ChemComm

ROYAL SOCIETY OF CHEMISTRY

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2017, 53, 3094

Received 12th January 2017,
Accepted 15th February 2017

DOI: 10.1039/c7cc00288b

rsc.li/chemcomm

Reversible DNA micro-patterning using the fluorous effect†

Gabriella E. Flynn,[a] Jamie M. Withers,[b] Gerard Macias,[a] Justin R. Sperling,[a] Sarah L. Henry,[a] Jonathan M. Cooper,[a] Glenn A. Burley*[b] and Alasdair W. Clark*[a]

Open Access Article. Published on 23 February 2017. Downloaded on 12/03/2017 11:20:33.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

We describe a new method for the immobilisation of DNA into defined patterns with sub-micron resolution, using the fluorous effect. The method is fully reversible *via* a simple solvent wash, allowing the patterning, regeneration and re-patterning of surfaces with no degradation in binding efficiency following multiple removal/attachment cycles of different DNA sequences.

Of central focus to many chemical studies is molecular surface patterning, due to the potential impact of this research in scientific fields ranging from biosensing and diagnostics to computing.[1] Of particular interest is the patterning of biomolecules, more specifically DNA. This is due to its propensity for specific molecular recognition and its ability to self-assemble.[2] As such, its unique structural and chemical properties have seen it employed in an increasingly diverse range of applications. From biological screening to materials assembly, the integration of micro- and nano-patterned DNA with solid supports is being used to develop novel devices that bridge the gap between organic and inorganic engineering.[3–5] As a result, efficient and flexible surface immobilization chemistries are paramount to the future development of DNA-based technologies. These chemistries must adhere to a number of stringent requirements: accessibility of the surface-bound DNA strands; intact functionality of the DNA base-pairing mechanism; high density of attachment; low non-specific binding; and high array stability. However, many immobilisation strategies fail to meet these requirements.[6] Additionally, they tend to be static in nature, providing no opportunity to modify the composition of the DNA pattern after initial immobilisation.[7–9] This restricts the potential applications of these surfaces, particularly in biosensing and diagnostics, where such devices are limited to a single use.[10] Therefore, the development of chemistries that enable reversible and rewritable patterning would provide a route towards more versatile systems and devices. To that end, reversible DNA patterning has been demonstrated as a means to create complex micro-arrays for reusable screening applications.[11,12] Furthermore, dynamic DNA patterns could provide a route towards a surface-based implementation of the transformable nanoparticle systems, which has been demonstrated recently in solution, for applications in reconfigurable nanophotonics.[13]

Currently, reusable DNA microarray chips typically rely on a stripping mechanism to refresh the surfaces between each use.[14] This denatures the double stranded DNA leaving only the covalently bound single strand on the surface, which is then available to bind once more to the target. This process has many benefits, including reduced cost and higher consistency between experiments.[15] Typically, high temperature detergents are required to strip the surface, which are potentially detrimental to the integrity of the chip. Furthermore, this technique only allows for the same probes to be screened multiple times.[14] As a result, investigations concerning the development of new re-writable DNA platforms have become more widespread, and have concentrated on the use of disulphide bonds as reversible anchors for DNA immobilisation, as these covalent linkages are capable of reversible cleavage.[16,17] Although these methods offer strong binding, they require a large excess of reagent to achieve an efficient regeneration, and are often limited by surface degradation after multiple cycles (due to, for example, thiol oxidation on surfaces).[17] In contrast, fluorous surfaces are chemically inert, and the non-covalent nature of the ‘‘fluorous effect’’ allows for the complete removal of surface-bound biomolecules using simple solvent washes.[18] In this paper, we show for the first time the implementation of the fluorous effect for the reversible immobilisation of DNA micro-patterns on solid supports.

The fluorous effect refers to the observation that highly fluorinated or perfluorinated compounds have a tendency to exclude themselves from both aqueous and organic phases.[19] The resulting effect of this phenomenon is the product of reduced London dispersion forces between per-fluorinated compounds as a consequence of the extremely low polarizability of the C–F bond.

