Supramolecular NanoStamping (SuNS)
fabricating nano/bio devices using DNA as movable type

by

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Supramolecular Nano-Stamping (SuNS): using DNA as movable type for fabricating multi-component devices

by

Arum Amy Yu

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ABSTRACT

When a useful device is developed, it always requires a mass-production technique to industrialize it. In the era of nano/biotechnology, the development of printing techniques has not followed the speed of the inventions of novel devices. One of the main challenges is handling at the same time the resolution and the chemical complexity of these nano/bio-devices.

Here a new stamping technique, Supramolecular Nano-Stamping, SuNS, capable of reproducing surfaces containing DNA-features is presented and discussed. SuNS is based on the combination of contact and supramolecular interaction between complementary DNA strands. It can replicate in a single cycle features made of DNA of arbitrary chemical complexity.

SuNS is a versatile technique, masters can be fabricated with various fabrication techniques, ranging from hard lithography to soft lithography. It was used to print on multiple substrates, hard (gold, silicon), soft (Poly-methyl-methacylate or Poly-dimethyl-siloxane) or even liquid. The technical specifications of the printing process depend on the substrate material. As an example SuNS can achieve state-of-art printing feature and point-to-point resolution (< 50 nm) when printing onto a hard substrates, or large area printing coverage (> 25 cm^2) when printing onto a liquid prepolymer. In SuNS a copy has the potential to be used as another master to generate more copies. Lastly and most importantly, SuNS can replicates features composed of DNA of different sequences in a single printing cycle while keeping the chemical differences between the patterns. SuNS is still in its infancy and far from complete, it is expected that it will be extended/improved in the future.

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Finally, the day has come to me. I look back the 4.5 years I spent at MIT with very complex feeling; it sometimes gave me so happy time whereas it sometimes gave me so hard time. A clear fact is that I would not have been able to survive here without the people who were beside me spatially and mentally. Here, I would love to appreciate to them.

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Chapter 1. Introduction

1.1. Introduction

Compared to 1950, the world has changed at an enormous rate, which is even getting faster and faster. (For example, the internet, originally designed for sharing data among a few universities or government agencies in 1960, spread from 1995 to the public becoming one of the most essential components in everyday life.) At the center of the change, there have been computers and, more generally, electronic devices, which have enabled the automation of industries and the handling of large amount of data. The development of computers was thanks to microelectronic technology, based on semiconductor; thus, it could be said that the recent 50 years has governed by "Micro" technology.

Now the trend is moving from "Micro" to "Nano". It seems very natural that "Nano" became one of the most popular words in scientific field because people have realized that "decreasing size" doesn't mean only "smaller" but also "newer and possibly better". Taking the trend of "Nano", numerous researches have been carried out for understanding the phenomena in nano-scale; for controlling the character of nanomaterials; and for patterning with a high resolution. As a result, various nano-devices with unique advantages started to be fabricated in the laboratory.

However, the industrialization of those devices is still far away from the current stage due to the absence of a relevant manufacturing approach for mass-production.

1.2. Stamping/Printing techniques

The definition of stamping/printing is making copies of a pattern from a mold. Printing is distinguished from lithography in that it makes a duplicate in parallel way: all shapes on the mold are fabricated at the same time. On the contrary, lithography produces patterns in serial ways, one by one. (Many people use the term "lithography" as more general meaning including not only serial fabrication technique but also parallel fabrication technique. I will use the word "lithography" as the meaning of serial approach in this sec.1.2 to explain what is printing – parallel production technique.) For example, if Prof. Ceder makes the solution of a problem set for his Thermodynamics class by solving the problems by hand, he makes it with lithography technique. If he makes 50 copies of the solution by using a copy machine to distribute them to his students, he makes them with printing technique. Lithography is used for making one of a kind, whereas printing is used for making a number of copies of the original one fabricated with lithography. This is the best strategy for mass-production of an item.

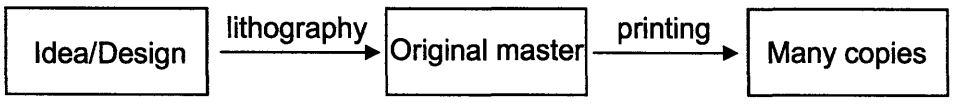

Figure 1.1 Simplified drawing of the role of lithography and printing in the industrialization of an item.

Human-beings have known this strategy since long time ago. Indeed, the history of stamping/printing dates as far back as 3000 BC. At that time, stamps (from a ring or a hand) were mostly used to sign important documents. Two documents stamped with one identical seal that contained a complex feature, implied they were from a same person. This identification method was much more efficient than drawing the same complex features twice on each of two documents by hand. The same concept - making a copy from a mold - was applied for fabricating many identical goods in industries with less cost and time. Nowadays, numerous industrial products such as cups, computers, and many others are made with printing/stamping techniques in which a mold is fabricated followed by producing its copies.

Another important example is Gutenberg's movable type, which induced a press revolution. The effect was enormous considering the fact that one book written by hand was as expensive as a good sized farm before he developed the movable type; todays, newspapers or magazines are so inexpensive that they can be easily thrown away after reading them.

1.3. Nanofabrication techniques

Currently, there are various nanofabrication techniques : some are modified/improved ones from conventional techniques developed for micro-scale,[1-6] (top-

down approach) and some are completely novel ones building a nanostructure by controlling molecular level interaction.[7-15] (bottom-up approach)

The items for the characterization and the evaluation of the nanofabrication techniques are the following.

i) Lithography vs. Printing

As described above, a nanofabrication technique is classified into one of two categories: serial production techniques or parallel production techniques.

ii) Patterning materials

Some techniques produce a physically defined pattern (e.g. nanoimprinting,[5,16,17] e-beam lithography,[1] optical lithography[18]), normally fabricated on inorganic material while some techniques produce a chemically defined pattern using soft organic molecules. (e.g. microcontact printing,[19,20] Dip-pen nanolithography(DPN),[7,21] replacement lithography[14]) In this thesis, the former one will be called "hard lithography" and the latter one will be called "soft lithography".

iii) Resolution

There are two kinds of resolution. One is feature resolution, which implies one feature size limit to be fabricated; the other is point-to-point resolution, which implies the spacing limit between two features that can be fabricated. In usual cases, point-to-point

resolution is lower than the feature resolution when using the same technique. For instance, the feature resolution of nanoimprinting is below 5 nm but the point-to-point resolution is only around 20 nm. (see sec.1.4.1)

iv) Process cost

The fabrication cost is always an important factor to evaluate the production technique. The techniques that use expensive facilities such as vacuum based equipments (e.g. e-beam lithography, x-ray lithography) or surface probe microscopes (e.g. DPN, replacement lithography, nano-grafting), originate a high price of the final products. In fact, nanoimprinting also requires costly facilities to apply uniform pressure (for details, see sec.1.4.1) However, if considering process cost per one final product, it is not so high.

Table 1.1 Some examples of nanofabrication techniques.

	Serial/Parallel	Patterning material	Resolution	Process cost per one item
E-beam lithography	Serial	Hard	~10 nm	High
DPN		Soft	~20 nm	High
Replacement lithography		Soft	~20 nm	High
Optical lithography	Parallel	Hard	~200 nm	Medium
Microcontact printing		Soft	~100 nm - ~500 nm	Low
Nanoimprinting		Hard	~10 nm	Medium

1.4. Nano-printing techniques

Unfortunately, while there are many serial fabrication techniques with high resolution, there are only few printing techniques that practically produce nano-patterns beside nanoimprint lithography. As mentioned in sec.1.2, serial nanofabrication techniques are too slow to produce a large volume of nano-devices. For example, electron-beam lithography is a successful top-down nanolithography technique based on silicon substrates. Although the technique is highly reproducible and the resolution is also great, it cannot be used for industrialization of the products. A simple calculation will be helpful to understand this point: the estimated time required to fabricate an array composed of 20 nm dots with 100 nm spacing on 10 cm X 10 cm size substrate is approximately 31.7 years, assuming that the exposure time for one dot is only 1ms. This example clearly states the reason why a relevant printing technique is essential for mass production. Indeed, the golden age of microtechnology would have been impossible without optical lithography (it is a printing technique in spite of the name), which copies a huge amount of devices on silicon substrates using a photomask.[18] In the same manner, the development of printing techniques satisfying the high resolution must follow the development of nanolithography for the mass-production of nano-devices. Currently, a number of research projects are being carried out for the purpose.

Among the various printing approaches introduced so far, I will review only contact-based printings, the techniques that transfer the information (i.e. the pattern on the master) through physical contact. (Optical lithography is excluded from the review for this reason) Roughly, they can be divided into two categories: techniques transferring 3-D spatial information (hard lithography, e.g. nanoimprinting)[16] or techniques transferring 2-D spatial information and chemical information. (soft lithography, e.g. microcontact printing)[22].

1.4.1 Nanoimprint Lithography (NIL)

Nanoimprint lithography (NIL), developed by Chou's group[5,16,17] is a low cost stamping technique reproducing 3-D physical topology with high resolution (<10 nm). To the best of my knowledge, this is the only printing technique able to reproduce the ultimate resolution of electron-beam, ion beam or x-ray lithography. It is based on hot embossing process: applying thermal energy and pressure on photoresist polymer placed on a hard master. As a result, a relief structure is left on the polymer by the permanent deformation and this pattern can be converted into inorganic structure by etching process. Due to this heating-cooling cycle, it's important to choose a combination of mold and the substrates with matched thermal coefficients and thermal durabilities.[5]

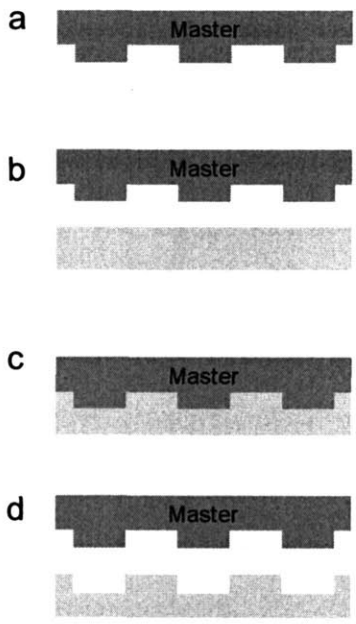

Figure 1.2. Schematic drawing describing the procedure of nanoimprinting. Breifly, it is composed of (a) preparation of a master with hard materials (b) contact with another substrate spin-coated with photoresist (c) embossing by heat and pressure (d) cooling and separation.

The most probable drawback on NIL is the tendency of adhesion of printed polymer on the master. This problem could be relieved in most cases by introducing anti-adhesive layer (generally, fluorinated silane monolayer).[5,23] Additionally, if the glass transition temperature (T_g) of the polymer to be patterned is low, the thermal stability of graved patterns decreases. For example, the imprinted pattern on poly(cyclohexyl acrylate) (T_g =

19°C) relaxed at room temperature after 10 days.[5] However, the polymer with very high T_g cannot be used considering the processing cost. Some researchers tried to improve this by using monomer or incorporating solvent.[5] However, the latter case requires a significant time to dry solvent.

Although NIL can reproduce extremely small features, not all patterns can be printed. Specifically, if the pattern contains a mixture of large features and small features on a large area, printing efficiency drops and various defects are generated on the printed polymer.[5] It is because the high viscosity of polymer prevents sufficient displacement of polymer under large sized patterns so that even distribution of polymer is impossible causing defects. An alternative against this problem is the combination of combination of nanoimprint and photolithography (CNP).[5] Using CNP, a large area pattern can be copied through photolithography using mask and a small sized pattern is copied through NIL.

UV nano-imprint lithography (UV-NIL)[24] is a slightly different version of the conventional NIL. Basically, in UV-NIL, UV curable thermosetting polymer is used as resist on instead of thermoplastic polymer. After the contact with the mold, the resist is cured by UV light and the relief pattern is left on the resist. The imprinted pattern onto the resist film can be etched for pattern transfer onto another substrate such as Si wafer. There are two ways to apply the resist on a substrate to be etched. One is spincoating of UV curable polymer with relatively higher viscosity. The other is introducing less viscous resist fluid into a gap between the mold and the substrate allowing for the fluid to spread out and

fill the gap due to the capillary force, which is known as Step-and-flash imprint lithography (S-FIL), developed by Willson's group.[24]

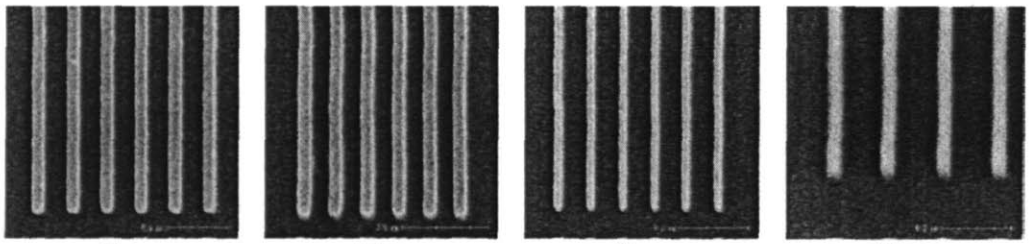

Figure 1.3. Scanning electron microscopy images of printed features with high resolution imprinting technique. The highest feature resolution of imprinting that has been achieved is below 10 nm. Courtesy of reference 6

UV-NIL has several advantages compared to the Chou's NIL. First, it does not require high pressure or high temperature inducing pattern distortion. Second, printed patterns are more thermally stable. Additionally, the resist film is less viscous (liquid-like), the even distribution is easier during imprinting steps. However, due to the low viscosity, the resulting profile of printed patterns is dependent on the roughness of the mold surface and it affects on the pattern transfer quality done by etching. This problem can be improved by using pre-exposure of the resist onto UV light before applying the mold on top. But this solution is sacrificing some advantages of UV-NIL coming from the low viscosity of resist film enabling the even distribution no matter what kind of features exists on the mold.

1.4.2. MicroContact Printing (μCP)

The most popular contact-based printing enabling print chemically defined patterns is MicroContact Printing (μCP), developed by Whitesides group.[19,20] It is a simple and versatile printing technique for soft molecules since it requires neither expensive equipments nor harsh chemical treatment (unless the chemically defined patterns needs to be converted into physical pattern with 3D profile). Basically, in μCP, micron-sized patterns are printed on other substrates using an inked stamp. In general, in μCP, an elastomer stamp, typically made of poly (dimethylsiloxane) (PDMS) is used to obtain conformal contact between the stamp and printed substrates since printing (the transfer of ink molecules) occurs only at the area in contact.

The elastomer stamp used in μCP is prepared by casting polymer on a mold that contains microsized patterns on relatively hard substrates such as SiO_2, Si_3N_4 or metal. For inking, a solution of ink molecules, for example, hexadecanethiol (HDT) in ethanol, is dropped on the stamp followed by blow drying.[25] When the inked stamp is placed on gold substrates, the HDT molecules form self-assembled monolayer (SAM) on the contact area owing to thiol-gold bond formation and autophobicity of HDT. If necessary, this HDT pattern can be converted into inorganic pattern through etching because HDT becomes a protection layer inducing different etching rate between printed area and non-printed area.

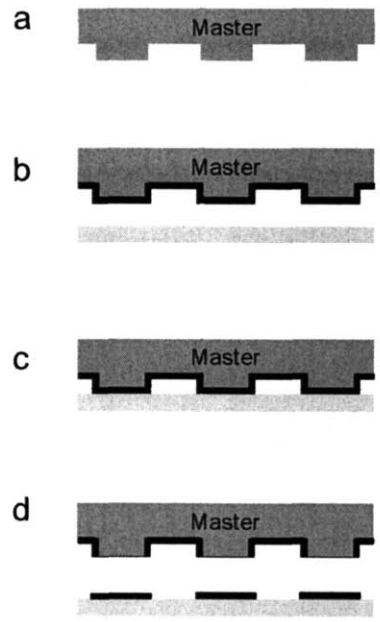

Figure 1.4. Schematic drawing of microcontact printing (μCP). The procedure of μCP consists of (a) preparation of PDMS master (b) inking of the master with the molecules to be printed (c) contact with another substrate (d) release the master from the printed substrate.

An elastomeric stamp made of PDMS, the key part of μCP, has several advantages: easy to release from the master, optically transparent, relatively stable against aging, and biocompatible. However, there are also significant drawbacks. In fact, the flexibility of PDMS (Young's modulus ~3 MPa) is the reason of low resolution of μCP although it is essential for the conformal contact during printing. Also, the possible patterns to be printed

and the geometry of the stamp are restricted due to the deformation of PDMS (pairing, sagging and roof collapse, see fig. 1.4).[19,20] With this reason, the patterns on a master for μCP is designed with aspect ratio in the range of 0.5~1 at optimum. Additionally, unmodified PDMS is hydrophobic so that only nonpolar molecules give a homogenous printing coverage since molecular inking is done by wetting. Another factor that affects μCP is the molecular weight of ink molecules.[25] The stability of printed patterns through μCP is controlled by diffusion. So if the printed molecule is volatile with low M.W (For example, dodecanethiol, M.W. = 158g/mol), the borderline of patterns are unclear and the patterns are diffused out soon while heavier ink molecules (eicosanethiol, M.W = 314g/mol) give a clear printing result.[25]

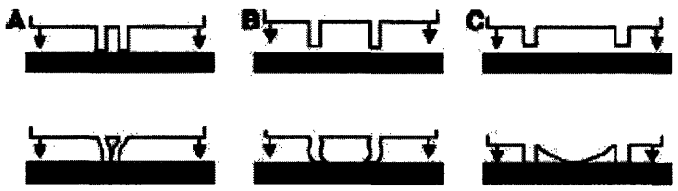

Figure 1.5. The most commonly observed stamp deformations: (a) pairing, (b) buckling, and (c) roof collapse. Courtesy of reference 20.

With μCP, a number of materials can be printed on various substrate systems. The starting combination was alkanethiol on gold substrates, studied well by many groups.[19,20,22,25-27] It was extended to printing silane molecules on SiO_2 inducing the

possible application area expanded into enormous fields including semiconductors, organic sensors, bioassays, which are based on silicon oxide substrates.[20,28-30] It is noticeable that the target molecules don't have to be functionalized with thiol or silane group to be printed on gold or silicon oxide surface; if the thiol or silane molecules contain another reactive functional group on the other end (e.g. carboxylic acid or aldehyde), it can make a bond with ink molecules transferred by a stamp.[20,31,32]

So far, not only small organic molecules but also big molecules including DNA,[33] proteins,[34] and polymers have been printed with μCP as well as inorganic materials such as metal ions, metal colloidal particles or nanotubes.[20,28,33] In order to print biomolecules, sometimes, a chemical treatment (e.g oxygen plasma treatment) is required to improve the wetting property of ink molecules. Also, through further modification, more complex structure can be fabricated by printing linker molecules without direct printing. (e.g. nanoparticle assembly by printing dithiol on gold substrates).

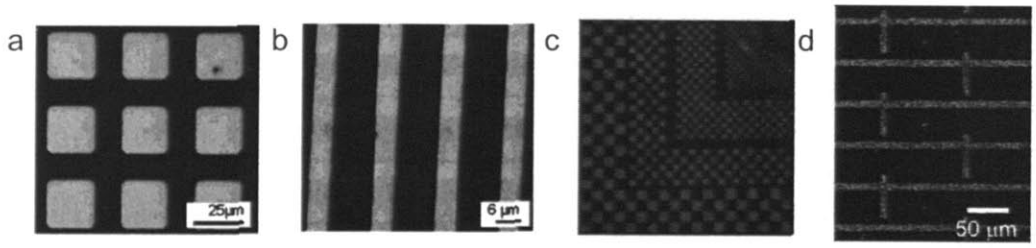

Figure 1.6. Fluorescence images of printed biomolecules (DNA in (a) and (b); protein in (c) and (d)) using μCP. Courtesy of reference 33 ((a) and (b)); and 34 ((c) and (d))

Since the μCP was introduced first, it has been analyzed and improved by Whitesides' group and many others.[2] First of all, the printing resolution could be substantially improved (~80 nm) by using a stiff mold.[2] (Young's modulus ~10 MPa) or composite stamp composed of two layer (hard thin film containing patterns assisted by a flexible pad).[35] Additionally, Pompe et al. introduced a "stamp pad" for μCP to minimize PDMS stamp swelling and to avoid excess deposition of ink molecules due to capillary condensation in the wedges of the stamp.[29] In order to increase the wettibility for hydrophilic molecules, oxygen plasma treatment is carried out, [36,37] polar molecules are coated on the stamp,[38,39] or polar polymer is used for stamp material (e.g. poly(ether-ester))[40].

1.4.3. Micromolding in capillaries MIMIC and μFN

Micromolding in capillaries (MIMIC) is another variation of soft lithography developed by Whitesides' group.[41] MIMIC is an inverted mode of μCP; a soft PDMS mold is placed on a substrate and then the area in contact is blocked; instead the intaglio part forms a microchannel with the substrate to be printed as another wall. If a prepolymer solution is applied on the end of channel that is open, the channel is filled with the solution by capillary force.[42] After curing, the PDMS stamp is released leaving a relief patterned polymer film. With the same strategy, beside prepolymers, ceramics, magnetic microbids and polymer bids can be patterned.[41] This procedure can be assisted by vacuum and the resolution can be improved by using two layer composite molds with the same reason of μCP case.

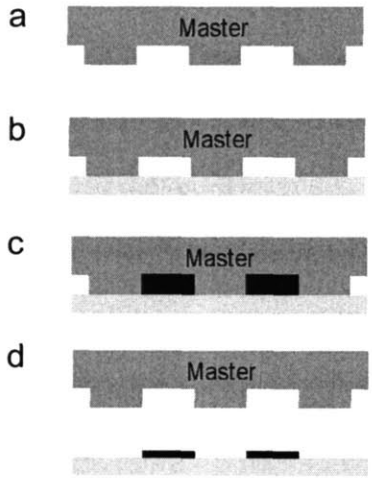

Figure 1.7. Schematic drawing of the procedure of MIMIC and μFN. (a) preparation of master (b) contact with another substrate (c) injecting ink molecules (d) release the master from the secondary substrate.

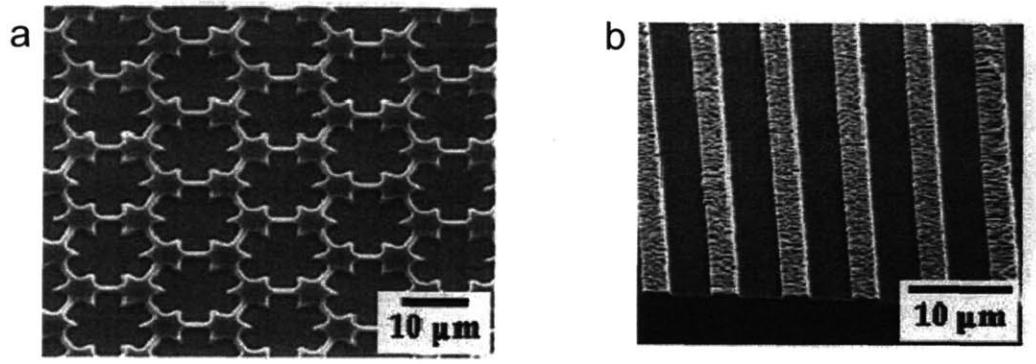

Figure 1.8. Scanning electron microscopy images of printed polyurethane (a) and amaranth (b) using MIMIC. Courtesy of reference 41.

MIMIC was extended to MicroFluidic channel or network (μFN) by Biebuyck et al. to fabricate biomolecules patterns.[43,44] The target biomolecules are injected into the microchannel with solvent by capillary force or additional pressure. The substrate to be printed is functionalized for the attachment. The biomolecules are transferred by laminar flow and immobilized by bond formation with the substrate. If each channel is isolated from the others, multi molecules can be patterned simultaneously by injecting different solutions into each channel.

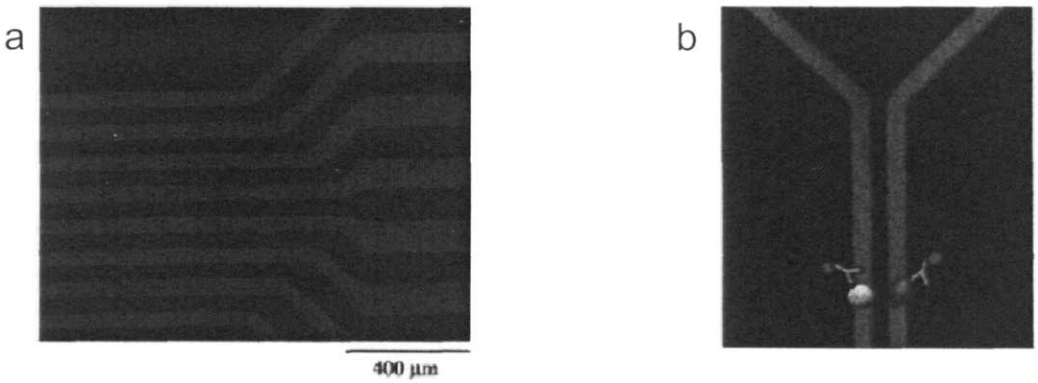

Figure 1.9. Fluorescence images of patterned protein (IgG) using μFN. In the image (b), it is shown that μFN can print two different molecules (two types of IgGs) in parallel way. Courtesy of reference 43 and 44.

