1. Modified probes immobilized on zirconated organophosphonate-treated slides. The phosphonate layer can be deposited using Langmuir−Blodgett methods (X is an aliphatic chain, ODPA) in which case the substrate is a hydrophobic surface, such as OTS-coated glass. Alternatively, it can be prepared by modifying a covalently attached layer, such as an aminopropylsilane film (X is aminopropylsilane).

be easily achieved using enzymatic (T4 polynucleotide kinase) and chemical (phosphoramidite chemistry) routes, we used such nucleotides to build microarrays on the ODPA−Zr surfaces using commercially available arrayers and scanners for the printing and imaging steps. The spotting conditions, described in the Experimental Section, were optimized using a fluorescent 5′-Cy3 tagged 33mer oligonucleotide with a 3′-phosphate modification, **5′Cy3−O33(W)−OPO₃H₂**. The bulky Cy3 label can only be introduced in the 5′-position, so the phosphate group was introduced in the 3′-position for optimizing the spotting conditions. Normally, 5′-phosphate modification is preferable and other experiments described here use 5′-modified probes. Several buffers were screened, including SSC (saline sodium citrate) with or without EDTA, phosphate buffer saline (PBS), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), acetate with or without NaCl, tris(hydroxymethyl)-aminomethane (Tris), carbonate, and TE (Tris-EDTA buffer). The highest fluorescence signals were consistently obtained using 1× SSC at pH 6. In general, basic buffers such as carbonate (pH 10) gave poor results. Phosphate buffers are expected to compete with the phosphate-modified oligonucleotide for binding the zirconated surface, and indeed, the probe binds at least 25 times better when spotted in 1× SSC (pH 6) than in 1× PBS.

As indicated, above, active zirconium phosphonate surfaces can be prepared in ways other than the Langmuir−Blodgett method.[40,42−52] For example, in one approach, an (aminopropyl)-silylated silicon oxide surface is treated with $POCl_3$ and a tertiary amine, followed by hydrolysis and treatment with aqueous $ZrOCl_2$,[40] resulting in zirconium phosphonate groups covalently tethered to the surface. These surfaces can also be used to form microarrays. However, in our hands the zirconium phosphonate substrates prepared using LB methods have been more reliable and the arrays formed from them more reproducible. To illustrate the concept and to explore a wide range of experimental variables, LB-prepared substrates have been used in the experiments reported here.

Table 1. Fluorescence Intensity[a] for 5′-Phosphorylated and Nonphosphorylated Probes after Hybridization with Comp-5′CY3–O33(X) (5 μM)

concentration of spotted oligonucleotide	O33(X)	5H₂O₃PO–O33(X)
50 μM	4300	27 000
20 μM	3900	26 000
5 μM	2200	15 500

[a] Slides were scanned at 80% of laser power and 70% of photomultiplier power.
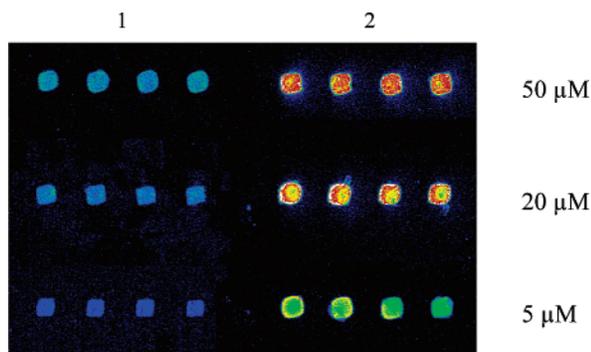
Figure 2. Fluorescence map of a zirconium phosphonate slide spotted with **O33(X)** (column 1) and **5′H₂O₃PO–O33(X)** (column 2) after hybridization with **comp-5′CY3–O33(X)** (5 μM). The different spotting concentrations are shown on the right. The fluorescence intensities are color-coded, varying from blue (low) to green, yellow, red, and then white (saturation). Spot sizes are 150 μm.