[a] Biomedical Engineering Research Division, School of Engineering, University of Glasgow, Rankine Building, Oakfield Avenue, Glasgow, UK. E-mail: Alasdair.clark@glasgow.ac.uk
[b] WestCHEM & Department of Pure & Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow, G1 1XL, UK. E-mail: glenn.burley@strath.ac.uk

† Electronic supplementary information (ESI) available: Experimental procedures and further data. See DOI: 10.1039/c7cc00288b

 This journal is © The Royal Society of Chemistry 2017

Open Access Article. Published on 23 February 2017. Downloaded on 12/03/2017 11:20:33.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

Molecular tagging with fluorous "ponytails" has the effect of drawing compounds into fluorous layers – a feature that has been widely exploited in synthetic chemistry to facilitate product purification.[19] "Fluorous affinity" tags have also been used as an effective means of immobilising carbohydrates and peptides on surfaces, and in screening protein-small molecule interactions.[18–26] Existing examples employ mechanical means to introduce samples, relying upon printing, stamping, or direct spotting of fluorous-tagged compounds in order to create defined, two-dimensional microarrays of molecular information.[21–26] The major advantages of this technology are the low sample volumes required and the ability for multiplexing, whereby multiple targets can be screened for on the same chip. However, a new printing/stamping step is required each time the surface is to be re-used. This is also true of the fabrication of many DNA microarray chips.[27] As a consequence, these techniques are often slow, are difficult to scale-up, and may rely on expensive printing devices.

In this manuscript we demonstrate that (i) short fluorous tags immobilise water-soluble oligodeoxyribonucleotides (ODNs) from aqueous solutions onto lithographically-defined, micro-patterned fluorous surfaces without the need to direct sample spotting; (ii) ODNs immobilised by this method remain available to hybridise with a complementary ODN; and (iii) the genetic information can be completely removed using a simple organic solvent wash, and fully replaced with different genetic information during a short incubation step. This was done 5 times with no degradation of the surface throughout the subsequent immobilisations steps.

To demonstrate the specificity of the fluorous effect to immobilise ODNs into defined regions, without preventing the ability of the strands to participate in hybridisation events, we fabricated micro-patterned fluorous surfaces using standard optical lithography. These surfaces contained arrays of 50 × 50 μm squares modified with (heptadecafluoro-1,1,2,2-tetrahydrodecyl)trimethoxysilane, while the regions surrounding the squares were modified with *n*-decyltrichlorosilane (Fig. 1). A 1 μM solution of **F-DNA1** (a 16-mer ODN containing an 8 carbon fluorous tag, see ESI†) was added to the surface and incubated for 2 hours before washing using TE buffer (10 mM TRIS, 1 mM EDTA (pH 8.0)). A 1 μM solution of the fluorescently labelled (TAMRA) complementary sequence, **cDNA1**, was then added to the surface and incubated in a humidity chamber for 2 hours. The surfaces were then rinsed in turn with TE buffer and DI water, and imaged using fluorescence microscopy at 20× magnification (0.4 NA). As Fig. 1b demonstrates, the fluorous immobilisation technique leaves the **F-DNA1** strands available for hybridisation, and the **F-DNA1**/**cDNA1** duplex was confined to the patterned fluorous areas.

One important issue in DNA microarray technology is non-specific binding, as this will ultimately limit both the sensitivity and the specificity of an assay.[6] To test the extent of non-specific binding on our novel surfaces, we introduced a fluorescently labelled (TAMRA), non-complementary strand (**ncDNA1**) to a substrate modified with a fluorous-**F-DNA1** micro-pattern. No fluorescence was observed upon addition of the non-complementary sequence to the surface, Fig. 1c, demonstrating that **cDNA1** adhesion was mediated solely by hybridisation with the fluorous **F-DNA1**, see Fig. 1 and 2.

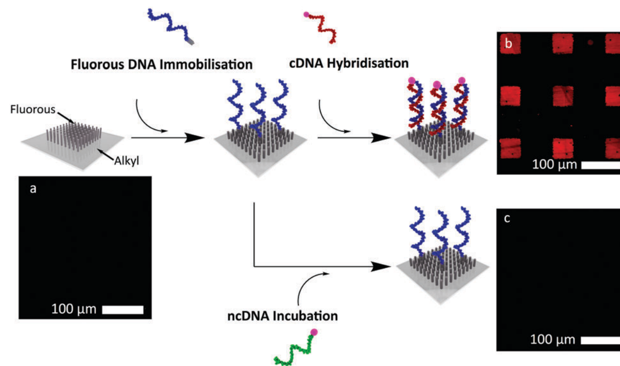


Fig. 1 Schematic of fluorous immobilisation of the **F-DNA1**/**cDNA1** duplex on glass substrates in defined arrays. Schematic shows the immobilisation of **FDNA1** onto fluorous regions. The surface is then either incubated with the complementary strand (**cDNA1**) or a non-complementary strand (**ncDNA1**). Fluorescence images were obtained (a) before immobilisation; (b) after immobilisation of **cDNA1**; and (c) after incubation of **ncDNA1**.