Various proteins such as immunoglobulin G [43]or streptavidin and DNA have been patterned on gold, glass, or polymeric substrates with μFN.[44-48] Since the biomolecules are immobilized sequentially from the entrance of the channel to the end, the depletion of ink molecules may occur when the solution arrives close to the end. It can be improved by using a reservoir pad of ink solution at the both end of channels. Also, another drawback is

channel wall adsorption of ink molecules. It could be solved by using μFN molds coated with polyethylene glycol (PEG), hydrophilic & non specific absorption repelling polymer, or other bio passivation layer. [46,49]

1.4.4. LISC, LISA and LCW

A few parallel process lithography techniques are assisted by the induced contact or wetting by the system favorable to low surface energy. Although in these techniques, there is no contact at the first time when the master is place on the second surface to be patterned, there exists a contact before release and the pattern on a master is copied through this contact. Lithographically induced self-construction (LISC) developed by Chou's group[50] is a technique that copies a physically defined pattern using a thermally induced contact. A protrusion pattern is placed a little distance above on a polymer coated substrate that is initially flat. If this system is heated up above T_g of the polymer, the polymer becomes soft and attracted to the protruded pattern. As a result, when the system is cooled down, mesas patterns that are identical with the protruded pattern on the master are left on the polymer film. If the master contains a relatively large protruding step, a periodic pillar array is formed under the large pattern. (Lithographically induced self-assembly, LISA).[51] The printed results of both LISC and LISA are homogenous polymer pattern with height profile like NIL's case.

Lithographically controlled wetting (LCW) developed by Cavallini et al[52] is similar with LISC but enables to make a copy pattern consisted of another component from the background. To generate chemically defined patterns on a surface, a stamp containing protruding part is placed on a liquid solution thin film, then menisci of the liquid form between the protrusion and the substrates due to capillary force. After evaporating the solvent of the solution, the solute pattern is left on the spot where the menisci formed. If the viscosity of the solution is low, thinner pattern can be obtained by the menisci formation on only the edge of the original protruding pattern.

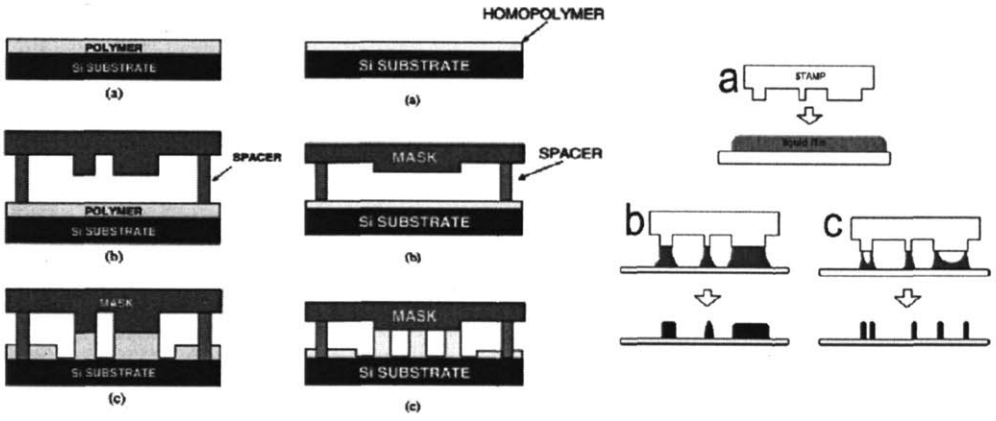

Figure 1.10. Schematic drawing of LISC, LISA and LCW. All techniques are based on the induced contact assisted by heating (LISC and LISA) or capillary force (LCW). Courtesy of reference 50,51 and 52.

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Chapter 2. Supramolecular Interactions in Printing.

2.1. Supramolecular interactions

Currently, the equipments for patterning have reached the level of manipulating molecules or atom. However, manipulating such a small unit is too challenging and time and cost-wasting way to fabricate devices. Researchers started to approach with a smarter way, such as taking an advantage of the forces in nature. One example is supramolecular interaction, a set of non-covalent intermolecular forces that works among two or more chemical species including hydrogen bonds, electrostatic force, hydrophilic/hydrophobic interaction, and Van der Waals (VdW) force.

In fact, supramolecular interaction is the main force working in biological systems such as protein folding, enzymatic reaction, immunological antigen – antibody reaction, translation or transfer of genetic information between DNA – DNA/RNA and cellular recognition.[1] Beside of bio-molecules, transition metal can be involved in this kind of interaction to form coordination metal complexes,[2] and many other organic molecules (e.g. dendrimers[3]) also form a supramolecular structure such as micelle or bilayer. Moreover, not only imitated molecules from the existing one in nature can be used, but also completely

new molecules can be synthesized with a design that maximizes the recognition efficiency to build the inventors' own supramolecular system.

Indeed, "Self-Assembly" assisted by supramolecular interaction is the most efficient and economical technique for manipulating big organic/bio molecules. It is hard to say what is the clear definition of self-assembly but it is generally accepted that self-assembly is the formation of super-molecular structure through intermolecular bonds (i.e. supramolecular interaction) that induces "selectivity/recognition power".[1,4] The selectivity enables a rapid conversion of starting materials into a final product with fewer steps, low error and high efficiency.

Recently, self-assembly has been exploited as tool to make complex structures in the nano-micro lithography field. The strongest advantage of self-assembly for patterning is that the precise construction of complex structures is capable simultaneously no matter how large is the system. The goal of lithography using self-assembly has been from the fabrication or modification of nano/bio devices[5-11] to the information storage. [11,12]

2.2. Supramolecular interactions in contact printing

Here, some approaches of combining contact printing techniques using supramolecular interaction will be reviewed. In order to apply supramolecular interaction, soft molecules should be involved in the printing techniques. Thus, only soft printing techniques are described here not hard printing techniques such as NIL, LISA or LISC.

In general, one cycle of contact printing in which soft organic molecules are involved as ink to be printed is composed of three steps: Inking -> Contact -> Release (in the case of fluidic channels, Contact -> Inking -> Release). I classified the supramolecular approaches by when the supramolecular interaction is applied among these three steps in printing process.

2.2.1. Supramolecular modification after printing (release).

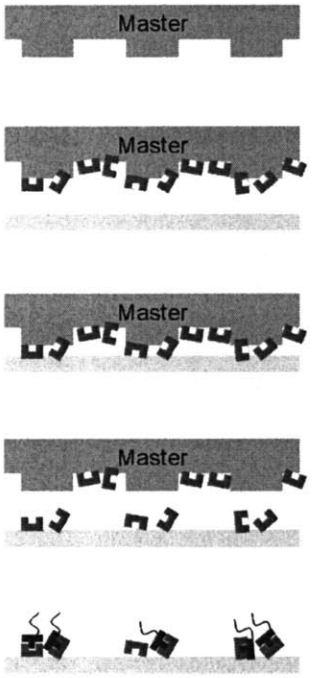

Figure 2.1. Schematic drawing of the procedure of supramolecular modification after release of a master in contact printing.

The simplest application of supramolecular interaction on contact printing is supramolecular modification on the printed pattern. Especially, this approach gives several advantages on fabricating patterns of biomolecules. To make bioactive patterns is attractive in many areas such as biosensors, characterizing cell-adhesion or bio-assay systems. However, controlling and handling soft biomolecules is always challenging for micro/nano

fabrication people. For this reason, it can be a good strategy to pattern the corresponding ligand molecules, more robust and simpler than the target biomolecules, first as precursors. Also, it can be expected that the biomolecules connected to the printed ligand will have more conformational freedom inducing higher activity compared to directly deposited biomolecules on surface because the ligand will act as a linker molecule.

As the most popular example, the specific interaction between biotin – avidin/streptavidin has been broadly used to make avidin/streptavidin pattern as final product by starting from printing biotin molecules. [13-15,16,17] Since a native PDMS stamp used as master is hydrophobic, the surface of PDMS master is often oxidized before inking to improve the wetting of biotin molecules. For better printing coverage, reactive functional groups have been broadly introduced on various substrates system such as self assembled monolayer on gold substrates,[13] chemically modified polymeric system (microstamping on activated polymer surface, MAPS)[14,15] or functional group containing polymer spin-coating.[16] Some researchers used dendrimers containing active functional groups to avoid the PDMS oxidizing step.[17] The wetting property of dendrimers on nonpolar PDMS is good due to many hydrocarbon branches even if they have polar groups in the end.

In order to keep away the soft molecules to be patterned from the harsh printing process, another useful strategy is backfilling. The negative pattern of a designed pattern is made by a cycle contact printing using another kind of molecules. Then, the area exposed with the ink molecules is passivated except the area that is supposed to be filled with the

actual target molecules. The printed surface is immersed into a solution of the target molecules to allow for forming self-assembled monolayer spontaneously.[18] Similar approaches can be applied to make two effective components pattern or to minimize non-specific binding during further process.[19] Specifically, for biomolecules, it is common to introduce (polycationic) poly ethylene glycol (PEG) layer, a hydrophilic and repulsive polymer to non-specific adsorption, subsequently after printing. [20,21]

Layer-by-layer assembly is a powerful technique to make ultra-thin film on surface. The thin film can be generated by electrostatic force, hydrogen bond, or metal coordination.. The same strategy can be applied on only printed region selectively. For example, if positively charged polymer is patterned on the substrate through contact printing process, a negative charged polymer thin film is able to be introduced on the pattern.[22,23]

2.2.2. Supramolecular Contact

Supramolecular interaction can be utilized during "contact" step in printing procedure for increasing the stability[24-26] and/or the function efficiency[27] of printed molecules, or controlling printing area.[28]

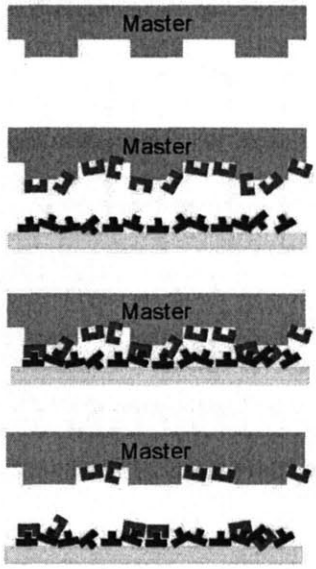

Figure 2.2. Schematic drawing of the procedure of supramolecular modification after release of a master in contact printing.

In the case of printing biomolecules on their corresponding ligand molecules, the activity of biomolecules can be more efficiently preserved due to the longer anchor, giving more conformational freedom. It was proved by printing avidin onto a polymeric substrate containing biotin moiety[27], by printing horseradish peroxidase (HRP) on bovine serum albumin (BSA) precursor layer on quartz[28], or by printing PEG on poly (methacrylic acid) (PMAA).[29] After printing, the functionality of printed molecules was characterized and they showed higher activity compared to direct deposition on a substrate without the complementary ligand. Also, the stability of printed patterns can be controlled changing the

condition affecting the involved supramolecular interaction such as temperature and pH.[28,29]

Without using conventional host-guest type molecules in nature, specially designed molecules for this purpose can be used for contact printing. Supramolecular lithography uses molecular printboards containing self-assembled monolayer (SAM) of molecules that have specific recognition sites for the target ink molecules.[24-26] In general, b-cyclodextrin (b-CD) SAM on gold or glass (SiO_2) substrates are prepared as printboards and ink molecules (sometimes polymer) containing hybrophobic guest moiety such as adamantly group or p-tert-butylphenyl group are printed on the boards through μCP or dip-pen nanolithography (DPN). These printed patterns remained without damage after rinsing with phosphate buffer or NaCl solution, or after long time had passed, compared to printed same molecules on –OH terminated SAM or PEG coated substrates, which implies higher stability of printed patterns due to the strong interaction between the designed host-guest molecules.

2.2.3. Supramolecular Inking

Among the three ways to apply supramolecular interaction on contact printing, the highest level of application is "supramolecular inking" in that the master has the information of recognition and the information is preserved through all printing steps and finally, copied on the printed substrates.

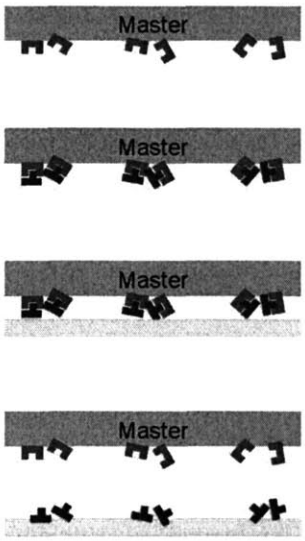

Figure 2.3. Schematic drawing of the procedure of supramolecular modification after release of a master in contact printing.

Most contact printings require physically defined masters (i.e height profile- defined masters) since their inking step is based on mostly weak Van der Waals force assisted by kinetically hindered mobility of molecules. Consequently, the molecules only on the higher part to be contact are transferred onto a secondary substrate. This printing depends on the mechanical property of materials consisting masters; if the masters are deformed, the printed patterns are also distorted. It is more probable when the master is soft such as PDMS.[30] However, if the master contains chemically defined patterns and if the patterns can be solely inked with the molecules to be printed, the effect of mechanical distortion can

be minimized. Supramolecular interaction can be applied to inking step. For selective inking, a master is fabricated with one component of supramolecular pairs and the ink will be the other part. The "smart" masters can capture its own ink molecules selectively and transfers them onto another surface (at this time, a stronger bond between the ink molecules and the surface is required than the affinity interaction) Another key advantage of this approach is that simultaneous multi-component printing is possible if the master is composed of two of more kinds of supramolecular pairs. Also, further supramolecular modification is always open after printing.

Affinity contact printing (αCP) is the first supramolecular printing technique based on selective inking assisted by the specific affinity between an antigen and the corresponding antibody.[31,32] A PDMS master containing antigen or ligand against specific biomolecules captures the target ink molecules from mixture solution and transfers the target onto another substrate. During the release step, the force stabilizing printed molecules on the secondary substrate is just Van der Waals adhesion force. It can be explained that since the printed molecules are big and the contact area is also enough large, the force required for detaching them is larger than the affinity force between the antigen and antibody.

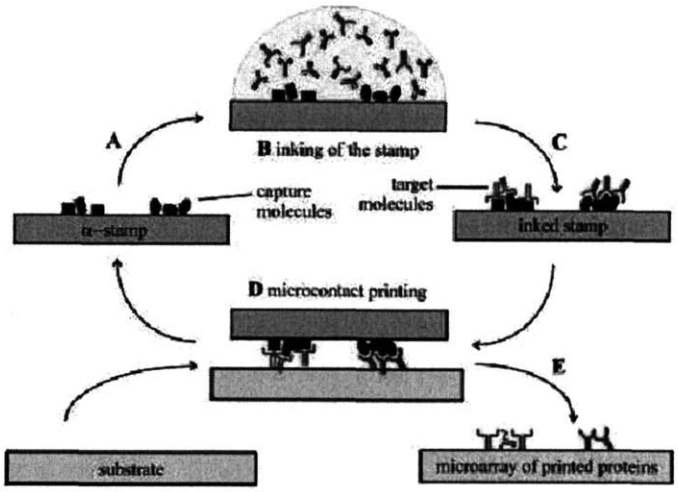

Figure 2.4. Schematic drawing of the procedure of αCP for printing two different proteins. The antigens immobilized on a master selectively capture the corresponding antibodies in solution (supramolecular inking) and transfer them onto another surface. Courtesy of reference 31.

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Chapter 3. Supramolecular Nano-Stamping (SuNS)

3.1 Introduction/ goal of the thesis

As described in chapter 1, various contact-printing techniques to industrialize nano/organic devices were recently developed, each with its own advantages and disadvantages. NIL,[1] LISA[2] and LISC[3] produce only inorganic patterns due to the harsh process, normally combined with heating and/or pressure. NIL is the only one that can obtain very high resolution.[1] Techniques in the soft lithography contact printing family including μCP, MIMIC and μFN[4,5] can fabricate "chemically defined patterns" using soft organic/bio molecules, allowing to fabricate more complex devices. Unfortunately, using those techniques, it is not easy to obtain such a high resolution below submicron.

In these days, more and more complexity is required to fabricate devices with high resolution. In semiconductor industries, the complexity of circuits has increased at an exponential rate in said in Moore's law[6]; with a similar trend, bio-devices, which have been emerging recently in many applications for research or clinical purposes,[7-9] are getting more complex. The complexity of a device is related to the information in the devices; more complex devices contain more information. The information in a device can consist of the patterns on the surface and the material used to make a specific component. Unfortunately, there are few fabrication/printing techniques that can put the large

information required for high function easily and this problem is more critical in the fabrication field of bio-devices due to the difficulty for handling fragile biomolecules, requiring complex processes.

In this thesis, I will describe the effort to develop a new printing technique that is able to reproduce organic/bio patterns with high resolution and high complexity. In order to achieve the goal, I took an advantage of a famous molecule with supramolecular interaction that works with an extremely high efficiency in nature: DNA.[10]

3.2. Review of DNA properties

Before introducing the new printing technique our group developed, I would like to explain some of the basic physical/ chemical properties that characterize DNA

DNA is a bio-molecule that contains genetic information in our body. It exists as a double helix, composed of two complementary chains; each is a template for the other in DNA replication. In living creatures, replication of the genetic information inscribed in DNA, done by an enzyme (DNA polymerase), is very accurate and the error frequency is less than 1 in 100 million nucleotides.[11] (Note that this is the error calculated after all error correction mechanisms) It implies that DNA is one of the most suitable medium for storing and transferring information.

3.2.1. Structure[11]

The following paragraphs are mostly derived from ref. 10. Please read them if you need to know about the DNA structure and chemical properties in depth.

DNA is made of a large number of deoxyribonucleoides, each composed of a base, a sugar, and a phosphate group. The bases of DNA molecules carry genetic information, whereas their sugar and phosphate groups perform a structural role. The 4 bases in DNA are adenine (A) and guanine (G), which are purines; and thymine (T) and cytosine (C), which are pyridines. (fig. 3.1) The variable part of DNA is its sequence of four kinds of bases (A, G, C and T).

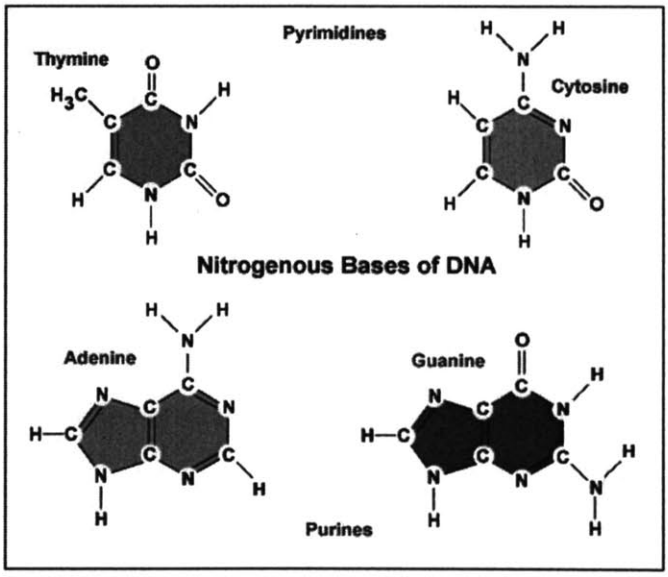

Figure 3.1. The chemical structure of the four bases in DNA: adenine (A), guanine (G), and thymine (T), and cytosine (C).[12]

The base pairs and sugar-phosphate backbones form a double helix, suggested by Watson and Crick as very well known. (fig.3.2) The important features of DNA double helix structure are the following: [11]

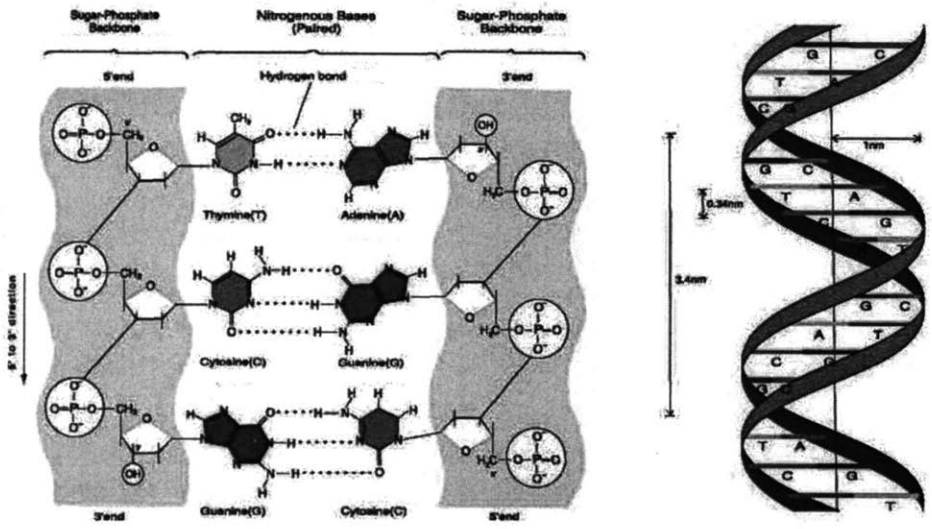

Figure 3.2. Double helix structure of DNA.[13]

i) Two helical polynucleotide chains are coiled around a common axis. The chains run in opposite directions.

ii) The purine and pyrimidine bases are on the inside of the helix, whereas the phosphate and sugar units are on the outside.

iii) The diameter of the helix is 20 Å. Adjacent bases are separated by 3.4 Å along the helix axis and related by a rotation of 36 degrees. Hence, the helical structure repeats after 10 residues on each chain, that is, at interval of 34 Å

iv) The two chains are held together by hydrogen bonds between pairs of bases. Adenine (A) is always paired with thymine (T); guanine (G) is always paired with cytosine (C) because of steric and hydrogen bonding factors. (fig.3.3) It should be noted that two hydrogen bonds form between A – T pairs whereas three hydrogen bonds form between G –C pairs

v) The sequence of bases along a polynucleotude chain is not restricted in any way. The precise sequence of bases carries the genetic information.

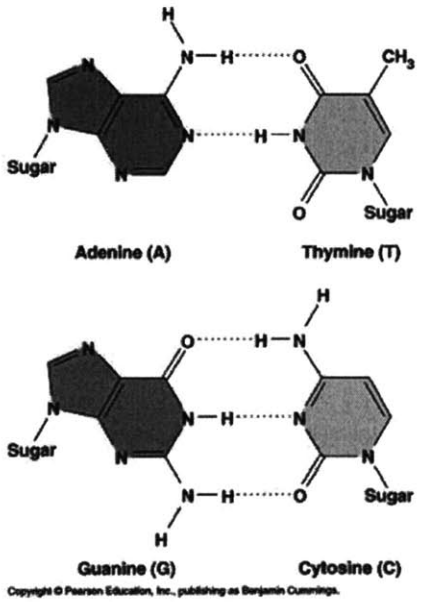

Figure 3.3. Hydrogen bond formation of an adenine-thymine base pair and a guanine-cytosine base pair.[14].

3.2.2. Hybridization/Dehybridization.

As shown in sec.3.2.1, two DNA strands with complementary sequences to each other bind together and form a double helix spontaneously assisted by hydrogen bond at ambient temperature. (Hybridization) However, if the hydrogen bonds between the paired bases are disrupted, the two strands separate. (Dehybridization) The two states (double helix and two single strands) can be reversibly switched by changing, for example, temperature or pH. The temperature at which half the helical structure is lost, is defined as melting temperature of DNA (T_m) or dehybridization temperature. Some people call dehybridization "melting" since it occurs abruptly at a temperature. It implies that the DNA double helix is a highly cooperative structure, stabilized by not only base pairing but also the stacking of bases.[11] Indeed, dehybridization of dsDNA is commonly explained with a unzipping process, which is composed of formation of defects (bubbles) at specific regions and their propagation.

Here, I will explain hybridization (dehybridization) process of DNA thermodynamically using a simple model. Two single stranded DNA molecules complementary to each other (A and A') exist in a bulky solution at a low concentration. (In this case, it is expected that the activation barrier for hybridization/ dehybridization is very low) The free energy change of hybridization process can be written as:

$$\Delta G_{hyb\_solution} = \Delta H_{hyb\_soltion} - T\Delta S_{hyb\_solution}$$

$$\text{At } T = T_m, \Delta G_{hyb\_solution} = 0, \quad \text{Thus } T_m = \Delta H_{hyb\_solution} / \Delta S_{hyb\_solution}$$

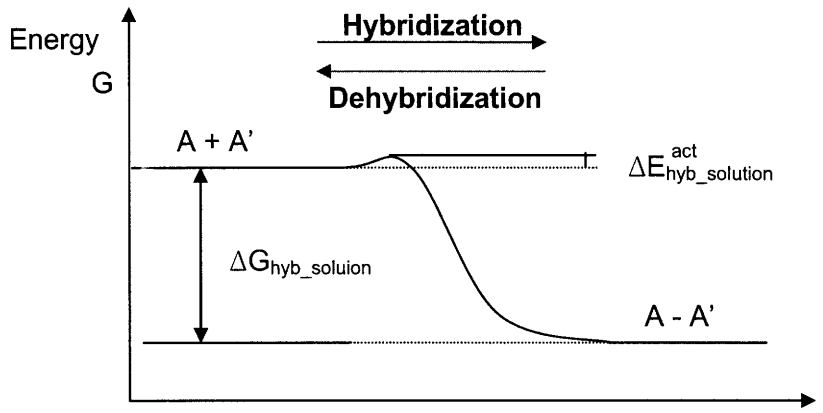

Figure 3.4. Free energy change during hybridization/dehybridization reaction that occurs in a bulky solution. ($T < T_m$)

The main factors that contribute this free energy change are i) hydrogen bond formation, ii) stacking of base pairs, and iii) electrostatic repulsion between phosphate backbones, negatively charged.[15-17] Thus, the free energy can be expressed as:

$$\Delta G_{hyb\_solution} = \Delta G_{hydrogen\ bond} + \Delta G_{stacking} + \Delta G_{electrostatic(A\text{-}A')}$$

In this equation, $\Delta G_{hydrogen\ bond}$ and $\Delta G_{stacking}$ assist hybridization whereas $\Delta G_{electrostatic(A\text{-}A')}$ is a unfavorable factor for hybridization.