Specific Binding of the Terminal Phosphate. Previously, Xu et al.[55] reported the immobilization of double-stranded DNA at aluminum(III) alkanebisphosphonate thin films on electrode surfaces via interaction of the internal phosphate diester groups of the DNA backbone with the aluminum phosphonate sites. To establish that the modified oligonucleotides attach specifically to the zirconated surface through the terminal phosphate group, competition experiments were performed with phosphate-modified and unmodified probes. The phosphate modified **5′H₂O₃PO–O33(X)** and the unmodified analogue **O33(X)** were arrayed in quadruplicate for each of three spotting concentrations 5, 20, and 50 μM, on a total of six ODPA–Zr slides. After rinsing and centrifugation, the slides were stored for 24 h at ambient temperature and humidity. One-half of the slides were then hybridized with a Cy3-labeled 33 mer target carrying the complementary base sequence (**comp-5′Cy3–O33(X)**) and the others with a noncomplementary Cy3-labeled oligonucleotide. After rinsing, the slides were analyzed using a fluorescence slide scanner.

Fluorescent signals above background were observed only for the slides processed with the complementary target. The fluorescence intensity of spots with the modified and unmodified probes is compared in Table 1 and Figure 2. For each spotting concentration, the intensity from spots of the nonderivatized probe was approximately 15% of that measured from the probe with the 5′-phosphate modification, implying specific binding of the terminal phosphate to the zirconium phosphonate surface. The low but noticeable amount of target adsorption at the nonderivatized oligonucleotides likely arises from weaker binding of phosphodiester groups of the probe to the surface.[55]
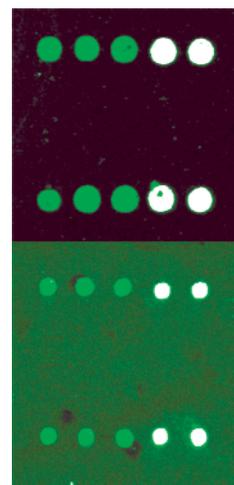

Figure 3. Fluorescence maps of zirconium phosphonate slides showing the increased signal-to-noise ratio upon treating with BSA prior to hybridization (top two rows) compared to the same system without BSA treatment (bottom two rows). The slides were spotted with **5′H₂O₃PO–O33(X)** at different concentrations and hybridized with **comp-5′CY3–O33-(X)** (5 μM). The fluorescence intensities are color-coded, varying from blue (low) to green, yellow, red, and then white (saturation). Spot sizes are 150 μm.

For both the modified and unmodified probes, the fluorescence of the spots begins to saturate at spotting concentrations above 20 μM.

Although binding of the unmodified probes as reported in Table 1 and Figure 2 is small, it is still measurable, indicating that in hybridization experiments nonspecific binding of fluorescent targets will lead to significant background. The low fluorescence background that arises from nonspecific binding of targets can be reduced by treating the slides with BSA solution prior to hybridization to saturate the nonspotted areas. The procedure is described in the Experimental Section, and fluorescence micrographs of segments of test arrays with and without the BSA treatment are shown in Figure 3. A significant decrease in the fluorescent background is evident. The BSA treatment inhibits nonspecific adsorption of target oligonucleotides but does not displace the covalently attached probes.

Including a Spacer between the Probe and the Surface. Enhanced hybridization is expected when the probe oligonucleotide is distanced from the support surface by a tether.[10,32,33,56,57] This observation, made for several other systems, is normally attributed to increasing the availability of the probe by relieving steric crowding.[10] A tether may also reduce nonspecific binding of the probe to the surface. To investigate this effect with the zirconium phosphonate surfaces, a spacer separating the probe from the terminal phosphate was introduced on the 5′-position. Oligomers of single bases were chosen as spacers because they are easily introduced via a synthesizer. Spacers of n-mer adenine $(A)_n$, guanine $(G)_n$, thymine $(T)_n$, and cytosine $(C)_n$ were introduced between the probe strands and the 5′-phosphate. Somewhat surprisingly, the identity of the spacer is significant.

Three sets of 33mer probes, **5′H₂O₃PO–(B)₁₁–O33(X)**, **5′H₂O₃PO–(B)₁₁–O33(Y),** and **5′H₂O₃PO–(B)₁₁–O33(Z),** were studied with 11-mer spacers of each of the bases, and hybridiza-

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Table 2. Fluorescence Intensity as a Function of the Nature of the Spacer Present on the Probes **5′H$_2$O$_3$PO−(spacer)−O33(X)**, **5′H$_2$O$_3$PO−(spacer)−O33(Y)**, and **5′H$_2$O$_3$PO−(spacer)−O33(Z)** after Hybridization with the Appropriate Cy3-Labeled Complements[a,b]

spacer	5′H$_2$O$_3$PO–(spacer)–O33(X)[c]	5′H$_2$O$_3$PO–(spacer)–O33(Y)[d]	5′H$_2$O$_3$PO–(spacer)–O33(Z)[e]
none	13 500 ± 500	17 000 ± 500	9000 ± 1500
(G)$_{11}$	35 000 ± 3000	40 000 ± 6000	21 000 ± 1500
(A)$_{11}$	11 000 ± 2000	16 000 ± 500	10 000 ± 1000
(T)$_{11}$	4500 ± 500	14 000 ± 1000	6500 ± 1500
(C)$_{11}$	4500± 500	8000 ± 1000	7000 ± 1500

