

General approach for the application of
Supramolecular NanoStamping (SuNS) to surfaces
of all types

by

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Ingénieur de l'Ecole Centrale Paris

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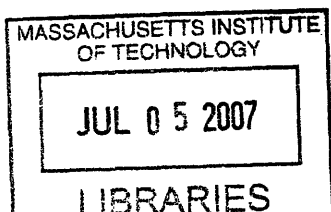
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Abstract

To novel ideas must correspond novel fabrication techniques, that enable the transfer of technologies from laboratories to the market. The success of microelectronics for example can not be separated from the success of the revolutionary manufacturing technology that has fed its expansion. The same is now true for nano- and biotechnologies that, to a large extent, have yet to find the technologies that will best answer their processing needs.

The question is to find an approach that will enable the production of devices with the required resolution, complexity and versatility, together with the necessary reliability and potential for high-throughput. Supramolecular NanoStamping (SuNS), a DNA based lithography technique developed in our group, is trying to answer to this set of requirements.

In this thesis, I present a new development in this lithography technique, expanding its application to a broad new range of substrates in a substrate-independent fashion. This work, which I conducted during the course of my master, proves the ability of SuNS to adapt to very diverse environments and applications.

Thesis Supervisor: Francesco Stellacci
Title: Assistant Professor

Acknowledgments

The work done for this thesis covers one year and a half spent in the Supramolecular NanoMaterials Group, SuNMaG, and I want to acknowledge first all of its members, whose backgrounds, personalities and research interests have made my experience at MIT so much richer. Although I have always been focused on SuNS, the momentum given by the diversity of research projects and the intensity of exchange in the group has been an important drive, and I have greatly benefited from this setting. This is also true for the MIT community as a whole, which has proven beyond my expectations in terms of diversity, intensity and scientific quality.

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Chapter 1

Motivation and Background

1.1 SuNS's principle

Supramolecular NanoStamping (SuNS) was introduced a couple of years ago in our group[1, 2, 3]. The same concept was simultaneously developed in Crook's group[4]. Its concept is to imitate on a surface the DNA replication that occurs naturally in our cells, in order to replicate 2-dimensional DNA patterns.

DNA molecules are long twisted helices made of two complementary strands held together by weak hydrogen bonds. These two strands are themselves long chains made of four different chemical groups called bases (adenine, cytosine, thymine, guanine), organized in a specific sequence, and attached to a sugar phosphate backbone (the DNA structure is schematically represented in figure 1-1). The complementarity of the two strands making up a DNA molecule is defined by the one-by-one complementarity of their constitutive bases: adenine can only bind to thymine, and cytosine can only bind to guanine. Two free complementary strands standing in one of our cells, or in a solution if it is in the laboratory, can recognize each other and hybridize, leading to the creation of the DNA double helix. On the contrary when heated up the weak hydrogen bonds that hold the two strands of the helix together can break and the two DNA strands separate, or dehybridize.

SuNS exploits this triple mechanism: recognition, hybridization, dehybridization, to reproduce 2D DNA structures.

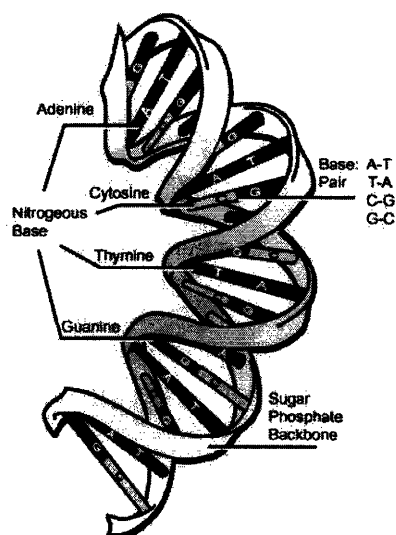


Figure 1-1: Schematic structure of a DNA double-helix (taken from www.scq.ubc.ca)

SuNS's method is comprised of three steps, shown in figure 1-2, starting with an original template that consists of a patterned monolayer of single-stranded DNA (ssDNA) molecules, also called oligonucleotides. The ssDNA of this pattern is first hybridized to its modified complement (noted cDNA), then brought into contact with another substrate, usually referred to as secondary substrate. The functionalized cDNAs end, pointing upwards, allows it to bind to the secondary substrate. Upon heating of the two substrates the dehybridization of the two DNA strands occurs, allowing the two substrates to come apart, with the cDNA staying attached to the secondary substrate in a pattern that is a perfect replica of the master's pattern. The master and replica obtained at the end of the process can then both be reused to start a new cycle and print onto other surfaces.

SuNS has first been reported with the printing of DNA patterns from gold substrates onto gold[1] or poly(methylmethacrylate) (PMMA)[2]. DNA lines were successfully printed on these substrates, with up to 40 nm in resolution on PMMA (see figure 1-3)[2]. Printed substrates were also proven to be reusable as masters to print onto other substrates, as can be seen in figure 1-4[2].

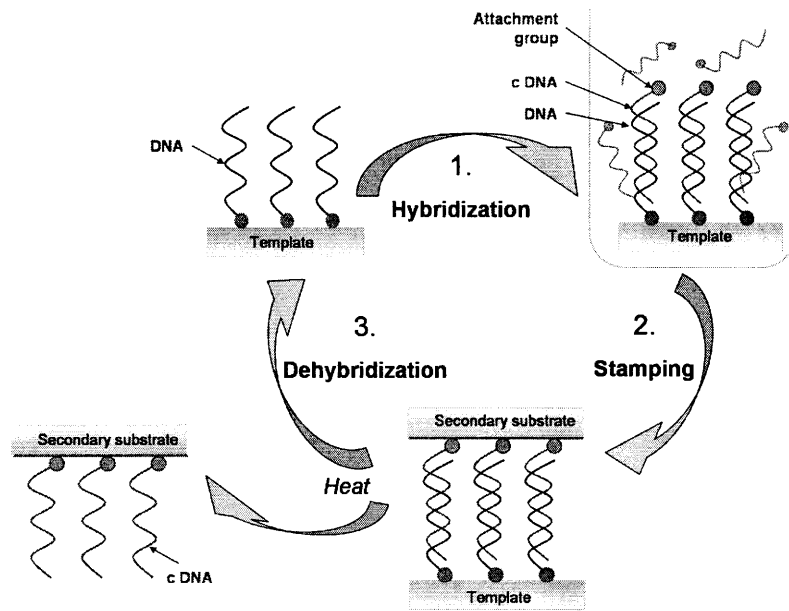


Figure 1-2: Schematic of the working principle of SuNS

SuNS's ability to reproduce patterns with multiple molecules was then demonstrated by printing two spots, each composed of a different sequence, A and B, in a single iteration. The printed sample was hybridized to the fluorescence-labelled DNA strands complementary to A and B, giving the result shown in figure 1-5.[1]

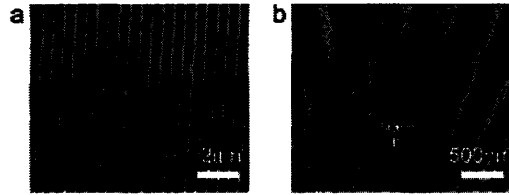


Figure 1-3: AFM images of DNA wires printed on a PMMA substrate. The arrows in (b) indicate the thinnest continuous part of the wire that was successfully printed. In an isolated case, we could print down to a thickness of 25 nm. From [2]

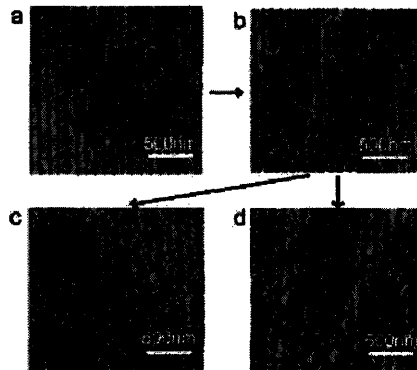


Figure 1-4: AFM images of DNA wires. a) DNA assembled on a series of SiO₂ parallel wires that are 50 nm thick and coated with gold. The wires become 70 nm thick after DNA assembly, probably due to assembly at the edges. b) DNA wires printed on a PMMA substrate, the average thickness is 75 nm. c) and d) DNA wires printed on gold-on-glass substrates using the sample shown in (b) as a master. (c) Printed first, and (d) printed second after rehybridization. [2]

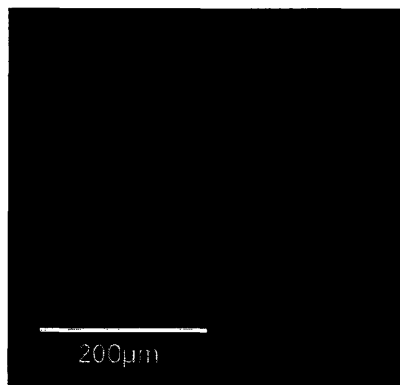


Figure 1-5: False color overlay of two fluorescence microscopy images obtained from one sample printed via SuNS. The two dots shown are about 0.45 mm apart. [1]

1.2 One more lithography technique?

The development of reliable and scalable nano- and microfabrication techniques is critical for the future of nanoscience and nanotechnology, and a set of promising fabrication tools has been reported over the past decades. This chapter will identify the main trends, without trying to be exhaustive, and will see how SuNS as a lithography technique is positioned in this environment.

1.2.1 "Hard" lithography, following the path of photolithography

The leading technique in micro- and nanofabrication remains photolithography, the workhorse of microelectronics since its invention in 1959, whose demise yet has been predicted for many years. Photolithography is the process of transferring geometric shapes from a mask first to a light-sensitive resist, using the light to selectively remove either the part shown through the mask or the part hidden by the mask, then to the surface of a solid wafer (usually silicon) by etching the areas where the resist has been removed (working scheme shown in figure 1-6)[5]. It is therefore a parallel process, able to reproduce large patterns in a single iteration, comprised of 3 steps. The standard area patterned in a single iteration through photolithography in the electronic industry is 30 cm in diameter.

Photolithography, though, suffers from an intrinsic limitation in resolution, imposed by the diffraction limit, which states that the minimum distance between two optically resolvable points scales with the wavelength of the light used. As the demand for ever higher resolution grows, the end of photolithography is predicted. There are means, though, to postpone the end of photolithography, which namely consist in finding ways to shorten the wavelength of the radiation used[6]. This involves first going into the deep-UV regime[7], then the X-ray regime[8], or exploring other kinds of radiations, which offer the advantage of shorter wavelengths for lower energies, such as electron-beams[9]. Electron-beam lithography, nevertheless, in spite of its superior resolution (linewidths of about 20 nm have been produced since the beginning of the

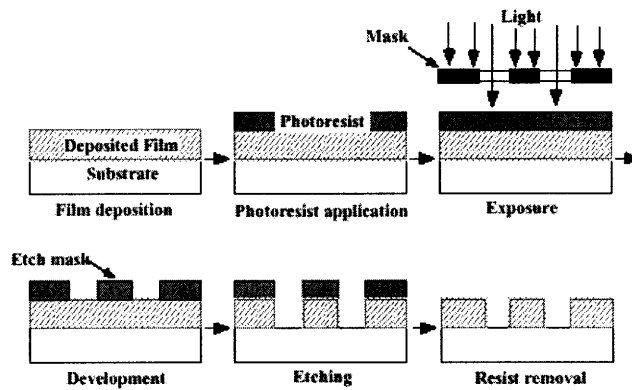


Figure 1-6: Working principle of photolithography. From www.hitequest.com

1990's) is devoid of one of the main qualities of conventional lithography, which is parallel processing. In electron-beam lithography the beam must be scanned over the surface to create a pattern, which makes for very slow processing times and very expensive products. For this reason, the primary use of electron-beam lithography today is the fabrication of masks for photolithography.

An entirely different approach, going back to the ancient techniques of printing and embossing, appeared in the 1990's, that reunites the need for very high resolution and parallel processing, with the additional low-cost advantage. The technique, called nanoimprint lithography, was developed by Chou's group and is based on the physical deformation of the resist with embossing to create a resist pattern, rather than on the modification of the resist chemical structure with radiation[10, 11]. The pattern made in the resist can then be transferred to an underlying substrate through etching, as it is the case in photolithography (scheme shown in figure 1-7). The difference in principle makes nanoimprint lithography capable of producing sub-10 nm features over a large area[12].

1.2.2 Soft lithography

Hard lithography, even if it manages to process devices shrinking to the required lengthscales while keeping a high throughput, does not answer all the needs of current

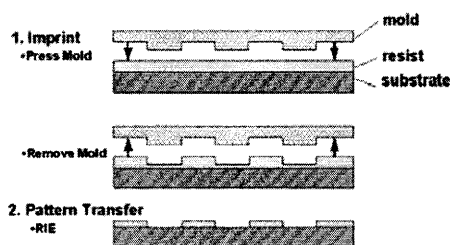


Figure 1-7: Schematic of nanoimprint lithography process: a) imprinting using a mold to create a thickness contrast in a resist, and b) pattern transfer using anisotropic etching to remove residue resist in the compressed areas. RIE = Reactive Ion Etching. From [12]

and future micro- and nanotechnologies, which have grown way outside the boundaries of microelectronics, to enter the fields of microsensors, microanalysis and more broadly biotechnologies in general. There, the inability of hard lithography in introducing chemical functionalities, its limitation to the patterning of resists that integrate very well with silicon but not always with other materials such as glass, carbon, plastics and ceramics, become a severe hindrance that has pushed researchers to look for alternative techniques. A whole set of these alternative approaches are grouped under the label "soft lithography", which owes its name to the fact that all these approaches rely on the use of flexible molecules and materials, as opposed to the rigid inorganic materials commonly in use in the microelectronics industry[14]. The leading technique in the field is microcontact printing (μ CP), developed in Whitesides's group, where an elastomeric stamp (e.g. Poly(dimethylsiloxane) (PDMS)), usually prepared by replica molding (shown in figure 1-8), is inked with molecules which are then transferred to the substrate by contact (see figure 1-9).[13, 15]

Microcontact printing is a parallel method, low cost and high throughput, that introduces chemical information in the patterning of substrates, and has therefore been a very successful approach in laboratories. Nevertheless it remains limited in resolution (about 100 to 200 nm), and cannot easily transfer multiple molecules at a time.