There are several factors that determine T_m. First of all, higher ratio of the G-C pairs forming 3 hydrogen bonds (c.f. A-T pairs form 2 hydrogen bonds) in the sequence, higher T_m. Also, shorter DNA has lower T_m (in the range 1 - 100 base pairs). However, T_m cannot

keep increasing by the length of DNA. The plot of T_m vs. the length of DNA is shown in Fig. XX in which $T_{m,sat}$ depends on the G-C composition [%] of the sequence.

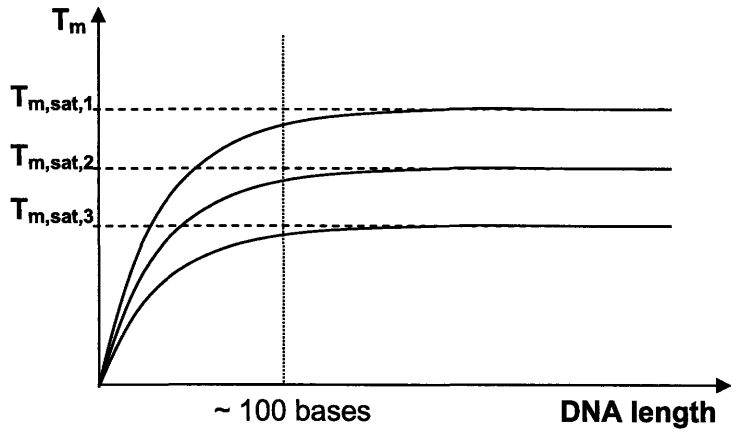

Figure 3.5. Plot of the melting temperature of DNA depending on the DNA length. The relation of G-C composition among sequence 1,2 and 3 is $(\%GC)_1 > (\%GC)_2 > (\%GC)_3$

$\Delta G_{electrostatic(A\text{-}A')}$ is originated from the negatively charged phosphate backbone of ssDNA molecules complementary to each other. To make hybridization preferable, $\Delta G_{electrostatic(A\text{-}A')}$ in the whole system should be smaller. A buffer with strong ionic power helps hybridization by shielding the electrostatic repulsion between two DNA strands. In fact, T_m of DNA is also a function of the ionic strength of the buffer used for hybridization and the standard T_m used officially is the melting temperature adjusted in 0.05M NaCl solution: if the concentration of Na^+ is higher, T_m is also higher; if it is lower, T_m is lower.

In general, the T_m is calculated in the following equation.

$$T_m = 81.5 + 16.6 (\log [Na^+]) + 0.41 (\%GC) - (\frac{500}{\text{Length of DNA}})$$

This equation shows G-C portion dependence, DNA length dependence, and salt concentration dependence of T_m, described above.

This information about DNA is extremely important to understand the technique and the results that will be described from the next section.

3.3. Supramolecular NanoStamping (SuNS).

Supramolecular Nano-Stamping (SuNS)[10,18] is a high throughput printing technique using both printing concept and supramolecular interaction - a smart stamping technique with supramolecular inking (see section 2.3.3). SuNS is based on the specific supramolecular interaction between complementary DNA strands for selective inking. Crooks' group also independently developed a new printing technique with a similar approach. [19-21] His results are reviewed in depth in chapter 10.

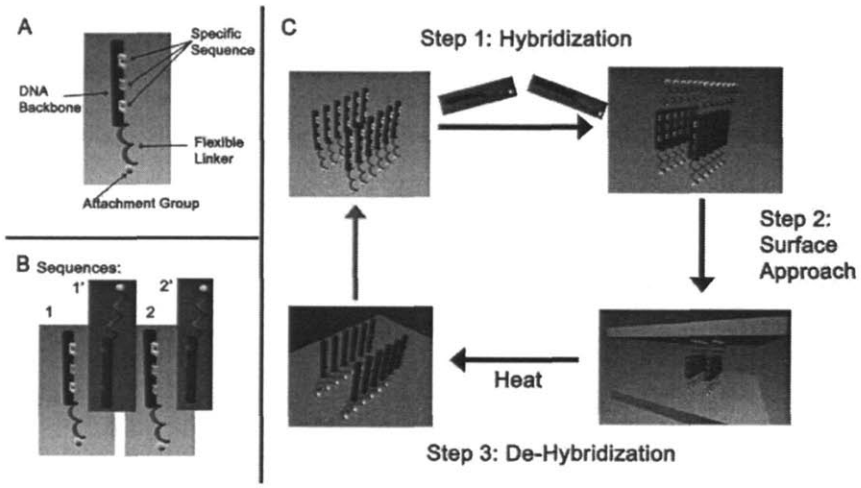

Figure 3.6. Schematic drawing of the printing procedure of SuNS. SuNS reproduces a pattern composed of different DNA strands using the supramolecular specific recognition interaction between complementary DNA strands.

Briefly, SuNS's working principle is the following (each step will be described in detail in sec.3.2.1 - 3.2.4): a starting master of SuNS, containing a patterned single stranded DNA (ssDNA) monolayer, is hybridized in a solution of mixture of complementary DNA (cDNA) molecules of which the end is modified with a chemical group that adhere to another surface. After hybridization, another substrate is placed on the master to allow for bond formation between cDNA molecules and the secondary substrate. The two substrates bonded by the double stranded DNA (dsDNA) can be separated by heating up to the dehybidization temperature of dsDNA. A copy composed of cDNA is left on the printed

substrate. Crooks' group uses mechanical force for dehybridization instead of thermal energy.[19,21]

3.3.1. Master preparation

The prerequisite of all printing/stamping (parallel reproduction) techniques is a master (mold/mask) to be copied. Although this is not a step in the printing "cycle" (The cycle to be repeated for reproduction should start with "hybridization" of the master), it should be described since it is an essential part of SuNS. The masters of SuNS are a patterned ssDNA monolayer on substrates. I classify the approaches to fabricate a master composed of a patterned DNA monolayer into three kinds.

The first is patterning using DNA molecules directly using soft lithography such as DPN.[22] This technique is straight forward and it does not need any more treatment but in many cases, it requires specific conditions for the handling of the sensitive biomolecules.

The second category is to make a pattern of reactive material using various lithography techniques followed by assembling a DNA monolayer on the pattern. For example, if a gold pattern is made with electron beam lithography, this pattern can be converted into a DNA pattern by immersing the substrate into a solution of thiolated DNA molecules. At this time, the primary patterning can be done by either soft-lithography or self-assembly. For example, if a pattern is fabricated using organic molecules with epoxy-ending group, the chemical pattern can be converted into DNA pattern by treating amine-terminated DNA molecules. The advantages of this approach are that it can avoid complex

fabricating conditions required for patterning biomolecules and the feature resolution is higher because of handling smaller units for patterning procedure.

The last one is backfilling: passivating a reactive surface using other molecules with a pattern, followed by treating the unpatterned part with DNA molecules. For example, after making octadecane thiol pattern on a gold substrate using μCP, the bare gold part can be occupied by DNA molecules by immersing the substrate into a thiolated DNA buffered solution. Generally, backfilling condition allows more ambient conditions for biomolecules.

In this thesis, various methods used for fabricating masters for SuNS will be described in detail in the following chapters.

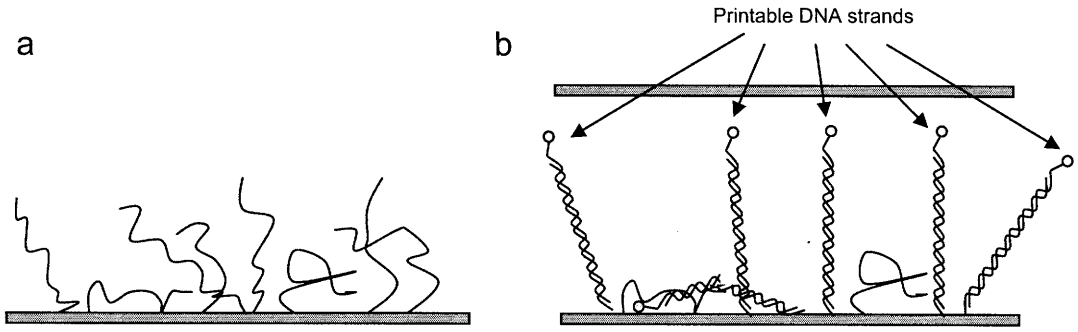

Figure.3.7. Schematic drawing that describes the morphology of a master (a) before hybridization and (b) after hybridization. Only cDNA with a functional group at distal position can be effectively printed.

Another important issue for fabricating masters is the morphology of DNA SAM on masters. SuNS works under the assumption that cDNA molecules that assemble on the

original DNA pattern are pre-oriented and the attachment group on cDNA molecules are at distal position, so the attachment group can make a chemical bond with the secondary substrate without unspecific binding of primary DNA on that surface. (fig.3.7) There are various methods to make DNA more accessible to cDNA for hybridization such as applying the electric field; covering all surface other molecules except the chemical binding site of immobilized DNA; or immobilizing DNA, which is hydrophilic, on hydrophobic polymeric substrates.[23-26] In fact, hybridization itself helps the DNA strands to stand up (the persistence of dsDNA is around 80nm[27] whereas the persistence of ssDNA is around 1nm[28]) so more DNAs become more available for contact step in SuNS. However, we still need to control the morphology of a master since it also affects on the hybridization efficiency of cDNA molecules. (See sec. 3.3.2) In order to obtain a good morphology of DNA patterns on masters, the chemistry to be used for the formation of DNA SAM should be carefully chosen and the reaction should be kinetically controlled.[15,26,29-31] In fact, it was reported that not all immobilized DNA molecules on surface are reactive to hybridization.[15,26,29-32] Indeed, the hybridization efficiency ranges from 5% ~ 100%, depending on the immobilization chemistries, the geometry of the surface and the density of immobilized DNA.[24,33-41] (see sec. 3.3.2) The unreactive DNA molecules to hybridization cannot take part in the printing process in SuNS undoubtfully and the DNA molecules that lay down on the surface after hybridization are not effective for printing, either. (fig.3.7)

3.3.2 Hybridization

In SuNS, "Hybridization" is the first step of the printing cycle. When a master prepared in one of the methods described above, is placed in a solution of cDNA molecules tagged with a functional group that is reactive to another surface.

Hybridization between complementary DNA strands is well understood in a bulk solution state as described in sec. 3.2. However, it should be noted that the information given for solution hybridization is not enough to explain "surface hybridization" in which one part of DNA pairs is tethered on a surface. Indeed, hybridization between DNA immobilized on a surface and cDNA existing in a solution is different from the hybridization between two complementary DNA freely moving in a solution in many aspects.[15,26,29,31,42] For example, the equilibrium binding constants for surface hybridization can differ by many orders of magnitude relative to solution values.[42] Also, this hybridization/ dehybridization process occurring on surface is not as fast as the same one that occurs in a solution. The main reason of this difference is that there are significantly increased energy barrier and the additional free energy required for cDNA to binds on the immobilized DNA on surface for hybridization. I propose that the free energy change for surface hybridization can be written as following (It is assumed that the units of free energies used in this equation is as normalized as possible);

$$\Delta G_{hyb\_surface} = \Delta G_{hydrogen\ bond} + \Delta G_{stacking} + \Delta G_{electrostatic\ (A\text{-}A')} + \Delta G_{penetration}$$

$$\Delta G_{penetration} = \Delta G_{steric} + \Delta G_{electrostatic(A'\text{-}\ surface)}$$

$\Delta G_{penetration}$ is the additional free energy required for cDNA molecules to penetrate into the layer of DNA bound on surface for hybridization and it is a function of the morphology and the density of DNA immobilized on a substrate.[42] $\Delta G_{penetration}$ can be approximated to the sum of ΔG_{steric}, the energy to overcome the steric hindrance for cDNA to approach to DNA; and $\Delta G_{electrostatic\ (A'\text{-}surface)}$, the energy to overcome the electrostatical repulsion between cDNA and a negatively charged surface due to immobilized DNA strands.[15,31,32] Since ΔG_{steric} and $\Delta G_{electrostatic\ (A'\text{-}surface)} > 0$, the equilibrium of this process is not as preferable to hybridized state as the case for solution case. In order to estimate $\Delta G_{penetration}$, the factors to be considered are the morphology of immobilized DNA, the surface geometry, and the immobilized DNA density,[24,33-42] which are dependent on the immobilization chemistry and the condition.[26,30]

The space constraint originated from the structure of immobilized DNA affects not only the equilibrium determined by $\Delta G_{penetration}$ but also the hybridization rate determined by the activation barrier $\Delta E^{act}_{hyb\_surface}$. Indeed, $\Delta E^{act}_{hyb\_surface}$ is much larger than $\Delta E^{act}_{hyb\_solution}$ because the cDNA molecules should plow their way through the layer of primary DNA molecules and form hydrogen bonds with one of them, stuck on a surface. This makes surface hybridization process slow down and it is same for dehybridization, too.

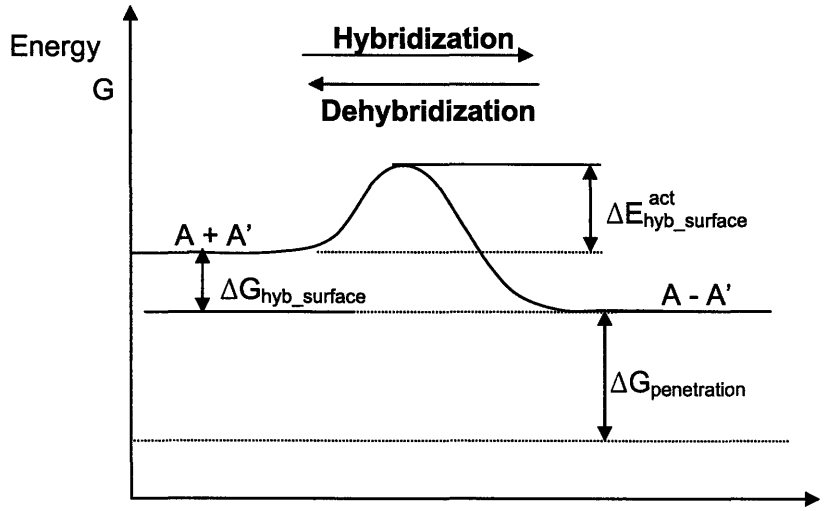

Figure 3.8. Free energy change during hybridization/dehybridization reaction that occurs in a solid surface. ($T < T_m$)

The analysis written above gives an idea on how the efficiency of the hybridization can be improved by adjusting the variables such as temperature, the composition of buffers and the time. I define the hybridization efficiency as the following equation.

$$\text{Hybridization Efficiency} = \left. \frac{\#\text{ of DNA hybridized with cDNA}}{\#\text{ of total DNA molecules deposited on surface}} \right|_{t = 2hr}$$

In this equation, hybridization efficiency implies how much portion of DNA molecules among the immobilized ones on surface are successfully hybridized within a certain time. (Because I believe that the time required for hybridization is also important for practical applications, I set the time not as infinite one but as 2 hour.)

The hybridization temperature is important for minimizing the probability of mismatching between DNA strands especially a master composed of two of more kinds of DNA is hybridized. Moderately higher temperature increases the hybridization rate and the recognition accuracy between DNA molecules complementary to each other, but too high temperature decreases the efficiency of hybridization. A high ionic strength of buffer is required for hybridization to shield the electrostatic repulsion from the negative charge in DNA backbone (phosphate). Additionally, it is recommended that to use detergent in the buffer for hybridization to relieve the Van der Waals force between DNA – DNA, immobilized on surface with entangled structure; DNA – cDNA; and cDNA – surface. Also, the time to be allowed must be adjusted to optimize the printing process since the hybridization of DNA is a saturation reaction. The rinsing method after hybridization to minimize unspecific binding is another factor to be considered. Fortunately, a number of studies have been already carried out by many biologists who are involved in DNA microarrays.[43] However, the data cannot be directly applied because the all parameters are dependent on the morphology of the DNA monolayer on the master, the DNA length and the sequence composition.[42] For instance, if the DNA pattern on a DNA microarray is like entangled spaghetti structure (the persistence of ssDNA is 1 nm[28]), it can be expected that the activation barrier for hybridization or dehybridization ($\Delta E^{act}_{hyb\_surface}$, $\Delta E^{act}_{dehyb\_surface}$) is higher than the case of well-organized DNA SAM. Thus, if the master is composed of a thiolated DNA SAM on gold surface, generally with a well aligned structure, I believe, the rinsing using a buffer with low ionic strength should be gentler and the hybridization time may be shorter (since hybridization/ dehybridization is faster) compared to the condition

optimized for the DNA microarray fabricated using a quick chemistry that results a complex structure.

3.3.3. Contact

After hybridization, a secondary substrate is placed on the master containing a patterned dsDNA monolayer for contact. In SuNS, two kinds of approaches can be used to obtain a good contact between the master and the secondary substrate, inducing a bond formation between cDNA molecules and the applied surface.

3.3.3.1. Capillary approach

A gentle approach using capillary forces can be used in the following way. After hybridization step, the master is gently washed with a low-concentrated buffer solution and DI water briefly. (Although washing hybridized masters with DI water may induce the loss of cDNA molecules hybridized on the masters, it is necessary to prohibit the salt formation on the surface after the buffer solution dries during contact process.) When the surface is still wet, a secondary substrate is placed onto the master. Then, a thin water film instantly forms between the master and the secondary substrate to minimize the exposed area with high surface energy. While the water evaporates, the spacing between two substrates decreases and capillary forces push the secondary substrate onto the master, and eventually induce a gentle contact.

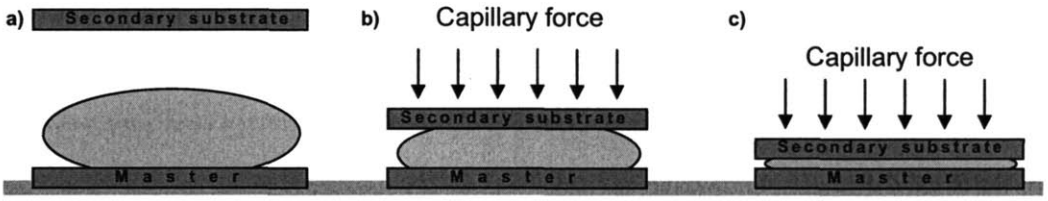

Figure 3.9. Schematic drawing describing the capillary approach

Capillary approach is effective when the involved substrates have a relatively high surface energy (hydrophilic) and at least one substrate is originally flat, light and flexible.

3.3.3.2. Mechanical approach

If the substrates involved in printing process are hydrophobic, rough and very rigid, their spacing cannot be effectively controlled only by the capillary force. In those cases, good spacing between the master and a secondary substrate for effective contact should be obtained with another approach. In fact, applying mechanical force is commonly used in many other printing techniques to control the contact. Although the concept is simple, it is challenging to obtain a contact with i) an appropriate degree ii) homogeneously over a large area using a mechanical force. (This is a critical problem of nanoimprinting technology, requiring expensive facilities[44,45]) Thus, in order to introduce the mechanical force for contact in SuNS' process, the following issues should be considered.

First of all, the amount of pressure to be applied should be optimized. It depends on the mechanical property of the substrate materials and the temperature of the condition. The correct condition can be found by a series of printing trials that carried out within the

expected range from the information of the material properties. Without optimization, it should be regarded that imprinting or non-contact is possible. However, the cDNA may be successfully transferred without damage even if there occurs imprinting moderately.

Second, it is also important to obtain a homogenous contact over a large area. Inhomogeneous pressure results a partial overprinting and a partial non-printing. In SuNS, this is mainly due to inhomogeneous pressure source or the surface mismatch originated from rough morphology of involved substrates. For this issue, it is helpful to introduce a flexible pad[46] between the master and the pressure source; or to increase the temperature to make the substrates softer allowing conformal contact. (especially, when the substrate is thermoplastic)

3.3.4. Dehybridization.

For the complete printing, it is necessary to separate the secondary substrate containing a copy composed of cDNA from the master as the last step: it is done by dehybridization of dsDNA between the two substrates attached together. There are several known methods in biotechnology to disrupt the hydrogen bonds between complementary DNA strands forming a double helix.

One is changing pH. If the concentration of H^+ (H_3O^+) increases, the Lewis base parts of the hydrogen bonds constructing dsDNA structure are blocked. As a result, the hydrogen bonds interaction decreases and then dsDNA molecules separates into two ssDNA molecules. The same result can be obtained when pH increases because the system will lose protons for forming hydrogen bonds.

The second method is increasing temperatures to give some energy for overcoming the hydrogen bond interaction and the stacking energy. (see sec.3.3.2) The dehybridization temperature (T_m) is a function of DNA length, the composition of the sequence and the ionic strength of buffer solution. Since the dehybridization occurs on surface in SuNS' process, the T_m is different from the calculated value. However, in this thesis, it is assumed that $T_{m\_surface}$ is similar with $T_{m\_solution}$.

The third way is introducing an enzyme that helps dehybridization of dsDNA. Indeed, this way is carried out to copy the genetic information in DNA to RNA in living creatures in which the pH and the temperature are constantly conserved.

Beside the known methods in the biotechnology described above, a mechanical force can be applied for dehybridization as another possible way. This approach cannot be used in DNA solution state but used only when DNA molecules are linked to another solid support (e.g AFM tips or other substrates). For successful dehybridization, the amount of the applied mechanical force for dehybridization should be larger than the interaction between complementary DNA strands used in SuNS, but smaller than the strength of the chemical bond formed between DNA and the solid substrates.[47] (Fig. 3.10)

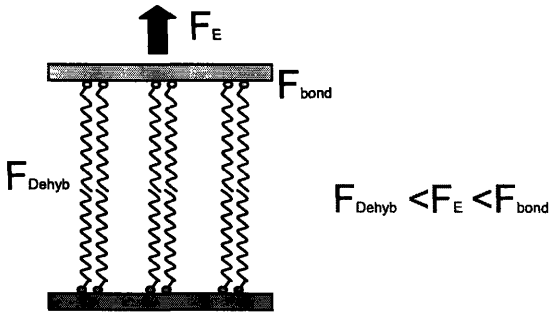

Figure 3.10. Requirement about the amount of force applied for dehybridization step in SuNS.

Among these four ways for dehybridization, only second one and the last one are acceptable for SuNS due to the diffusion limit (In SuNS, the dsDNA molecules should be dehybridized within an extremely small space – between two substrates apart to each other with around 10nm scale spacing.). For these reasons, I applied mostly a thermal energy (see chapter 6 and 7) and, sometimes mechanical energy additionally in combined way with thermal energy if necessary (especially, when the adhesion force between two substrates is big) (see chapter 8 and 9), for the last step of SuNS - to take the copied pattern composed of cDNA molecules apart from the original pattern composed of primary DNA molecules. In fact, Crooks' group used mechanical force for dehybridization in their approach. [19-21]

3.4. Key advantages

SuNS is a unique printing technique with some exclusive key advantages as following:

3.4.1. Growth of the master

In normal printing techniques, a master can be easily distinct from a printed pattern from the master. On the contrary, in SuNS, a printed pattern is functionally identical with the master because it is a mirror image of the original pattern composed of cDNA so a copy made through SuNS is active in reproduction in the same manner with the master. In more details, if a copy is hybridized with primary DNA molecules with a chemical binding group, the same pattern composed of the primary DNA can be printed onto another surface through SuNS. I define 1^{st} generations are copies from an original master and 2^{nd} generations are copies from 1^{st} generations. In short, a copy (1^{st} generation or n^{th} generation) made through SuNS can be used as another master to make another copy (2^{nd} generation or $(n+1)^{th}$ generation) while the original master makes other copies. This character accelerates the printing rate of SuNS enormously since the number of master gets increased by the number of printing cycles: theoretically, it increases exponentially. To the best of my knowledge, SuNS is the only printing technique that has this character.

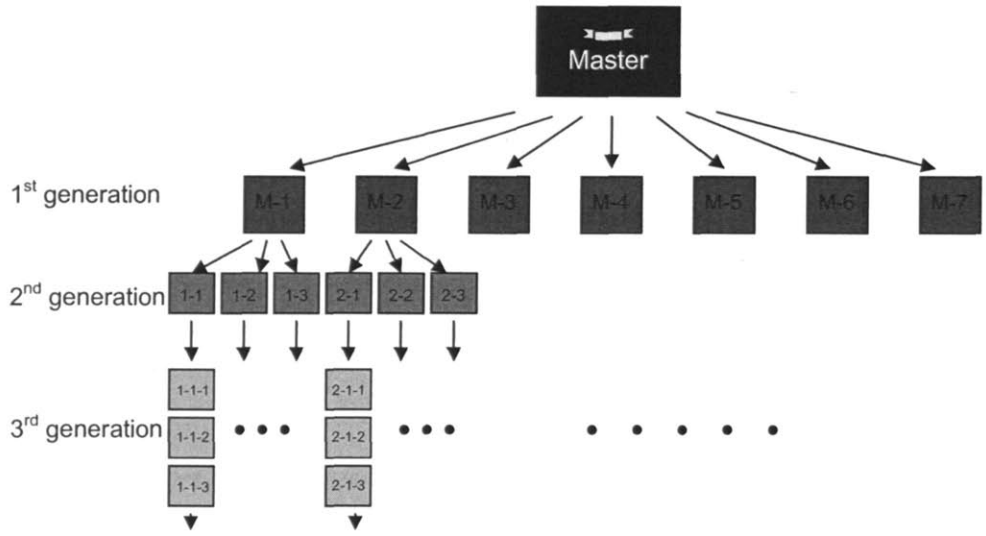

Figure 3.11. Schematic drawing of the growth of master in SuNS.

3.4.2. Versatility in master

The masters for SuNS can be fabricated with various techniques as far as it contains a patterned ssDNA monolayer. As described in section 3.2.1, masters can be prepared either soft lithography or hard lithography that is followed by converting the pattern into the one composed of DNA molecules. Many other printing techniques require a specific master for their own methods. For example, the master for microcontact printing must be a PDMS stamp with a geographic restriction. Since there is no specific material or design for fabricating master in SuNS, the different characters of various masters (e.g. resolution) can be copied through SuNS.

