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Selective binding of oligonucleotide on TiO₂ surfaces modified by swift heavy ion beam lithography

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Abstract

We have used swift heavy-ion beam based lithography to create patterned bio-functional surfaces on rutile TiO₂ single crystals. The applied lithography method generates a permanent and well defined periodic structure of micrometre sized square holes having nanostructured TiO₂ surfaces, presenting different physical and chemical properties compared to the surrounding rutile single crystal surface. On the patterned substrates selective binding of oligonucleotides molecules is possible at the surfaces of the holes. This immobilisation process is only being controlled by UV light exposure. The patterned transparent substrates are compatible with fluorescence detection techniques, are mechanically robust, have a high tolerance to extreme chemical and temperature environments, and apparently do not degrade after ten cycles of use. These qualities make the patterned TiO₂ substrates useful for potential biosensor applications.

Keywords

Titanium dioxide, Bio-functional surface, Swift heavy ion beam lithography, Nano-topography, Dip-Pen Nanolithography.

1. Introduction.

Interest in the biocompatibility of titanium dioxide (TiO₂) [1] is continuously growing due to it being a natural oxide layer on Ti and Ti-based body implants. Besides the main interest of TiO₂ as a substitutive and repairing material, this material offers interesting surface characteristics [2, 3] suitable to improve commonly used biomedical applications in *e.g.* immunology, oncology and

molecular biology [4-6]. Previous studies have focused on different aspects of the sintering process, phase, composition, micro- and nanostructure of TiO₂ for specific application as a biomaterial.

It has been reported that deposited nanostructured TiO₂ is a suitable platform for biochip applications to bind proteins, nucleic acids, antibodies and even cells [7-11]. Recently different fluorescent tracers have been immobilized on to single-crystalline rutile TiO₂ [12], using restricted UV exposure through a mask. Furthermore, experimental studies describe the direct adsorption of DNA oligonucleotides to anatase TiO₂ nanoparticles [13] and mineral rutile TiO₂ [14]. Theoretical approaches highlight the relevance of the surface chemical properties for the absorption of tripeptides on TiO₂ rutile single crystal [15]. Since the surface characteristics of TiO₂ play a key role in the interaction with biomolecules and cells, new processes for generating controllable changes of TiO₂ surface properties, such as topography, morphology and surface chemistry, can be advantageous in biosensing and 3 dimensional (3D) cellular scaffolding [16] applications for near future analytical, diagnostics, and imaging techniques.

UV light exposure is frequently use for sterilization of surfaces and it has also been employed satisfactorily for photo-immobilization [17]. UV light can also promote chemical changes of the exposed materials. It is known that the chemical properties of TiO₂ surfaces change due to UV irradiation [18]. This is for example of importance for the use of TiO₂ in photocatalysis. The exposure of TiO₂ substrates to UV light can also be a key factor to promote selective binding of molecules to surfaces [12]. Notwithstanding the rather well know surface chemistry of TiO₂ [3], some aspects related to UV exposure and the role of the chemical and biological properties of TiO₂ surfaces are still unclear. This is especially the case for TiO₂ nanostructures [19, 20].

Typical lithography processes for creating user defined patterns on a substrate, having different suitable chemical or morphological properties, require the application of several production steps, which increases the complexity, the fabrication risk, and the economic cost for a single assay. Therefore, if the substrate is kept permanently predefined with stable areas for easier standard protocol activation, in addition to its ability to resist well established sterilization process allowing multiple uses, the assay process becomes more accessible for routine application also outside state-of-art infrastructure laboratories.

In this work we have employed a lithography method based on a permanent 3D patterning process that utilizes the changes in chemical and physical properties induced by high energy (MeV) heavy-ion beam irradiation via so-called ion track formation [21, 22]. Chemical etching of the ion track damage is applied for various applications [21, 22], e.g. the fabrication of microfiltration membranes used in biology and medicine [23]. MeV ion-beam irradiation is often referred to as Swift Heavy Ion beam (SHI) irradiation, and we will use this nomenclature here.

We take advantage of the sensitivity of single crystalline rutile TiO_2 to SHI irradiation [24]. The cumulative effect of ion track formation following high ion fluence irradiation by such ions leads to localized damage and amorphous volumes in rutile single crystals [25-30]. Most of the damaged volume can be selectively etched away by an aqueous solution of hydrofluoric acid (HF) [24,26]. The etching process, however, leaves behind a thin surface layer of remaining damaged TiO_2 in the irradiated and etched areas [24,25].