To answer to the resolution requirement, together with the transfer of complex

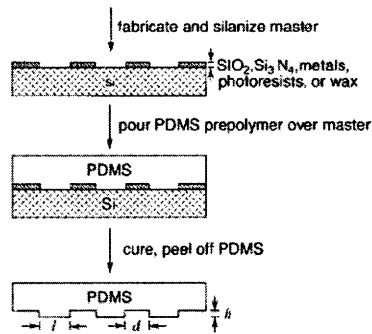


Figure 1-8: Schematic illustration of the procedure for casting PDMS replicas from a master having relief structures on its surface. From [13]

chemical information, mainly one approach had been developed so far, dip-pen nanolithography, which involves the transfer of an ink to a substrate directly from a coated atomic force microscopy (AFM) tip. The resolution obtained is the resolution of an atomic force microscope, on the order of a couple of nanometers, and since its inception dip-pen has been extended to a variety of surfaces and molecules[16, 17]. Unfortunately, this remains a serial method, the patterned surface needing to be scanned with the AFM tip, a long and therefore expensive process.

1.2.3 SuNS's contribution

It appears above that a gap needs to be filled, which requires a method for the parallel, high throughput reproduction of patterns that have both the required resolution and the ability to reproduce complex chemical information, while being compatible with biotechnology applications. SuNS's approach fulfills this requirement, as can be seen point by point from the advantages it offers, described below:

1. It is a stamping technique, and as such it is intrinsically a parallel method that can produce large patterns in a single step.
2. SuNS is a "color" printer, able to print with high information density, since it can print different DNA sequences at a time. This ability to reproduce chemical information in addition to topological information (the geometry of a pattern) is

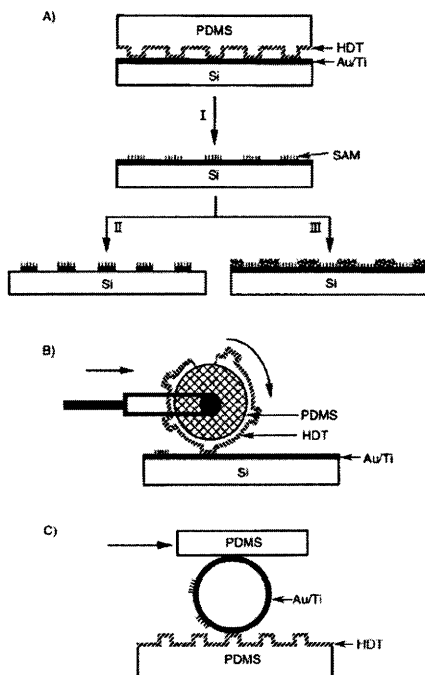


Figure 1-9: Schematic illustration of procedures for μ CP of hexadecanethiol (HDT) on a gold surface: A) printing on a planar surface with a planar stamp (I: printing of the SAM, II: etching, III: deposition); B) large-area printing on a planar surface with a rolling stamp; C) printing on a nonplanar surface with a planar stamp. After the "ink" (ca. 2 mM HDT in ethanol) was applied to the PDMS stamp with a cotton swab, the stamp was dried in a stream of N₂ (ca. 1 min) and then brought into contact with the gold surface (ca. 10 + or - 20 s). From [13]

critical for many applications in view.

3. The molecular recognition on which it is based allows very high resolutions. A resolution of up to 40 nm has already been demonstrated, in a laboratory setting, without any optimization of the protocol.

Until now, the main limitation of SuNS, which is also a limitation for most of the lithography techniques mentioned above, lied in the substrate-dependence of its protocol and on the very small number of substrates on which it had proven successful. The first results with SuNS were obtained using gold as a substrate, as well for the template as for the replica[1]. Then a new protocol enabled its extension to a new replica substrate, a cheap transparent polymer this time, poly(methyl methacrylate)

(PMMA)[2]. The following sections will present the possible strategies to extend SuNS further to other surfaces, and how we have chosen the one that is the subject of this thesis.

1.3 Motivation for the extension of SuNS to new surfaces

To understand how the extension of Supramolecular NanoStamping to other substrates than gold and poly(methyl methacrylate) (PMMA) is critical, we will review here its main potential applications. The need for scalable and reliable surface DNA patterning technologies is obvious in the variety of DNA-based devices that have been developed over the two decades for use in genetic analyses and medical diagnostics. We will distinguish here, and consider successively, the applications to DNA biosensors, DNA microarrays and "lab-on-a-chip" configurations. Other interesting uses of 2-dimensional DNA patterns, such as DNA-directed molecular self-assemblies and DNA computing, won't be considered here due to their lower level of maturity.

1.3.1 DNA biosensors

A very active area is the field of biosensors, which exploit the powerful recognition abilities of bioreceptors (e.g. gene probes, but also antibodies and enzymes) for the detection of molecules, with applications in drug development and medical diagnostics. Biosensors couple a biological recognition element and a transducer, that translates the biorecognition event into an electric signal (schematically represented in figure 1-10)[18].

In the case of DNA biosensors, ssDNA probes, attached to the transducer's surface, recognize and hybridize to their complementary DNA strands contained in the analyte, and the hybridization is then detected and translated into an electrical signal by the transducer. The hybridization recognition methods are numerous (optical, electrochemical, mass-sensitive...) and each favors a set of different surfaces and sur-

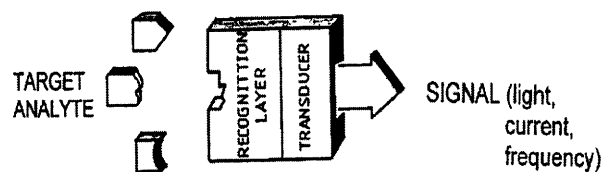


Figure 1-10: Conceptual diagram of the biosensing principle. From [19].

face chemistries for the immobilization of DNA probes, mainly focused on the use of gold, carbon and quartz[19].

1.3.2 DNA microarrays

DNA arrays, gene chips, or biochips are often intermixed terms that refer to a class of multiple DNA detection systems which enable the rapid, miniaturized and accurate analysis of nucleic acid samples. These DNA microarrays have revolutionized many aspects of genetic analysis, from the diagnostics of genetic diseases, the measurement of differential gene expression (e.g. figure 1-11), to drug screening, personalized medicine and forensic analysis[20].

DNA chips, that are usually 1 to 2 cm² in size, can be comprised of millions of reaction zones, each of them exhibiting oligonucleotide probes of different sequences, that can recognize and hybridize to their complementary strands in the analyte solution. Mass spectroscopy, and to a greater extent fluorescence imaging are the most common hybridization detection methods. In the case of fluorescence imaging, the scanning of the surface enables the detection of fluorescence-labeled DNA strands from the analyte, hybridized at specific spots on the array. These hybridization chips are usually fabricated on glass, silicon or plastic supports[21, 22].

One variant of the biochips applications is the idea to produce microchip biosensor devices where the sensors, detectors, amplifiers, and the logic circuitry are integrated on a single chip (see figure 1-12). Like a conventional DNA array the integrated biochip allows simultaneous detection of multiple DNA targets, but the miniaturization of all the detection and analysis steps in an integrated circuit allows its appli-

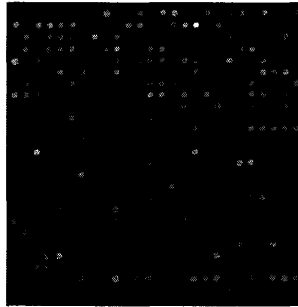


Figure 1-11: Example of microarray hybridization. A representative portion of a microarray shows the differential signals from two RNA samples. One RNA sample was reverse transcribed into cDNAs labelled with red fluorophore, the other RNA sample into cDNAs labelled with green fluorophore, and the cDNA mixture was hybridized to spots of DNA representing different genes. Selective hybridization of cDNA from either RNA sample to a DNA spot produces red or green signal; hybridization of cDNA from both RNA samples produces yellow signal. In this example, red spots represent RNAs enriched in hermaphrodites with wild type germ lines, and green spots are RNAs enriched in *glp-4(bn2)* mutants with greatly diminished germ lines. From [23].

cation under "in-the-field" clinical conditions[24]. This requires the surface used for the DNA immobilization to be amenable to integration into a microelectronics format and suggests the use of silicon.

1.3.3 "Lab-on-a-chip"

Another active field is the development of the so-called "Lab-on-a-chip" configuration, which integrates multiple processes, from sample collection, DNA extraction and amplification, to hybridization and detection, on a single microfluidic platform the size of a credit card[25, 26, 27] (see figure 1-13 for an example of a lab-on-a-chip layout). The integration of all these steps on a single chip offers tremendous advantages in terms of sample and reagent consumption, contamination, efficiency, speed and cost. In addition, the miniaturization and ease of use enable the analyses to be transported from the laboratories to "in-the-field" conditions.

The technology is far from being mature, and although there have been efforts to mimic the monolithic approach of the silicon microelectronics technology[28], a

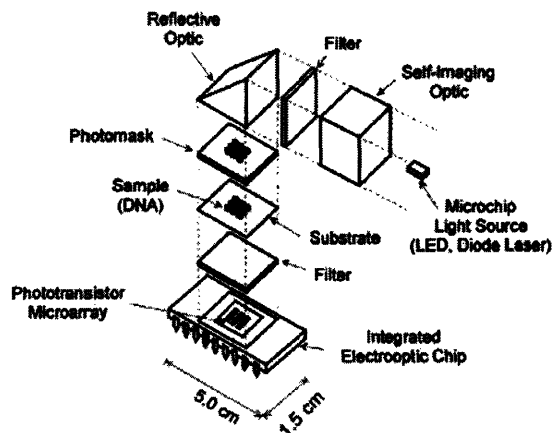


Figure 1-12: Schematic diagram of a DNA biochip concept. From [24].

variety of material technologies, with different levels of compatibility, are under development, corresponding to the different requirements of microfluidics, optical and electrical detection. However, some common materials and processing techniques are evolving, often on the basis of silicon compounds and polymers. Semiconductors and metals are obviously necessary components of electrical detection schemes and wiring, while polymers are attractive for microfluidic uses[29]. They are cheap, easily manufactured, and can be bonded to other surfaces, such a silicon, to form fluid channels.

The set of examples presented above, which give an overview of the state of technologies and research in the field of DNA detection, is representative of the wealth of materials approaches currently under development. Technologies may in the end not convergence fully, and the variety of tools now available may find its application in the engineering of very diverse devices meeting the needs of specific diagnostics and analyses. We can presume that this diversity will translate in a diversity of materials solutions. Developing a technology for the patterning of DNA that can encompass many different materials environments is therefore critical, with a first emphasis on silicon and silicon-derived materials, and commonly used polymers such as PMMA and PDMS.

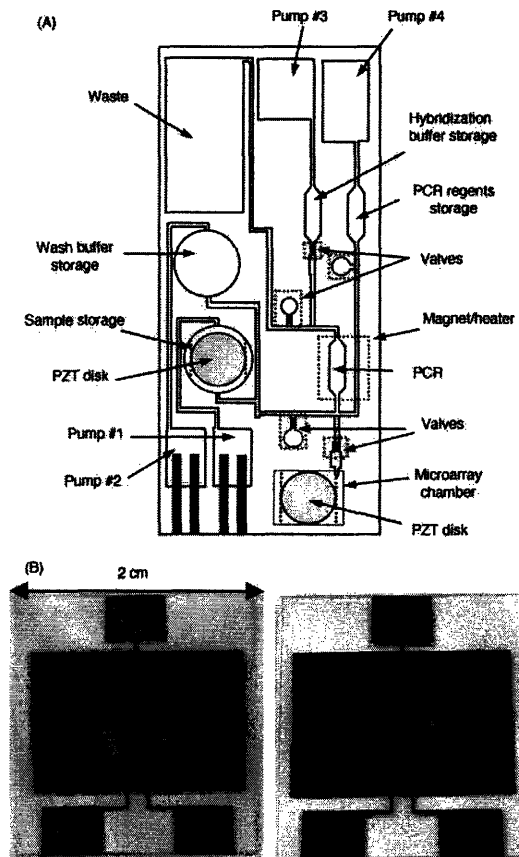


Figure 1-13: A) Schematic of the polycarbonate fluidic chip developed by Motorola Labs. B) Photos showing the top (left) and bottom (right) views of the integrated PCR/electrochemical chip. WE: gold working electrode; CE: Pt counter electrode; and RE: Pt pseudo reference electrode. From [26].

1.4 Immobilization of DNA on surfaces

Reproducing DNA patterns on surfaces using SuNS requires the ability to direct efficiently the immobilization of DNA strands on surfaces of our choice. This implies the choice of an immobilization strategy, usually the prior preparation of the substrates used and their chemical derivatization in the case of inert substrates like the silicon-derived ones.

Characteristics of patterns printed with SuNS are strongly associated with the properties of the surface used, such as the chemical and physical structure, the surface architecture, surface tension, hydrophobicity and hydrophilicity. Therefore special care must be taken in the choice of an immobilization strategy.

1.4.1 DNA modification for attachment on surfaces

The negative charge of the DNA backbone, or the many free amine groups provided by the bases A, C and G in single stranded DNA, provide ways to attach unmodified DNA strands to a range of reactive surfaces. Nevertheless, the use of unmodified DNA, that links to surfaces through intra-chain bonds, has important drawbacks. Mainly, some of the bases are linked very closely to the surface and are likely to be less available for hybridization, which may destroy the hybridization properties of the DNA strand. This is even more critical for SuNS, which not only requires a good hybridization efficiency of the attached DNA strands, but also needs that, once hybridized, the DNA helices have a free end able to bind to another surface. Therefore, although it increases sensibly the processing costs, it is usually advised[30] to use modified DNA strands, with functional groups attached to their 3' or 5' end that can bind specifically to surfaces.

For this purpose, we have been using commercially available 5'-modified DNA strands with two types of modifications: thiol and amine.