3.4.3. Massive information transfer

Most importantly, SuNS has a potential to transfer a huge amount of information contents. Usual printing techniques can transfer physical information only, defined by size, shape & location. (Exceptionally, affinity contact printing[19-21] can transfer a chemical information) However, thanks to the specific interaction of complementary DNA strands, - the key part of SuNS' working principle - SuNS can transfer chemical information inscribed in the DNA sequences used for the pattern with physical information together. For instance, if a master is composed of various DNA strands with different sequences, SuNS can print all kinds of the corresponding cDNA molecules simultaneously by hybridizing the master in a solution containing cDNA molecules mixture and transferring them onto another surface in a single cycle. On the contrary, using other conventional printing techniques, multi printing cycles must be required for the same case. This character allows for SuNS to be the most reliable technique for printing multi-component devices. Indeed, printed DNA patterns can be converted into other components (e.g. nanoparticles or protein): if the desired component is tagged with a DNA fragment complementary to the DNA that is already printed on surface, the printed DNA can be easily converted into the target component by immersing the printed substrate into a solution containing the target molecules. At this time, a single DNA sequence works as a zip-code and it directs further modification of other materials tagged with cDNA so we can even establish a true multi-component fabricating process using bio-chemically directed self-assembly through SuNS.

3.4.4 Good chemical property of ink molecules.

Among fragile bio-molecules, DNA is the best starting/final material to print. Indeed, using DNA as ink molecules has several advantages compared to affinity contact printing (αCP)[48,49] that uses ligand-protein interaction. First, DNA molecules (oligonucleotides) are smaller than proteins. This gives SuNS a better printing resolution and less non-specific binding due to the smaller Van der Waals force. Second, since DNA is more stable than proteins, the overall printing process is more reliable. Also, more chemical modifications can be introduced to DNA molecules so that various chemistry and substrate systems can be applied in SuNS. (And this affects on the printing quality) Third, there are a lot of possibilities on sequential variation using a combination of specific number of four bases (A, T, G, and C). Each variation of sequence has the unique chemical affinity attraction but with approximately identical other properties. On the contrary, for αCP, it's not easy to find many kinds of compatible protein that can be printed together because it will be challenging to handle various proteins in one uniform printing condition. Lastly, printed DNA patterns can be converted into ones composed of protein or any other components (see section 3.3.3).

These advantages described here will be proved in the following chapters with the corresponding key results.

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Chapter 4. Possible applications for SuNS

4.1. Introduction

Based on the unique advantages of SuNS, an easily achievable product using SuNS will be a pattern composed of many kinds of DNA molecules, which is hard to obtain with other conventional printing techniques. Indeed, there is a real product composed of many different DNA pieces, extremely useful for medical/clinical purpose or biological research: DNA microarrays. In chapter 4, the current DNA microarray fabrication techniques and the important factors to be considered for DNA microarrays fabrication and characterization will be reviewed.

4.2. DNA microarrays

A microarray is a microminiaturized system containing many dots (probes) each of which is composed of different species (in most cases, bio-molecules) on a solid support. A microarray is a high-throughput device that allows a parallel analysis of a number of bio-elements in a single experiment.[1-3] It is a tremendous advantage considering the fact that most of bio-experiments are time consuming and very sensitive to the variables from the experimental environment so that it is hard to analyze all elements with the exactly identical experimental condition.

The working principle of microarrays is the following: the kinds and the location of all the probes on a microarray are already known. After the microarray is exposed to target molecules modified with a tag, the target molecules can be identified using the signal from the tag detected from the location of the corresponding probes that interacts with the target. Since it is based on the specific interaction between biomolecules, the technique can be applied only to the molecules that have a specific interaction with another: DNA - cDNA, DNA –RNA, RNA - protein, antibody – antigen.[3-6]

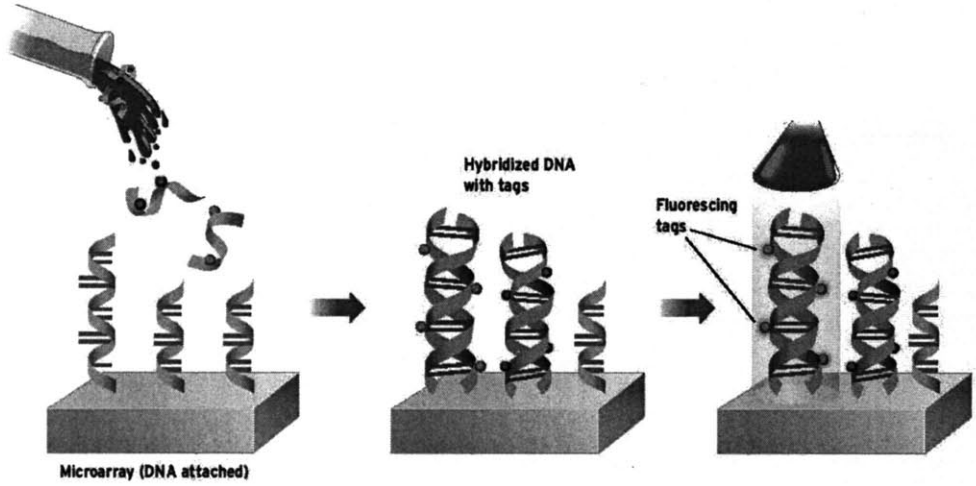

Figure 4.1. Schematic drawing that shows how oligonucleotides molecules are analyzed by using DNA microarrays. Extracted from reference 3

Among many kinds of microarray systems, DNA microarrays are a good example of successfully developed multi-component bio-devices that are currently commercially available.[3,4,7] It has been shown that DNA microarrays are applicable for enormous bio/medical research field including gene expression, mutation detections, single nucleotide

polymorphism, cancer research, microbial diagnostics, infectious diseases detection, genomic disease detection, drug discovery and food testing.[1,4,7,8]

Since they were introduced first time in early 90s, the techniques involved in DNA microarrays have progressed in an enormous rate due to the special interest and efforts on the emerging new technique, capable of opening the new era of genomic information analysis. For example, when the first DNA microarray was introduced by Schena and his colleagues, it contained only 48 probes,[9] but one year after, DNA microarray composed of 1046 dots were made,[8] and soon after, 10^5 different oligonucleotides spots could be fabricated on a single substrate by using photolithography based fabrication technique.[10]

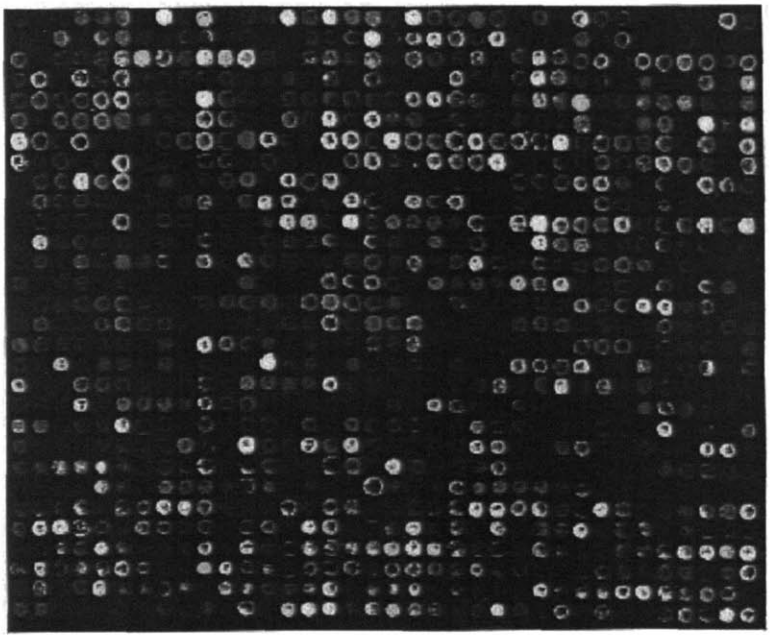

Figure 4.2. Fluorescence image of a DNA microarray[11]

Also the surface chemistries used for immobilizing DNA molecules (oligonucleotides) on a substrate have been expanded by many groups. [4,9,11-15] The DNA microarray in the early stage was made on a polyacrylamide gel,[9,11] but it was moved to more robust substrates such as glass substrates. Currently, beside of glass, silicon, silicon oxide, fused silica, gold, PMMA, PDMS, and SU-8 are used as substrate materials,[4,12-15] depending on the fabrication method and the applications.

As the tag attached to target molecules for analysis after hybridization, fluorescence molecules are more commonly used instead of radioactive molecules.[4] Using fluorescence molecules allows simultaneous co-hybridization of multi-target molecules so that the number of analyzing steps decreases.[4] Due to this advantage, many of standardized DNA microarray scanners are based on the fluorescence microscopy. However, it set the minimum of one probe spot size to the resolution limit of optical microscopy. For the reason, miniaturizing DNA microarrays should be followed by the equivalent improvement of fluorescence microscopes to be used for analysis. In the ultimate case of fabricating high resolution array below optical limit, it is necessary to develop/design a new analysis technique with higher resolution. (e.g AFM based method)

4.3. Current fabrication techniques of DNA microarrays

The two major categories of current DNA microarray fabrication techniques are i) in-situ synthesis of oligonucleotides and ii) Deposition of presynthesized oligonucleotides.

4.3.1. In-situ synthesis

In-situ synthesis is an efficient approach to obtain high density microarrays. [1,10] It is used for fabricating microarrays composed of relatively short oligonucleotides since the error on the sequences synthesized increases by the number of process steps, proportional to the length of DNA, and there is no way to purify the synthesized molecules after processing.

Photolithography, borrowed from the semiconductor industry, could be successfully applied for DNA microarray fabrication.[1,10] For example, Affymetrix produces DNA microarrays (Genechips®) with extremely high probe density using photolithography. It is a highly parallel process that synthesizes all probes on a substrate base by base simultaneously. (Note that it still needs a serial process to attach each base to make the complete sequence.) It is composed of repeating the selective light exposure through an optical mask onto oligonucleotide spots with photolabile protective group on top, and the addition of new nucleotides with protective group on the activated spots. The fabricating procedure needs 100 optical masks and 100 cycles for fabricating 25 bases length microarrays no matter how many spots are on one substrate. This approach allows an ultimate high density DNA microarrays (10^6 probes in $1.28cm^2$) with high accuracy on spot geometry.[4] However, there are also drawbacks of this photolithography based technique : i) there need a significant time and cost for designing/fabricating of masks, ii) all process has to be done in clean rooms using expensive facilities, and iii) only short DNA

oligonucleotides can be synthesized (maximum 25 bases). Recently, as a more cost-effective way, a modified photolithography method, directing the light using mirrors not using masks, was developed. [4]

Another in-situ synthesis technique is using inkjet (Agilent, HP) printers that deposit 4 kinds of phosphoramidite nucleotide (A, T, C, G) on a specified location in a certain order, depending on the sequences to be desired.[4] This technique cannot reach such a high probe density of ones fabricated using photolithography (around 10^4 on a slide) but the process of simpler since it does not require many optical masks and working in a clean room. Also, a microarray composed of longer DNA probes can be synthesized due to higher photo-sensitivity of phosphoramidite group. (50-100bases) [4]

4.3.2. Deposition (contact & non contact)

Another popular approach is depositing presynthesized DNA on a substrate using a printing facility using a relevant surface chemistry. In most cases, the surface of these substrates needs to be functionalized with a chemical group and modified or unmodified DNA can be deposited on those substrates. The applicable chemistries are listed on table 1.

Table 1. The list of commonly used chemistries in DNA microarray fabrication. Courtesy of reference 3.

DNA modification	Substrate modification
None	Polylysine
	Amine
	Epoxy
	Diazonium ion
	SU-8
	Unmodified glass
	Agarose film
	Membrane
Silanes	Unmodified glass
Thiols (–SH)	Gold
	Mercaptosilanes
	Maleimide
	Iodoacetyl
Amines ($-NH_2$)	Aldehydes
	Epoxy
	Isothiocyanate
Phosphates (PO_3)	Aminated surfaces
Biotin	Avidin

The most noticeable advantage of this category is that there is no restriction of the length of DNA to be printed: it can be used to fabricate microarrays containing 3kb long DNA molecules. However, the printing resolution of DNA spots and the fabricated probe density are much lower than photolithography based method. (around 10^4 on a slide)

These spotted DNA microarrays can be fabricated either contact printing or non-contact printing. Contact printing based on mechanical microspotting or wetting uses pins or tweezers. Because this technique delivers DNA drop onto another substrate by contact,

the wettability of DNA solution on the substrate is a crucial parameter. Basically, when DNA is to be printed on a hydrophobic surface, contact printing has a potential to generate defect spots with less printed DNA. In the worst case, the DNA solution may be completely retracted when the pin is detached from the surface; as a result, the printing is unsuccessful. On the contrary, with non-contact based method such as inkjet, bubble jet, or piezo electric technology using nozzles, the amount of deposited DNA solution does not depend on the surface character of the substrates to be printed. [4]

Another reason of probable defects of these physical delivery techniques is the evaporation of DNA solution printed after deposition before printing procedure is not complete. In order to decrease the evaporation of deposited DNA solution, betaine or DMSO contained buffer is used to make the evaporation slow down. [4]

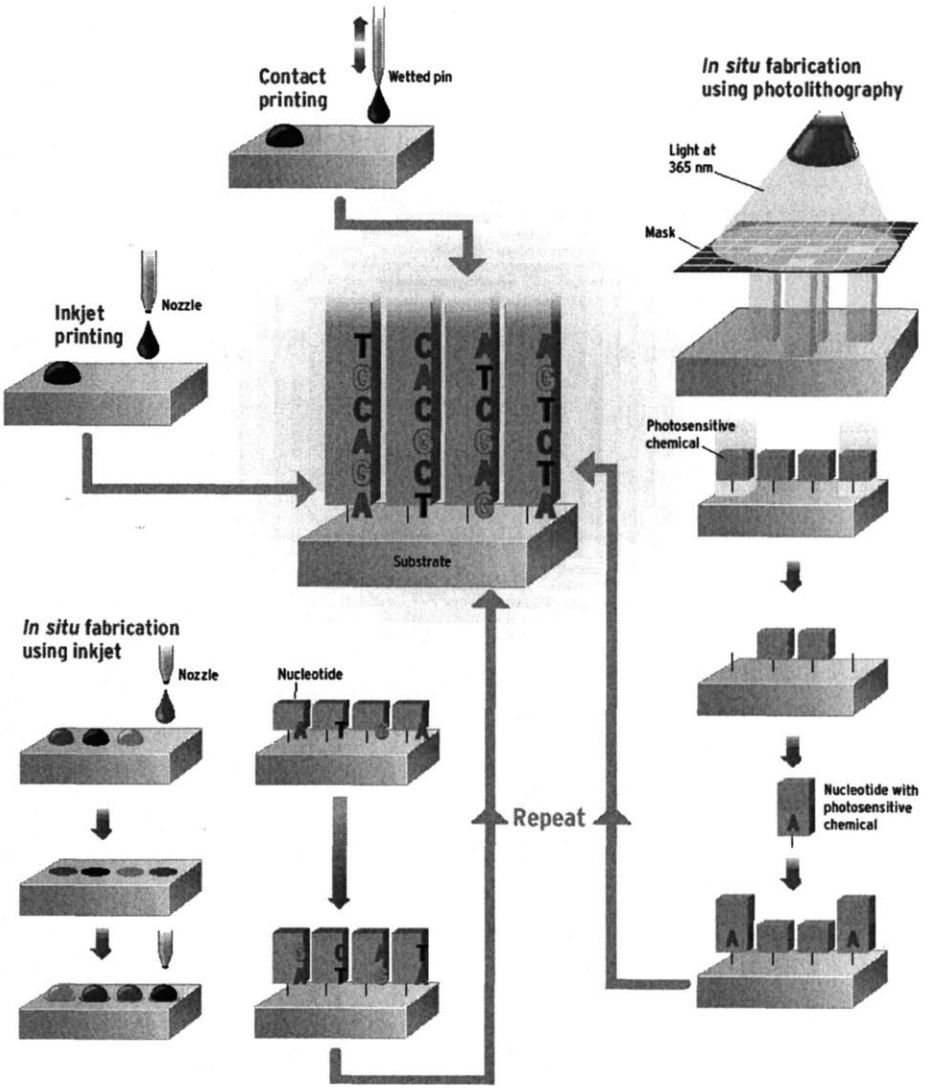

Figure 4.3. Simplified drawing that shows the processes of various fabrication techniques of DNA microarrays. Courtesy of references 3.

To optimize the function of DNA microarrays is still very challenging since there are too many parameters affecting the performance of DNA microarrays.[4] There are several factors to be considered for a good performance of DNA microarrays. And many of these are overlapped with the ones in SuNS procedure.

First, the parameters involved in the fabrication techniques are i) the concentration of DNA probes immobilized on the solid support, DNA length and the sequence composition to be synthesized or printed, ii) fabrication facilities, iii) printing temperature, spotting buffer, and humidity, and iv) used surface chemistries, and blocking techniques.

Second, the parameters involved in hybridization steps are i) target concentration, ii) hybridization buffer composition (the strength of ionic power), iii) hybridization temperature, and iv) rinsing method.

Lastly, the parameters involved in analysis are i) the kinds of used fluorescence molecules, and ii) the optical performance of microscopy.

Table 2. Possible factors affecting to DNA microarray performance. [4] Courtesy of reference 4

	Array geometry	Spot density	Spot performance			Background	Specificity
			Morphology	Probe density	Hybridised density		
Robotics	Yes	No	No	No	No	No	No
Spotter type (pin, inkjet)	No	Yes	Yes	Yes	Yes	No	No
Pin type	No	Yes	Yes	Yes	Yes	No	No
Humidity	No	Yes	Yes	Yes	Yes	No	No
Temperature at spotting	No	Yes	Yes	Yes	Yes	No	No
Probe concentration	No	Yes	Yes	Yes	Yes	No	No
Spotting buffer	No	Yes	Yes	Yes	Yes	No	Yes
Immobilisation chemistry	No	Yes	Yes	Yes	Yes	Yes	Yes
Blocking technique	No	No	No	No	No	Yes	No
Stringency during hybridisation/washing	No	No	No	No	Yes	No	Yes
Hybridisation conditions (diffusion/mixing)	No	No	Yes	No	Yes	Yes	Yes
Probe sequence	No	No	No	No	Yes	No	Yes
Target preparation	No	No	No	No	Yes	No	No

4.4. Other Possible Applications for SuNS

Although reproducing DNA microarrays is the most easily achievable goal where SuNS is utilized, there are much more possible applications of SuNS considering that DNA patterns can be easily converted into many other components (see section 3.3.3). Thus printed DNA patterns using SuNS can be multi-component devices such as protein microarrays, organic integrated circuit containing the mixture of inorganic and organic components. Especially, for protein (antibody) microarray, it was reported that protein patterns can be made from DNA patterns by hybridizing with cDNA tagged with target proteins. This approach is expected to allow more conformational freedom to the immobilized proteins due to the enough long linker molecules (DNA), compared to the one directly immobilized on a surface. Indeed, it was reported that proteins with DNA linker shows higher function activities than the one without linker group.[16] In conclusion, SuNS can be used to fabricate protein arrays through the bio-chemically directed assembly of various proteins after printing DNA as coding materials.

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Chapter 5. Printing Homogeneous DNA Monolayers Using SuNS

5.1. DNA self assembled monolayer (SAM)

There are various ways to immobilize DNA molecules on a surface. For example, silanization is the most common method to immobilize DNA on glass or silicon substrates: the kind of silane to be used may be different by the kind of the functional group on DNA molecules.[1-3] Coating the substrates with a polymer containing a reactive group or activating the surface of polymer substrates can be also another possible approach.[4-9] Recently, another method/chemistry for the immobilization of DNA has emerged: immobilizing thiolated DNA on gold surface.[10-12] (In general, various molecules containing thiol or modified with thiol have been patterned on gold surface using soft lithography techniques.[10,13-15]) Thiolated DNA molecules form a self assembled monolayer (SAM) on gold surface as many other short thiol molecules do. Using this chemistry, DNA could be patterned on gold substrates using μCP, DPN or nanografting. [16-19]

A homogeneous SAM made of thiolated DNA molecules on a gold substrate has been widely used for the AFM characterization for the measurement of the interaction between complementary DNA strands or the study of the morphology of SAM or the fluorescence analysis to check hybridization efficiency.[20,21] Since the whole substrate is evenly covered by DNA molecules, any part of surface can be chosen for evaluation and it

is convenient for constructing statistical data. Moreover, a thiol SAM on gold surfaces provide have been widely studied due to the high degree of control over surface physical properties;[22] as a result, a thiolated DNA SAM on gold substrates has been relatively well characterized including hybridization efficiency/rate and the morphology structures of ssDNA SAM. [20-29] For these reasons, I chose a homogenous SAM of thiolated DNA as the simplest master for proof-of-concept of SuNS and for evaluating the fatigue of masters and the printing efficiency by the number of printing cycle. Also, most of the masters described in this thesis were made of a thiolated DNA monolayer on gold substrates because of the many advantages of thiol SAMs mentioned above.

5.2. Proof-of-Concept

5.2.1. AFM study

To prepare a master, a thermally evaporated gold-on-mica substrate was immersed in a 5 μmol 18mer thiolated ssDNA (**HS-18A**) buffered solution (0.5M KH_2PO_4, pH 3.8) for at least 3 days. The buffer with strong ionic power helps the formation of a dense monolayer by shielding the negative charge of each ssDNA molecules effectively. (It has the same effect as the "salty" buffer used for the hybridization of DNA microarrays) After rinsing with DI water, the substrate was placed in 1mM merchapto-hexanol for 1 hour to minimize the unspecific binding of DNA molecules and make DNA stand up from the surface.[21] After thorough cleaning the master, it was placed in 5 μmol thiolated cDNA (**HS-**

18A') buffered solution (0.5M NaCl/TE buffer, pH 7). After rinsing with a low-concentrated buffer solution followed by rinsing with DI water briefly, (when the substrate was still wet,) another gold-on-mica substrate was place onto the hybridized master for capillary contact approach. At the dehybridization temperature, the attached substrates the master and the secondary substrate was spontaneously separated. The master and printed substrate were imaged with the Tapping Mode Atomic Force Microscopy (Tapping mode AFM) at each step. (See chapter 11 for experimental details)

In the all AFM images, a terrace feature of gold (111) surface, the typical micro-structure of gold evaporated on mica substrates, is detected. On the surface of the master before printing (a), there is a densely packed DNA SAM (**HS-18A**) with defects, shown as dark red spots in the AFM image.[21] After hybridization step, these red spots shrank due to the formation of additional monolayer composed of cDNA (**HS-18A'**). (b) After printing, it was detected that the transferred DNA monolayer has somewhat lower density compared to the one of the master on the AFM image of the printed one; whereas the master is almost preserved. However, it is challenging to judge the printing efficiency using AFM when a dense monolayer without pattern is used as master.

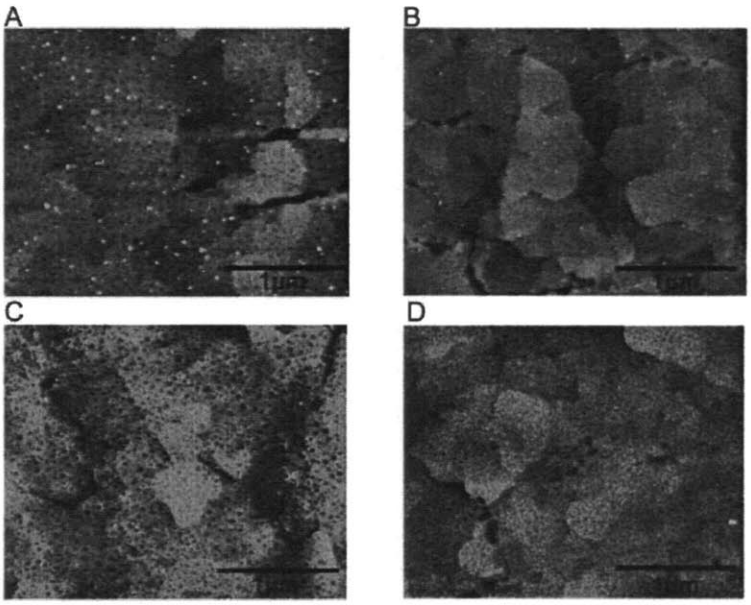

Figure 5.1. Tapping mode AFM images that show the proof-of-concept of SuNS. (A) the master composed of a thiolated ssDNA SAM of original sequence (18A) on a gold-on-mica substrate. (B) the master after hybridization with the complementary DNA (18A'). (C) the printed substrate. (D) the master after printing.

5.2.2. fluorescence studies[30]

In the AFM images (fig 5.1), it was detected that a number of DNA molecules were transferred to the secondary gold substrate and the master still had a DNA SAM on the surface after printing. However, this AFM study could not prove that the transferred DNA molecules were composed of single stranded cDNA (**18A'**) as designed in the mechanism

of SuNS (Chapter 3) or they were composed of double stranded DNA (dsDNA), mechanically stripped from the master. To ensure this point, after printing, both the master and the printed substrate were immersed into a solution of the ssDNA primary that was functionalized with fluorescence dye (**RhoG-18A**). If the printed pattern was composed of cDNA (**18A'**), more fluorescence modified DNA would bind to the surface compared to the master while if it was composed of dsDNA, there would be no difference with the master.

Figure 5.2 shows the result of this control experiment using fluorescence. A piece each cut from the master and the printed substrate was kept as unexposed to the fluorescence molecules and it was also imaged as control sample to calculate a dark count of the fluorescence images. (baby blue line and yellow line). Compared to the dark count, it is obvious that very little fluorescent DNA was found on the master. Considering that the unspecific binding of fluorescence modified DNA molecules contributes the slight amount of fluorescence, it is reasonable to conclude that the master did not nearly contain complementary DNA molecules. On the other hand, the strong fluorescence signal from the printed substrate implies that the printed SAM is mainly composed of cDNA. Indeed, after subtracting the dark count, the fluorescence intensity of the printed substrate was about 10 times that of the one of master. Hence, the working principle of SuNS could be confirmed.