Combining SHI with a lithographic mask makes it possible to obtain localized 3D patterning on rutile TiO_2 single crystals, see Fig. 1. A variety of structures in TiO_2 with lateral dimension down to

70 nm and depth of 1 μm have been created by this method over cm^2 areas [25-27,29,30]. There are several advantages of using swift heavy-ion beam based lithography compared to other lithography methods. Such ions can modify or transform materials that are otherwise insensitive to electron or photon irradiation and they can induce structures with much higher aspect ratio and resolution. In contrast to Focused Ion Beam (FIB) technique, which is a serial patterning method based on local material removal by sputtering, the current ion beam-based projection method, gives directly an ordered array of ion irradiation spots inducing deep material modification, allowing the possibility of increasing the patterned area and reducing the processing time.

Here we show results on the use of swift heavy-ion beam based lithography to create bio-functional patterned surfaces on rutile TiO_2 single crystal. The patterned substrates are shown to enable localized binding of oligonucleotide molecules. Two ways of depositing the oligonucleotides were utilized: (i) A simple pipette method, and (ii) and micro-spotting techniques using quill like microchannel cantilevers in a Dip Pen Nanolithography (DPN) setup. While the first method is an easy way of depositing the same oligonucleotide in all wells of the substrate, the latter allows for the individual addressing of specific wells. This can become important for many applications, especially if the deposition of more than one type of oligonucleotide molecules is desired. In addition, the substrates are shown to not degrade, even after several consecutive cycles of sterilization and uses. These observations present suitable characteristics of the substrates as platforms for biosensor applications.

2. Materials and Methods

2.1 The ion beam lithography process:

The cleaning process of the substrates prior to mask positioning and ion irradiation was as follows: Immersion in acetone, isopropanol, ethanol and deionized water followed by dry blowing in N₂. The masks used for the lithography process were metal TEM (Cu) grids. Masks with square holes of 37 μm in size and 25 μm of separation between them, having a thickness of 25 μm in average, were placed over 5×5 mm square rutile TiO₂ single crystal substrates having (100), (110) and (001) surface orientation, a thickness of 0.5 mm and density of 4.25 g cm⁻³ (MTI corporation, USA). The samples were uniformly irradiated with 25 MeV Br⁷⁺ ions at normal incidence up to a total fluence of $8 \cdot 10^{13}$ ion cm⁻² using the 5 MV Tandem accelerator at Centro de Microanálisis de Materiales de Madrid. Typical irradiation times ranged from 2 to 5 minutes per sample, yielding a patterned area of 25 mm². Larger areas can be obtained by increasing irradiation time and repositioning the substrate. The applied mask thickness completely stops the ions between the square holes so that the substrate is only exposed to the ion beam in the areas represented by the mask holes. After irradiation the masks were removed, and the samples were etched in a HF solution (20 w%) for 25 minutes.

Helium Ion Microscopy (HIM) and Atomic Force Microscopy (AFM) techniques were employed for the characterization of the surface morphology. HIM was performed with an ORION PLUS microscope from Carl Zeiss SMT using 30 keV He⁺ ions with beam current between 0.7 and 4 pA. The imaging was performed by detecting secondary electrons, using an Everhart-Thornley detector. One sample was mechanically cleaved along the micro-pattern to obtain a cross sectional view of

the generated micro-wells. AFM (Nanotec Electrónica S. L., Spain) was performed after the chemical etching process to characterize the surface structure and morphology of the samples. The AFM images were obtained in dynamic mode at ambient condition using commercial probes with a constant force of $K \approx 1.5 \text{ Nm}^{-1}$ and tip radius of $R \approx 10 \text{ nm}$ (Nanosensors PPP-FM, Germany).

To further investigate the structure of the surface following irradiation and HF etching, Grazing Incidence X-Ray Diffraction (GI-XRD) was carried out. This was performed on samples exposed to the same irradiation and HF etching process, but without the use of masks. This generates a continuous modified surface which is easier to analyze. GI-XRD was performed in a Panalytical mod. X'Pert Pro, using $\text{Cu K}\alpha$ radiation.