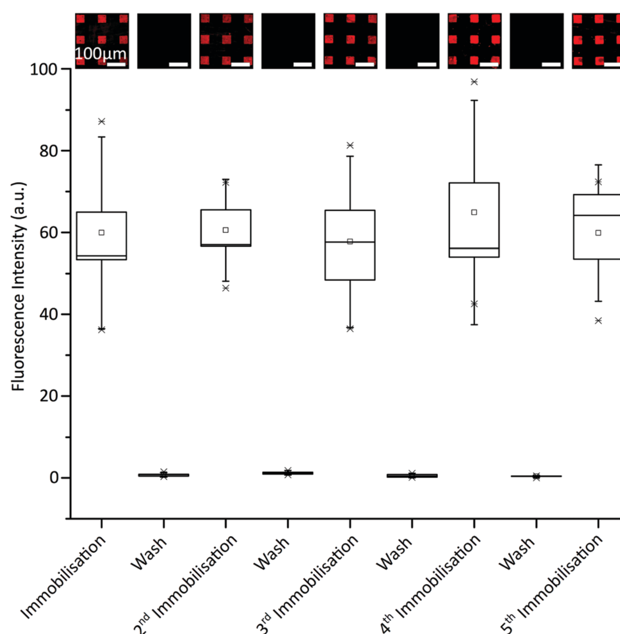


Fig. 2 Following the immobilisation of **F-DNA1** and its hybridisation to **cDNA1**, the substrates were imaged using fluorescence microscopy. Fluorescence images were then taken following the complete removal of immobilised DNA after each washing step. This was repeated 5 times. Each image corresponds to the box plot beneath. The graph shows the change in fluorescence intensity of the different immobilisations and removals of the **F-DNA1**/**cDNA1** duplex from the surface.

We next investigated the reversibility of the immobilisation and the durability of the fluorous surface over five DNA removal-replacement cycles. A range of solvents were screened to determine the optimal washing protocol, which was found to be a solution of 50% v/v MeOH in phosphate-buffered saline (PBS) (1 M, pH 7.4) followed by a methanol rinse. No loss of fluorescence intensity was observed after 5 cycles of immobilisation and washing with MeOH/PBS (Fig. 2).

In order to assess if more complex information could be interchanged in place of less complex information on a surface,

Open Access Article. Published on 23 February 2017. Downloaded on 12/03/2017 11:20:33. This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

we synthesized a 32-mer ODN, **F-DNA2**, which contained a 5′fluorous tag and a complementary sequence, **cDNA2**, which contained an Alexa Fluor 488 tag. Further to this, we fabricated more intricate patterns using electron-beam lithography (Fig. 3), which contained feature sizes as small as 500 nm. Using our optimised conditions, described above, **F-DNA1** was immobilised, then hybridised with its complement **cDNA1** on the more intricate patterns. The duplex was then washed from the surface followed by the immobilisation of **F-DNA2** and hybridisation with **cDNA2**. Here we see the potential of this immobilisation chemistry for applications in re-writable DNA microarray technology, where the same surface can be used multiple times to detect different targets. Further to this, in the case of the **F-DNA2**/**cDNA2** duplex, the eight-carbon fluorous tag comprised less than 2% of the total mass. However, it was shown to be capable of immobilising the water-soluble ODNs with sufficient strength to remain bound to the surface through aqueous washes.