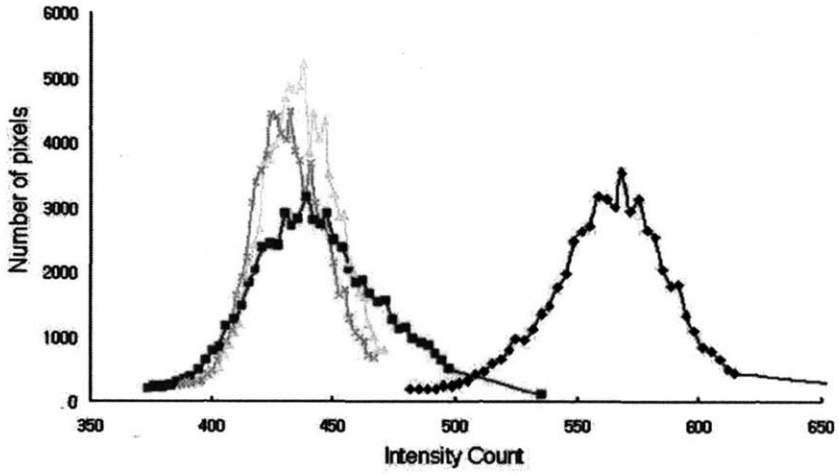

Figure 5.2. Plot of the number of pixels vs. the fluorescence intensity of four kinds of sample analyzed for the proof of concept of SuNS using fluorescence. The pink and the blue curves are plots from a master and a printed substrate treated with primary DNA labeled with Rhodamine green respectively. The pink and the baby blue curves are made of the data collected from a piece of master and a piece of the printed substrates that were not exposed to any fluorescent dye, thus these represent the background signal of the microscope used for this experiment.

5.3 The fatigue of a master

The most important prerequisite to be a printing technique is that a master should be able to be reused to make more copies: it is the virtue of printing. In order to evaluate the reprinting efficiency of SuNS, it was repeated to print using one master containing a homogenous DNA SAM onto another gold-on-mica substrate. In the early trials, the masters were fabricated on gold-on-mica substrates. However, after 3-4 times of printing, it

was found that the gold layer containing a DNA SAM on top was often peeled off from mica substrates. This is possibly due to the thermal stress during heating and cooling cycle originated from the difference in thermal expansion coefficients of gold and mica, or the mechanical stresses that applies on the gold surface during the contact and dehybridization process. Moreover, this happening is very likable since there is no strong adhesion force between gold and mica. In order to prevent the detachment of gold from its substrate, a smart technique introduced by Ulman was applied to transfer the gold layer from mica to a glass slide coated with an epoxy layer.[31] The resulting gold-on-glass substrate made using the technique, significantly improved the master's mechanical properties and consequently provided a better reprinting efficiency.

Thanks to the improved mechanical property, I could print multiple times using one master. The printed substrates were imaged with Tapping mode AFM and the obtained phase images were analyzed to estimate the density of the printed DNA SAM. The reason of analyzing not height images but phase images is that a phase image is more sensible to recognize organic pattern from inorganic hard surface since it is originated from the viscoelastic behavior of the sample. This character of phase images gives us the information about the DNA morphology excluding the roughness of the gold substrate contributing the height images. The images were analyzed using a function in AFM software that can report the particle density on a surface by considering DNA as particles. Although it does not give an absolute density value of printed DNA patterns, it can be still effective for comparing the printing efficiency between printing steps. In order to obtain

more confident data, I used several images taken at different regions in one sample and calculated the average.

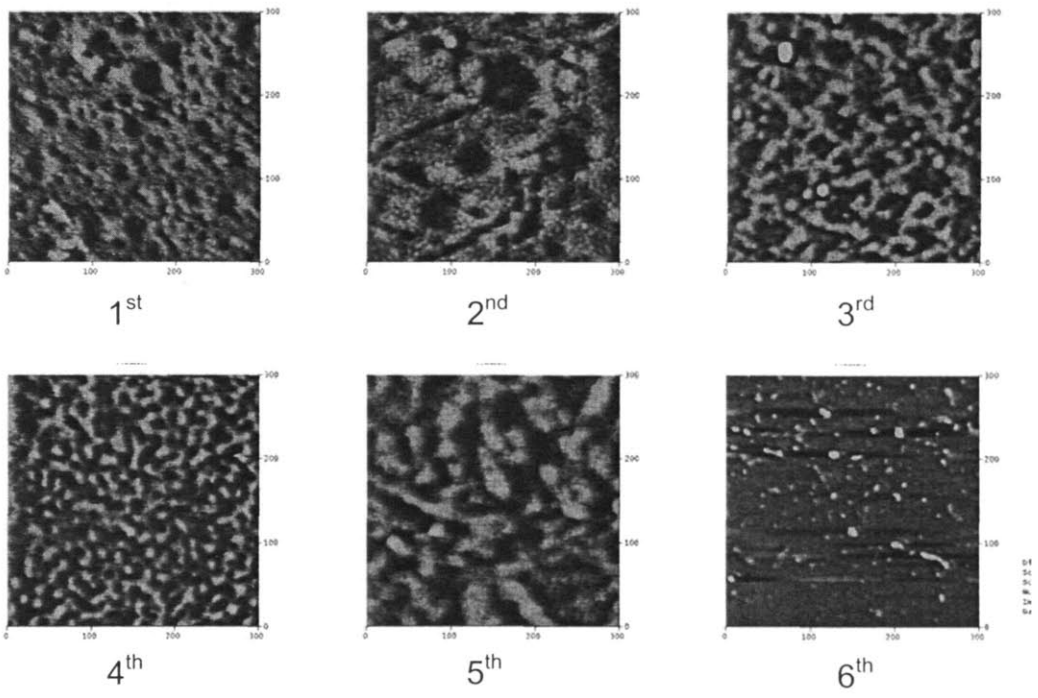

Figure 5.3. Representative Tapping mode AFM phase images of printed patterns in series from one master (scan size: 300nm)

From this analysis, it was found that there is a constantly decreasing trend in printing density after each printing cycle (Fig. 5.4). The displayed data offer the information about the relative density of each printed DNA monolayer measured against the first printed one. (The coverage of the first printed monolayer is set as 1.) These results indicate that the density of the printed SAM slowly decreases for the first five printed samples but then

suddenly drops in the sixth sample. By observing the master, it was found that this is not due to the loss of primary DNA from the master but due to the macroscopic roughness of the master's surface originated from the repeated heating/cooling cycle in printing process, which induces imperfect contact between the master and the secondary substrates. This result shows that a good mechanical property of the masters is critical in SuNS.

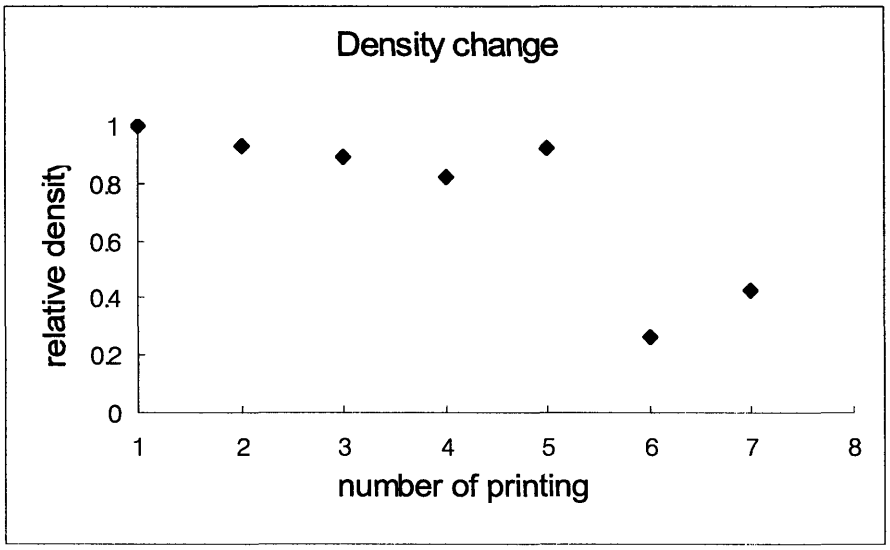

Figure 5.4. Plot of the density change of printed patterns as a function of printing cycle in series from one master

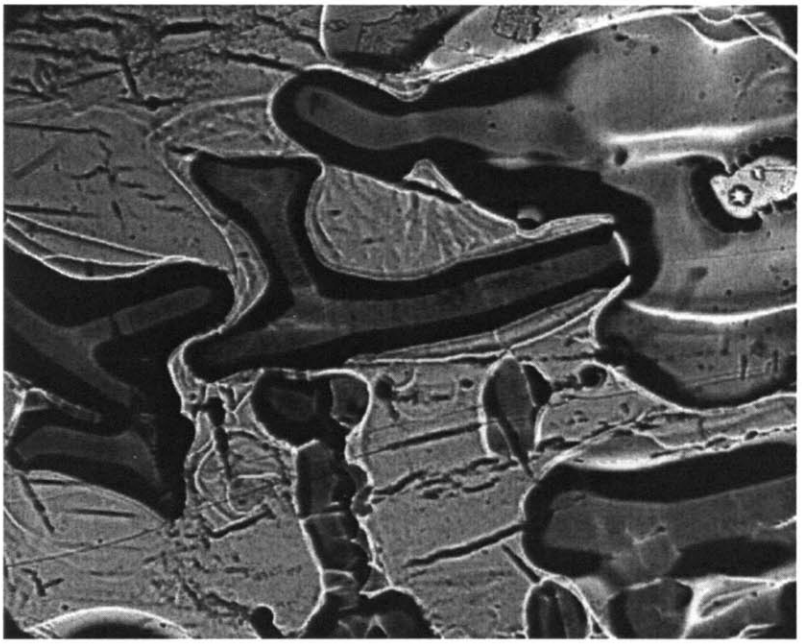

Figure. 5.5 Optical microscopy image of a master with fatigue after more than 7-8 printing cycles. It is obvious that the surface lost the flatness due to the formation of wrinkles.

5.4 2nd generation printing

One of the unique advantage of SuNS is that a copy can be potentially another master to produce more copies since a copy made using SuNS is also composed of single-stranded DNA monolayer. To prove this point, a copy from the original master was hybridized with thiolated primary DNA (**HS-18A**) and used to print on another gold-on-mica substrate. It is called the 2nd generation. By repeating the same printing process, I could make up to 4th generations. All printed patterns were analyzed with the same method described previously. The density change among different generations showed a similar trend with the one shown in figure 5.4, which keeps decreasing.

Lastly, one fact must be pointed out: the preliminary results shown in this chapter were obtained when all the printing parameters were not optimized yet. (Even now they are not perfectly optimized.) I believe that current performance of SuNS is better and there is more space to be improved.

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Chapter 6. Printing onto Gold Substrates using SuNS

6.1. Introduction

In chapter 5, it was already shown that using SuNS, a homogeneous DNA SAM was successfully printed on gold surface with the gold-thiol bond formation. In chapter 6, the experiments of printing patterns onto gold substrates after hybridization with complementary DNA (cDNA) modified with a thiol ending group are described.

6.2. High feature resolution printing and the condensed water effect [1]

One of the main advantages of printing from gold substrates to gold substrates is the high resolution. Since gold is a hard material, the features on the master are preserved during contact and transferred onto a secondary substrate without a serious loss of resolution.

A master containing a grating pattern (parallel lines) with a pitch of 450 nm was fabricated on a silicon-oxide substrate using UV lithography by Joy Cheng in Ross group. (fig 6.1) After the top of the surface was coated with gold (adhesion layer: chromium 5 nm), the master was immersed into a 5 μM thiolated DNA (sequence 18A: **ACG CAA CTT CGG GCT CTT)** buffered solution (0.5M KH_2PO_4, pH 3.8) for 5 days. After

exposing the substrate to 1mM 6-merchapto-1-hexanol for 1 hour, it was placed in a 2 μM thiolated cDNA (sequence 18A': **AAG AGC CCG AAG TTG CGT)** buffered solution (0.5M NaCl/TE buffer, pH 7). After rinsing with a low-concentrated buffer solution (0.1M NaCl/TE buffer, pH 7) and DI water briefly, when the substrate was still wet, another gold-on-mica substrate was placed onto the hybridized master. Similarly to the case of printing a homogeneous SAM, the capillary approach was used. After allowing time for the water film between the master and the secondary gold substrate to be dried, the attached substrates were separated at dehybridization temperature. (75°C) However, in the first 20-30 trials, the grating patterns were not printed; instead, DNA molecules were observed over the whole area of the printed surface in the AFM images like a homogeneous SAM printing (Fig. 6.2),

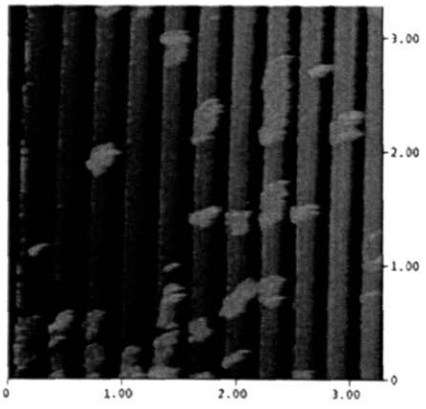

Figure 6.1. Tapping Mode AFM image of the master with grating pattern (450 nm pitch). A DNA monolayer was introduced on the top of the pattern.

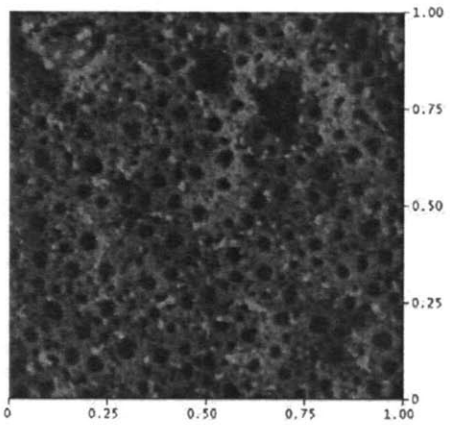

Figure 6.2. Tapping Mode AFM image of the printed pattern under the wet condition. The condensed water between the master and the secondary substrate transferred the dehybridized cDNA molecules to the secondary substrate and they formed a homogeneous SAM on the secondary substrate.

My understanding about the reason why the area that had no contact with the master had a transferred DNA monolayer is following: because DNA is an extremely hydrophilic molecule, the water applied for the capillary approach cannot be completely dried. Thus water would exist between the master and the secondary substrate during dehybridization process and it would become a medium that transfers cDNA molecules dehybridized from the master to the secondary substrate. In fact, this master has a DNA monolayer not only on the top of the pattern but also on the bottom of groove because the both of them were coated with gold. Therefore, the cDNA molecules on the bottom of groove could also be transferred to the secondary substrate by water during dehybridization and form a bond

with the secondary substrate. Moreover, I believe, the water induces for the printed cDNA molecules diffused on the surface, too.

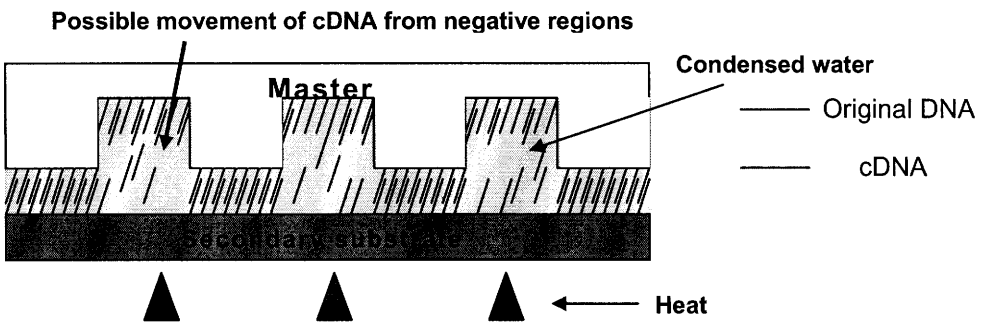

Figure 6.3. Schematic drawing of possible situation during dehybridization process under the wet condition when the master shown in figure 6.1 was used. The whole surface of the master is covered with a DNA monolayer and the dehybridized cDNA from the master would be transferred to the secondary substrate although they are not located on the region in contact when the water exists between two substrates.

To avoid this undesirable transfer under the explanation described above, an additional drying step to remove the condensed water between the master and the substrate to be printed was added between "Contact" and "Dehybridization" in the printing process of SuNS. (fig 3.1) After drying the samples in a desiccator for overnight, the attached substrates were separated by dehybidization step. Fig 6.4 shows the AFM image of printed pattern with the drying step; the printed pattern is clearly defined and perfectly reproduced from the master.

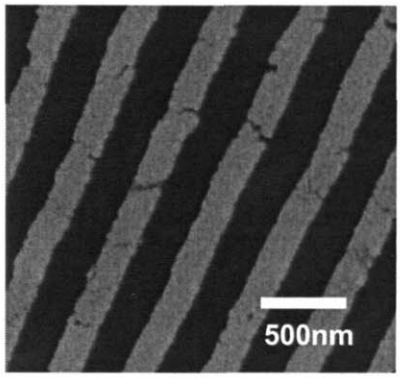

Figure 6.4. Tapping mode AFM height image of clearly printed grating lines from the master shown in figure 6.1 with drying step.

After this finding, the drying step has been added for printing a high resolution pattern. For example, the next master chosen for proving the high printing resolution of SuNS, containing a grating pattern with a thickness of 50 nm and a pitch of 100 nm, could be successfully printed with this additional drying step. This master was fabricated with Achromatic Interference Lithography (AIL) on a silicon substrate and also top of the pattern was coated with gold (Adhesion layer: titanium). After introducing two different DNA SAMs (18A, 50A) on the top of two identical pieces of the master respectively followed by hybridization, the grating pattern was printed on another gold substrate. Due to the roughness of gold substrates, only high terraces of gold-on-mica substrate have printed features. Also, in the AFM images, the printed lines are thinner than the lines on the master; especially, the printed lines using 18mer are even thinner than the printed lines using 50mer. The reason seems that the top morphology of the evaporated gold pattern on the master has

a curvature and only high part of the curved line can contact the secondary substrate. If the DNA molecules are longer, the printable area becomes larger due to the flexibility of DNA molecules. As a result, the thinnest line obtained using 18mer and 50mer was each 22nm and 38nm respectively and this is the best printing resolution when using soft biomolecules. However, for the same reason, the printed lines are more often disconnected when a shorter DNA is used.

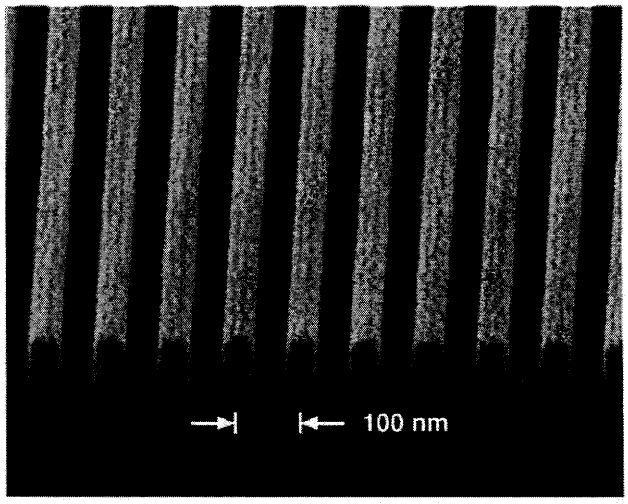

Figure 6.5. SEM image of the master used for high resolution printing of SuNS. It is composed of 50nm thick lines with 100nm pitch). After coating the top of pattern with gold, a DNA SAM was introduced on the surface by immersing it into a thiolated DNA solution to be a master for SuNS.

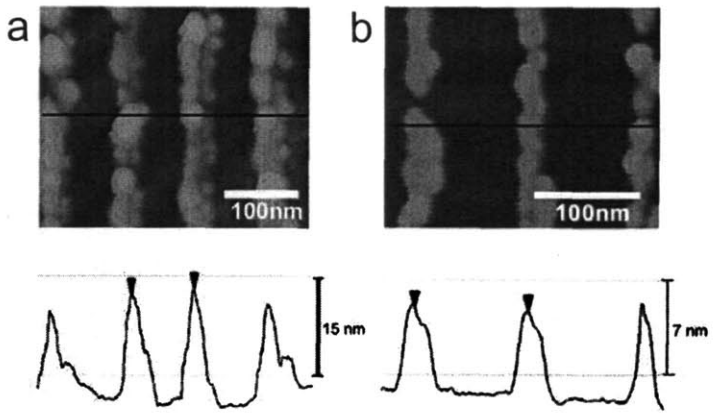

Figure 6.6. Tapping Mode AFM height images of high resolution SuNS printed patterns using the master shown in fig.6.5. The thin lines were printed with (a) 50 mer DNA; and (b) 18 mer DNA respectively with a drying step. When 18mer was used as ink molecules, the resulting lines are thinner and the profile is lower than when 50mer was used.[1]

When a short drying time (around 4 hour) was allowed, it was detected that the printed substrate contained a somewhat unclearly defined pattern but higher printing coverage was obtained. Fig 6.7 (a) was the phase image of Tapping Mode AFM of the printed result with shorter drying time. Although the phase image shows clear lines because phase images are more sensitive to detect DNA molecules, it is obvious that the resulting printed lines with less drying time are significantly diffused out from the desired location in the corresponding height image (fig 6.7 (b))

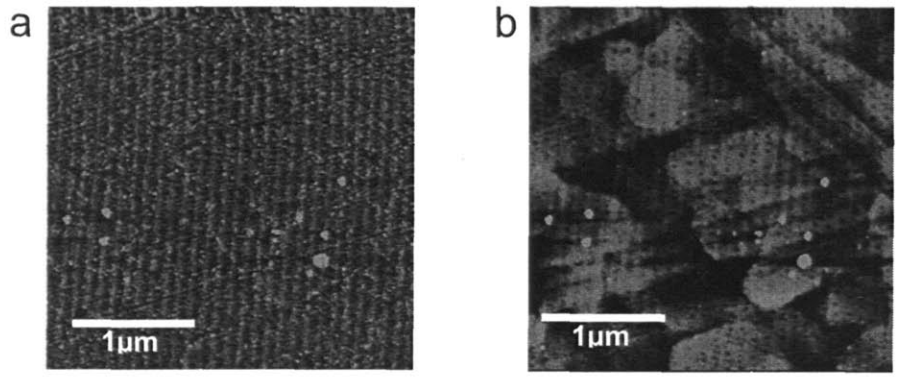

Figure. 6.7. Tapping mode AFM phase image (a) and height image (b) of printed grating pattern from the master shown in figure 6.1 with shorter drying time. It was shown that the printing coverage was improved while the printing resolution was sacrificed due to the remaining water.

In summary, the condensed water should be removed for reproducing efficiently high resolution patterns on a gold surface using SuNS. Unfortunately, in the dry condition, only relatively limited area that is in good contact with the master is printed since the gold substrate is rigid. On the contrary, in humid condition, it can be expected that water, existing between the master and the secondary substrate, can help to overcome the roughness of the substrates because it transfers DNA molecules located on the bad contact region to the secondary substrate. Therefore, if higher resolution is demanded, SuNS should be carried out in a low humidity condition; if higher coverage is necessary while resolution is not a big issue, it is better to keep the water without drying step or with a short period of drying step.

6.3. Soft lithography pattern printing [1]

The masters of SuNS can be various patterns, fabricated not only with hard lithography like e-beam lithography or UV lithography but also with soft lithography. Among the soft nanolithography techniques, Dip-pen nanolithography (DPN),[2] developed by Chad Mirkin is one of the most promising techniques to fabricate organic pattern with high resolution. To prove that SuNS can reproduce patterns fabricated with soft lithography, I used a master containing a box pattern (40μm X 40μm) composed of octadicane-thiol (ODT) fabricated with DPN. The master does not have any physical profile defined as height difference but has only a chemically defined pattern. By immersing this master into a thiolated ssDNA DNA solution (sequence: 18A), the whole area was expected to be covered with DNA molecules except the square pattern made of ODT. After hybridization with cDNA (sequence: 18A') modified with thiol, another gold substrate was placed onto the master and printed. After separation, the printed pattern was exposed to a solution of primary DNA functionalized with fluorescence (Rhodamine green – 18A) and imaged by the fluorescence microscopy. (fig. 6.8)

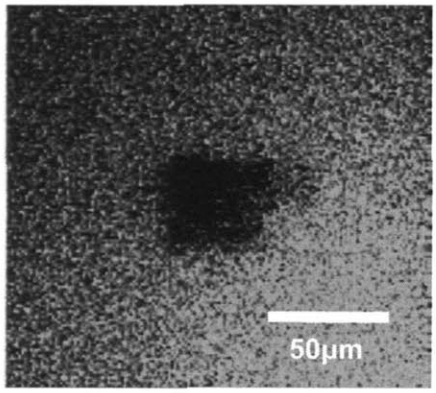

Figure 6.8. Fluorescence image of a printed box (negative pattern) using SuNS. The original box pattern on the master was made of ODT using DPN and a DNA monolayer was introduced except the box.

Figure 6.8 shows a homogeneous fluorescence signal from overall area except a square, shown as dark color, corresponding to the ODT pattern on the master. This result has a significant meaning in that SuNS is the first printing technique that can reproduce a chemically defined pattern fabricated with soft lithography on the best of my knowledge.