1.4.2 DNA immobilization chemistries on solid surfaces - Common approaches

Choosing a strategy for the immobilization of DNA involves the choice of a combination surface / modification chemistry. Although the possibilities seem very broad, the number of commonly used surfaces and their modifications is actually quite limited, a small number of techniques being widely adopted, usually because of their simplicity and robustness.

The most widely used surfaces for chemical derivatization are gold, and silicon and silicon-related substrates. On gold the DNA immobilization is mostly based on thiol compounds, while the methods are more diverse on silicon, either relying on silane compounds for oxidized silicon and glass, or on the direct attachment of functional groups through Si-C bonds to unoxidized silicon.

Gold

In the case of gold substrates, oligonucleotides are usually modified with a thiol-linker, which covalently binds the strands to the surface. This immobilization method has been extensively studied, and the mechanisms of interaction of gold and thiol-modified oligonucleotides are now well-known[31]. In addition, both these studies and our experience show that this attachment method is very favorable to SuNS. First, the gold-thiol bond is reversible, therefore when a gold surface is in a solution of thiolated oligonucleotides a dynamic equilibrium takes place between the attached molecules and the molecules in solution, where molecules constantly adsorb and desorb from the surface. This favors the formation of high quality monolayers, the oligonucleotides having higher chances to tether to the surface in the most favorable conformation, since this offers a better stability. The quality of the monolayer can then be increased further by the use of a "backfilling" by a short thiol-ended molecule, like mercaptohexanol. A dense sublayer of these spacer molecules helps to remove the weakly unspecifically bound oligonucleotides, and to make the attached nucleotides stand up with a preferred orientation toward the substrate normal.[32, 33]

The good control that we have of DNA immobilization on gold surfaces, together with the fact that the DNA conformation is very favorable to SuNS, have made of gold our surface of choice for the fabrication of templates. I have experienced important difficulties printing from non-gold custom-made templates, whereas those same substrates, after being printed via SuNS from gold templates, could then be used themselves successfully as templates. This suggests that the conformation of the oligonucleotides was more favorable to SuNS on gold, and also that this conformation was retained on the printed substrates.

Oxidized silicon and glass

Surfaces of glass and silicon with a layer of native oxide share the same chemical properties. In order to enable covalent immobilization, a chemical modification of the surface is necessary, so that the functionally inert silanols (Si-OH) of the glass or oxidized silicon surface are modified to possess functionalities that react with the modified oligonucleotides. Glass slides being the most common substrates for DNA microarrays due to their optical properties, mechanical and chemical stability, a family of surface derivatizations has been extensively used for their modification, involving a monolayer of functionalized silane compounds, which at one end bind to the silanol groups of the surface and at the other end can bind either directly or through linkers to the modified oligonucleotides. The most common functional groups for the attachment of oligonucleotides are thiol[35], aldehyde[36] and epoxy[37], shown in figure 1-14.

The amino group's modification is advantageous in that it offers several kinds of immobilization strategies, using homo- or heterobifunctional linkers. On the other hand, thiol, aldehyde or epoxy groups can bind covalently, without the assistance of a linker, to thiol- or amino-modified oligonucleotides. Because of this advantage, although immobilization of capture probe DNA molecules was reported to be less efficient on these surfaces[34], they are widely used in the fabrication of DNA microarrays.

The silicone elastomer poly(dimethyl siloxane) (PDMS), which combines the prop-

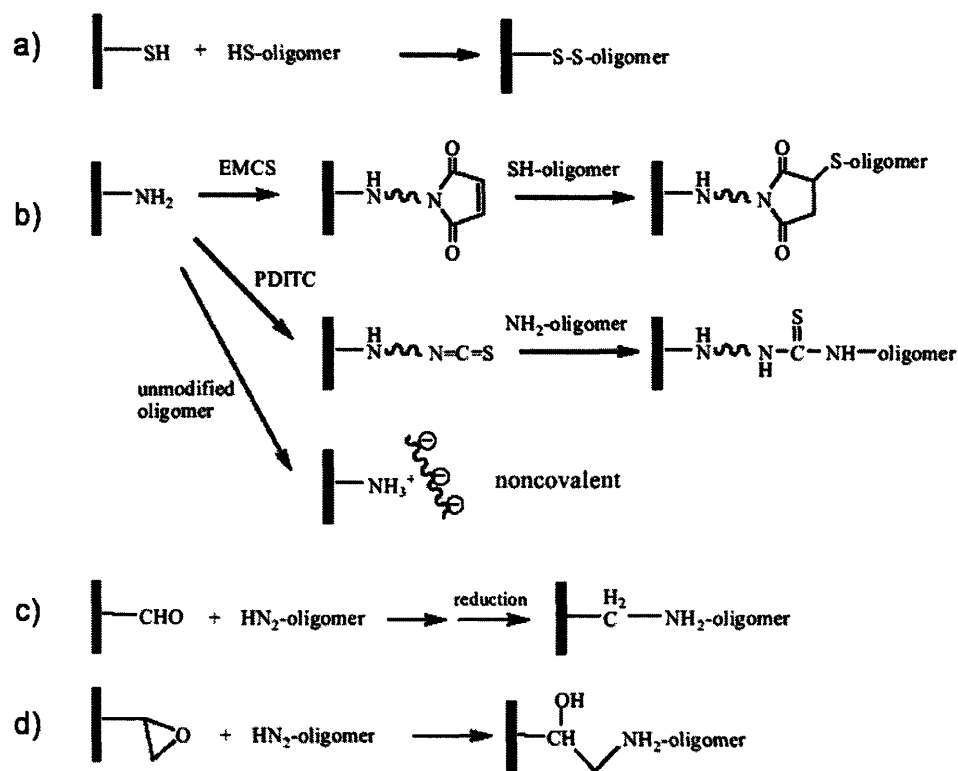


Figure 1-14: Surfaces modified with a thin layer of various functional groups and immobilization methods of DNAs on the surfaces. DNA molecules can be immobilized on a) thiol, b) amino, c) aldehyde, d) epoxy surface either covalently or ionically. From [34].

erties of both plastic and silica-based substrates, can also be silanized after plasma oxidation. Its modification was reported using 3-mercaptopropyltrimethoxysilane[38] resulting in active thiol groups, or 3-aminopropyltriethoxysilane[39] resulting in active amine groups.

Unoxidized silicon

Other, less common methods involve the direct functionalization of silicon substrates without an oxide layer. These new attachment methods provide modified silicon surfaces through direct carbon-silicon bonds. Two especially interesting approaches result in surfaces with amino[40] or N-hydroxysuccinimide[41] groups, to be cou-

pled with thiol-modified oligonucleotides using a linker and amine-modified oligonucleotides, respectively. Although these techniques are still in their infancy and have been used in very few DNA array laboratories, they are very advantageous in that they avoid the inhomogeneity and chemical variability of oxidized surfaces, and they therefore look very promising.

1.4.3 Using silicon and glass silanization for SuNS

Being the most widespread and well-documented method for the functionalization of oxidized silicon, silanization was our natural choice for SuNS. It first appeared in the 1980's in the work of Sagiv[42] and has since then proved very attractive mainly thanks to the simplicity of its mechanism and to the stability of the resulting molecular platforms.

The silanes most currently used for surface derivatization are trifunctional, and have the general formula RSiX_3 , with $\text{X} = \text{Cl}, \text{OMe}, \text{OEt}$. Their structure, shown on figure 1-15, can be divided into three parts:

- a trifunctional head group is responsible for binding the molecule to the surface and to the adjacent molecules.
- an alkyl chain, of variable length, assists in the formation of ordered molecular structures through the inter-chain van der Waals interactions.
- a terminal functional group is chosen to attach subsequently to other molecules, in our case oligonucleotides.

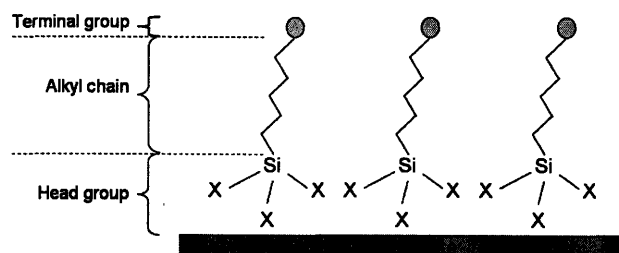


Figure 1-15: Schematic of the structure of silane molecules

The mechanism of the formation of a self-assembled monolayer of silanes is gen-

erally considered to take place in four steps, represented schematically on figure 1-16[43, 44]:

1. The silane molecules are physisorbed at the hydrated silicon surface.
2. In presence of the adsorbed water layer on the surface the silane groups $-\text{SiX}_3$ then hydrolyse into highly polar trihydroxysilanes $-\text{Si}(\text{OH})_3$.
3. The $-\text{Si}(\text{OH})_3$ groups form covalent bonds with the hydroxy groups on the SiO_2 surface.
4. A compact monolayer is formed, driven by the lipophilic interactions between the alkyl chains.

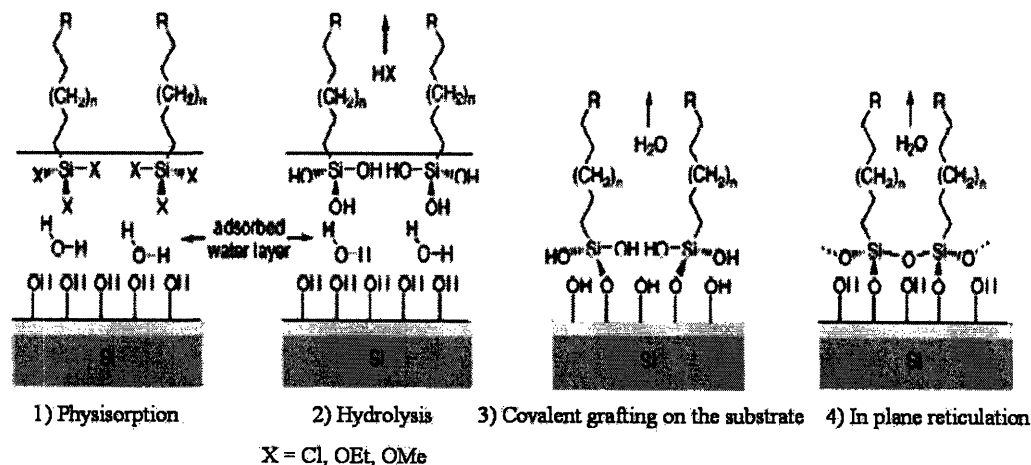


Figure 1-16: Schematic of the steps involved in the formation of a silane self-assembled monolayer on a hydrated silicon surface. Taken from [43].

The three silanol groups present in the trialkoxysilane trifunctional molecules are not all reacting with the surface, and can react with adjacent molecules, leading to the polymerization of the siloxane layer, which gives the monolayer a better resistance to hydrolysis. Therefore, the formation of a silane monolayer is the result of two competing phenomena: the condensation of the surface silanols with the silane molecules' silanols, and the polymerization of siloxanes, both via Si-O-Si bonds. Both occur in the presence of water, which is why a layer of water on the surface is necessary, but the presence of water multilayers is likely to lead to the nonuniformity of the silane

layer, since the silane monomers polymerize before reaching the surface.

This is why the simplicity of the silanization reaction is only apparent. Although the silanization of surfaces has been extensively used and reviewed[45, 46], and there has been some efforts in the past decade to study the reliability and stability of silanization conditions[47], the exact mechanisms and parameters are still not fully understood. First, the original silicon oxide surface itself is prone to inhomogeneity and variability in the relative number of Si-O-Si and Si-OH linkages. Then the level of polymerization of the siloxane layer(s), as described above, is extremely hard to control. This chemical variability can lead to a lack of reproducibility and stability, especially when the silanization is carried out in liquid phase[50]. For this reason, although silanization in liquid phase is easy and extensively used, it has often been preferred anhydrous gas-phase reactions which, without solving all the problems related to silanization, have proven to give better and more reproducible results[51].

In my attempt to immobilize DNA on silanized silicon and glass, I chose to silanize in gas-phase, using mainly two apparatus:

1. The first apparatus used an argon flow to carry the silanes to the surface, similar to what was used by Hong et al.[50], and shown in figure 1-17.
2. The second apparatus used vacuum to vaporize the silanes and deposit them on the surface. The samples and a beaker containing pure silanes were simply placed in a vacuum desiccator before vacuum was applied.[52, 53]

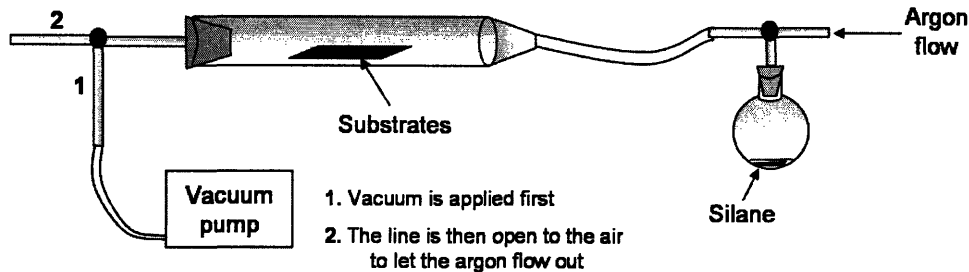


Figure 1-17: Schematic of the apparatus using an argon flow for the vapor phase deposition of silanes on oxidized silicon

The silanization was conducted in parallel on silicon wafers covered with a layer of

native oxide and on glass, and two types of silanes were used, 3-glycidoxypropyltrimethoxysilane (GOPS)[37] and triethoxysilylbutyraldehyde (TESA) (formulas shown in figure 1-18), whose terminal functions both react with amines.