[a] Slides were scanned at 75% of laser power and 65% of photomultiplier gain. [b] Average of three experiments. [c] 10 μM spotting concentration. [d] 50 μM spotting concentration. [e] 5 μM spotting concentration.

Table 3. Fluorescence Intensity[a,b] as a Function of the Length of the Poly-G Spacer Present on the Probe **5′H$_2$O$_3$PO−(G)$_n$−O33(X)** (20 μM spotting concentration) after Hybridization with **comp-5′CY3−O33(X)** (5 μM)

n	5′H$_2$O$_3$PO–(G)$_n$–O33(X)
0	17 000 ± 1000
1	13 000 ± 1000
3	11 000 ± 1000
5	27 000 ± 2000
7	44 000 ± 5000
9	42 000 ± 7000
11	36 000 ± 2000

[a] Slides were scanned at 75% of laser power and 65% of photomultiplier gain. [b] Average of three experiments.
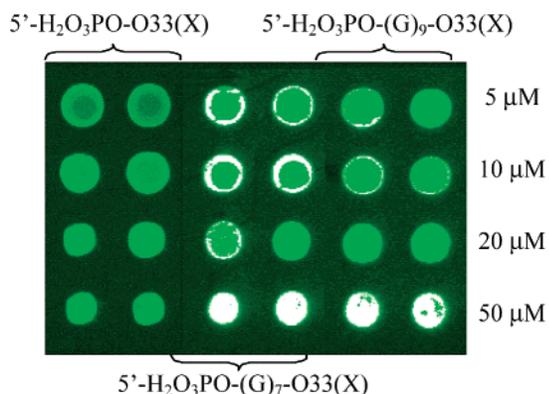
Figure 4. Fluorescence maps after hybridization comparing intensities of probes **5′H$_2$O$_3$PO−O33(X)**, **5′H$_2$O$_3$PO−(G)$_7$−O33(X)**, and **5′H$_2$O$_3$PO−(G)$_9$−O33(X)** each hybridized with **comp-5′CY3−O33(X)** (5 μM). The concentrations of the spotting solutions are indicated at the right. A significant increase in fluorescence intensity is seen for probes with a poly-G spacer. The fluorescence intensities are color-coded, varying from blue (low) to green, yellow, red, and then white (saturation). Spot sizes are 150 μm.

tion with the appropriate Cy3-labeled complements, **comp-5′Cy3-O33(X)**, **comp-5′Cy3-O33(Y)**, and **comp-5′Cy3-O33-(Z)**, was compared to the same probes without the spacers. The results, summarized in Table 2, clearly show that the nature of the spacer is critical. For each probe, a polyG spacer increases fluorescence after hybridization by a factor of 2 relative to the cases where no spacer is present. In contrast, the polyA spacer has essentially no effect while the polyT and polyC spacers actually lead to decreased hybridization relative to probes with no spacer. The same trend was observed for spotting concentrations from 5 to 50 μM, so the influence of the spacer is independent of the identity of the probe and its concentration. The influence of the length of the spacer was also studied. A series of polyG spacers, (G)$_n$, from $n = 1$ to 11 were compared (Table 3 and Figure 4), and the most pronounced effects were observed for $n = 7-11$.

Unlike other homopolymers, polyG does not normally exist in a single-stranded form, and this feature may be responsible

for the observed behavior. Studies have shown that single strands associate in parallel or in a variety of antiparallel orientations.[58−64] In the presence of potassium or sodium ions, such as is present in the sodium citrate buffer, strands of polyG can form stable four-stranded helices.[64] A possible consequence of the tendency of polyG segments to associate is an increase in surface coverage of the probes within a spot, either by inducing the probes to pack tighter or by forming multidentate aggregates that raise the avidity for the surface relative to single probes. Alternatively, the aggregates may be responsible for increased hybridization efficiency by providing rigid tethers that better orient the oligonucleotide for access by the targets. More detailed studies are required to fully understand the mechanism of the polyG effect.