6.4. Multi-DNA printing

The most important and unique advantage of SuNS is the capability of printing multi kinds of DNA in a single cycle. SuNS is a true printing technique that transfers high-information. [3]

Using gold-thiol chemistry, a part of a DNA microarray could be printed on gold substrate. The DNA microarray containing DNA dots composed of two different sequences (50A, and 50B) used as master was hybridized in a mixture solution of thiolated

cDNA (50A' and 50B'). Through the SuNS's printing process, this multi-DNA pattern was printed on a gold substrate. The result is shown in fig 6.9 where the printed two dots are shown as two different colors representing a different DNA sequence by hybridization with primary DNA mixture each of which was modified with different fluorescence dye. At this time, the printed pattern was made not only of the spatial information (i.e. size, shape and location) but also of the chemical information (i.e. DNA sequences). That is the reason why SuNS is a high information density printing that can transfer the chemical information.[3] In fact, this is the first result of printing multi kinds of DNA simultaneously by selective inking. Also, it proved that SuNS has a strong potential to be able to reproduce a whole DNA microarray, composed of a number of different DNA pieces.

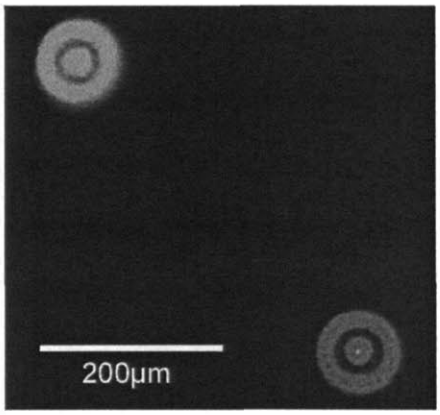

Figure 6.9. Fluorescence image of printed pattern containing high information (i.e. various DNA sequences). This image was made by overlaying two fluorescence images obtained using a red filter and a green filter each. The two different DNA dots blinking with two

different colors were printed using SuNS simultaneously, which implies SuNS can replicate patterns composed of various DNA in a single printing cycle.

6.6. References.

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3. Yu, A. A. & Stellacci, F. Stamping with high information density. *Journal of Materials Chemistry* **16**, 2868-2870 (2006).

Chapter 7. Printing on PMMA substrates using SuNS[1]

7.1 Introduction

In chapter 6, printing on gold substrates using SuNS was described. By using gold substrates, SuNS can duplicate a pattern with the ultimate resolution, considering the fact that the molecules to be printed are big biomolecules: DNA. However, there were several drawbacks on gold substrates to be the most reliable substrate for SuNS.[1] First, gold is not optically transparent: it is a critical problem especially for bio-array systems, which are normally analyzed with the fluorescence microscopy. Second, gold substrates and most of gold-coated substrates are not flexible.[a] This property allowed for the high resolution for SuNS but also the hardness made it difficult to obtain a good contact between the master and a secondary substrate to be printed on a large area. In usual case, the surface of either masters or secondary substrates cannot be perfectly flat due to the intrinsic (microscopic and/or macroscopic) roughness of substrates or dusts on the surface. Moreover, SuNS' procedure is composed of many steps of soaking substrates in aqueous solutions: this is the one, avoided by the people, involved in the semiconductor fabrication because wetting the substrates with water induces significant contamination by dusts due to the high surface

[a] Note that the total flexibility of a gold-coated substrate depends on the solid support on which the gold film is deposited. However, when the experiments described in Chap.5 and Chap.6, it was presumed that there would not be possible to obtain a flat gold surface on soft substrates; this became the main reason of moving toward other substrates. Now it turns out that it is possible to obtain a flexible substrate with a gold thin film for large printing area. [Jung, Yu, Akbulut and Stellacci, unpublished result]

energy of water. In order to overcome the roughness, the flexibility of the master or secondary substrates is required. Third, the process for obtaining very flat/fresh gold substrates requires relatively complex facilities. Finally, gold coated substrates tend to be expensive.

For those reasons, I tried to find alternative substrates. In fact, the first alternative substrate material tried was glass – microscope cover slips. In order to print on glass substrates using SuNS, the surface needed to be silanized with epoxy terminal group (however, there are many other possible terminal groups),[2] and amine functionalized cDNA or biotin functionalized cDNA with streptavidin treatment was used. However, the printing often failed since the cover glasses were not so flat macroscopically. As the evidence, when I placed a functionalized glass substrate on a hybridized master, inhomogeneous diffraction patterns on the overall area of contact region were detected, which means the gap between the master and the secondary substrate is not even along the area. As a result, a good contact could not be easily obtained. Only large sized features were sometimes printed. Moreover, handling a cover slip was very difficult because it is brittle and it's hard to find them in an aqueous solution.

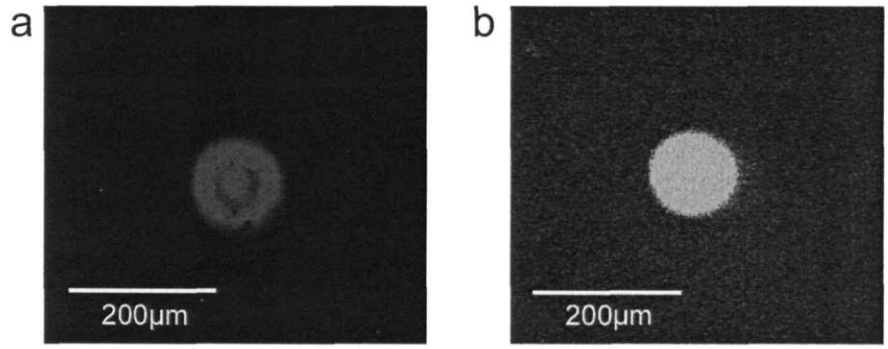

Figure 7.1. Fluorescence images of printed DNA dots on epoxy terminated glass substrates (a) using amine modified cDNA; (b) using biotinylated cDNA followed by streptavidin treatment.

An alternative substrate material was polymethylmethacrylate (PMMA), a popular acrylic polymer that is often used as the replacement of glass. To be the secondary substrates of SuNS, the surface of PMMA must be functionalized. (See 7.2) [1]

PMMA has several advantages as an alternative substrate for SuNS.[1] First, it is optically transparent. Second, it is inexpensive: the price of PMMA substrates is 1/70000 of the one of gold-on-mica substrate of the same size. Even after considering the cost for additional treatment of PMMA, it is still a much more economical option compared to printing on gold. And most importantly, PMMA has a medium glass transition temperature (T_g) (around 100°C). Thus it is mechanically robust at room temperature but it becomes soft at slightly higher temperature to obtain a conformal contact with the master, inducing a large printing coverage.

In this chapter, I will describe how PMMA is introduced in SuNS and what are the main advantages of using PMMA.

7.2 Printing Procedure

PMMA sheets commercially available were obtained from J. Freeman. Inc. (MA, USA), a distributor for Plexiglass® (Rohm, Germany) and cut into 5 mm X 5 mm squares with a die-saw. Since methacrylate groups, dominantly covering the surface of PMMA, are very stable, they should be activated and functionalized with a more reactive group. A slightly modified functionalization method developed by Fixe and his colleagues[3] was applied for preparing secondary substrates of SuNS. The basic scheme is shown in fig 7.2. After washing in isopropanol (99%) for 30 min and in Millipore water for 30 min, the cut substrates were immersed in 10% (w/v) hexamethyldiamine in 100mM borate buffer (pH 11.5) for 8 hours. They were cleaned with Millipore water twice (15 min), and immersed in 2.5% v/v glutaraldehyde in 0.1M phosphate buffer (pH 7) for 8 hours. The modified substrates were cleaned in Millipore water twice (10 min), and dried in a desiccator at room temperature. Then, amine-terminated cDNA molecules can be printed on this aldehyde terminated PMMA substrates using SuNS.

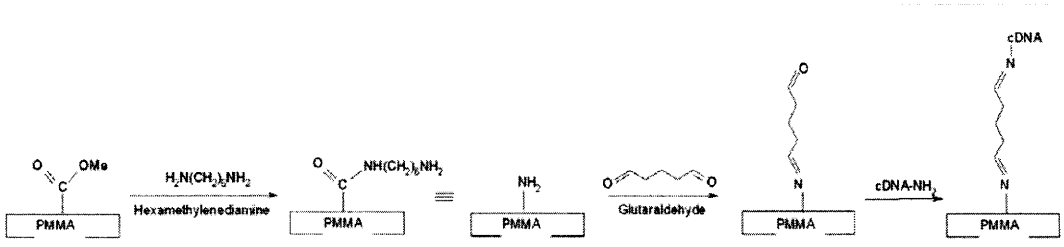

Figure 7.2 Scheme of PMMA functionalization for SuNS. [1]

The basic printing process is similar with the one of printing on gold substrates with a few changes. First, capillary force approach is not used but mechanical approach is used since the PMMA substrate used is too thick (6.5mm) to expect an effective capillary force. In most cases, enough pressure could be applied just by placing a standard microscope glass slide on the top of the two substrates in contact. Second, a heating step was added between "contact" and "drying" before dehybridization. In order to induce the optimal contact, the coupled substrates were placed in an oven for 20 min at 75°C. At this temperature, the slightly softened PMMA substrate is able to conform to the surface profile of the master, originated either from master's intrinsic physical morphology or from the impurities such as dusts. This is the main reason why I used PMMA substrates for better printing coverage. Here, it should be noted that the temperature is high enough to make the PMMA substrates soft to overcome the small roughness (the size of dusts), but I believe, in macroscale, the PMMA is still rigid and it does not destroy the original pattern composed of DNA molecules on the master. As evidence, I could reuse a same master to print multiple copies with this process.

7.3. Proof of concept

In order to test whether the PMMA substrates functionalized with the scheme shown in fig. 7.2 are effective for SuNS or not, a homogeneous monolayer made of 50mer DNA (**HS-50A)** was prepared on a gold substrate; and after hybridization with amine-terminated cDNA (**H_2N -50A'**), the monolayer was printed on an aldehyde functionalized

PMMA substrate. After deactivating unused aldehyde groups in $NaBH_4$ solution or ethanol-amine solution, the printed PMMA substrate and unprinted PMMA substrate were immersed into a solution of DNA of primary sequence labeled fluorescence dye (**RhoR-50A**). After cleaning, the two substrates were checked with a fluorescence microscope. (Since the background intensity of gold and the one of PMMA are different, I didn't compare the printed one with the master in this homogenous printing) The result is shown in figure 7.3.

The detected fluorescence intensity of the printed PMMA substrate (blue line) is from effective hybridization of printed cDNA (**H_2N -50A'**) with original DNA with fluorescence tag (**RhoR-50A**), unspecific binding of **RhoR-50A** and the dark-count of the fluorescence microscope used; whereas the detected fluorescence intensity of the unprinted PMMA substrate is only from unspecific binding of **RhoR-50A** and the dark-count. Here, the dark-count is same for the both samples since the substrate material and the exposure condition are identical. Additionally, it is supposed that, after deactivation treatment to get rid of effective aldehyde groups on surface, the amount of unspecific absorption of fluorescence modified DNA molecules (**RhoR-50A**) on the printed PMMA substrate and that of the unprinted one are also same. Then, the difference of fluorescence intensity between the two substrates is from the effective hybridization, implying the amount of transferred cDNA (**H_2N -50A'**) onto the printed PMMA substrate. Indeed, the printed PMMA has a much stronger fluorescence intensity compared to the control sample. It implies that the functionalized PMMA is an effective substrate system for SuNS.

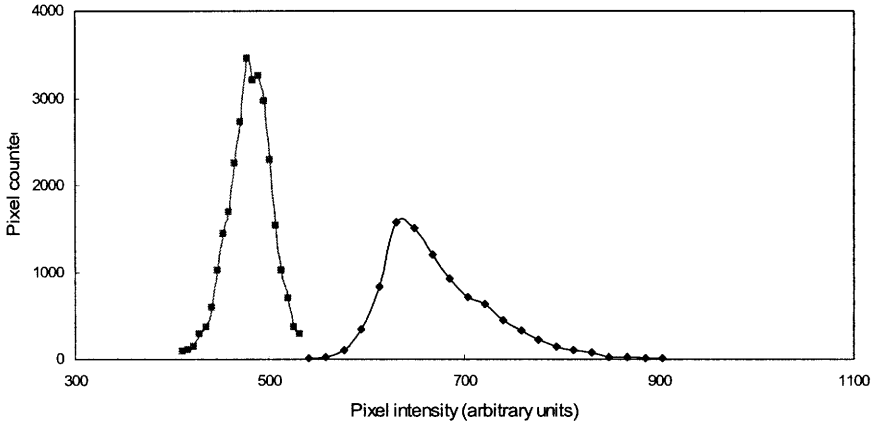

Figure 7.3 Plot of the number of pixels vs. the fluorescence intensity of a printed PMMA substrate (blue line) and non-printed one (pink line) after hybridization with primary DNA with fluorescence dye. The pink line represents the sum of the background signal of the microscope used for this experiment and unspecific binding of fluorescence modified DNA molecules on surface.

7.4. Printing Coverage [1]

In order to test the coverage of SuNS provided by PMMA substrates, a silicon substrate containing various patterns dispersed in a large area (2 mm X 2 mm), fabricated by electron beam lithography (EB) was tried as a master. To place a ssDNA monolayer on the patterns, the master containing gold-coated patterns was immersed in a 5μM 5'-thiolated DNA (**HS-50A**) solution for 5 days. After hybridizing the original ssDNA monolayer on the patterns with amine terminated cDNA (2μM, **H_2N -50A'**), a

functionalized PMMA substrate was placed on the master. In order to obtain the optimal contact, these coupled substrates were placed in the oven for 20min at 75°C under a light pressure (2 X 10^3 Pa) provided by a piece of glass slide. At this temperature, the slightly softened PMMA substrate is able to overcome the roughness of the surface, originated from the master itself or dusts. I believe that this treatment accelerates the rate of bond formation reaction between cDNA molecules and the PMMA substrate and it will be discussed more later. After the heating, the sample is placed in a desiccator for overnight to make the reaction irreversible by removing water, another product of the reaction. For dehybridization, the coupled substrates were placed in the oven at the temperature of 90°C followed by placing a small amount of dehybrization buffer (0.1M NaCl/TE buffer) on the substrates. After cleaning the separated substrates, the printed patterns were imaged with the Tapping mode AFM. Figure 7.4 shows that very different shapes of patterns were successfully printed through SuNS with high fidelity. Each image of Figure 7.4 (a), (b) and (c) was randomly obtained in a different area, which implies the higher coverage of PMMA substrates. Moreover, it should be noted that the printing feature resolution (<50nm) was not compromised much by using PMMA (fig 7.4 (c).) [1]

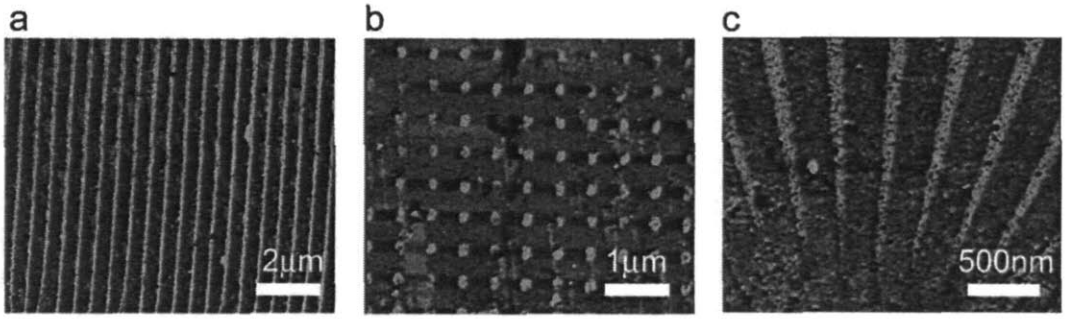

Figure. 7.4. Tapping mode AFM images of printed patterns on PMMA substrates. These images show very improved printing coverage of SuNS by introducing PMMA as secondary substrate system. [1]

7.5. Heating step and the bond formation rate.

In SuNS applied on PMMA, the heating process was originally designed for contact optimization between a master and a secondary substrate (PMMA). But it's probable that this thermal energy accelerates also the bond formation reaction between the functional group (amine) on cDNA and the aldehyde group on PMMA surface.[1] In order to evaluate this idea, a control experiment, printing patterns without heating using the same master, was performed.

The same master containing various gold-coated EB patterns used in sec.7.3 was used as a master. Since the silicon substrate is very flat, good contact could be obtained by simply applying higher pressure (~10^5 Pa) without heating. After 10 hours, the substrates were dehybridized and the printed PMMA substrate was imaged with the Tapping mode AFM. (Fig. 5)

Printed patterns were found all over the surface of a printed PMMA substrate but the density of printed DNA monolayer (the percentage of the pattern part that gave higher profile than the background – unprinted part) was not high. This result implies that the transfer of pattern was not done with high efficiency even though there should have been a good physical contact. It can be concluded that heating induces not only the conformal physical contact but also an effective "chemical contact" (i.e. chemical bond formation)

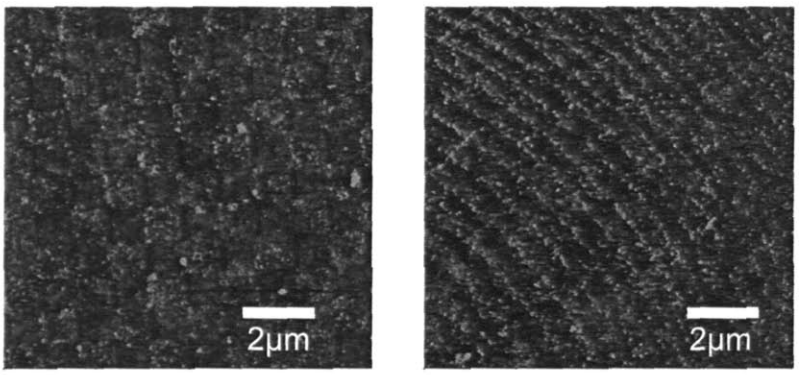

Figure 7.5. Tapping mode AFM images (height) of printed patterns without heating process. A good physical contact was obtained by a higher applied pressure on a large area but the good density of printed DNA monolayer could not be obtained.

7.6. 2nd generation printing[1]

As described in chapter 3, one of significant advantages of SuNS is the exponential growth of masters, which means that a printed pattern (1st generation) can be used as a new

master to make another copy (2^{nd} generation). As an example of the experiments that show this capability of SuNS, I used a grating pattern with 100 nm pitch, fabricated with Achromatic Interference Lithography (AIL), as a master (Fig 7.6 (a)). Similarly with the case of e-beam sample, the master contained a DNA monolayer on top (**HS-50A**). After hybridization with amine terminated cDNA (**H_2N-50A'**), the grating pattern was printed on aldehyde functionalized PMMA substrate (Fig 7.6 (b)). This printed pattern was hybridized with 5'-thiolated DNA (**HS-50A**) followed by stamping 2^{nd} generation on a gold-on-glass substrate.[4] (see Chap. 11 for experimental details) After stamping once, the new master, copied from AIL was hybridized with 5'-thiolated DNA (**HS-50A**) again and reused to stamp another 2^{nd} generation of copies. These 2^{nd} generation of copies from AIL pattern were also imaged by TMAFM. (Fig 7.6 (c) and (d)) The sharpness of the pattern was somewhat lost due to the stamping cycle including heating, possibly deforming the pattern on PMMA substrates. However, the AFM images clearly show that the thin grating lines could be printed with the exactly same pitch (100nm) from the first printed one. To the best of our knowledge, this is the first result to obtain 2^{nd} generation of copy from 1^{st} generation of copy using exactly identical principle and with identical character. It implies that the SuNS makes a copy, functionally identical complementary, with the master, as like a duplication of DNA for transcription occurring in our bodies.

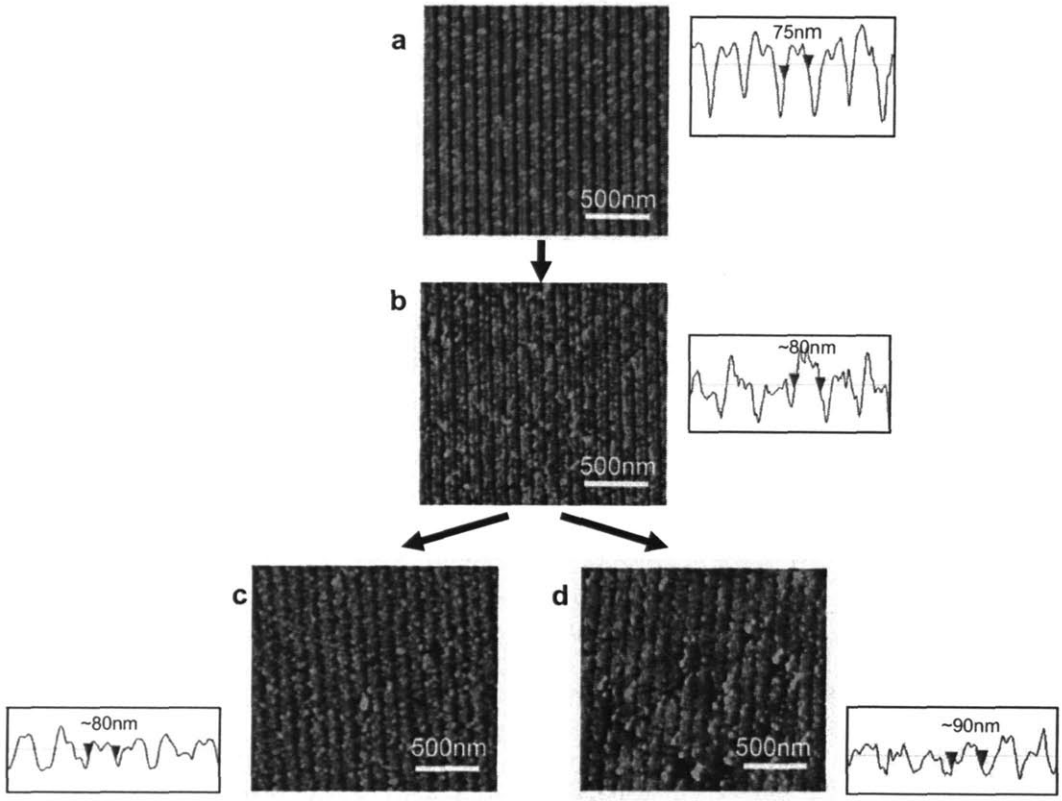

Figure 7.6. Tapping mode AFM images of (a) a master; (b) first generation printed from (a) onto a PMMA substrate; (c) and (d) second generation printed from (b) onto gold substrates.[1]

7.7 Combination with imprinting[1]

A major advantage of PMMA is that, at reasonably low temperature, it becomes soft. This character allows for SuNS to be combined with imprinting technique. A substrate made of a 1 μm thick Si_3N_4 membrane containing hexagonal arranged holes (diameter: 50um, spacing: 100 μm), placed on a gold surface. A ssDNA monolayer was allowed on

the exposed gold bottom of these holes. Efficient printing was obtained by placing the PMMA substrate on the master using the usual pressure of 2 X 10^3 Pa and then heating at 75°C for 60 min. The combination of pressure and thermal energy applied for longer time allows the PMMA substrate to forms posts that reach the DNA on the gold surface. The printed sample was placed in a solution containing DNA molecule of the original sequence 5'-modified with Rhodamine Green and imaged with the confocal microscopy. The bright dots shown in fig. 7.7 represent the transferred DNA monolayer.

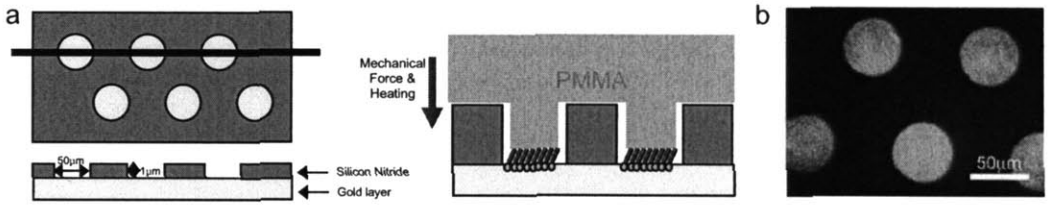

Figure 7.7 Schematic drawing of (a) the master used for the combination of SuNS and impriting; and imprinted PMMA substrate (b) the resulting fluorescence pattern printed on a PMMA substrate. [1]

7.8. References

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Chapter 8. Printing with a large area coverage for DNA microarrays: printing from/onto PDMS

8.1. Introduction

In chapter 6 and 7, it was shown that SuNS can reproduce high resolution features using gold and PMMA substrates. This can be a promising result for fabricating nano-devices composed of soft molecules. However, as described in chapter 3, because of the unique advantages of SuNS, the quickest application easily predicted would be printing DNA microarrays, [1-3] which are one of the most complex devices and currently fabricated with costly methods. Unfortunately, there were a few problems to be solved for directly applying SuNS to copy DNA microarrays. The most critical issue is that it is difficult to obtain enough large printing coverage as much as the size of a DNA microarray (~ 1 $inch^2$) by using "hard" substrate such as gold or PMMA. After a number of failures, I concluded that at least one substrate between either a master or a secondary substrate must be soft.

Several factors should be considered for choosing a substrate for a large printing area: i) methods of functionalization for the attachment of DNA molecules, ii) flexibility and iii) stability at dehybridization temperature. Polydimethylsiloxane (PDMS) is a popular silicon elastomer in various applications such as microfluidics[4] and micro-contact

printing.[5,6] While it is very flexible, it keeps its shape at high temperature due to crosslinking bonds. It is obvious that printing resolution will be sacrificed by using PDMS due to the deformation of PDMS during contact. However, it should be pointed out that such a high resolution is not required to print DNA "micro"-arrays since the smallest dots that can be fabricated with current techniques is only ~1μm. (because of the resolution limit of DNA microarray scanner) In general, in SuNS, the printing resolution and the coverage have been always in the relationship of compromise like the case of printing on gold vs. printing on PMMA or the case of printing with high humidity vs. printing with low humidity.