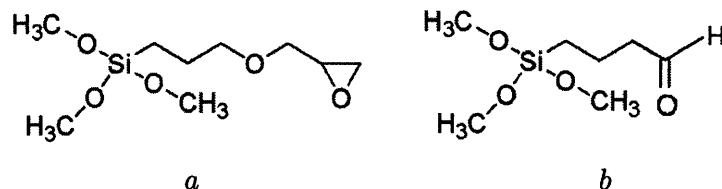


Figure 1-18: Formulas of a) 3-glycidoxypropyltrimethoxysilane (GOPS); and b) triethoxysilylbutyraldehyde (TESA)

The DNA immobilization and control were carried out following the pattern below:

1. The oxidized silicon surface was functionalized by silanization.
2. DNA dots were spotted on the surface, which was incubated in a humid chamber during the time of the DNA immobilization (between 2 hours and overnight).
3. The surface was passivated in a solution of triethanolamine to prevent the subsequent non-specific attachment of nucleotides.
4. The surface was hybridized with fluorescently labelled complementary DNA.

Details are available in the experimental section.

The results were controlled mainly by fluorescence imaging of the resulting immobilized DNA strands, after they were hybridized with their fluorescently labeled complementary strands. AFM images were also taken at the different steps of the silanization and DNA immobilization, but this imaging didn't give consistent results, due to the inhomogeneity of the surfaces.

To face the difficulties that I encountered immediately with the immobilization of DNA on the silanized surfaces, I tried to take advantage of the wealth of approaches proposed in the literature[47, 48, 49], and worked on the optimization of the main reaction parameters:

- The pre-silanization treatment, meant to provide a clean oxide layer with a high density of silanol groups on the surface (Si-OH)

- The silanization method, mainly its temperature and reaction time
- The post-silanization method, aiming to improve the cross-linking of the silane layer: with or without a baking step, with or without sonication

In spite of these long efforts spent in optimizing the reaction, the immobilization and hybridization of DNA gave extremely variable and inconsistent results. This could be attributed to two different causes: first, during the silanization reaction itself, in addition to the precited parameters there were other parameters that I poorly controled in the laboratory, mainly the humidity level and the level of cleanliness of the samples. Then, given that the fluorescence control used occurred only at the last stage of a long succession of events, the failure to obtain consistent levels of fluorescence could be due to the inefficiency of steps subsequent to the silanization. Most probably, the lack of control of the immobilization conditions, and particularly of the humidity level, was responsible for a part of the lack of reproducibility of my experiments.

1.5 A substrate-independent approach to surface modification: reactive polymer coatings

Another approach for the immobilization of DNA exists, contrasting with the complexity of the conventional DNA immobilization strategies presented above. The latter involve, as was said, a strategy of selective couplings, between surfaces and the functionalizations they can bear, and imply trade-offs that need to be reconsidered differently for each material. The other approach we came to consider, developed in Lahann's group[54, 55, 56, 57], is based on thin reactive polymer coatings, that can be used on a variety of substrates. The method is substrate-independent, and can therefore be used for any substrate envisioned for SuNS. What is more, the failure in using silanes for silicon-derived surfaces pushed us to consider seriously this method that offers very high reproducibility.

The technique is based on the chemical vapor deposition (CVD) polymerization

of substituted [2,2]paracyclophanes, to yield functionalized poly(p-xylylenes) coatings (scheme shown in figure 1-19). The coatings can exhibit a wide variety of chemically active groups, such as amines, esters, alcohols, aldehydes and anhydrides, thus able to bind to DNA in different manners. The choice of a functionalization is therefore decoupled from the choice of a substrate, and both can be chosen among a wide spectrum. Moreover, this simple, one-step surface modification offers accurate control of the composition and of the architecture of the films, and an excellent adhesion.

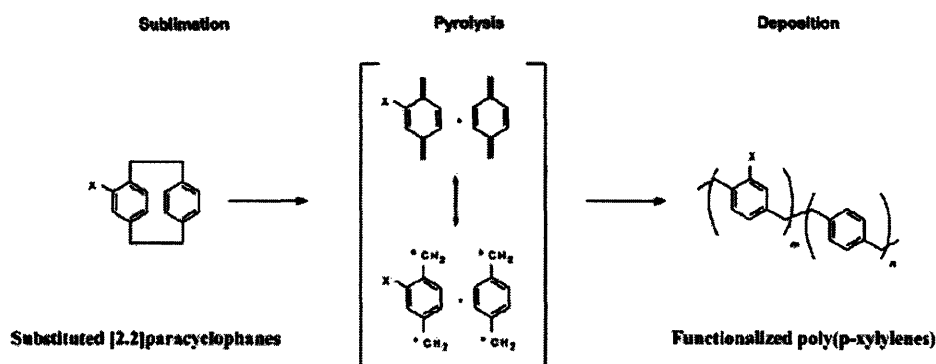


Figure 1-19: Schematic of the CVD polymerization of substituted [2,2]paracyclophanes, to yield functionalized poly(p-xylylenes). From [57]

For all these reasons, and keeping in mind the wealth of applications that could benefit from the extension of SuNS to new materials, we have turned to these reactive polymer coatings, creating a collaboration with Prof. Lahann's group. The objective was to use Lahann's coatings to functionalize and print a variety of substrates with a unique protocol. The experimental work done with these coatings and the results obtained will be the focus of the following chapter.

Chapter 2

Printing on CVD coated substrates

This chapter presents the results obtained with the printing of various functionalized poly(p-xylylenes) coated substrates using SuNS's method. Five substrates could be printed thanks to this new approach, covering a very broad spectrum of material types: silicon, quartz, polystyrene, acrylic and PDMS. In addition, different kinds of patterns, with different scales, were used to print on these substrates, and 3 different functionalizations of the polymer coatings were tried.

2.1 Methods

All the substrates used as templates in the results presented here have a gold surface, to which single stranded DNA (ssDNA) binds via a thiol bond. A homogeneous DNA monolayer is obtained by soaking the substrates 4 days in a solution of thiol-modified ssDNA, followed by a 1 hour immersion in mercaptohexanol to backfill the DNA monolayer and avoid the non-specific attachment of other DNA strands during subsequent steps.[32, 33]

In a humid chamber the attached ssDNA is then left to hybridize under cover-slip in a solution of amine-terminated cDNA. The functionalized poly(p-xylylenes) coated secondary substrate, whose function is chosen to bind to the amine end of the cDNA, is then brought into contact with the template, and a slight pressure is applied and maintained overnight while the substrates are kept in a vacuum desiccator. After this

stamping step the two substrates are then heated at 90°C to dehybridize the DNA strands, and separated.

2.2 Printing on silicon in the sub-micrometer range

Printing in the sub-micrometer range, with patterns that have features in the order of hundreds of nanometers, in addition to the fact that it's a lengthscale that fits many device applications, has another advantage: it makes it possible to check for pattern transfer over very small areas, on the order of a few micrometers using atomic force microscopy, therefore circumventing the problem of low printing coverage. This proves very valuable at the early stages of development of a new method for SuNS, when the printed areas are too small to make a large pattern transferrable.

2.2.1 Masters used

To print in the sub-micrometer range two types of templates were used. They were gold coated silicon gratings with 50 nm deep trenches and a 100 nm or a 700 nm pitch, schematically represented in figure 2-1.

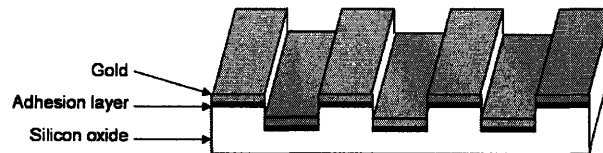


Figure 2-1: Schematic of the gold coated silicon gratings used as templates for nanoscale prints

- The 100 nm pitch silicon gratings were fabricated in the NanoStructures Laboratory at MIT. The initial grating pattern was produced using a technique called Achromatic Interference Lithography (AIL)[58]. The initial pattern was then transferred into silicon by reactive-ion etching. The silicon grating was then coated, by e-beam deposition, with 3 nm of titanium followed by 7 nm of gold. A Scanning

Electron Microscopy image of one of these templates can be seen in figure 2-2.

- The 700 nm pitch silicon gratings were made in Prof. Ross's group, then coated, by e-beam evaporation, with 5 nm of chromium followed by 5 nm of gold. Figure 2-3 shows an Atomic Force Microscopy image of one of these gratings.

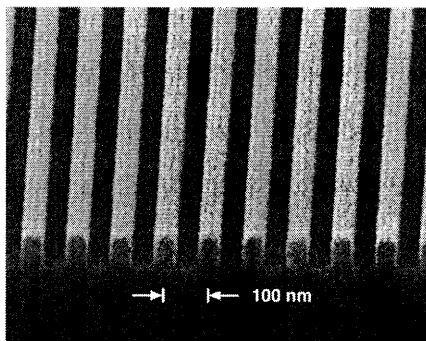


Figure 2-2: Scanning Electron Microscopy image of a 100 nm pitch gold coated silicon grating fabricated using AIL

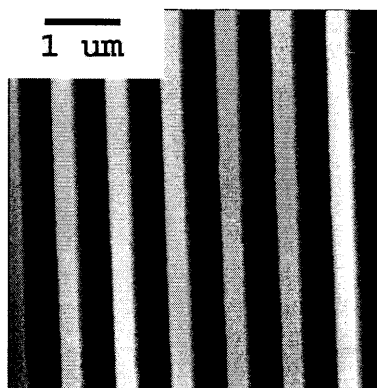


Figure 2-3: Atomic Force Microscopy image of a gold coated 700 nm pitch silicon grating. Z range = 80 nm

2.2.2 Printing patterns with a 700 nm pitch onto aldehyde functionalized coated silicon

3 functionalizations of the poly(p-xylylenes) were tried, all able to bind to the amine-modification of our cDNA (see figure 2-4 for the molecular structures):

- aldehyde: poly(4-formyl-p-xylylene-co-p-xylylene) (noted ppx-cho)
- pentafluorophenol ester: poly[p-xylylene carboxylic acid pentafluorophenol ester-co-p-xylylene] (noted ppx-pfp)
- anhydride: poly(p-xylylene-2,3-dicarboxylic anhydride) (noted ppx-anhydride)

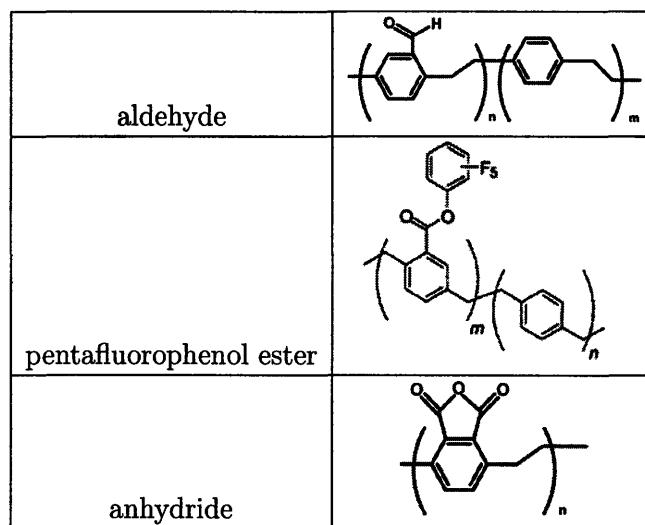


Figure 2-4: Formulas of the poly(p-xylylenes) used on the secondary substrates

The best results were obtained with the aldehyde functionalized coatings, but it's only when special care was given to the handling of the poly(p-xylylenes) coated substrates to ensure their cleanliness and smoothness, both during their fabrication in the laboratory of our collaborators and during the shipping, that I started obtaining results.

I evaluated as about $50 \mu\text{m} \times 50 \mu\text{m}$ large the printed areas, and these printed areas were found mostly near the corners of the substrates, where the slight damage of the polymer coatings suggested that the pressure was the highest. Images of prints on aldehyde functionalized coated silicon are shown in figure 2-5 and 2-6.

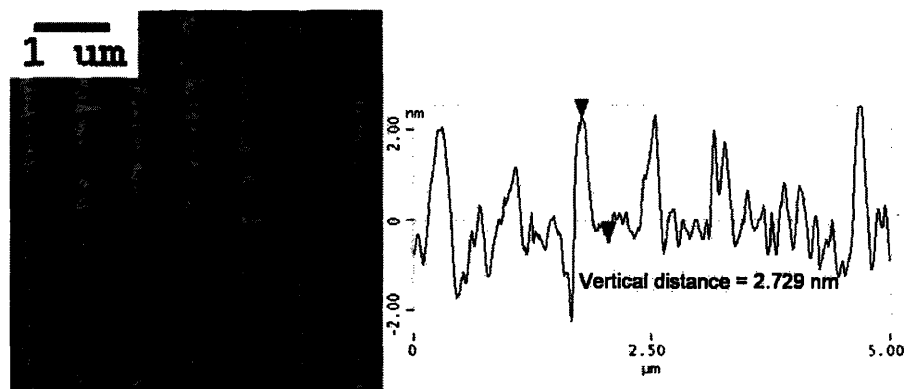


Figure 2-5: Atomic Force Microscopy height image and section of a ppx-cho coated silicon substrate after it was printed with a 700 nm pitch master. Z range = 5 nm

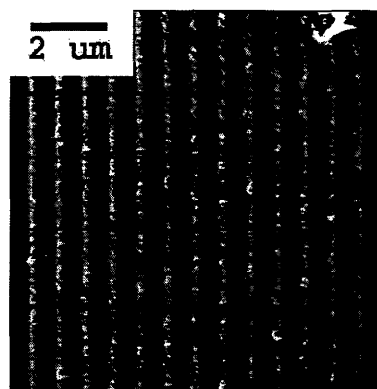


Figure 2-6: Atomic Force Microscopy height image of a ppx-cho coated silicon substrate after it was printed with a 700 nm pitch master. The sample is the same as the one shown in figure 2-5. Z range = 5 nm

2.2.3 Trying other functionalizations of the polymer coatings

As was said above, the results were not as satisfying using other functionalizations of the coatings, anhydride and pentafluorophenol ester, as with the aldehyde modification. Moreover, a difficulty appeared in the control of the results, due to a combination of the printing of DNA and of the imprinting of the polymer coating. Imprinting means the topological modification of the underlying coating due to a too high pressure of the grating onto the surface[59]. An imprinted pattern can be up to 50 nm deep, while the length of a single DNA strand is about 20 nm and a DNA

line rarely goes above 12 nm in height. When the pressure applied is too high and imprinting occurs, it is hard to determine to what extent the substrates were printed: the presence of patterns is not anymore an evidence for the presence of DNA.