To obtain further understanding of the nature of the fluorous immobilisation, we monitored the binding events using a Quartz-Crystal Microbalance (QCM) (Fig. 4). This enabled us to investigate the binding events as a reduction in the resonance frequency of an oscillating 5 MHz crystal due to an increase in mass.[28–30] To determine the specificity of the interaction, immobilisation of **F-DNA1** was investigated using an unmodified silica QCM chip and a chip with a fluorous surface. Solutions of DNA (3.3 μM) were introduced to the chip using a flow cell at 40 μL min^{-1} and 20 °C. A decrease in frequency, representing an increase in surface mass, was observed upon addition of **F-DNA1** and adsorption was complete within 30 minutes for both surfaces.[31] Although **F-DNA1** was adsorbed onto both fluorous and silica surfaces, after the introduction of the complementary sequence **cDNA1**, hybridisation was only observed in the case of **F-DNA1** immobilised onto the fluorous QCM chip. In the case of **F-DNA1** immobilised onto fluorous surfaces, the hydrophilic single-stranded DNA was directed away from the hydrophobic surface and toward the bulk solution, where it remained accessible to **cDNA1**. In the case where **F-DNA1** was immobilised on silica, it is possible that charge-inversion of the silanol surface by cations present in the buffer results in the single-stranded DNA lying flat against the surface, where it was inaccessible to hybridisation.

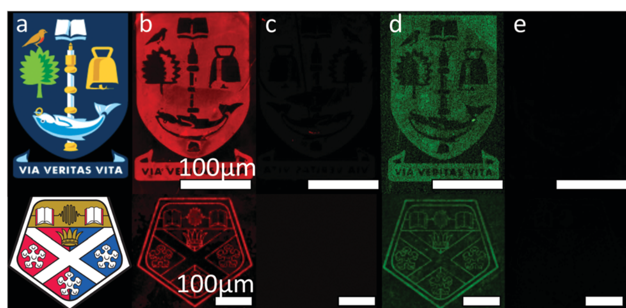


Fig. 3 Fluorescence images taken following; (b) immobilisation of **F-DNA1**/**cDNA1** duplex; (c) complete removal of immobilised DNA by the washing step; (d) immobilisation of the **F-DNA2**/**cDNA2** duplex to the same pattern; (e) 2nd complete removal by washing. (c) and (e) are the combined images of the surface taken with filters for both TAMRA and Alexa Fluor 488. (a) Shows the original templates used for electron beam lithography.

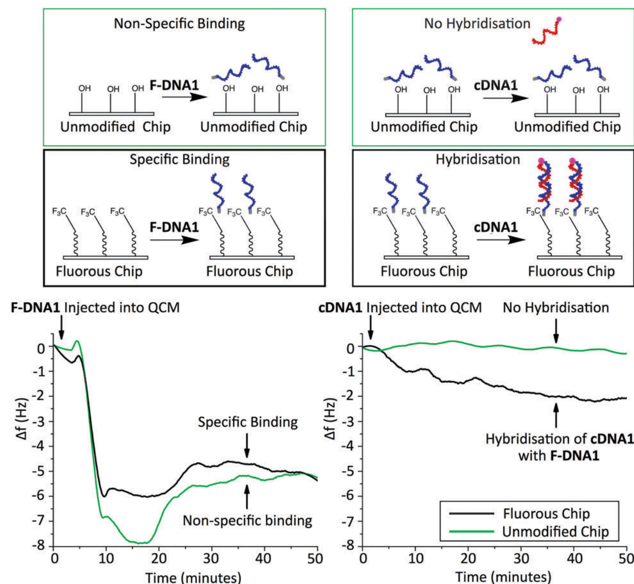


Fig. 4 Schematics and real-time QCM measurement of DNA binding. Left: Comparison of **F-DNA1** binding to a fluorous modified surface (black) and a control surface with no fluorous modification (green). Right: Hybridisation of **cDNA1** to each surface after **F-DNA1** modification. A negative change in frequency (Δf) represents an increase in mass on the surface.

In summary, we have demonstrated a novel method for the reversible immobilisation of DNA information onto surfaces. By developing lithographically patterned fluorous surfaces we remove the need to repeatedly direct the fluorous-modified biomolecules onto that surface for each subsequent "re-use". The use of fluorous molecular tags enabled the immobilisation of ODNs to fluorous surfaces, and when used in conjunction with alkylated regions, allowed for the preparation of fluorescent patterns with low non-specific binding of the non-complementary strand to the sensing region and low non-specific binding of the target complementary strand to the non-sensing regions. ODNs immobilised using this method remained capable of hybridisation with complementary strands and displayed complete removal and replacement characteristics. This method enables the replacement of one DNA sequence for another, by a process that is repeatable and with no associated degradation of the efficiency of binding, with time. This reversible immobilisation chemistry could in the future be exploited across many research fields, particularly in DNA microarray development, where progress is already being made in fabricating re-usable sensing platforms.[32]

This work was supported by The EPSRC Centre for Doctoral Training (EP/F500424/1), the Royal Academy of Engineering (grant 10216/103), the EPSRC (grants EP/P51133X/1 and EP/N016874/1), and The Leverhulme Trust (grant RPG-2014-343). JC also acknowledges support from a personal EPSRC Fellowship (EP/K027611/1) and Biophononics ERC Advanced Investigator Award. The authors also wish to thank all the staff working in the James Watt Nanofabrication Centre for their support. All data relating to the work outlined in the article can be found here: http://dx.doi.org/10.5525/gla.researchdata.380.