Additional acids baths in H_2SO_4 (98% w) and piranha solution ($\text{H}_2\text{O}_2:\text{H}_2\text{SO}_4$) were performed to test the resistance of the patterned substrates without noticeable effects on the pattern.

2.2 DNA oligonucleotides deposition and photo-immobilization:

Single-stranded oligonucleotides were designed using the Vector NTI software (Informax, Inc, USA), according to β -Actin gene sequence from rat (*Rattus norvegicus*). The oligonucleotide used consisted of the following amino acid sequence: 5'-GCCAACCGTGAAAAGATGACCCAGATCATGTTTGAGACCTTCAACACCCC-3', labelled at the 3'-end with Cy3 fluorescence dye. Storage was handled according to the supplier's and manufacturer instructions (Sigma-Aldrich Química, Spain). Two different techniques were employed for the oligonucleotides fixation experiments: (i) In the first approach direct deposition of 1 μl of β -Actin oligonucleotide at a given concentration was applied by a pipette onto the TiO_2 substrates patterned by the ion-beam lithography. After oligonucleotide deposition, the substrates

were dried for 10 minutes at 65°C and then exposed to UV light in a UV oven at a wavelength of 254 nm and 10 mW cm². The exposure time to UV light was 15 minutes. Once the exposure to UV light had been performed, extensive washes of the samples with 8.3 M urea at 65 °C and room temperature were made in order to remove excess of oligonucleotide not bound to the patterned TiO₂ substrates. The same procedures of deposition of oligonucleotides and washing were applied for a set of samples with deposited oligonucleotides, but not exposed to UV light. Additional none patterned substrates were exposed to UV under the same conditions, and examined and taken as control samples. (ii) The second approach consisted of depositing oligonucleotides by quill-like pens using a DPN system (NLP 2000 system, NanoInk, Inc.) into selected micro-wells and on virgin surfaces of the substrate, to allow a direct comparison of the binding capacities of both types of surface. The depositions were carried out at room temperature and with controlled humidity about 60% r.h. to avoid drying of the ink in the pen's reservoir. The quill-like pens were so-called "Surface Patterning Tools" (SPT-S-C10S, Bioforce Nanosciences, Inc.) [31]. The depositions were performed by single and double contacts and different flowing times ranging from 0.5 to 1 s. This allows the deposition of different volumes of oligonucleotides on selected types of surfaces. The used solution (or ink) was made by mixing oligonucleotides in pure water (concentration 100µM) with glycerol (87%) in proportion 1:1. The glycerol prevents drying of the small volume (1µl) of ink in the SPT reservoir during the lithography. For fixation of oligonucleotides the same previous method of UV photo-immobilization was applied (15 minutes of UV exposure at wavelength 265 nm).

The cleaning protocol employed to reuse the substrates after the oligonucleotide fixation experiment was as follows: baths in H_2SO_4 and H_2O_2 (2:1) solution (*'piranha solution'*) for 10 minutes and subsequent bath in deionized water.

2.3 Detection techniques and statistical analysis

Deposited oligonucleotide was analysed by densitometry measurements using the software Image Quant TL v2003.02 (GE Healthcare, UK). Wide-field fluorescence microscopy was done in a Nikon Eclipse 90i (Nikon Instruments, UK) microscope with a Cy3 specific filter. All images were acquired with a 40x Plan-Apo objective. The same fields were also acquired with differential interference contrast (DIC) technique to visualize the patterned substrate structure. Images were processed with Nis-Elements software from Nikon. Statistical analysis of the data was performed by Student's t-test and $P < 0.05$ was considered significant. All values are expressed as mean $\pm$ standard error of the mean.

3. Results

3.1 The ion-beam lithography process

The patterned TiO_2 substrates obtained by the swift heavy-ion beam based lithography consist of an array of square micro-holes or micro-wells, reproducing the lateral dimensions of employed masks. An optical microscope image of a patterned sample employing a mask of $37\text{ }\mu\text{m}$ squares is presented in Fig 1 a). In order to clarify the results and discussion, we will use the definitions given in Fig. 1 b) to describe the different zones of the patterned substrate. The depth of the micro-wells (or etching depth) resulting after the localized SHI irradiation and subsequent HF

etching, is related to the ion energy and fluence, in addition to the fact that there is a certain chemical etching threshold for rutile TiO_2 [24-28,32]. For the present applied ion energy and fluence a characteristic depth of 2 μm is obtained [28], which is the maximum depth obtainable with the used ion. It is important to note that only damaged and amorphous TiO_2 is affected by HF etching, crystalline (virgin) rutile TiO_2 is not [24-26, 32]. Therefore, the surface between the micro-wells (or top of the walls) is still crystalline rutile as it has not been exposed to ion irradiation.