Figure 2-8 shows how the image analysis is complicated by the imprinting. On image 2-8 the 14 nm depth measured for holes present in the lines (shown in the section of image 2-8) suggests imprinting, while the complex texture of these lines, compared to a simple imprint like the one shown in figure 2-7, suggests that DNA was nevertheless transferred.

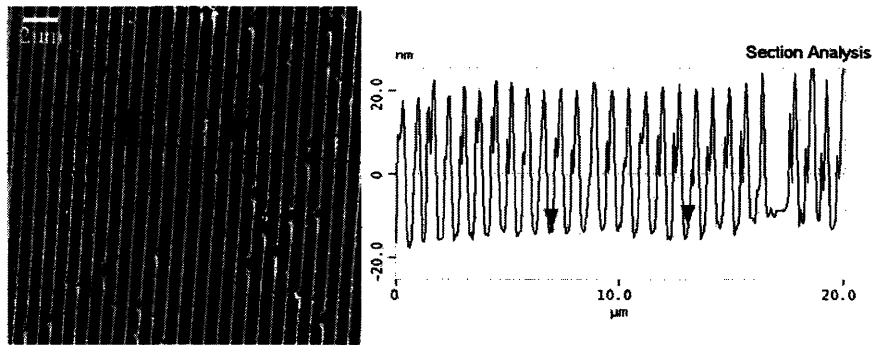


Figure 2-7: AFM image and section of an ppx-anhydride coated silicon substrate which was imprinted when stamped from a 100 nm pitch master. Z range = 50nm

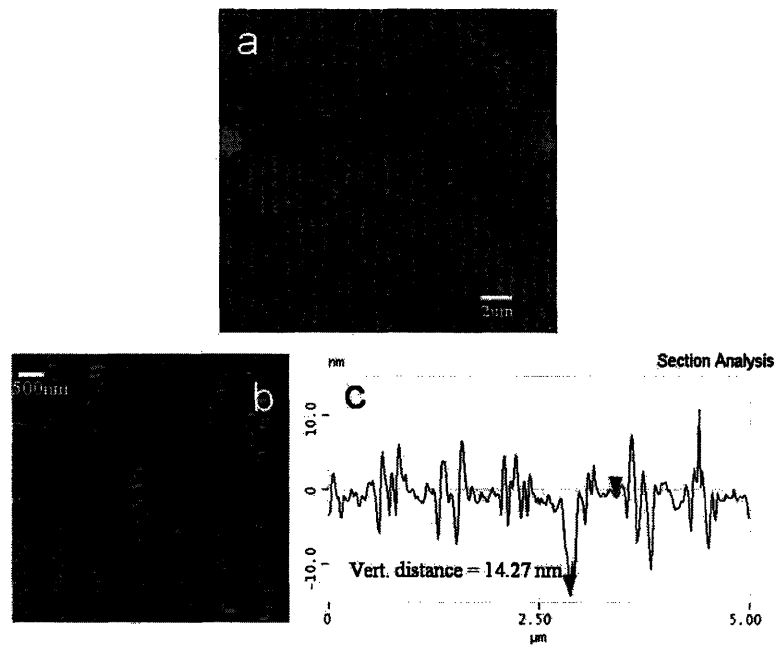


Figure 2-8: AFM images of a ppx-pfp coated silicon substrate printed with a 700 nm pitch master. Z range: a)=10nm, b)=30nm. b) is a close-up view of what is seen on (a). c) shows a section measured in (b).

2.2.4 High resolution printing

Following the results obtained with the aldehyde functionalized (ppx-cho) coatings they became our coatings of choice for all the subsequent printings. They were used to test the resolution of our new printing approach, by printing from 100 nm pitch masters. Results such as those shown in figure 2-9 were obtained.

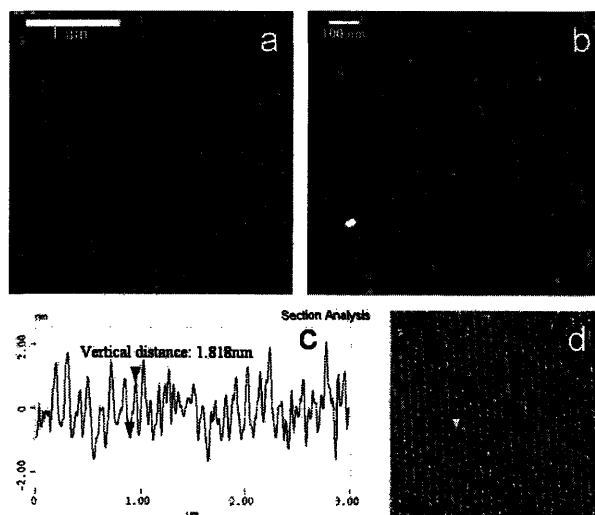


Figure 2-9: AFM images of a ppx-cho coated silicon substrate printed from a 100 nm pitch master. a) z range=5nm; b) z range=10nm. c) section analysis along the black line shown in (d)

There were variations from print to print in the density and continuity of the DNA lines. In figure 2-10 for example the conformation is unusual, with a high density as shown by the average height of the printed lines (9 nm), but many discontinuities in the pattern. Such differences between prints obtained from a same template on a similar type of surface have to be attributed to unequal qualities of contact during the stamping step.

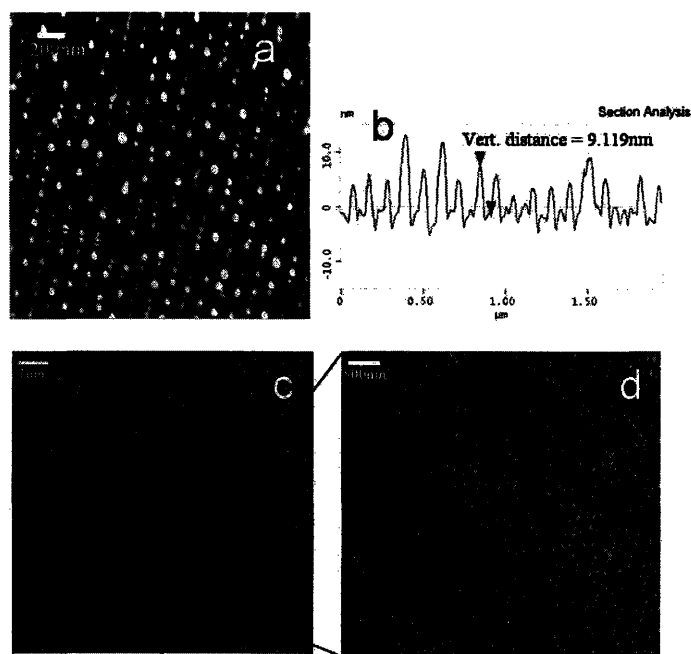


Figure 2-10: AFM images of a polymer coated silicon substrate printed from a 100 nm pitch master. a) and c) z range=15nm. d) z range=10nm. b) shows a section taken in (a). d) is a close-up view of the bottom left corner in (c).

2.3 Printing onto various other substrates

The success of SuNS on aldehyde functionalized poly(p-xylylenes) coated silicon substrates suggested that these coatings could enable the stamping of all kinds of other substrates. As was said above, the chemical vapor deposition technique used to obtain these polymer coatings provides reactive surfaces of equal quality independently of the substrate chosen. The adhesion of the coating can vary from one substrate to another, but silicon, which is usually the most problematic in terms of adhesion, proved safe for SuNS.

Four materials: quartz, polystyrene acrylic and polydimethylsiloxane (PDMS), in addition to silicon, were coated with aldehyde functionalized poly(p-xylylenes) and successfully used as replica surfaces for SuNS to print in the sub-micrometer or micrometer range.

The results are shown in the following figures, on quartz, polystyrene, and acrylic, printed from gold coated silicon gratings identical to the ones used to print on silicon.

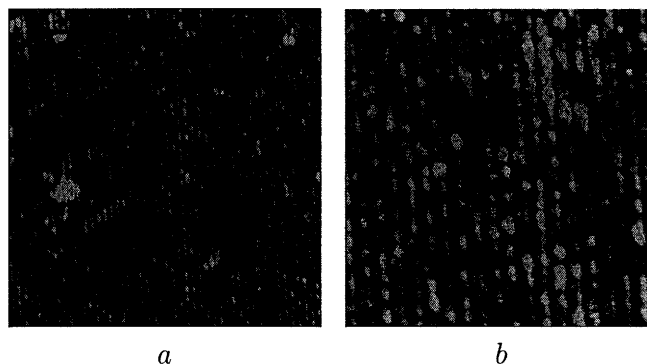


Figure 2-11: AFM images of ppx-cho coated quartz printed with a 100nm pitch master. a) Z range = 15 nm. b) Z range = 6 nm

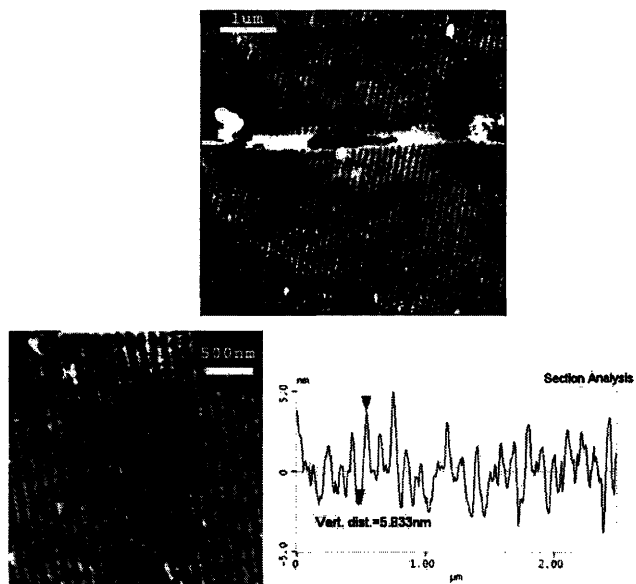


Figure 2-12: AFM images of a ppx-cho coated polystyrene substrate printed with a 100nm pitch master. Z range=10nm

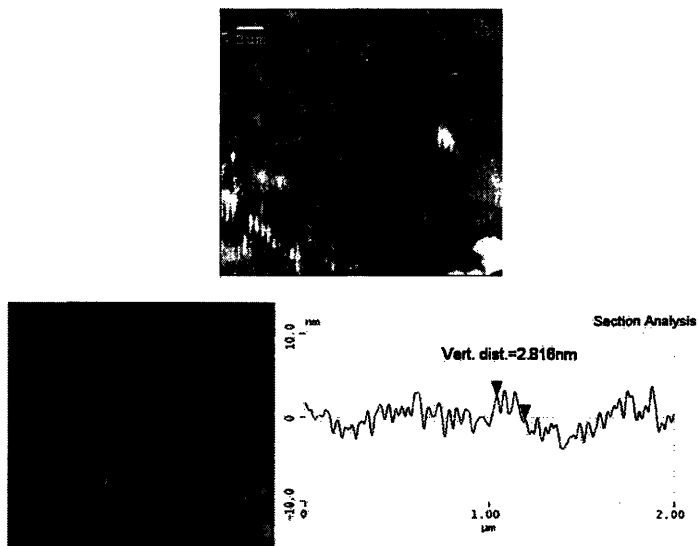


Figure 2-13: AFM images of a ppx-cho coated acrylic substrate printed with a 100nm pitch master. Z range=20nm

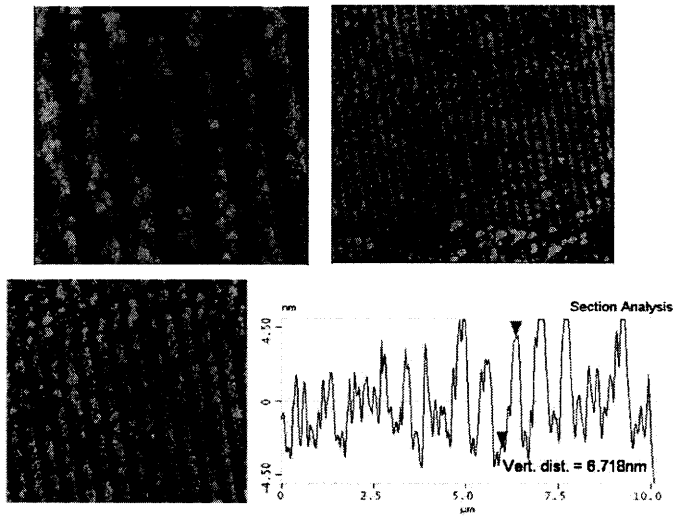


Figure 2-14: AFM images of a ppx-cho coated acrylic substrate printed with a 700nm pitch master. Z range=10nm

2.4 Using SuNS in the micrometer range

2.4.1 Motivation

Printing in the micrometer range enables the control by fluorescence microscopy, which brings with it two advantages:

1. As opposed to scanning probe microscopies like AFM, which can only scan surfaces on the order of 10 μm wide at a time, optical fluorescence microscopy can give us information over large areas, therefore providing us for SuNS with insight in the printing coverage.
2. The fluorescence control provides not only topographical information, but also chemical information: the fluorescence signal is emitted by fluorescence modified DNA strands that are hybridized to the ssDNA previously printed on the surface. Therefore the fluorescence detection depends on the ability of the printed ssDNA to hybridize with the expected DNA strand, which provides us with information on its sequence.

These two advantages make the printing of micrometer scale patterns extremely attractive for SuNS. This section presents the results obtained at that scale, together with the controls made on our printed patterns, which exploit the quality of the information obtained by fluorescence microscopy.

2.4.2 Printing onto polystyrene on PDMS in the micrometer range

To print in that range a large contact area is needed during the printing step, therefore the best candidates as substrates for replica were polymers that can conform to the roughness of the template's surface, such as acrylic, polystyrene or PDMS. Acrylic and polystyrene, although they are hard and brittle at room temperature, can indeed become soft if heated up to 60°C. We use this property by heating up to 60°C for 15 minutes the template and replica just after they were put in contact in the press. During this heating step the surface of acrylic or polystyrene conforms better to the

surface of the master, increasing the contact area. The samples are then cooled down and left overnight in a vacuum desiccator as in a regular stamping protocol.