Open Access Article. Published on 23 February 2017. Downloaded on 12/03/2017 11:20:33.
This article is licensed under a Creative Commons Attribution 3.0 Unported Licence.

Notes and references

1 P. S. Cremer, *J. Am. Chem. Soc.*, 2011, **133**, 167–169.
2 A. M. Hung, H. Noh and J. N. Cha, *Nanoscale*, 2010, **2**, 2530–2537.
3 A. W. Clark and J. M. Cooper, *Angew. Chem., Int. Ed.*, 2012, **51**, 3562–3566.
4 A. W. Clark, D. G. Thompson, D. Graham and J. M. Cooper, *Adv. Mater.*, 2014, **26**, 4286–4292.
5 J. Zhang, S. Song, L. Wang, D. Pan and C. Fan, *Nat. Protoc.*, 2007, **2**, 2888–2895.
6 S. B. Nimse, K. Song, M. D. Sonawane, D. R. Sayyed and T. Kim, *Sensors*, 2014, **14**, 22208–22229.
7 D. I. Rozkiewicz, J. Gierlich, G. A. Burley, K. Gutsmiedl, T. Carell, B. J. Ravoo and D. N. Reinhoudt, *ChemBioChem*, 2007, **8**, 1997–2002.
8 C. Wendeln, I. Singh, S. Rinnen, C. Schulz, H. F. Arlinghaus, G. A. Burley and B. J. Ravoo, *Chem. Sci.*, 2012, **3**, 2479–2484.
9 I. Singh, C. Wendeln, A. W. Clark, J. M. Cooper, B. J. Ravoo and G. A. Burley, *J. Am. Chem. Soc.*, 2013, **135**, 3449–3457.
10 C. M. Niemeyer, L. Boldt, B. Ceyhan and D. Blohm, *Anal. Biochem.*, 1999, **268**, 54–63.
11 A. A. Lubin, R. Y. Lai, B. R. Baker, A. J. Heeger and K. W. Plaxco, *Anal. Chem.*, 2006, **78**, 5671–5677.
12 F. Huang, H. Xu, W. Tan and H. Liang, *ACS Nano*, 2014, **8**, 6849–6855.
13 A. Kuzyk, R. Schreiber, H. Zhang, A. O. Govorov, T. Liedl and N. Liu, *Nat. Mater.*, 2014, **13**, 862–866.
14 X. Zhang, W. Xu, J. Tan and Y. Zeng, *Anal. Biochem.*, 2009, **386**, 222–227.
15 P. L. Dolan, Y. Wu, L. K. Ista, R. L. Metzenberg, M. A. Nelson and G. P. Lopez, *Nucleic Acids Res.*, 2001, **29**, e107–e107.
16 L. Li, W. Feng, A. Welle and P. A. Levkin, *Angew. Chem.*, 2016, **128**, 13969–13973.
17 X. Du, J. Li, A. Welle, L. Li, W. Feng and P. A. Levkin, *Adv. Mater.*, 2015, **27**, 4997–5001.
18 R. L. Nicholson, M. L. Ladlow and D. R. Spring, *Chem. Commun.*, 2007, 3906–3908.
19 I. T. Horváth, D. P. Curran and J. A. Gladysz, in *Handbook of Fluorous Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, 2005, pp. 1–4, DOI: 10.1002/3527603905.ch1.
20 C. M. Santos, A. Kumar, S. S. Kolar, R. Contreras-Caceres, A. McDermott and C. Cai, *ACS Appl. Mater. Interfaces*, 2013, **5**, 12789–12793.
21 H. D. Edwards, S. K. Nagappayya and N. L. B. Pohl, *Chem. Commun.*, 2012, **48**, 510–512.
22 S. K. Mamidyala, K.-S. Ko, F. A. Jaipuri, G. Park and N. L. Pohl, *J. Fluorine Chem.*, 2006, **127**, 571–579.
23 H. D. Edwards, S. K. Nagappayya and N. L. B. Pohl, *Chem. Commun.*, 2012, **48**, 510–512.
24 B. Y. M. Collet, T. Nagashima, M. S. Yu and N. L. B. Pohl, *J. Fluorine Chem.*, 2009, **130**, 1042–1048.
25 N. L. Pohl, *Angew. Chem., Int. Ed.*, 2008, **47**, 3868–3870.
26 G.-S. Chen and N. L. Pohl, *Org. Lett.*, 2008, **10**, 785–788.
27 M. Dufva, in *DNA Microarrays for Biomedical Research: Methods and Protocols*, ed. M. Dufva, Humana Press, Totowa, NJ, 2009, pp. 63–79.
28 F. Caruso, E. Rodda, D. N. Furlong, K. Niikura and Y. Okahata, *Anal. Chem.*, 1997, **69**, 2043–2049.
29 S. Yamaguchi, T. Shimomura, T. Tatsuma and N. Oyama, *Anal. Chem.*, 1993, **65**, 1925–1927.
30 Y. Okahata, Y. Matsunobu, K. Ijiro, M. Mukae, A. Murakami and K. Makino, *J. Am. Chem. Soc.*, 1992, **114**, 8299–8300.
31 G. Sauerbrey, *Z. Phys.*, 1959, **155**, 206–222.
32 A. A. Lubin, R. Y. Lai, B. R. Baker, A. J. Heeger and K. W. Plaxco, *Anal. Chem.*, 2006, **78**, 5671–5677.

Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2017

Supporting Information: Reversible DNA Micro-patterning Using the Fluorous Effect

Experimental Section:

Materials. HPLC-purified DNA oligomers with several different modifications (Table 1) were purchased from Integrated DNA Technologies (IDT). Ultrapure water (18.2 MΩ.cm) was used for all solutions and protocols. All chemicals were obtained from Sigma unless otherwise stated. The oligomers were diluted to final concentrations in TE buffer and concentrations and purity was determined using UV-Vis spectrometry (Nanodrop 1000 Spectrophotometer, Thermo), prior to experiments.

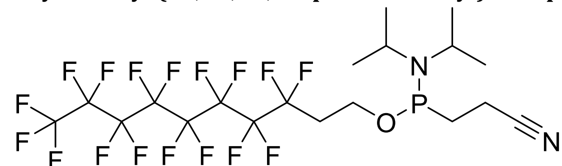
Probe	Modification	Sequence
FDNA1	R^F HHH	TGC AGA TAG ATA GCA G
FDNA2	R^F HHH	CAT CAT GAA TTC CAT AAG CTT CAT GGA TCC AT
cDNA1	TAMRA	CTG CTA TCT ATC TGC A
cDNA2	Alexa Fluor 488	ATG GAT CCA TGA AGC TTA TGG AAT TCA TGA TG
ncDNA1	TAMRA	ATG ATG AAG CTT ATG ATG

Table 1: List of oligomers used, their base pair sequence and any modifications present.

Fluorous-tagged DNA Synthesis. Fluorous-tagged oligonucleotides were synthesised using standard solid-phase methods on an Applied Biosystems 392 DNA/RNA synthesiser. DNA synthesis reagents and solutions were purchased from Link Technologies Ltd. Oligonucleotides were purified by RP-HPLC, and characterised by MALDI-MS.

Oligonucleotide FDNA1: 5′ – R^F HHH TGC AGA TAG ATA GCA G – 3′ where R^F is a fluorous nucleotide and H is hexaethyleneglycol (HEG). Calculated m/z: 6528.22, observed m/z: 6507.59.
Oligonucleotide FDNA2: 5′ – R^F HHH CAT CAT GAA TTC CAT AAG CTT CAT GGA TCC AT – 3′ where R^F is a fluorous nucleotide and H is hexaethyleneglycol (HEG). Calculated m/z: 11300.01, observed m/z: 11314.14.