System Sensitivity. The sensitivity of the system was then investigated using the three phosphorylated probes **5′H$_2$O$_3$PO−(G)$_{11}$−O33(X)**, **5′H$_2$O$_3$PO−(G)$_{11}$−O33(Y)**, and **5′H$_2$O$_3$PO−(G)$_{11}$−O33(Z)** and the unmodified analogues **O33(X)**, **O33(Y)**, and **O33(Z)**, each spotted at 10 μM on the same slide followed by treatment with BSA. For the hybridization step, the three complementary Cy3-labeled oligonucleotides were used individually and as a mixture of the three, each at a concentration to 100 nM (compared to 5 μM used in the previous experiments described in Tables 1−3 and Figures 2−4). Fluorescence was only detected at spots exposed to the appropriate complement. After hybridization, there is no significant decrease in the fluorescence intensity at the phosphorylated probes when using 100 nM target relative to the 5 μM target solutions, indicating good sensitivity of the ODPA−Zr chips (Figure 5). However, the most striking feature is that the intensity ratio measured for the phosphorylated oligonucleotides versus the unmodified probes is close to 1000. This result further illustrates the specific grafting of the oligonucleotide through the (G)$_{11}$–PO$_3$H$_2$ moiety. Also, the BSA treatment, in addition to passivating the unspotted regions of the slide, appears to wash off physisorbed probe molecules and those bonded only via the phosphate diester groups, leading to greater contrast between the modified and unmodified spots (compare Figures 2 and 5).

Conclusion

In conclusion, we demonstrated a new approach for covalently immobilizing oligonucleotides on glass slides for microarray

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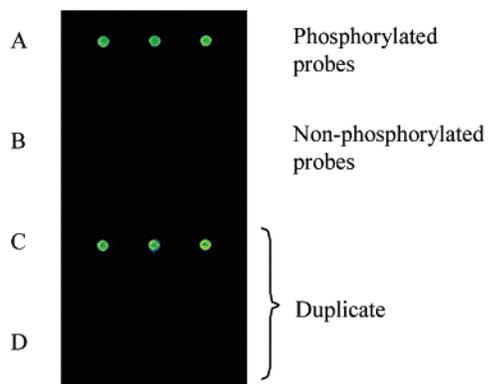
Figure 5. Fluorescence map of zirconium phosphonate slides comparing modified and unmodified probes after BSA treatment and hybridized with corresponding Cy3-labeled complements. The slides were spotted with **5′H₂O₃PO−(G)₁₁−O33(Z)** (lines A, C) and **O33(Z)** (lines B, D) and hybridized with 100 nM complements **comp-5′CY3−O33(Z)**. Spot size is 100 μm. The slides were scanned at 75% of laser power and 60% of photomultiplier gain. The fluorescence intensities are color-coded, varying from blue (low) to green, yellow, red, and then white (saturation).

applications that takes advantage of mixed organic/inorganic zirconium phosphonate chemistry. The fundamentally different attachment chemistry complements existing technology and may offer advantages for some applications, especially as the demands for oligonucleotide arrays become more varied. The zirconium phosphonate supports are stable for months or more and can be used directly, without needing activation prior to use. The process relies on modifying the oligonucleotides in the 5′-position by a free phosphate group, an easy modification that can be performed by enzymatic routes. The phosphate-modified probes can be used directly, and the only required postspotting treatment is rinsing. High specificity of the oligo-nucleotide anchoring onto the support has been demonstrated, along with good sensitivity of the resulting immobilized probes for detecting complementary targets. Although not yet inves-tigated, the methodology should be readily adapted to iono-covalent anchoring of phosphorylated double-stranded DNA prepared by PCR, a process that is harder with the more common organic covalent anchoring mechanisms because they require nonnatural modification of the probes.

While not required, improved signal-to-noise ratio is obtained by postspotting treatment with BSA to passivate the unspotted regions of the arrays. Signal-to-noise ratios as high as 1000 have been demonstrated after hybridizing with fluorescent targets. The BSA treatment displaces physisorbed probes and protects the array from nonspecific binding of targets. The BSA also provides a biocompatible hydrophilic surface. Further improve-ments in performance were observed when the probe is linked to the terminal phosphate via a spacer. Oligomeric guanine spacers of 7−11 units are optimal. The nature of the linker is important, as polyA, polyC, and polyT spacers did not show the same enhancement.