The masters used to print in that range were silicon substrates patterned with photolithography, resulting in a pattern of 1 μm large gold squares on which a monolayer of thiolated ssDNA was assembled. After being printed (cDNA of sequence A' is expected to be transferred) the samples were hybridized with fluorescent DNA strands of the same sequence as the one present on the master (sequence A), and imaged with a fluorescence microscope. Some results obtained with PDMS were extremely promising, showing a very high fluorescence intensity contrast as can be seen on figure 2-15, but the results were on a whole very hard to reproduce with some consistency, probably due to the lack of a good method to avoid the trapping of air bubbles between the template and the replica. This will be treated in chapter 3, covering the issues of contact printing and our approaches to solve that problem. On polystyrene, nevertheless, good results could be obtained with consistency, as can be seen on figure 2-16, where the fluorescent squares show that the printed DNA hybridized successfully. This proves first that the transferred DNA is in a conformation favorable to its rehybridization and can be used actively for further experiments, and also shows that it has the sequence expected according to SuNS's principle.

Printed areas up to 3 millimeter in diameter were obtained on polystyrene using this method, with very shallow or no imprinting and damaging of the underlying polymer coating.

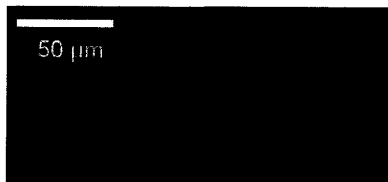


Figure 2-15: Fluorescence microscopy image of a printed PDMS sample, after its hybridization with fluorescently labeled complementary DNA strands

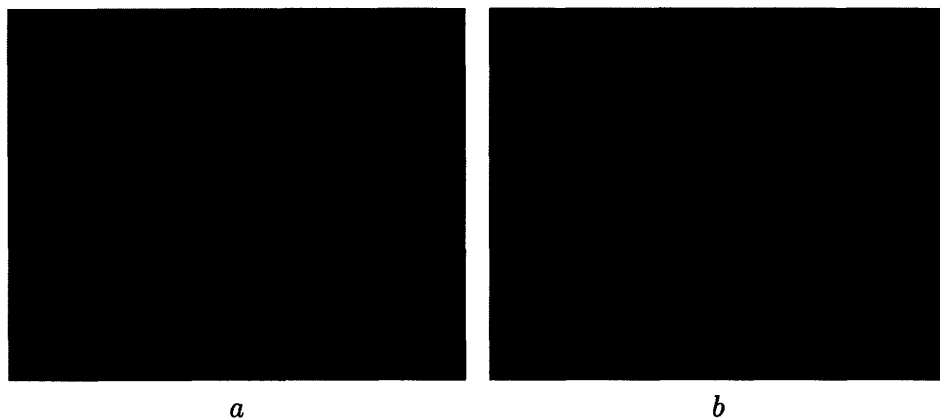


Figure 2-16: Fluorescence microscopy images of a polystyrene substrate, taken after hybridization of the printed DNA strands with fluorescently labeled strands

2.4.3 Control of the nature of the printed patterns

As was said above, the fluorescence imaging of the printed DNA patterns intrinsically provides us with information on the sequence of the transferred DNA . To control this, a pattern of printed DNA underwent two cycles of hybridization-dehybridization:

1. The printed ssDNA pattern (expected sequence A') was hybridized with fluorescently labelled DNA strands having the same sequence as the DNA present on the master (sequence A), then imaged with a fluorescence microscope, and the fluorescence intensity was measured along a line.
2. The DNA was then dehybridized by heating up the sample at 90°C in deionized water, it was imaged and the fluorescence intensity was measured along the same line as before.
3. The ssDNA pattern was then rehybridized, this time with fluorescently labelled DNA having a sequence different from the one present on the master (sequence B). Again the sample was imaged with a fluorescence microscope and the fluorescence intensity was measured along the same line as the previous times.

All the fluorescence measurements were taken using the same parameters of exposure. The comparison of the three measurements done is presented in figure 2-17.

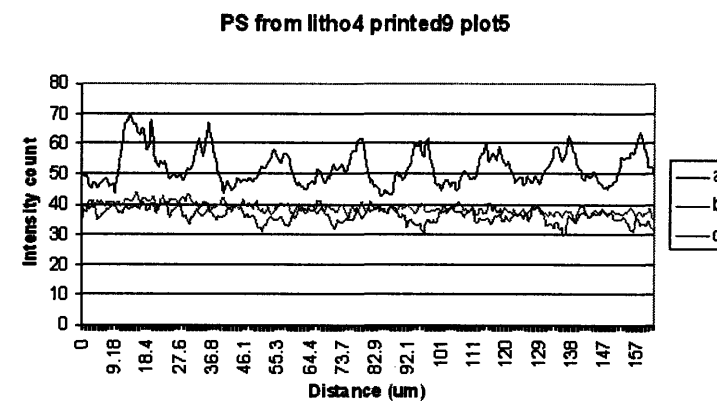


Figure 2-17: Superposed plots of the fluorescence intensity count measured along a same line in three different cases: a) The sample is hybridized to fluorescently labelled DNA strands with the same sequence as the ssDNA present on the master. b) The sample is dehybridized. c) The sample is hybridized with DNA strands of another sequence.

2.5 Reprinting from a replica

Being able to use a printed substrate as master is one of the main specificities and advantages of SuNS. Furthermore, the ability to reprint from a replica constitutes a proof that the stamped pattern consists of DNA strands of the expected sequence in a conformation that leaves them active for further manipulations. A nanoscale pattern previously printed onto a silicon substrate coated with aldehyde functionalized poly(p-xylylenes) (ppx-cho) was reprinted onto a polystyrene substrate also coated with ppx-cho, following the same method described earlier. The pattern had a 100nm pitch and could be reprinted with clarity over areas in the 50 μ m range. Results are shown in figure 2-18.

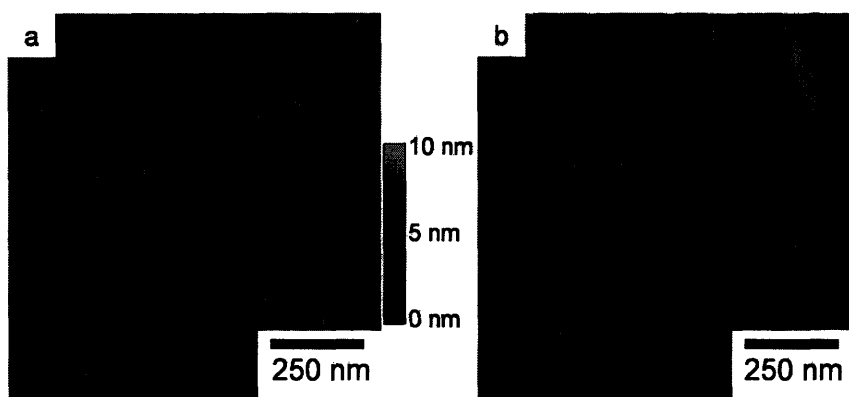


Figure 2-18: Second generation printing. a) Tapping mode AFM image of a printed ppx-cho coated silicon substrate used as a master b) Tapping mode AFM image of a ppx-cho coated polystyrene substrate reprinted from the printed silicon substrate shown in (a).

2.6 Printing on patterned coatings

Combining the pattern transfer ability of SuNS with the use of pre-patterned secondary substrates is a straightforward way to multiply the possibilities and increase the complexity of the produced patterns (the principle is schematically represented in figure 2-19). The CVD coatings from Prof. Lahann's group, due to the ease with which they can be architected, were good candidates to put this idea into practice.

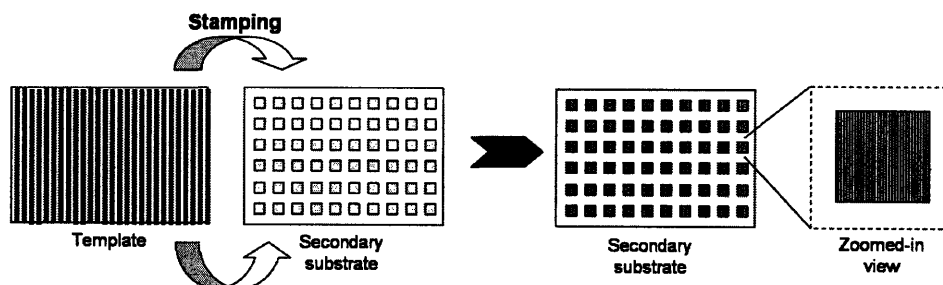


Figure 2-19: Principle of the cross-sectioning of SuNS's transferred patterns with patterns on coatings. Figures are not to scale.

Two methods were used by Hsien-Yeh Chen in Prof. Lahann's group to produce patterned coatings:

- The samples are covered with a PDMS membrane that acts as a mask during the polymer deposition, allowing the coatings to form only in the $200\ \mu\text{m}$ to $500\ \mu\text{m}$ wide squares left open in the membrane. The membrane is then peeled off, leaving only a pattern of poly(p-xylylenes) coated squares on the substrates. This way the DNA can only be printed in the squares. This method has one practical advantage for SuNS: the fact that the coating is discontinuous, present only on the squares, creates a difference in topology that makes the squares' edges visible (though very dimly) with an optical microscope. This helps greatly to find the printed patterns with atomic force microscopy. This advantage, though, can turn into a major disadvantage: due to the small size of each piece of coating it seems to be less firmly adhering to the underlying substrate, the edges of each square uneven and usually damaged during the stamping step, and the whole square's surface slightly undulating, which makes the AFM imaging delicate.

- The samples are uniformly coated during the CVD step, but the coating is then deactivated partially by microcontact printing (μ CP), using biotin hydrazide as ink. Biotin hydrazide reacts with the aldehyde in the contact areas, which are evenly distributed $100 \mu\text{m}$ large squares, thus passivating the squares and leaving a grid with active aldehyde functionalization. This way the DNA can only be printed out of the squares. This method avoids any topology-related defects since the coating is homogeneous, and although it is less practical for SuNS than the previous method since the patterns are harder to find with AFM, it should theoretically be preferred.

Yet, for practical reasons (the patterns couldn't be found using the μ CP coated substrates) it is on a substrate patterned using a PDMS membrane that successful results could be obtained. One such pre-patterned polystyrene sample was printed from a 100 nm pitch grating master, and the printed DNA was hybridized with its fluorescently modified complement. Fluorescence microscopy enabled me to check that the DNA was transferred only in the coated squares (figure 2-20), while using atomic force microscopy I could observe the nanometer scale printed patterns in the coated squares (figure 2-21) and check that no pattern was visible out of the coated squares.

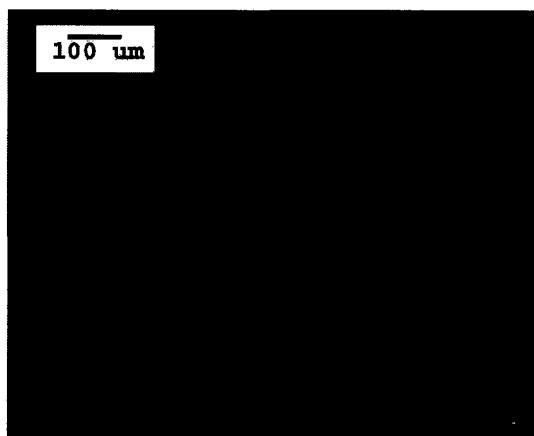


Figure 2-20: Fluorescence microscope image of a pre-patterned polystyrene substrate, after it was stamped with a 100 nm pitch master and hybridized with fluorescently labelled complementary DNA strands. The square shown is $200 \mu\text{m}$ wide.

We have proven here that substrates of very different types, silicon, quartz, polystyrene,

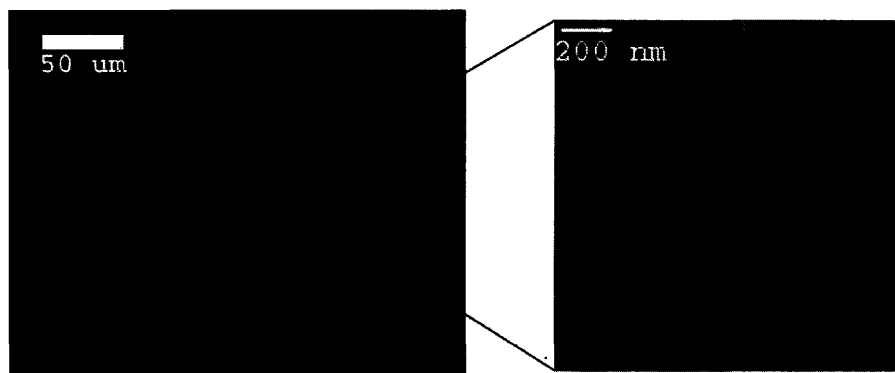


Figure 2-21: Fluorescence microscope image of a square element of a pre-patterned polystyrene substrate, after it was stamped with a 100 nm pitch master and hybridized with fluorescently labelled complementary DNA strands, and atomic force microscope image of the printed pattern inside a square. Z range = 10 nm

acrylic and PDMS, could be successfully printed using SuNS's approach thanks to the poly(p-xylylenes) coatings deposited in Prof. Lahann's laboratory. All these stampings could be performed using a single protocol, which provides us with a universal approach to the stamping of DNA patterns. Furthermore, fluorescence imaging on large area prints with patterns in the micrometer range enabled us to control the sequence of the printed DNA and its ability to hybridize to its complementary DNA strand. To this first proof of concept was added the successful stamping of another coated substrate using a previously printed coated silicon sample as master. This success, in addition to confirming the nature of the printed patterns, proves that the DNA conformation on the printed ppx-cho coated substrates enables them to successfully act as masters. This is very promising and could be used for further developments of SuNS. Indeed we have shown that we can produce complex patterns on these ppx-cho coated substrates by combining SuNS's pattern transfer with the patterning of the coatings themselves. This combination gives us a lot of freedom for the fabrication of ppx-cho coated masters with complex DNA architectures, often needed for SuNS's other projects and hard to obtain otherwise.