The bottom and inner walls of the etched micro-wells are composed of damaged TiO_2 . This was clearly seen by GI-XRD of a continuous irradiated surface etched in HF, which has also been presented in [25]. In addition, to a broadening of the original rutile single crystal peak, additional features associated with amorphous and polycrystalline rutile phases were observed. Thus even after chemical etching there is still remaining ion beam induced damage at the surfaces [24, 25].

AFM measurements show an increased roughness of the surface following irradiation and etching. The roughness at the bottom of the micro-wells was measured by AFM, see figure 1 d) giving a mean RMS value of 4.5 nm as compared to 0.2 nm of the virgin surface (profiles in Fig. 1 d).

This roughness was further investigated by HIM. The primary benefit of HIM as compared to Secondary Electron Microscopy (SEM) is that it allows for significantly higher image resolution, enhanced surface sensitivity, a better contrast and a greater depth of focus. The images in a HIM thus provide more detailed information on sample topography than SEM imaging [33-35]. In addition, charging of insulators, as in our case TiO_2 , can be suppressed by the use of an electron flood gun.

Figure 2 shows the surfaces of the bottom and walls of the micro-wells as examined by HIM, revealing topography with distinct nanostructured features. The bottom of the wells has a very rough or ‘hairy’ appearance with features having tens of nm in height and diameter. Similar characteristics are observed for the side walls. HIM images revealed a higher height or roughness of the nanostructure than that measured by AFM. This difference in measured roughness is caused due to the size of the AFM tip which limits the full penetration to resolve the whole nanostructures.

The observed roughness is due to the high etching selectivity of damaged TiO_2 , which makes the etching more sensitive to non-uniformities in damage volumes induced by the swift heavy ions. The non-uniform etching is probably attributable to spatial fluctuations of the deposited energy in TiO_2 along the ion path due to the statistical nature of the energy deposition [36, 37]. Especially when the etching occurs close to the chemical etching threshold (corresponding to the bottom of the wells) these density fluctuations may be rather large, reflecting a large dispersion in the diameter of the damage volumes or latent tracks induced by individual ions [37,38]. In addition, partial overlap between individual ion impacts, caused by lower ion fluence, will also lead to non-uniformities in damage volume. This may occur near the mask edges due to multiple scattering [39]. A stochastic variation in ion impingement will give rise to a strong nanometer-scale texture of the walls in the etched micro-wells. A longer etching time does not remove the surface structures.

The patterned substrates show a resistance to the applied cleaning protocol without losing their surface structure. They are also transparent to visible light, and no fluorescence signal was observed to emerge from the substrates with the employed excitation and detection wavelengths.

3.2. DNA oligonucleotides adsorption.

Figure 3 shows fluorescence and differential interference contrast (DIC) microscopy images of deposited oligonucleotides by pipette over the whole surface of the patterned substrate. After deposition and 15 minutes of UV exposure the oligonucleotides are more or less evenly distributed over the whole patterned surface, even though there is a higher concentration inside the micro-wells, Fig. 3(a). Following washes in urea the fluorescence signal only originates from inside the wells, Fig. 3(c), and no signal is detected on the virgin surface between the wells. This is taken as evidences of correct oligonucleotide immobilization, only inside the patterned micro-wells. Since the inner surface of the walls presents the same characteristics as the bottom of the micro-wells, the detection of a more intense fluorescence signal from the inner-walls of the micro-wells (see Fig 3(c)) can be explained by the light scattering from the inner-walls.

Fig. 4 shows densitometric measurements of samples after oligonucleotide deposition and successive washing in urea. Although absolute values of intensity could not be obtained due to inhomogeneous deposition, the linkage between DNA oligonucleotide and substrate was observed even after multiple rounds of strong washing using a strong denaturing buffer such as urea (8.3 M) at 65°C for 10 minutes and at room temperature overnight. The decrease in fluorescence after the first wash is due to the removal of excess oligonucleotide that was not bound in the micro-wells. Subsequent washes did not produce a significant decrease in fluorescence, indicating a strong linkage between oligonucleotide and the patterned TiO₂ surface. Samples covered with oligonucleotides, but not exposed to UV and samples taken as control did not show any detectable fluorescent signal after first washing. No remarkable differences in the magnitude of the

fluorescence signal were detected with respect to crystalline surface orientation of the substrates used, under identical conditions of UV exposure and oligonucleotide deposition.