2-cyanoethyl (*1H,1H,2H,2H*-perfluorodecyl) diisopropylphosphoramidite



Under an inert atmosphere, *1H,1H,2H,2H*-perfluorodecanol (500 mg,0.77 mmol) and diisopropylethylamine (180 mg, 1.4 mmol) were dissolved in dry acetonitrile (6 mL). 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (357 mg, 1.5 mmol) was added. The reaction was monitored for completion by thin layer chromatography using ethyl acetate/hexane (1:3) as mobile phase (R_f of perfluorodecanol ~ 0.5, R_f of product ~ 0.7) and staining with acidified $KMnO_4$. Starting material was consumed after 2 hours. The reaction mixture was loaded directly onto a silica gel chromatography column and eluted with hexane/ethyl acetate/triethylamine (72:25:3), affording the product as a colourless oil (614 mg, 88 %). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.20 (t, 12 H, J_{HH} = 7.3 Hz), 2.46 (m, 2 H), 2.64 (t, 2 H, J_{HH} = 6.4 Hz), 3.62 (m, 2 H), 3.78-4.02 (m, 4 H). ^{31}P NMR ($CDCl_3$, 200 MHz) δ: 148.6.

Micro-patterning of Substrates. Borate wafers (University wafers) were cleaned in toluene, acetone and IPA. They were then oxygen plasma ashered (2 minutes, 100 W) immediately before being transferred to a 1% v/v solution of n-decyltrichlorosilane (DTS) in toluene for 10 minutes. The surfaces were then cured at 100 °C for 30 minutes. Standard photolithography was used to micro-pattern the surfaces. Briefly, wafers were spin coated with S1818 (Shiply, USA) at 4000 rpm for 30 seconds to produce a ~1.8 μm thick layer. Micro-patterns were transferred onto the wafer by exposure to UV radiation (SUSS Microtec MA6, Germany) for 4.5 seconds through a chrome mask. The development of the S1818 resist was performed using a 1:1 ratio of Microposit developer (Shiply, USA) and RO for 1 minute. Samples were then dried under nitrogen. Following development, samples were oxygen plasma ashered for (2 minutes, 100 W) to remove the DTS silane from patterned areas. Samples were then sonicated in acetone for 5 minutes to remove the photoresist, rinsed with IPA and dried under nitrogen where they were then silanised, as described above, using (Heptadecafluoro-1, 1, 2, 2-tetrahydrodecyl) trimethoxysilane) (Gelest). To produce the crests, standard E-beam lithography was used, in place of photolithography, according to the procedure outlined in [1].

Immobilization & Hybridization of DNA. Immobilization of fluorous-tagged DNA (FDNA) on the surfaces was carried out in a simple humidity chamber. 1uM (unless otherwise stated) solutions of fluorous-tagged DNA were introduced into the chamber and incubated on FDTS micro-patterned surfaces at RT for 2 hours. Samples were rinsed with TE buffer and the complimentary DNA strand (1 uM unless otherwise stated) was introduced for 2 hours. Samples were then rinsed with DI water, TE buffer, dried under nitrogen then imaged. All experiments were carried out in triplicate.

Removal and Re-immobilization of DNA. Immobilized DNA was removed via the fluorous tag using a 1:1 solution of MeOH:PBS for 30 minutes under gentile agitation (Scheme 1). It was then rinsed with MeOH and dried under nitrogen. Re-immobilization was carried out using the same protocol as described above. Samples were protected from light to minimize photo-bleaching throughout the experiment.

QCM Apparatus. AT-cut quartz crystals with a fundamental resonance frequency of 5 MHz (25 mm) were obtained from Microvacuum Ltd (Hungry). These crystals were supplied with Au/SiO_2 coating that was used to graft silane molecules to using the same method outlined above. Crystals were mounted into an OWLS QCM-I (MicroVacuum, Hungary) attached to a mechanical pump to introduce the samples. DI water was flushed through until a stable baseline was established. The flow rate and temperature (40 μL/minute; 20 °C) remained constant throughout the experiment. Solutions were introduced slowly through an injection loop (total volume was 500 μL).

Fluorescence Microscopy. Surfaces were imaged using an Axio Observer Z1 (Carl Zeiss, Germany) under control of Axiovision software. Images were then analyzed using ImageJ software.