Chapter 3

Enabling good contact during printing - An engineering problem

SuNS is a printing technique very similar to its famous predecessor, the paper stamping press: an ink, for us the complementary DNA strands, previously deposited on the template (in our case, hybridized to the DNA strands attached to the master), needs to be transferred **through contact** to the "paper", or more generally to the secondary substrate of our choice. As in all the more conventional stamping techniques, the contact is therefore critical for the proper functioning of SuNS and requires careful engineering.

This engineering, which has been the subject of many generations of improvements in conventional paper stamping, could constitute a full project on its own and is for a big part out of the scope of our current research, which aims first at enlarging and defining new directions for the application of SuNS. Nevertheless, some technical considerations, looking at what has been and is currently done in the field of contact printing, can help us to improve sensibly our printing results in the laboratory and increase the reproducibility of our experiments.

3.1 Review of contact approaches

Supramolecular NanoStamping is only one in many different printing methods which all share the same concern for efficient contact during the printing step, although they may solve the contact issue in different ways depending on their specificities. What we present here is a brief review of these approaches to a successful contact, focusing on the nanoimprinting application, which requires both very large homogeneous stamping and very high resolution. The various approaches can be differentiated upon the way they apply pressure during the printing step, using either mechanical or air pressure.

3.1.1 Mechanical pressure

Mechanical pressure is what has been used in paper presses, and remains the most widespread and straightforward way to contact a template and a secondary substrate to print a replica. It is also the first approach that naturally came to our minds for SuNS. Mechanical pressure, though, represents a challenge in its implementation, since only the perfect parallelism of two smooth plates can guarantee the homogeneity of the pressure applied to the two substrates in contact, and parallelism is hard to obtain with simple tools. The failure to solve that mechanical issue leads to the kind of printing results shown in figure 3-1, where the uneven printed pattern reveals the non parallelism of the plates used to bring the template and the secondary substrate into contact. More dramatically, this lack of parallelism prevents the printing over large areas, and in my case, using mechanical vices with hard plates I most of the time printed only in thin bands of about 50 μm by a couple of millimeters.

Perfect parallelism is nevertheless achieved on a daily basis not only for industrial applications but also in research laboratories. Optical equipments can be a good source of inspiration, or the work of groups such as Willson's gives a good example of high precision home-built systems.[59, 60]

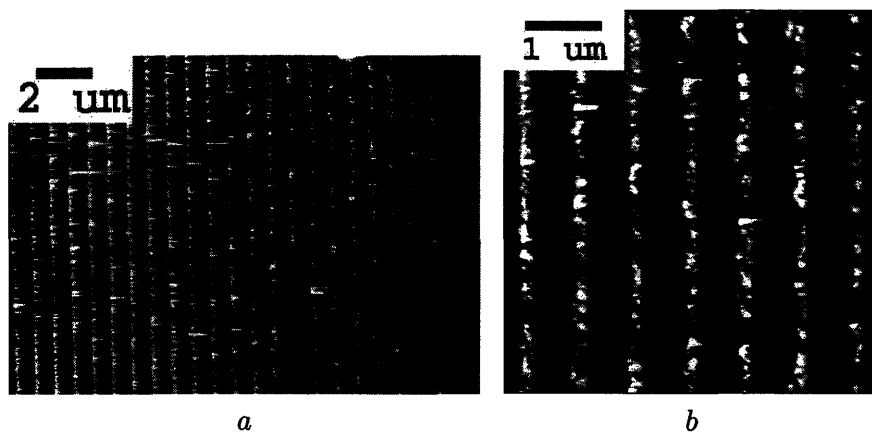


Figure 3-1: Atomic Force Microscope images of a ppx-cho coated silicon printed from a 700 nm pitch master, using mechanical pressure in a vice with an imperfect parallelism. Z range = 5 nm

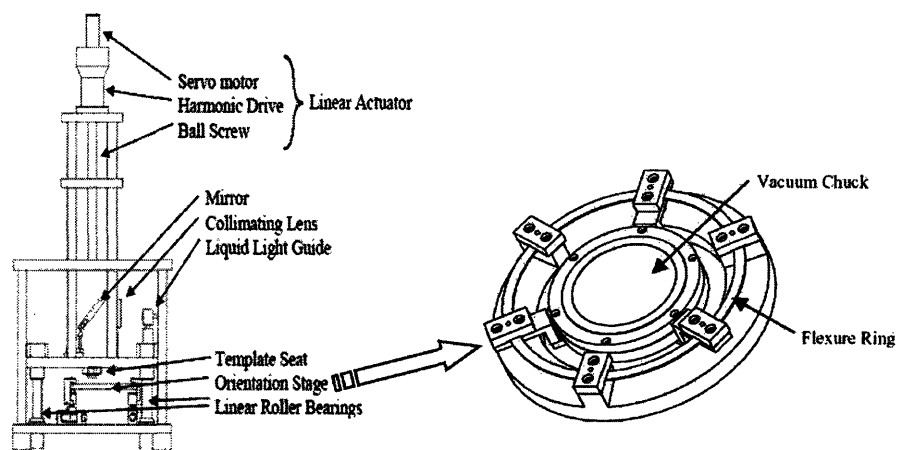


Figure 3-2: Step and Flash Lithographic Press, with detailed orientation stage. From [59]

Prof. Willson's group, for the Step and Flash Imprint Lithography (SFIL), needed as we do a good lithographic press, able to ensure the parallelism of the template and the secondary substrate during the imprint step. In their home-built system (apparatus shown in figure 3-2) much is done to ensure that parallelism is achieved. In addition to a precision positioner constituted of two horizontal plates and linear roller bearings they use an orientation stage to ensure the parallel contact of the two substrates. When the template approaches the secondary substrate with controlled force thanks to a linear actuator, the secondary substrate attached to the orientation stage is free to orient itself to correct the imperfection of the positioner and achieve parallel contact with the template. This is possible thanks to a flexure ring in the orientation stage, which is deflected by the moment generated about the center of the stage when the template contacts the secondary substrate.[59, 60]

With such a technique, Willson's group has been able to perform imprint lithography homogeneously over 200 mm wafers. As a conclusion, this approach shows that with the appropriate system the contact issue can be solved using mechanical pressure. Nevertheless the complexity of the engineering involved prompted us to look for alternatives.

3.1.2 Air pressure

The air pressure alternative is used in Chou's group for nanoimprinting. They have developed an "air cushion" system for nanoimprinting that takes advantage of the intrinsic uniformity of air pressure, without any need for smooth parallel plates as in the case of mechanical pressure[61]. Furthermore, not only does the use of air pressure simplify the engineering challenges of a printing press, it also has fewer requirements on the smoothness and flatness of the master and template.

The advantages of air pressure are summarized in figure 3-3, while figure 3-4 provides a comparison of the pressure distribution in the solid parallel-plate press (SPP) and the air cushion press (ACP). The pressure was measured using pressure-sensitive films when a nominal pressure of 1.38 MPa was applied, and the measurement shows that in normal conditions the actual pressure in the solid parallel-plate press varies

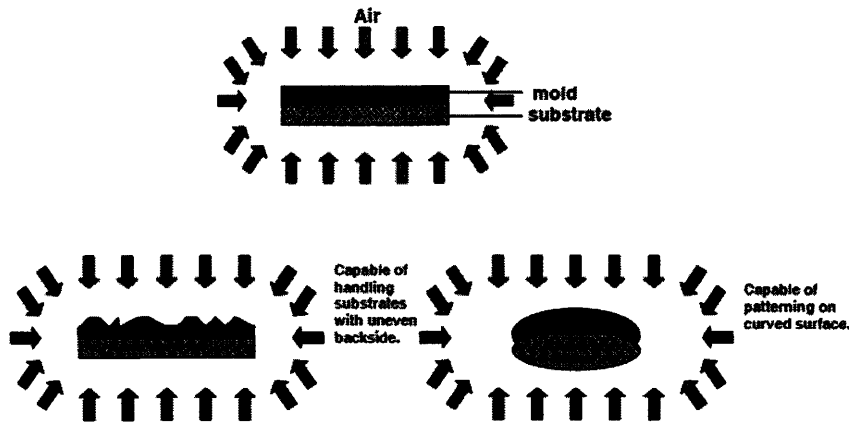


Figure 3-3: Schematic and advantages of the air cushion press (ACP) nanoimprint principle. From [61]

over a factor of 5 across the 100-mm-diameter nanoimprint field[61].

Practically the air cushion method for contact printing is composed of three steps:

1. The two substrates are brought into contact, sealed and placed in a chamber.
2. Low vacuum is applied to the chamber: the air is expelled from the region between the 2 samples. This is critical to avoid any air bubbles in the interstitial space between the samples, which would prevent the two substrates from being pressed against each other when pressure is applied to the chamber.
3. Air is introduced in the chamber to a pressure of typically 1.38 MPa without penetrating between the two substrates, thus pressing them together .

Figure 3-5 presents a schematic of the air cushion press setup.

This system has been in use since shortly after the introduction of nanoimprinting by Chou's group (1998). Since this change they have never come back to the more conventional solid parallel-plate press, and a company, Nanonex created by Prof. Chou, is now exploiting commercially the air cushion press system. This might explain why the sealing method that lets the air out of the interstitial volume but doesn't let it back in when pressure is applied remains unclear and could not be explained by students in Prof. Chou's group.

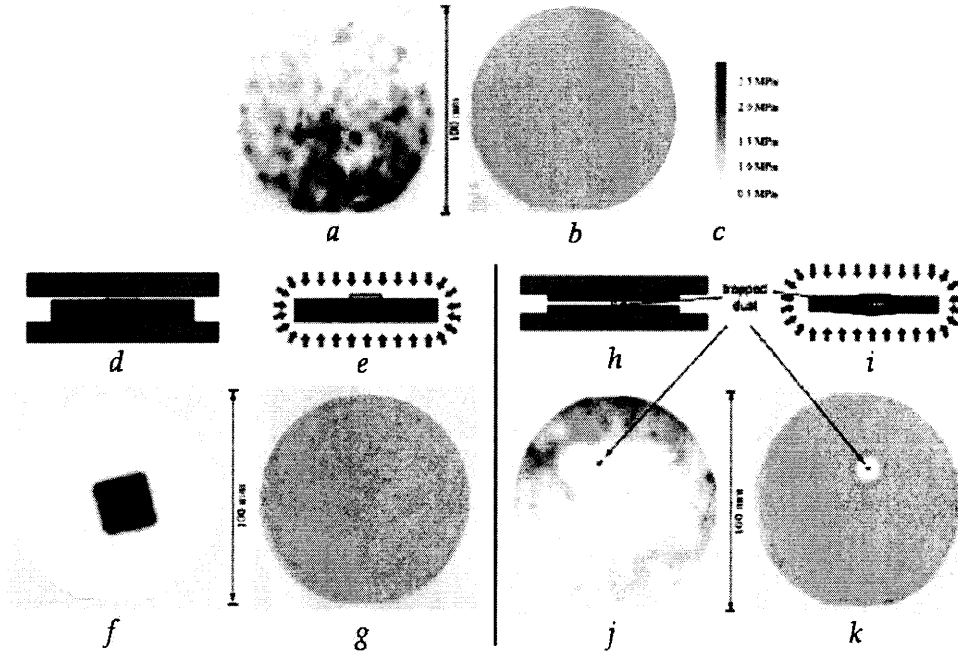


Figure 3-4: a) and b) Pressure distributions across a 100-mm-diameter imprint field when a 1.38 MPa nominal pressure is applied using SPP and ACP, respectively. c) Pressure vs color intensity calibration chart. d) and e) Schematics of experimental setups for studying the effects of backside dust/topology in SPP and ACP, respectively. f) and g) Pressure distributions across a 100-mm-diameter imprint field when a paper piece of $2.6 \times 2.6 \text{ cm}^2$ in area and 0.1 mm in height is inserted on the backside of the mold, and a 1.38 MPa nominal pressure is applied using SPP and ACP, respectively. h) and i) Schematics of experimental setups for studying the effects of trapped dust in SPP and ACP, respectively. j) and k) Pressure distributions across a 100-mm-diameter imprint field when a 0.1-mm high paper dust is trapped, and a 1.38 MPa nominal pressure is applied using SPP and ACP, respectively. From [61]

3.2 Using and improving mechanical pressure in SuNS's process

Over the past one and a half year of research, efforts have been made to implement better stamping conditions. Prior to this the only method used to provide good contact and a slight pressure on the samples during the printing step was to place a microscope glass slide on top of them. The first change was to switch to small mechanical vices (shown in figure 3-6) that were modified to improve the quality of

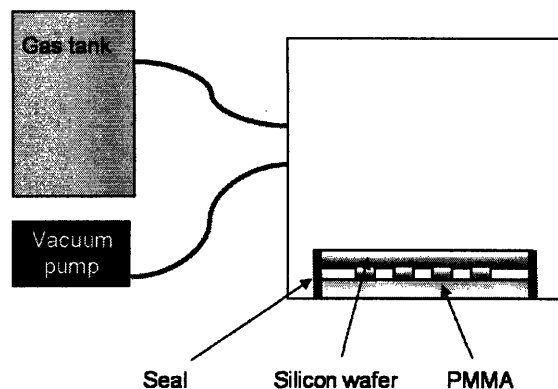


Figure 3-5: Schematic of the air cushion press setup

the contact they provide. Such vices were used for all the prints presented in this thesis.

Before using these rudimentary vices a first modification had to be made. It consisted in covering the original non-parallel rough plates with pieces of a silicon wafer and placing soft PDMS stamps between the original plates and the silicon ones to correct for the lack of parallelism. The resulting smooth and approximately parallel silicon plates were about 2 cm x 3 cm large. These were used for about one year, until pressure-sensitive films¹ enabled us to control the efficiency of our setup.