8.2. PDMS printing

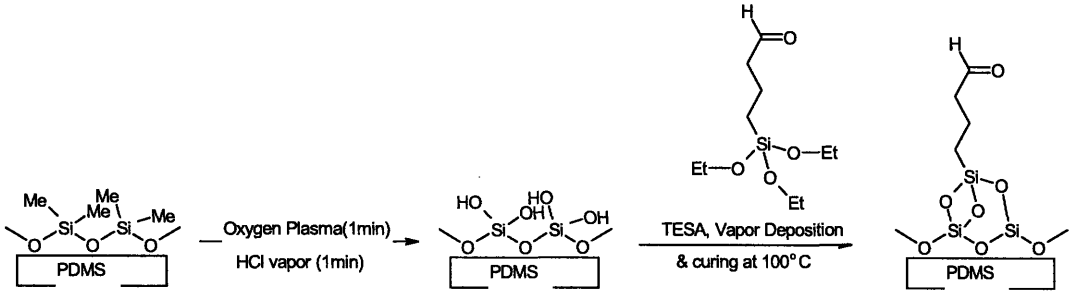

Figure 8.1. Schematic of a possible PDMS surface functionalization. [7]

Aldehyde functionalized PDMS films[7] with trenches were prepared following the protocol in Ref [7] with slight modifications. (Fig. 8.1) Silicon elastomer base and curing agent (Sylgard 184, Dow Corning) were thoroughly mixed with a weight ratio of 10:1.

This mixture was poured into a Petri dish containing a silicon substrate with grid pattern in micron scale (20 μm pitch, 10 μm thickness). It was allowed to settle in the Petri dish for 1 hour to remove trapped air bubbles followed by curing at 60 °C for 2 hour. After being released from the mold, the cured PDMS substrates with trenches was cut into 2 cm X 3 cm size. For oxydization, they were placed in the chamber of oxygen cleaning system oxygen plasma was applied for 1min under vacuum of 20 mmHg. The oxidized substrates were immediately exposed onto HCl vapor for 1min. They were placed in the oven where the substrates were exposed to the vapor of triethoxysilylbutyraldehyde (TESA) at the temperature of 100°C for 30 min under vacuum (~ 30 mmHg).

A 25μM aqueous solution of amine terminated DNA (**H_2N-50A'**) was dispensed on a functionalized PDMS substrate. After allowing incubation time (2 days) followed by aldehyde deactivation treatment, the master was hybridized with thiolated cDNA (**HS–50A**) and then printed on a gold-on-glass substrate. After hybridization with the original sequenced DNA modified with Rhodamine Green dye (**RhoG –50A'**), the printed substrate was imaged with a confocal fluorescence microscope. (Fig 8.2) Compared to the fluorescence image of the background, printed dots have much higher fluorescence intensity.

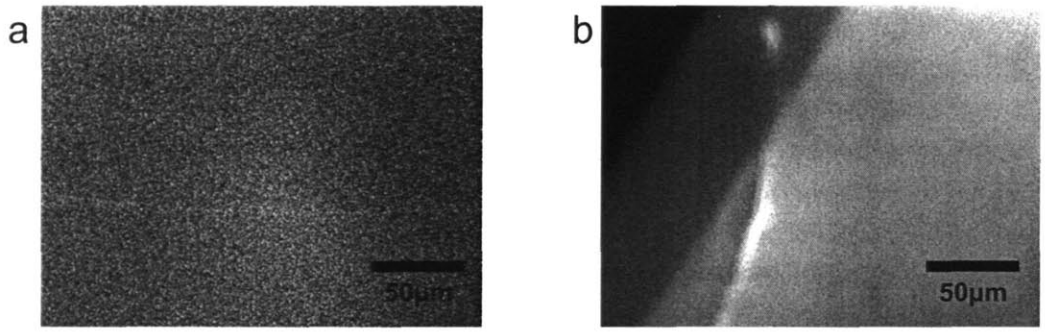

Figure. 8.2. Confocal fluorescence image of a printed DNA arrays on gold substrate. (a) unprinted region (background) (b) the edge of a printed dot, in which a pattern of the poses located on the master could be barely detected.

To print on optically transparent substrates, the PDMS master made of amine terminate DNA (**H_2N-50A**) was hybridized with amine terminated cDNA (**H_2N-50A'**), and then it was printed onto an aldehyde coated glass slide. After deactivating unused aldehyde groups by reduction in a $NaBH_4$ solution or treatment with ethanolamine, the printed slide was immersed in a solution of original sequenced DNA strands with Rhodamine Red (**RhoR- 50A**) and imaged with a fluorescence microscope. (Fig 8.3.)

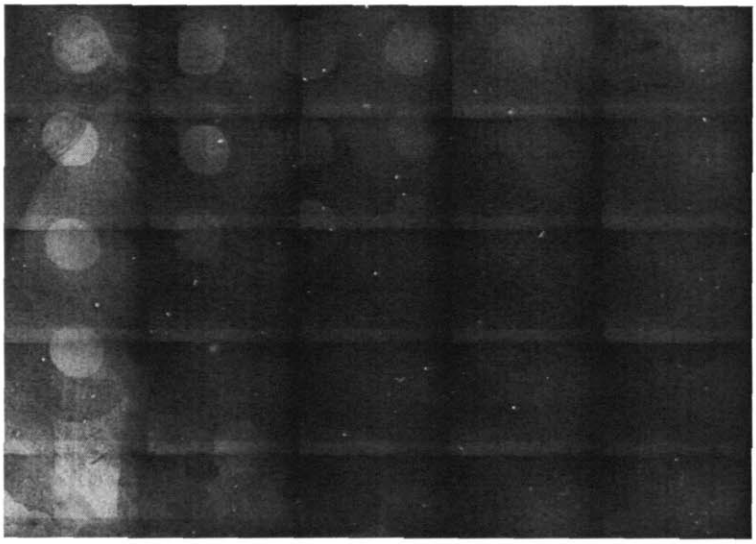

Figure 8.3. The mosaic image composed of fluorescence images of a printed DNA 2D array on an aldehyde coated glass slide using a master fabricated on PDMS. The printed dots on the top left triangle region have higher fluorescence intensity than the background while the ones on the down right triangle region have lower one.

Although it was the first successful result showing a true macroscale printing area, larger than $1cm^2$, the result is somewhat defective. First of all, in fig.8.3, some of printed dots show lower fluorescence than the background; sometimes, all printed dots had lower fluorescence intensity than the background. The reasons for this may be found from 1) the printing efficiency is not high enough; 2) the unspecific binding of fluorescence molecules on the unprinted part is more severe than on the printed part, probably because the deactivation process is not completely done. Additionally, very often, it was found that the

PDMS stamp was stuck on the functionalized glass slide permanently and did not separated during the dehybridization step.

8.3. Possible improvements.

From a series of the experiments, I concluded it is not a good option to use PDMS as the material for SuNS' master. (It implies only the methods of direct- depositing DNA molecules on a functionalized PDMS substrate to make a primary master.) I believe that the most critical problem on the approach using PDMS substrates described in this chapter was the master preparation. As explained in chapter 3, SuNS works under the assumption in which the functionalized cDNA molecules to be printed are pre-oriented. Because the morphology of hybridized cDNA molecules resembles that of the original DNA monolayer forming a pattern on the master, the morphology of original pattern must be well-organized to allow for SuNS to work as designed. Unfortunately, many chemistries used for DNA immobilization do not give consistent efficiency; they are highly dependent on many other parameters such as humidity, temperature, or drying time.[3,8] Specifically, the silanization, the most common immobilization method for DNA on PDMS, often forms a complex network on a surface, not a uniform monolayer of immobilized molecules.

From my experience, using gold-thiol chemistry is a good strategy to obtain a good master for SuNS because it produces well-defined ssDNA monolayers with a pre-orientated morphology and enough conformational freedom, favorable to step 1 (Hybridization) and

step 2 (Contact) in SuNS' working principle. (It should be noted that most of the successful results of SuNS have been obtained from masters fabricated on gold substrates using thiol-terminated DNA.) This is due to the formation mechanism of self assembled monolayer (SAM) of thiol, which is in the dynamic equilibrium allowing the rearrangement of thiol molecules. This is a slow procedure, but as a result, molecules are well aligned in SAM. On the contrary, most spotting techniques for DNA microarrays fabrication use quicker chemistries (e.g. epoxy-amine bond).[9] I believe, these kinetically controlled reactions leave DNA molecules immobilized with the entangled structure. Another advantage using gold substrate is that the interface energy between gold and many polymer or organic molecules coated substrates is small. Thus, detaching process is easier compared to many other functionalized substrates such as aldehyde coated glass slides.

For this reason, using gold- thiol chemistry will be good for fabricating DNA microarrays that are made of a better-defined DNA SAM to be a master for SuNS although it is not very common in current techniques used for DNA immobilization.

From the discussion written above, it can be expected that SuNS using PDMS substrates could be improved in many ways. For example, printing efficiency would be increased if a master is fabricated on a gold substrate and it is printed onto a functionalized PDMS as a secondary substrate because the quality of master is higher and the separation would be easier. Or it would be also a possible way to use thin film of gold coated PDMS substrates as master in order to obtain both the good quality of DNA monolayers on the master (from gold) and the flexibility (from PDMS).

I'm going to leave these issues as open questions and I think additional experiments should be carried out. Moreover, large scale printing was achieved by another approach, which will be described in chapter 9. Also the information that I learned from the series of printing large area for DNA microarrays using PDMS was reflected to design the new approach, Liquid Supramolecular NanoStamping (LiSuNS)

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Chapter 9. Liquid Supramolecular Nanostamping (LiSuNS)

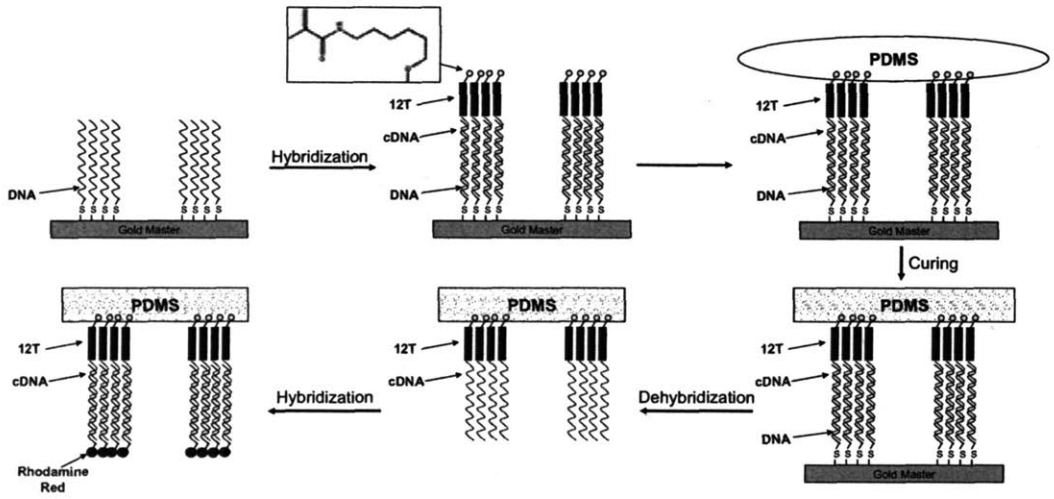
9.1 Introduction

The master of the conventional printing, used for a long time (since the ancient age), has been defined with physical shape on the surface and its duplicate patterns have been made from the contact between the master and another surface to be printed. At this time, not only the real pattern but also undesirable shape originated from the roughness of the substrates, the deformation of the substrates or the impurities on the surface are copied and it is the main reason of defect of printed result. This defect is negligible if the size of pattern to be printed is relatively big as far as moderately clean and flat substrates are given. However, as the scale of devices to be printed has become smaller (below milli-meter), this has became a much more critical issue in printing technology. Moreover, in such a small scale fabrication, it is harder to control such sources to contribute unexpected morphology since even one dust particle, is enough to disturb the best contact condition. For this reason, a major part of fabrication of semiconductor device despite the use of non-contact printing – optical lithography,[1] is carried out in a clean room to minimize the dusts. In contact printing techniques such as microcontact printing, the dependence of printing quality on the surface morphology is even higher.[2]

In spite of the difficulties, it is essential for the printing technology to reproduce the whole features on the master without significant defects no matter how difficult is to

control the sources affecting the printing quality in such a small scale. Thus, in micro/nano-printing process, special care has been required for obtaining clean and flat substrates (e.g using clean rooms) and this additional care contributes to the increase the printing cost. For example, the price of extremely flat and clean glass slide substrates for printing DNA microarrays are around 200 times that of normal glass slide substrates.[3]

Liquid Supramolecular NanoStamping (LiSuNS), a new derivative of SuNS,[4,5] is a novel printing technique that can handle any roughness of substrates. The key concept of LiSuNS is to use a liquid prepolymer solution as a secondary substrate to be printed. The basic working principle of LiSuNS is similar with the one of SuNS. (Fig 9.1) First, a substrate containing a patterned DNA monolayer, composed of one or more kinds of DNA is used as a master. Second, the master is immersed in a solution of complementary DNA (cDNA) that has a bulky protection group on its end. Third, a prepolymer solution is poured on the hybridized master followed by curing. Finally, the cured polymer substrate is detached from the master at dehybridization temperature. There will be many options by choosing different combinations of prepolymer, protection group, linker group and fluorescence. Figure1 shows one example of the scheme possible in LiSuNS.



Fiqure 9.1. Schematic drawing of a possible printing cycle of LiSuNS.

The main advantage of LiSuNS will be the extremely large printing coverage regardless of the surface roughness because LiSuNS uses a liquid with high mobility as the substrates to be printed on, inducing the best conformal contact with the master. Additionally, the immobilization reaction of printed DNA molecules on the secondary substrates is done with the polymerization reaction of secondary substrates: the functional group on cDNA take part in the polymerization, forming the cross-linking bond between cDNA and the cured polymer. Therefore it is not necessary either the surface functionalization of secondary substrates or the post-treatment to deactivate unused functional groups after printing. As a result, the whole process of LiSuNS is very simple and fast by eliminating the functionalization and deactivation process, for example, which take 24 hours in a case of printing on PMMA (see chapter 7)

9.2 Printing Coverage

In order to test the concept of LiSuNS and the printing coverage, several DNA arrays were fabricated by dropping a thiolated DNA solution **(HS-50A)** on gold-on-glass substrates (~ 2.5 cm X 2 cm each) using a micropipet. (one drop size is approximately 2-3mm in diameter) These array patterns were hybridized with cDNA **(Acrydite^{TM}-50A')** and dried. A polydimethylsiloxane (PDMS) prepolymer solution was poured on the petri-dish containing the master and followed by curing. After dehybridization, the printed PDMS substrate containing a copy composed of cDNA was hybridized with an originally sequenced DNA modified with fluorescence dye **(RhoR –50A)** for analysis. (fig. 9.2) The biggest substrate size tried to print using LiSuNS was 2 cm X 5 cm but I believe that the actual printing coverage would be much larger since all 6 substrates could be printed in the same Petri-dish successfully.

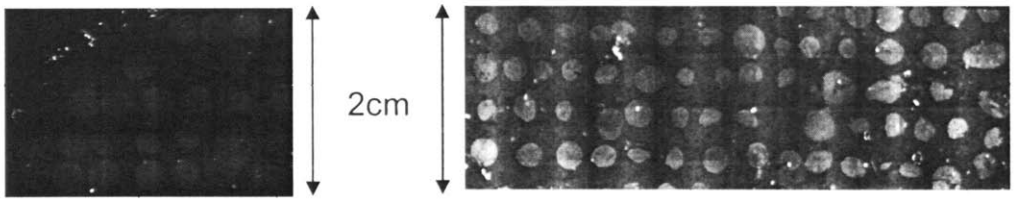


Figure 9.2. Mosaic images composed of fluorescence images of printed DNA milli-arrays on PDMS substrates using LiSuNS. The substrate size is approximately 2cm X 3cm and 2cm X 5.5cm each.

I could obtain a similar result using another polymer that is a urethane-based and UV curable. (Norland optical adhesive 63, NOA 63). After hybridizing a master with cDNA (**Acrydite^{TM}-50A'**), a small amount of prepolymer was applied onto the master followed by weak UV exposure for 20 min (wavelength 370nm)

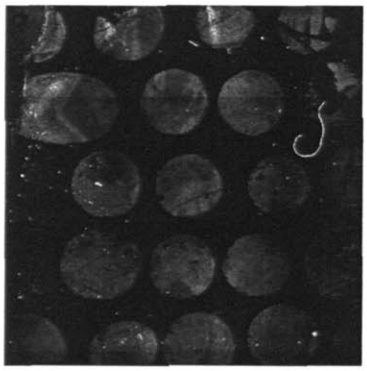


Figure 9.3. Fluorescence images of printed DNA milli-arrays on a photocurable polymer substrate using LiSuNS. The substrate size is approximately 2 cm X 2 cm.

9.3. Printing microsized patterns fabricated with various techniques.

After verification of working principle of LiSuNS, several masters containing micron-sized patterns of DNA were fabricated using a pico-liter dispense system of Hewlett-Packard (TIPS®) and a microfluidic channel (μFN). LiSuNS reproduced both patterns fabricated with soft lithography onto PDMS substrates successfully. (figure 9.4 and figure 9.5)

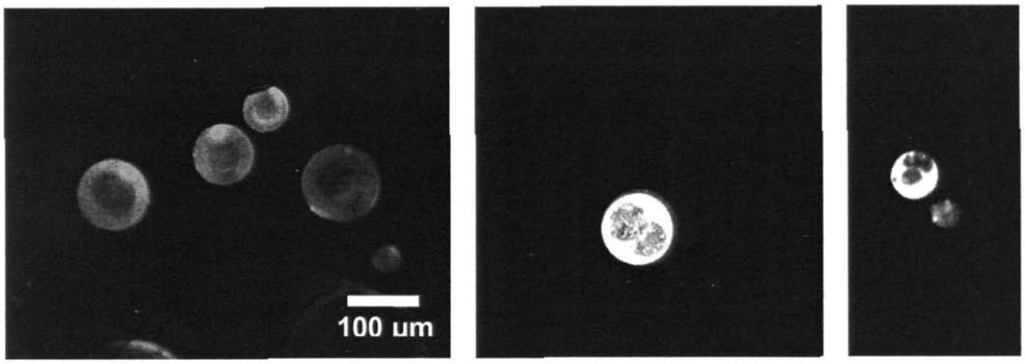


Figure 9.4. Fluorescence images of printed dots of which size varies from 20μm - 100 μm using LiSuNS, collected from three different area in one substrate. The DNA dots were fabricated on gold substrates using TIPS® system

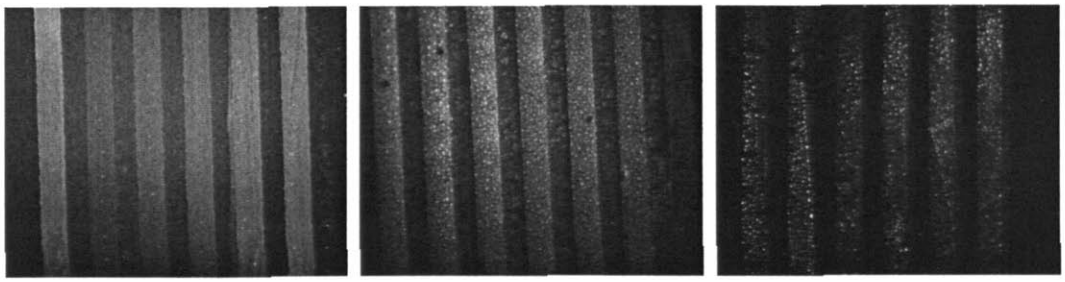


Figure 9.5. Fluorescence images of master, fabricated with uFN, (a), printed pattern first (b). printed pattern second (c).

As an example of hard lithography master, an array of micron sized squares (10 μm X 10 μm each) was fabricated using the optical lithography followed by introducing DNA

monolayer on top of the patterns. This array pattern was reproduced on PDMS substrates with LiSuNS. (figure 9.6)

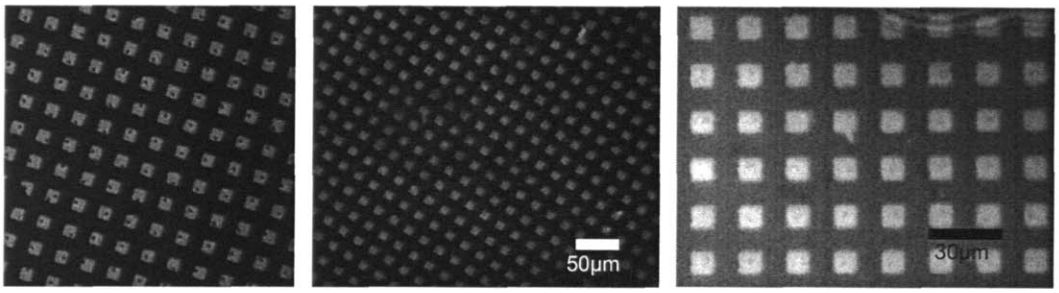


Figure 9.6. Fluorescence images of (a) master composed of 10 μm X 10 μm gold array fabricated optical lithography followed by introducing DNA monolayer on top; (b) and (c) printed patterns using LiSuNS.

Especially, the optical lithography patterns were replicated many times for studying the printing efficiency of LiSuNS and a related factor. It will be described more in Sec.9.7

9.4. Multi-DNA Printing

The best advantage of SuNS is that it can print two or more kinds of DNA at the same time.(See Sec.3.3.3) [5] Like SuNS, LiSuNS should be also capable of printing multiple DNA patterns simultaneously since they share the basic working principle using the specific interaction between the complementary DNA strands. To prove this capability, a line pattern composed of two different DNA was fabricated on gold substrates using a

microfluidic network (μFN). μFN is one of soft lithographic technique using a PDMS mold and it can fabricate a pattern of multi component by injecting different component solution into each channel isolated from each other. [6] Two different DNA solutions (**HS – 50A, HS – 50B**) were injected into 3 channels each, located alternatively. The master was printed on a PDMS substrate after hybridization with cDNA mixture solution (**Acrydite™-50A', Acrydite™-50B'**) using LiSuNS and the printed one were immersed into a solution of original sequenced DNA strands each of which was functionalized with Rhodamine Red and Rhodamine green (**RhoR-50A, RhoG-50B**), respectively. The fluorescence overlay image (figure 9.7(b)) of a image taken using a red filter and another image taken using a green filter proves that the two kinds of DNA were successfully printed using LiSuNS.

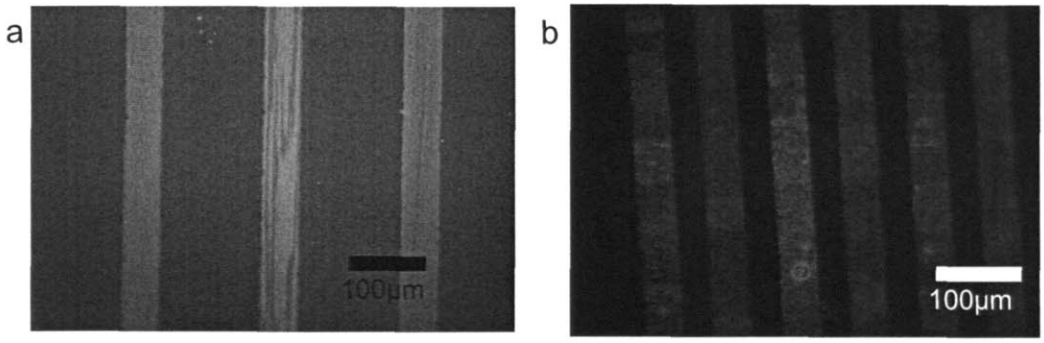


Figure 9.7. Fluorescence images of (a) master composed of two different DNA (A, B) fabricated with microfluidic network (b) printed pattern. In (a), only three lines composed of A are shown since the master was hybridized only with **RhoR-50A'**. After printing, the resulting pattern was hybridized with **RhoR –50A** and **RhoG –50B** proving that the lines composed of two DNA were successfully printed through LiSuNS.

9.5. 2nd generation printing

LiSuNS shares another unique advantage of SuNS: the growth of masters. (see sec. 3.4.1) An experiment showing this capability of LiSuNS was performed. After hybridization of the printed lines from a μFN pattern containing single kind of DNA (fig. 9.5 (b)); and the printed lines from a μFN pattern containing two kind of DNA (fig. 9.6(b)) with thiolated DNA with the original sequences, (**HS – 50A** ; or **HS – 50A** and **HS – 50B**) the patterns were printed on a gold substrate followed by hybridization with **RhoR-50A'**.

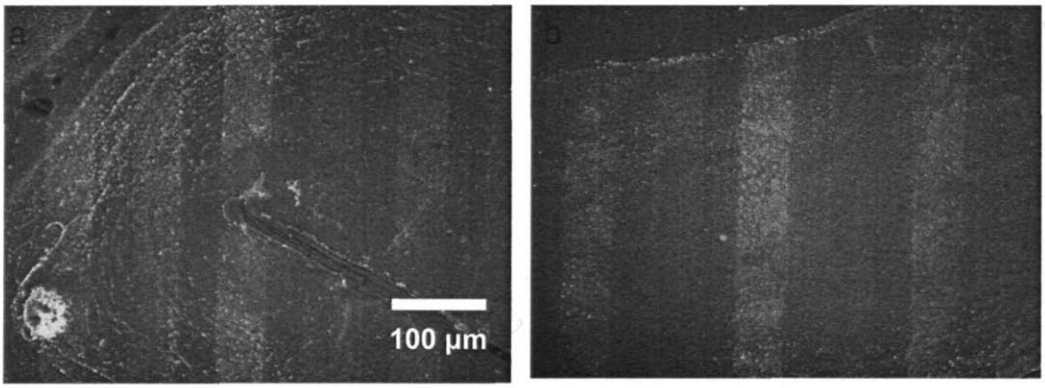


Figure 9.8. Fluorescence images of 2nd generation printed lines from a copy printed on PDMS with LiSuNS as the master. (a) 2nd generation printed from one kind of DNA pattern (b) 2nd generation printed from two kinds of DNA pattern. In the image (b), only three lines are clearly detected because only sequence 50A can be shown due to the hybridazation with **RhoR-50A'**.