Controls:

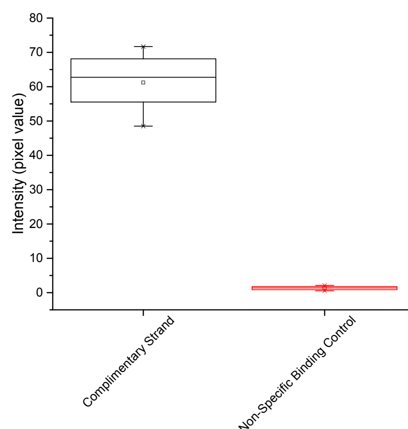


Figure 1: Fluorescence intensity observed from addition of non-complimentary sequence to fluorous micro-pattern.

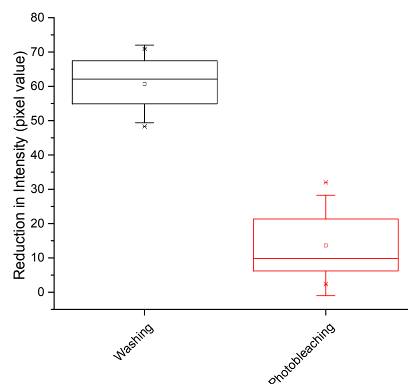


Figure 2: Reduction in fluorescence intensity due washing off fluorous tagged DNA using MeOH:PBS mixture and photobleaching.

Contact Angle Measurements:

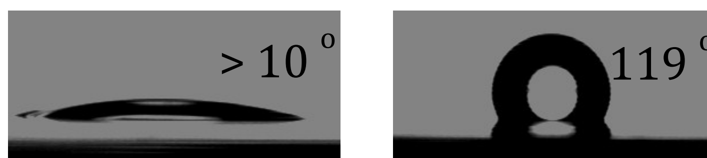


Figure 3: Contact angle measurements after cleaning surfaces, giving a contact angle of >10 º, and after silanising wafer with FDTS, giving a contact angle of 119º.

QCM Data:

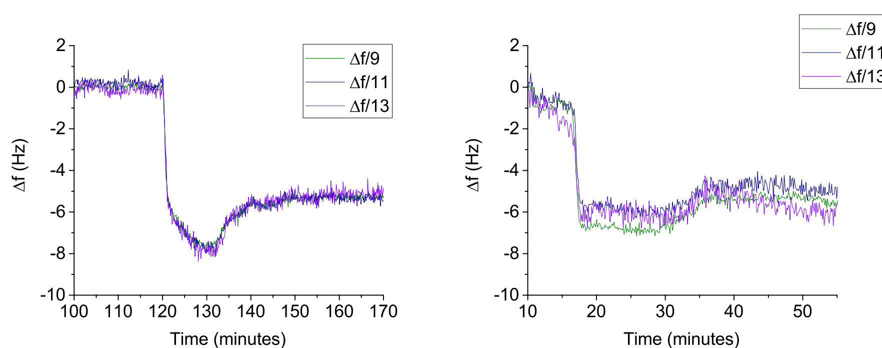


Figure 4: QCM data used in Figure 4 before smoothing. Plots show data from 3 overtones. (Left) F-DNA1 immobilisation onto unmodified QCM chip (Right) F-DNA1 immobilisation onto FDTS silanised QCM chip.

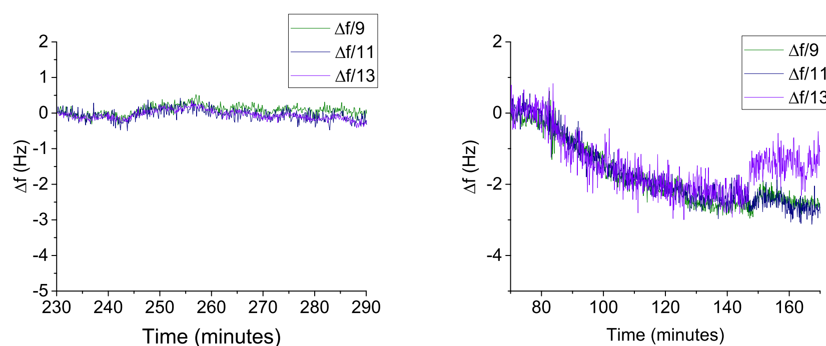


Figure 5: QCM data used in Figure 4 before smoothing. Plots show data from 3 overtones. (Left) cDNA1 injection into holder containing unmodified QCM chip (Right) cDNA1 injection into holder containing FDTS silanised QCM chip.

References:

1. Henry, S.L., et. *Organic & Biomolecular Chemistry*, 2016, **14**(4), 1359-1362.