Treatment of samples in a *piranha solution* eliminates any trace of fluorescence signal (drops to the same zero level as from control substrates). This led us to the conclusion that this cleaning procedure wiped out linked oligonucleotides to substrate. After the cleaning procedure, patterned TiO₂ substrates were reused to repeat the experiment, yielding similar results.

Depositions of oligonucleotides employing quill-like microchannel cantilever allowed controlled amounts of oligonucleotides to be deposited at selected places, see Figure 5. This method offers a direct comparison of the binding of isolated and limited amounts of oligonucleotides onto different TiO₂ surfaces compared to the continuum way of covering the whole substrate using pipette, and allows for the individually filling of each micro-well. With this method, solutions of oligonucleotide were deposited on the surface inside the micro-wells and on the virgin surface, see Figure 5. The size and depth of the micro-wells did not limit the access of the pens to the bottom of the micro-wells, allowing multiple depositions inside each micro-well.

The fluorescence signals disappear from the virgin single crystalline surface (top of wall) after UV exposure and washing in water, see Fig 5 c). The fluorescence signal from the immobilized oligonucleotides inside the micro-wells is, however, still visible. It decreases, nonetheless, compared to the signal detected right after photo-immobilization, due to the elimination of excess (not bound) oligonucleotides by the washing procedure. The registered decrease of the intensity after wash inside the micro-wells is similar to that observed by direct deposition by pipette after

washing in urea (Fig 4), showing the large amount of not reactive or weakly bound oligonucleotide to the surface.

The absence of any fluorescence signal from the depositions performed on the virgin surface illustrates again that the nanostructured surface inside the micro-wells has an increased ability of binding the oligonucleotides efficiently as compared to the smooth rutile surface between the holes.

4. Discussion

Previous reports [13,14] have described DNA adsorption on TiO_2 nanoparticles, thin films and even mineral surfaces, although the mechanism remains unexplained. Cleaves et al. [14] pointed out that the binding capacity of nucleotides with surface functional groups on rutile is promoted at kink and steps sites on the surface. Such defects behave as islands with different chemical behaviour compared to a defect free surface [15]. It is also known that TiO_2 surfaces change their chemical and physical properties by UV irradiation. For example TiO_2 surfaces changes from hydrophobic to hydrophilic behaviour [18], and at the same time their ability to attach molecules changes [12]. Such behaviour can be explained in terms of photoreduction of surface Ti^{4+} to Ti^{3+} and subsequent occupation of hydroxyl groups on the generated O vacancies [18]. Other authors proposed a different mechanism for rough amorphous TiO_2 films that consists of the generation of a Ti-O dangling bond on the TiO_2 surface [40]. The generation of Ti^{3+} -O vacancy complexes [41] and the absorption of water or hydroxyl groups [32], due to the induced defects after ion irradiation of rutile single crystals, have also been reported. Finally, it has also been observed that the wettability of smooth hydrophilic surfaces improves by roughening them [42]. However, these studies describe the surface of TiO_2 as hydrated or free of attached hydrocarbons. It was recently reported that TiO_2

surfaces, under standard atmospheric conditions and exposed to UV or plasma, shed most of the attached hydrocarbons [43] covering their surfaces. As a result these surfaces change their wettability behaviour due to the newly available surface bonds for hydration.

Our results show that after UV exposure of the whole patterned substrate there is a clear difference in chemical behaviour of the surfaces in the micro-wells created by swift heavy-ion beam based lithography compared to that of the surrounding virgin rutile. We did not detect, by fluorescence, any attachment of oligonucleotide molecules on the original smooth crystalline rutile areas between the micro-wells. It was only observed that the photo-immobilization of oligonucleotide by UV exposure take place in the micro-wells, *i.e.* at the localized highly damaged and nanostructured TiO₂ surfaces resulting from the ion irradiation and subsequent HF etching (Fig. 2 and 3).