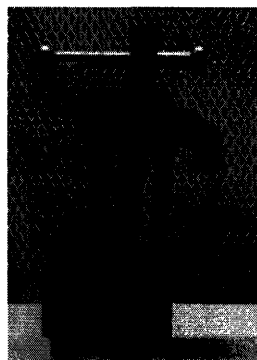


Figure 3-6: Vice used as a stamping press

The pressure-sensitive films provided us with a mapping of the pressure distribution in our samples during the printing step. They are composed of two layers

¹Pressurex tactile pressure sensor films from Sensor Products Inc.

of material, one containing embedded micro-capsules of color-forming material, the other one containing a developing product. When undergoing pressure, the micro-capsules are broken, and the color is developed. A higher pressure causes a higher density of broken capsules and therefore a higher color intensity. Therefore the color map, without any further quantitative analysis, gives us an estimation of the pressure distribution.

Using these films by placing them between the template and the replica substrates before applying pressure through our usual setup, a first observation was made: hard plates such as the ones described above were providing a highly uneven pressure, most of the time concentrated only along one edge of the substrates due to a lack of parallelism. Therefore we introduced an additional soft PDMS stamp between one hard plate and the samples, in order to obtain a better correction of parallelism. Up to now this little change in setup has brought very sensible improvement in the quality of the printing.

Looking for further improvement, once this correction made, we modified the press again and compared three different setups. Again, the comparison was obtained by placing pressure-sensitive films between the two substrates, before applying with the vice the same pressure as the one used in a real stamping trial. The results we obtained, together with the setup they are related to, are presented on figure 3-7.

These controls have shown that we could easily improve the quality of the contact by making small changes to our setup. A few characteristics appear critical:

1. A soft stamp like a piece of PDMS combined to a solid plate, instead of two hard plates, can greatly increase the contact area by correcting for the lack of parallelism or smoothness.
2. The plates should be of a size comparable to the size of the samples to provide better homogeneity.
3. An interesting alternative to flat plates and stamps in order to provide a good homogeneity in pressure can be to use the compliance of a material like PDMS in a pyramidal or conic shape to have the pressure start from the center and spread towards the edges of the sample.

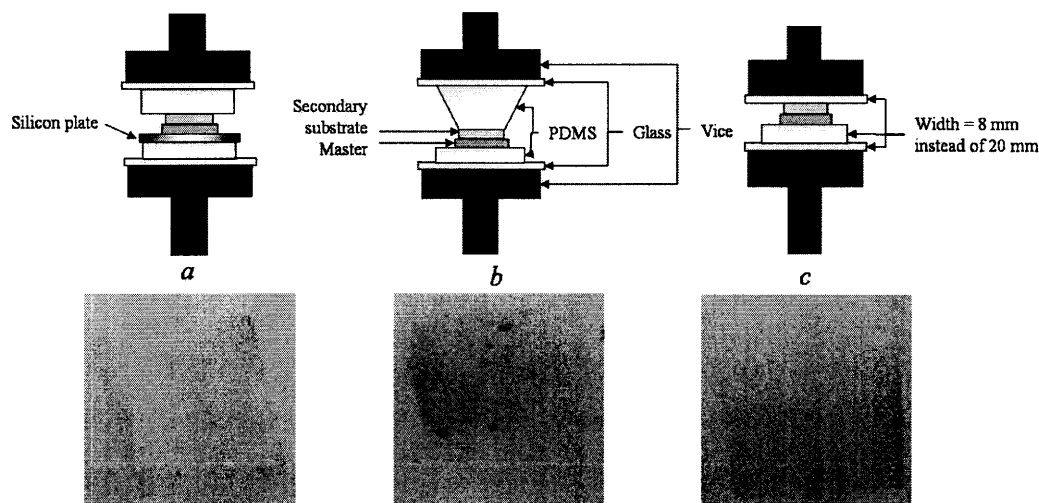


Figure 3-7: Schematics and results of modifications made to the stamping vices. a) No modification made. b) In addition to one PDMS stamp underneath the samples a pyramid-shaped PDMS stamp is added, that flattens at its apex and spreads onto the secondary substrate when pressure is applied through the vice, enabling the contact to spread from the center. b) In a setup similar to (a) all the plates are cut in order to be of a width that is comparable to the width of our substrates (8 mm), to be compared to the previous 20 mm-width.

3.3 Using air pressure for SuNS

As was said above, the use of air pressure in stamping could prove very valuable for SuNS. A first approach, circumventing the setup of a vacuum/pressure chamber, is to use inflatable balloons to apply air pressure.

The "balloon" approach is what I used to print onto PDMS. The specific advantage offered by an inflatable balloon in the case of PDMS is that the pressure it applies while being inflated starts from the center of the substrates and spreads towards the edges, which should avoid the trapping of air bubbles between the PDMS and the master it is printed from. A schematic of the setup is shown on figure 3-8.

Although this rudimentary setup doesn't provide a good reproducibility (difficulty to quantify the pressure applied, possible air leaks during the stamping), it offered some promising results for the printing of microscale patterns onto PDMS. The master used to print onto PDMS in that case was a silicon substrate patterned with

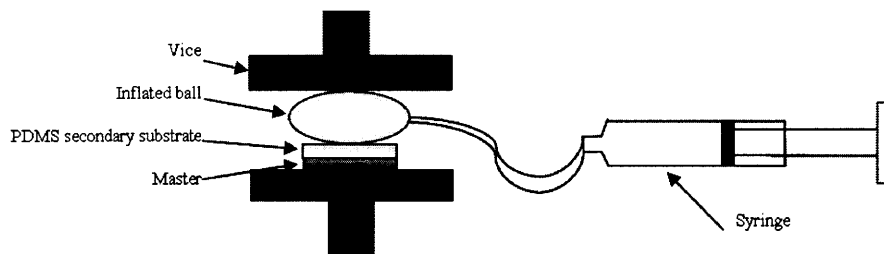


Figure 3-8: Stamping setup using an inflatable balloon

photolithography, resulting in a pattern of $1\ \mu\text{m}$ large gold squares on which a monolayer of thiolated ssDNA was assembled. After printing on PDMS and hybridizing the printed DNA with its fluorescent complement, images such as the one presented on figure 3-9 were obtained, very locally, on the PDMS sample. The area exhibiting the high contrast shown is about a millimeter in diameter, and corresponds to the area where the highest pressure was applied by the balloon. Around the printed area, the homogeneous fluorescence tends to prove that the poor quality of the contact enabled the formation of a water meniscus between the two substrates, in which the fluorescent complementary DNA was dehybridized from the DNA strands attached to the template and was transferred homogeneously onto the printed substrate. This was made possible by the fact that the stamping is performed out of a vacuum desiccator to limit the risk of leaks from the pressurized balloon.



Figure 3-9: Fluorescence microscopy image of a PDMS sample printed using an inflatable balloon setup and hybridized with fluorescently labeled complementary DNA strands.

What precedes is just a first attempt at using the possibilities offered by air-pressure stamping. Not enough has been done so far to really apply the advantages

of air pressure to our SuNS protocol, but it remains an area of interest that should be explored further.

3.4 Adapting the stamping method to the substrate's specificities

As shown in this thesis, SuNS can be applied to a broad variety of materials with a virtually identical protocol, thanks to substrate-independent reactive polymer coatings. Yet, one thing remains to be tailored depending on the kind of substrate we are printing onto: the method used to provide contact between the master and the secondary substrate during the stamping step itself. Our current substrates of choice exhibit very different hardnesses and roughnesses, and therefore require different approaches for the solving of the contact issue.

- Hard substrates like silicon and quartz are simply stamped using a mechanical vice adapted with soft PDMS plates.

- Polymeric substrates like polystyrene and acrylic, albeit also printed in the same kind of vice, are softened at the beginning of the stamping step by rising the temperature to 60°C, enabling them to conform better to the intrinsic roughness of the template's surface, thus increasing the printed area, as has proven our experience.

- A soft material like PDMS is stamped using air pressure, via an inflatable balloon placed between the coupled samples being stamped one onto another and the upper plate of the vice. As it is inflated, the balloon applies a growing pressure on the substrates that decreases gradually starting from the center of the substrates, therefore avoiding the trapping of air bubbles between the samples.

What precedes only constitutes an example of how we can creatively use the wealth of stamping approaches currently in use in many diverse applications, to tailor systems that best adapt to the materials we want to print onto. In the scope of this thesis, we have already proven how sensible improvements in the printing quality

and coverage could be obtained thanks to simple considerations and controls in the stamping techniques: on a hard polymer like polystyrene printing coverages of the order of 3 millimeter in diameter were obtained, while on a soft substrate like PDMS sensible improvements in the fluorescence contrast could be managed.

Chapter 4

Experimental methods

The substrates used are usually about 0.25-0.5 cm² in size.

DNA

The oligonucleotides are 50 mers purchased from Integrated DNA Technologies with commercially available 5' modifications.

Sequences used:

A: 5'- TCC CAA AGA ACA GTG GTG GCT CAA GCT ACG GCC CCT CAT
GAA AAT CCT GG -3'

Complementary sequence A': 5'- CCA GGA TTT TCA TGA GGG GCC GTA GCT
TGA GCC ACC ACT GTT CTT TGG GA -3'

Non-complementary sequence B: 5' TGA CTG CTG TAG TTC AGA AGA GGT
TTG GCT TTC CAG AGG GCA GTG TAG AG -3'

Modifications used:

5' thiol modifier C6 S-S for attachment on gold

5' amino modifier C6 for attachment on polymer coated substrates

5' RhodamineRed -X NHS Ester for fluorescence control

DNA immobilization on gold

The gold substrate is soaked 4 days in a solution of 5'hexyl-thiol modified 50mer single-stranded DNA of sequence A ($4 \mu\text{M}$ in 0.5 M potassium phosphate buffer). It is then rinsed with Millipore water, subsequently immersed for 1 hour in a 1 mM 6-mercapto-1-hexanol (bought from Sigma-Aldrich) aqueous solution to limit non-specific adsorption of DNA during subsequent manipulations, rinsed again abundantly, and dried with a dust remover.

DNA hybridization on gold

A few micro litres of a $1 \mu\text{M}$ solution of single-stranded complementary DNA (sequence A) in hybridization buffer are dropped on the substrate and covered with a glass cover-slip. The hybridization buffer is 1 M NaCl in TE buffer for hybridization on gold. The substrate is then left a minimum of 2 hours up to overnight in a sealed humid chamber. It is subsequently rinsed with 4xSSC buffer, diluted twice, followed by a brief rinse in Millipore water, and dried with a dust remover.

DNA hybridization on GOPS and TESA modified surfaces, or on poly(p-xylylenes) coated substrates

The sample is first passivated by putting it in a solution of 0.1 M Tris/0.05 M ethanolamine for 5 minutes at room temperature. After rinsing with Millipore water a few micro litres of a $1 \mu\text{M}$ solution of single-stranded complementary DNA (sequence A) in hybridization buffer are then dropped on the substrate and covered with a glass cover-slip. The hybridization buffer is 4xSSC, 0.1% SDS. The substrate is then left a minimum of 2 hours up to overnight in a sealed humid chamber. It is subsequently rinsed with 4xSSC buffer, diluted twice, followed by a brief rinse in Millipore water, and dried with a dust remover.

DNA dehybridization

The substrate is soaked twice 15 minutes in Millipore water at 90°C and rinsed abundantly.

Fabrication of the masters

The gratings were fabricated in the NanoStructures Laboratory at MIT. The initial 100 nm pitch gratings were obtained using Achromatic Interference Lithography (AIL)[57]. The pattern was then transferred into silicon by reactive-ion etching. The silicon grating was then coated, by e-beam deposition, with 3 nm of titanium followed by 7 nm of gold. The 700 nm pitch silicon gratings were made using interference lithography, then coated by e-beam evaporation with 5 nm of chromium followed by 5 nm of gold.

The square patterns were obtained from silicon substrates by photolithography, followed by the deposition of a 5 nm adhesion layer of chromium followed by 25 nm of gold, by e-beam evaporation.

General SuNS procedure

The patterned gold master is prepared as described above for the immobilization of thiol modified ssDNA of sequence A, followed by the hybridization with complementary amine terminated ssDNA (sequence A'). The aldehyde functionalized poly(p-xylylenes) coated secondary substrate is then brought into contact with the master, and a slight pressure is applied and maintained overnight while the substrates are kept in a vacuum desiccator. The two substrates, while still under pressure, are subsequently placed in an oven at 90°C for 30 minutes to dehybridize the DNA strands, the pressure is then released, and a droplet of 0.1 M NaCl in TE buffer is put on the coupled samples to help them to come apart. The samples are then rinsed with Millipore water and blown dry with a dust remover.

Characterization

Tapping Mode AFM images were obtained using a Digital Instrument MultiMode Nanoscope IIIa, using both a J scanner. All experiments presented in this paper were performed using Veeco Nanoprobe™ tips (Model nb: RTESP, length: 125 μm, resonance frequency: about 300 kHz).

Fluorescence microscopy images were obtained using a Zeiss AxioPlan 2 with a mercury lamp. The imaging was performed using Rhodamine Red modified oligonucleotides, and the filters used in excitation and collection had maximum transmittance at 546 nm and 590 nm, respectively.

Chapter 5

Conclusion

This thesis has shown how SuNS could be used to pattern a variety of substrates with very different properties: silicon, quartz, polystyrene, acrylic and PDMS, with a single protocol. This was accomplished thanks to functionalized thin polymer coatings realized in Lahann's group, from the University of Michigan. These coatings, whose chemical functionalities can be tailored to the needs of their specific applications, provide us with high quality molecular platforms independently of the underlying substrate. Therefore, the success of stamping using these coatings provides SuNS with a virtually infinite spectrum of substrates and chemical approaches to the binding of DNA. This versatility will probably prove critical for SuNS as it makes it capable to serve a very wide range of DNA-based applications, adapting to their specific environments, materials and chemistries of choice.

Nevertheless, to pursue this track and become a serious option for the many applications that could benefit from its unquestionable advantages, SuNS needs to become more reproducible and reliable. This implies first to work on identifying and understanding the parameters that determine its success, but also to tackle the mechanical engineering problems related to the stamping itself. The lack of a standardized process, at this stage, can only slow down the expansion of SuNS, to new materials, new lengthscales, and new applications.

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