Although we have not elucidated the nature of this peculiar behaviour, or the reason for the surface-molecule interaction, it is clear that the UV induced binding/photo-immobilization to the nanostructured surface inside the micro-wells is strong enough to resist washes with denaturing buffers such as urea. We assume that in our case the rough morphology (nano-topography) of the surface in the micro-wells in combination with the remaining surface damage after HF etching [24,25] and local stoichiometry changes may enhance the binding with the oligonucleotides following UV exposure. Even an enhanced detachment rate of hydrocarbons in the micro-wells, compared to the surrounding flat single crystal TiO₂, induced by the UV, may yield more available surface atoms for binding. In addition, the generation of dangling Ti-O bonds or Ti³⁺-O vacancies complexes at the damaged TiO₂ surface could play a key role in the oligonucleotide photo-immobilization.

The nanostructured surface clearly increases the active surface area for chemical process and may also enhance the linkage between surface and molecules due to a close interlocking and improved contact by the increased surface area. The increased chemically active area of the nanostructured and modified TiO₂ surfaces could, together with the energy from the UV light, thus promote a reaction leading to the formation of a relative strong bond between the surface and the oligonucleotide molecules. The actual binding mechanism is influenced by the chemical, physical and structural characteristics of the employed patterned TiO₂. Changes in e.g. band gap or photon adsorption efficiency could result in different activation energies for chemical reactions on the single-crystalline rutile surface compared to the damaged nanostructured TiO₂ surface in the micro-wells.

5. Conclusions

We have used swift heavy-ion beam based lithography as a method to obtain an array of nanostructured TiO₂ surface showing bio-functional properties. We were able to demonstrate that single stranded DNA (oligonucleotides) are immobilized inside the created micro-wells and selectively binds to the modified TiO₂ nanostructured surfaces following only UV exposure. This demonstrates that the modified TiO₂ surface, induced by swift heavy-ion beam based lithography, presents different chemical properties compared to surfaces of rutile TiO₂ single crystals. Two different deposition strategies were employed: a direct and non-selective deposition by pipetting and a more precise localized deposition by using quill-like microchannel cantilevers in a DPN setup. The first approach allows the complete filling of micro-wells with the same oligonucleotide,

whereas the second approach allows the selective deposition of even different oligonucleotides into given micro-wells. Repeated experiments with the same substrates after aggressive cleaning processes were performed several times yielding identical results, probing the stability of the material and its usability after extreme chemical conditions.

Although deeper studies are necessary to understand the induced chemical changes in the TiO₂ surfaces following SHI irradiation and UV exposition, the presented results, taken as a whole, open up the possibility to use permanently patterned TiO₂ substrates as transparent and re-usable platforms for biosensor applications.

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Figure captions:

Figure 1 (Colour on-line): The patterned TiO_2 substrates following swift heavy-ion beam lithography. a) Optical microscope image of the obtained pattern. Single-crystalline rutile TiO_2 zones are coloured in orange and modified TiO_2 in blue. b) Sketch and definition of different zones on the patterned samples. c) AFM measurement of the pattern. d) Topography measurements along a line at the bottom of a micro-well (blue), and along a line between wells (orange). Note the difference in the Z-scale.

Figure 2: a) HIM image from the patterned substrate showing the bottom, inner and top wall surface of a micro-well. b) HIM image with a detailed view of the nanostructured surface of the bottom of the micro-well.

Figure 3 (Colour on-line): a) Fluorescence image of a sample after deposition by pipette of Cy3-labeled oligonucleotide on whole patterned TiO_2 substrate. b) Fluorescence image of sample after wash in urea. c) and d) are differential interference contrast images corresponding to a) and b). Size of micro-wells is 37 by 37 μm .

Figure 4: Results of densitometric measurements with $n=3$ of Cy3-labeled oligonucleotide deposited on whole patterned TiO_2 substrates and attached by UV exposure of surface. Fluorescence of control substrate not exposed to UV after oligonucleotide deposition is 0. Line is only to guide the eye.

Figure 5 (Colour on-line): Fluorescence microscopy images showing the selective deposition and photo-immobilization of Cy3-labeled oligonucleotides inside the micro-wells. a) Oligonucleotides after deposition by DPN. The arrows indicate depositions on the virgin surface, which disappear

after washing. b) Detailed view of the marked area in a). c) Oligonucleotides after photo-immobilization and washing. d) Detailed view of marked area on c). Size of micro-wells is 37 by 37 μm .

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5