9.6. Printing extremely rough surface

LiSuNS' own advantage, distinct from the original SuNS, is the large printing coverage. It means that not only the area size but also the roughness that LiSuNS can handle is big. In fact, during the experiments in which patterns on "relatively flat" surface were printed, I found that they were successfully printed in spite of the existence of macroscopic obstacles such as human-hair or fabric debris. Indeed, the conformal contact can be achieved with any rough masters by using the mobility of liquid prepolymer. In order to test this idea, it was tried printing from a master fabricated on a coin, which is full of embossing features. To make a DNA pattern, a coin was coated with gold using e-beam evaporator followed by dropping a solution of thiolated DNA (**HS-50A**) by a micropipette, similar with the master used for proof-of-concept (sec 9.1). Unfortunately, the density of DNA monolayer forming pattern on the master was not high enough - based on checking the fluorescence intensity of the master after hybridization with **RhoR-50A'** - but it was used as it was.

After printing using LiSuNS, I found that the printed PDMS substrate obtained the embossing features, exactly same with the one on the coin, which implies the contact was conformal. After hybridizing the printed pattern with the primary DNA with fluorescence dye (**RhoR-50A**), it was imaged by the fluorescence microscopy. (fig 9.9) Although the fluorescence intensity of printed pattern is not high due to the low quality of the master, it is still possible to detect that DNA dots were printed independently from the physical features on the coin master.

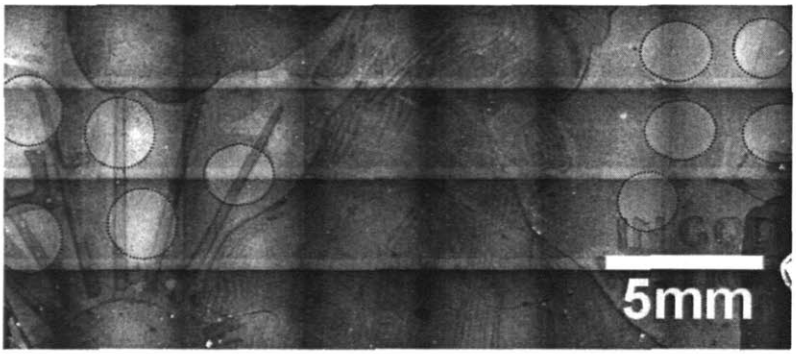


Figure 9.9 A part of the fluorescence image of printed DNA dots from a master fabricated on a gold coated coin. The circles drawn with dot lines are printed patterns from the coin master.

9.7 Humidity dependence of printing efficiency

More extensive studies on LiSuNS' printing condition of were carried out using the micro-array master fabricated with optical lithography. One of the biggest concerns about LiSuNS is the possibility of damage on the DNA molecules by the liquid PDMS prepolymer due to the high mobility of prepolymer solution. For example, if the PDMS gets into double strand DNA structure and reacts even with the original DNA molecules on masters during polymerization, the master would be permanently damaged and it cannot be reused with high printing efficiency. For this reason, I tried to print multiple times using one master to check the printing efficiency. After 10 times, the resulting printed pattern

had still 75% of fluorescence intensity of the one of master without significant decrease by the number of printing cycle. (figure 9.10)

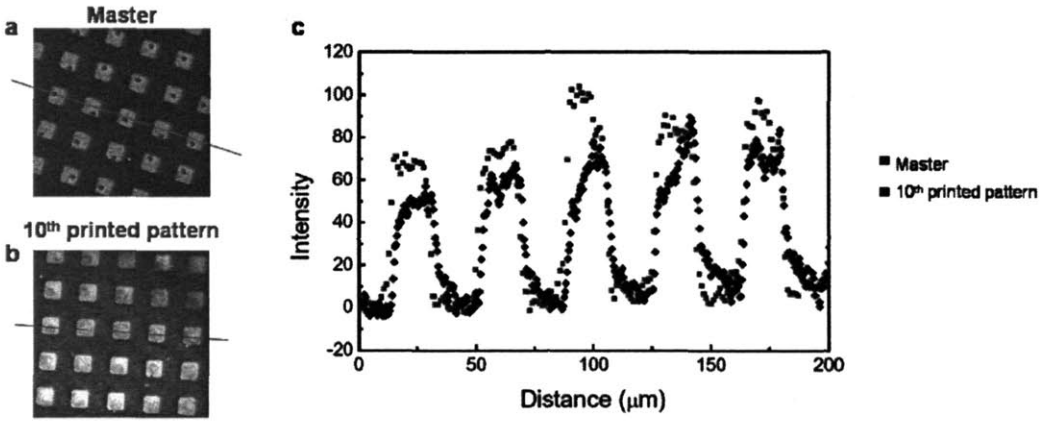


Figure 9.10. Fluorescence images of (a) a master and (b) 10^{th} printed pattern. (c) Plot of the relative fluorescence intensity (the fluorescence intensity of printed pattern – background) of the master (red) and the one of printed one (blue).

It was found that the printing efficiency is more dependent on the relative humidity of the printing environment. For example, at the printing trials that carried out at higher humidity than 50%, the fluorescence could never be detected which means printing was not successful. Additionally, the trials that carried out in a glove box with extremely low humidity were also failed. (Figure 9.11)

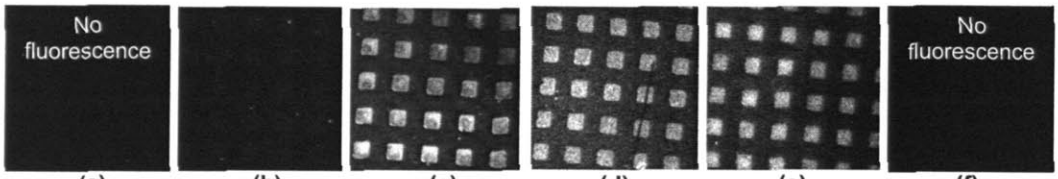


Figure 9.11. Fluorescence images of printed patterns obtained at different humidities. (a) > 50%, (b) 48% (c) 41% (d) 38% (e) 34% (f) ~0%.

A hypothesis was introduced to explain this symptom as following. Since DNA is an extremely hydrophilic molecule, a water film forms on DNA pattern to decrease interface energy. At this time, the thickness of water film would be determined by the relative humidity. If the relative humidity is too high, condensed water would cover the whole cDNA molecules including the binding sites so that cDNA molecules could not form a bond with PDMS structure. On the contrary, if the relative humidity is too low, the water film is very thin and not only cDNA but the primary DNA strands would also exposed to the PDMS prepolymer and join to the crosslinking reaction. In this case, a successful dehybridization could not be carried out and moreover, the master could not be reused due to the damage. Although the water film prohibits the successful printing at humid atmosphere, it helps to protect the DNA molecules from the harsh polymerization of the secondary substrates.

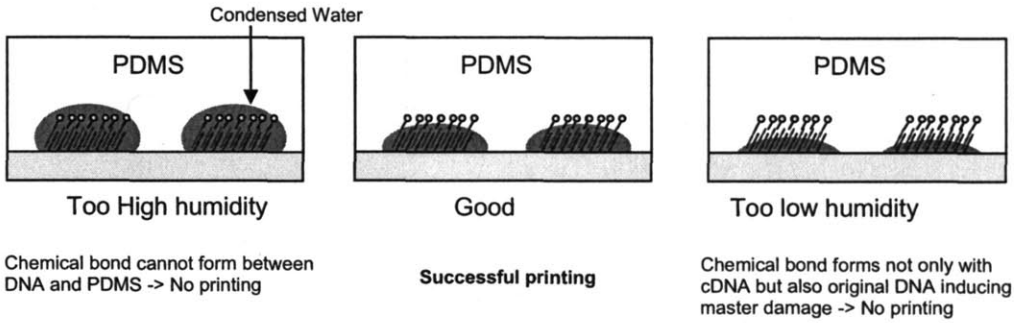


Figure 9.12. The schematic picture that describes a possible situation of the printing process in LiSuNS at different relative humidities.

9.8. Resolution Test.

Although LiSuNS is designed for high coverage printing, not for high resolution, it is still important to know the resolution limit of LiSuNS. In order to test the printing resolution of LiSuNS, a master containing various patterns fabricated with E-beam lithography, which was also used for printing on PMMA substrates (sec. 7.4 fig. 7.4) was tried to print using LiSuNS. (fig. 9.13)

Within a relative humidity range of 30% ~ 40% (see sec. 9.6), clear fluorescence signals were detected from the entire patterns. The printed patterns are composed of a series of lines with various thicknesses. Since the thickness of some printed lines were below the optical limit, it had to be assisted by Tapping mode AFM imaging to identify each lines. The corresponding Tapping mode AFM images show that the resolution limit of LiSuNS is at least 200nm.

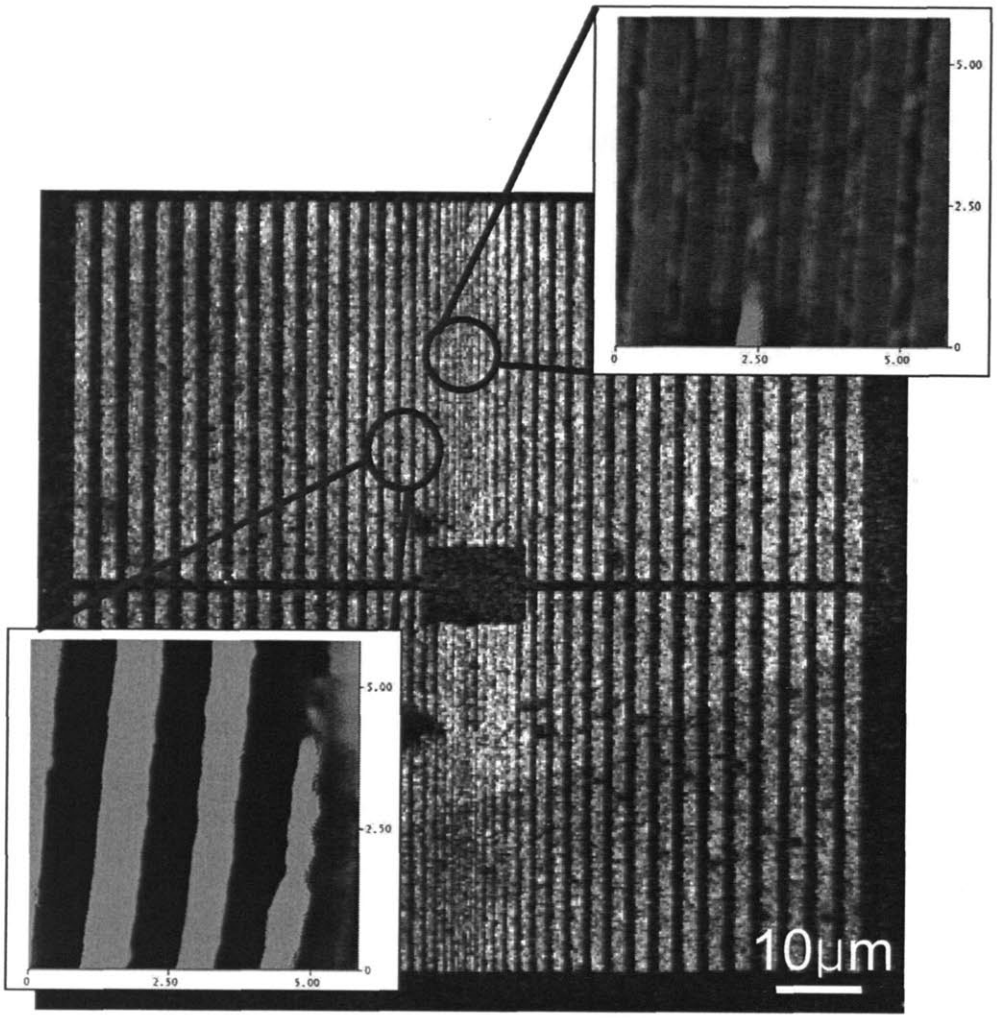


Figure 9.13. High resolution printing of LiSuNS. Many lines with different thickness were printed using LiSuNS. Since the printed thin lines, located on the center are below the optical limit, supplemental Tapping mode AFM images were given. From the analysis of the AFM images, the thinnest line printed with LiSuNS is 200 nm.

9.9. The role of functional group

Although a functional group containing acryl group was introduced into cDNA, **Acrydite™- 12T - 50A'** to be printed through LiSuNS, it is questionable whether it is really necessary because DNA molecules themselves have a lot of reactive functional groups that can join the cross-linking reaction of PDMS polymerization.

For the reason, it was tried to print patterns using unmodified cDNA only containing 12T as a protection group. After trying several times to construct statistical data independent from the many variables including humidity, it was found that the fluorescence intensity of printed pattern using unmodified cDNA is approximately 30% lower than the one of printed patterns.

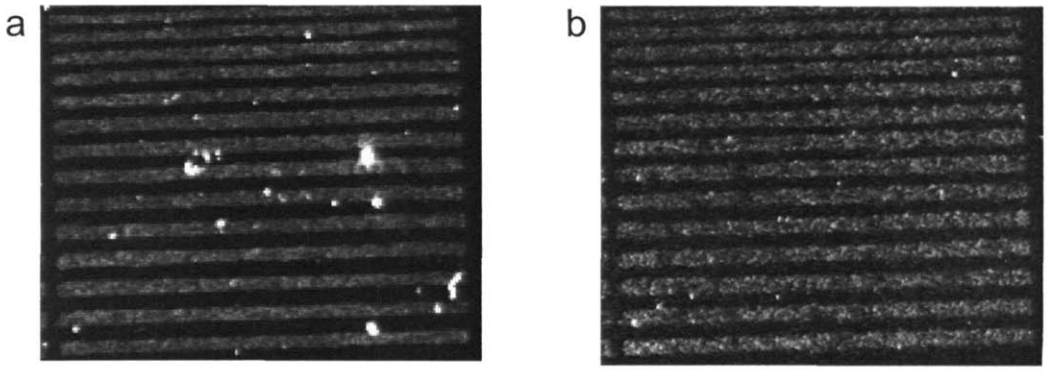


Figure 9.14. Fluorescence images of printed lines, a part of the resolution test patterns, using (a) vinyl terminated cDNA (**Acrydite™ – 12T – 50A'**) and (b) unfunctionalized cDNA (**12T – 50A'**). These images show that the printing efficiency is higher when the cDNA molecules to be printed contain a primary attachment group on their end.

This is a similar result with an analysis of DNA microarray fabrication: when DNA molecules are deposited on a substrate that is amine-reactive (e.g. aldehyde terminated substrates), printing DNA molecules functionalized with a primary amine in the end, gives a higher immobilization efficiency[7] although a DNA molecule has a number of amine groups, which can react with the surface. It can be understood by the mobility difference and the reactivity difference between a primary reactive group and a secondary reactive group, originated from the conformational difference.

9.10. Conclusion

In this chapter, it has been shown that LiSuNS is a reliable printing technique beyond the roughness of substrates and it is able to print a copy from various masters fabriciated with hard lithographies (optical lithography), with soft lithographies (μFN), or with direct deposit (similar with Ink-jet printing) (TIPS). Using LiSuNS, a chemically defined pattern on a master can be printed onto another surface no matter what is a physical profile of the master. However, it is noticeable that the physical profile is also copied on the printed substrate beside the chemical pattern so that it can be used another information if necessary. Indeed, LiSuNS will be the first printing technique that can transfer both 3-D physical information and chemical information while general printing techniques transfer 3-D physical information (e.g. imprinting); or 2-D physical information and chemical

information (microcontact printing). I hope that, with these unique advantages, LiSuNS will be a strong candidate of fabrication techniques for inexpensive bio-devices such as DNA microarrays with high resolution (below 1μm).

9.11. References.

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5. Yu, A. A. et al. Supramolecular nanostamping: Using DNA as movable type. *Nano Letters* **5**, 1061-1064 (2005).

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Chapter 10. Conclusion

In this chapter, the main results of my thesis will be summarized. However, before the summary and the conclusion, I would like to review the research that has taken place in Crooks' group in the recent 2 years, because I believe that it is possible to find a way to improve SuNS by looking at a different view, showing more scaled-up vision. Indeed, SuNS started from printing very small scale (~50 nm) and now it is going to print a large area (bottom-up approach); whereas Crooks' group started from printing very large features (~1mm) and is trying to print smaller features (top-down approach).

10.1. Review of Crooks' work

In 2005, Crooks and coworkers reported a method[1,2] very similar to SuNS developed independently: their method also uses the specific interaction of complementary DNA strands for replication of DNA microarray. Using this technique, they showed a microarray composed of up to three kinds of DNA could be printed multiple times.

Although the working principle of Crooks's method is the same of SuNS, there are a few differences in the detailed printing process. First, they use biotin-streptavidin attraction to transfer cDNA onto the secondary substrates;[1] a master is hybridized with cDNA modified with biotin and printed on streptavidin coated substrates[1]. In order to

utilize the biotin-streptavidin interaction, introducing a small amount of buffer or water is necessary before the master is placed on a secondary substrate.[1] Second, a secondary substrate, streptavidin coated PDMS, has 10 μm deep trenches.[1] They believe these trenches help to drain the solution away from the interface to facilitate contact.[1] Third, after contact, the two substrates are separated by mechanical force (Mechanical dehybridization).[1]

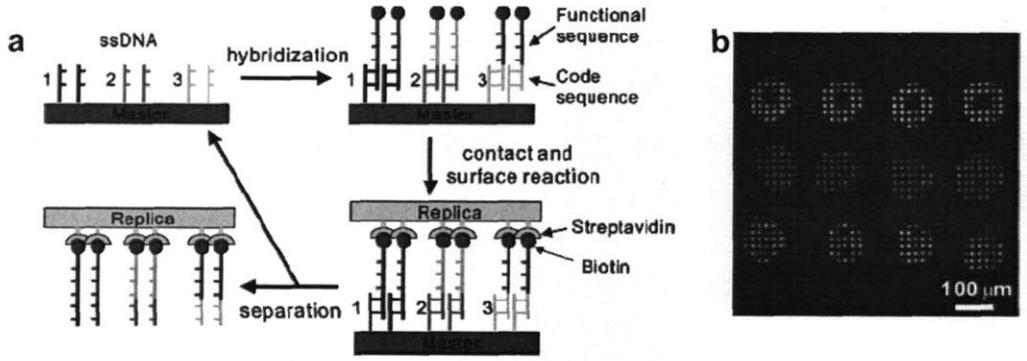

Figure 10.1. (a) Schematic drawing of the printing DNA microarray using zip-code. (b) Fluorescence image of a printed array. The square arrays in the printed dots are from the trench pattern on the PDMS substrate, used as the secondary substrate. Courtesy of reference 2

When they replicated features composed of multiple DNA, they used a zip-code master containing short DNA fragments inducing selective hybridization.[2] (fig.10.1) The target cDNA to be printed is composed of two parts: i) functional sequence and ii) zip-code sequence. Although the zip code consists of only 12 bases, it is enough to find the

complementary one. After the full cDNA molecules were printed with biotin-streptavidin interaction, the result was checked by hybridizing fluorescence tagged DNA complementary to the functional sequences. This approach allows facile dehybridization because the zip-code part for selective binding is short whereas the selectivity is not so strong.

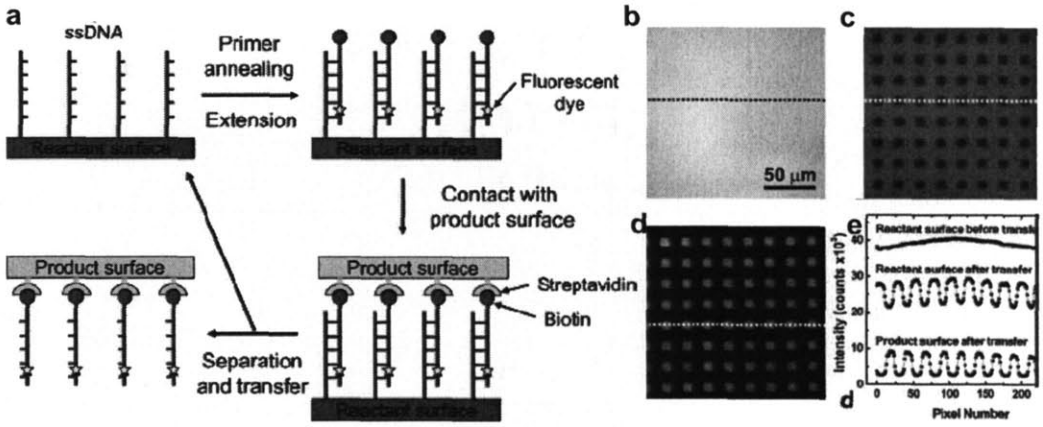

Figure 10.2. (a) Schematic drawing of the printing DNA microarray assisted by in-situ synthesis of cDNA to be printed using PCR; (b) Fluorescence image of a master after PCR incorporated fluorescence dye into synthesized cDNA, (c) Fluorescence image of the master after printing, (d) Fluorescence image of the printed pattern, and (e) Fluorescence intensity profile shown in (b)-(d). Courtesy of reference 3

The most remarkable result from Crooks' group is that they transferred cDNA synthesized in-situ using primary DNA strands on a master, as a template with PCR technique, onto a secondary substrate.[3] (fig.10.2) The advantage of this approach is that

there is no necessity to pre-synthesize cDNA molecules to be printed, depending on the DNA components on the master. With this approach, it is possible to print many different personalized DNA microarrays without modification.

However, there are several weak points in their results. First, most of their results were obtained from printing of a homogeneous pattern onto trenched substrates; the square arrays shown in the fluorescence images of replica are not from the master but from the trench that was on contact with master.

Second, when they printed multiple DNA dots, they used a master containing shorter DNA strands (They call them zip-codes) than printed cDNA stands. (fig.10.2) After printing, the result was checked by hybridizing printed pattern with dye-modified DNA complementary to the sequence that did not take part in the hybridization; there is no evidence to prove that the printing was done by the reversible supramolecular assembly (hybridization) and dissembly (dehybridization), or just by unspecific binding. Additionally, in the case of printing PCR products,[3] they did not prove that the cDNA to be printed was synthesized without significant defects; I do not believe that the synthesized cDNA has the complete complementary sequence toward the primary DNA because the access of cDNA molecules to immobilized DNA on surface is much more difficult than the case of free DNA in solution, which is the normal condition for PCR. It is mainly due to the negative charge of DNA and the complex morphology of DNA pattern on the master. (see sec.3.3.2)

Third, in their first report, only 20% of DNA,[1] located on top of a master, could be printed on the second surface, based on fluorescence study comparing the master and a

printed pattern; if considering the portion of DNA that couldn't be hybridized with cDNA, the printing efficiency will be even lower. It implies that their method of master preparation is not so effective for printing. (They fabricate the master by spotting amine-terminated DNA onto epoxy terminated glass substrates.)

In spite of these weak points, their work has a significant meaning in that they showed that it is possible to replicate a conventional DNA microarray using the supramolecular interaction of DNA, as shown in the scheme.

10.2. Conclusion

In this thesis, it has been showed that a DNA pattern can be duplicated on another surface using the supramolecular specific interaction of DNA with high resolution. At this time, SuNS can transfer not only spatial information as well as many other printing but also the chemical information inscribed in the sequence of DNA printed. This unique character of SuNS allows printing multi-DNA simultaneously and furthermore, the printed one can be multi-component devices with the following bio-chemical direct modification.

SuNS can be applied to various substrate systems: gold, acrylic polymer (PMMA), or silicon crosslinking polymer (PDMS). By using different substrates, the different advantages could be obtained; we can choose one depending on what aspect is important. If

a high resolution is necessary, gold or PMMA substrate should be used whereas if a high printing coverage is required, an approach using PDMS should be applied.

Although SuNS has shown promising results as the next generation of printing technique for multi-component bio-devices, many issues still have been left as open questions. Fabricating suitable masters to SuNS is always one of the most important task. Moreover, all parameters affecting printing quality have not been found and/or optimized. Additionally, it will be good to design and develop a new equipment that can be used for inducing a good/uniform contact between the master and a secondary substrate, subsequently allowing a gentle dehybridization. Finding more substrate systems is also an essential subject to be investigated.

I believe that SuNS can be improved and expanded in many ways. Indeed, SuNS is the only printing technique that can reproduce all information contained in master with high efficiency. The most recent Crooks' work about printing cDNA synthesized in-situ from the master[3] shows one of the possible progress of SuNS in future: if that approach is combined with (Li)SuNS, even a true self-replication of a master that copies the complete information of master including physical information and chemical information onto the secondary substrate is possible.

10.3. References

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3. Kim, J. & Crooks, R. M. Transfer of surface polymerase reaction products to a secondary platform with conservation of spatial registration. *Journal Of The American Chemical Society* **128**, 12076-12077 (2006).

Chapter 11. Experimental Section.

In chapter 11, detailed experimental descriptions are provided.

11.1. Common Materials

All modified DNA strands were purchased from Integrated DNA Technologies. All other chemical were purchased from Sigma-Aldrich, Fluka, Calbiochem Inc. and used as received. Millipore water (18.2MΩcm) was used to clean samples and make all required buffer solution. The following sequences were used in this thesis.

Code Name	Sequence
18A	5' - ACG CAA CTT CGG GCT CTT - 3'
18A'	5' - AAG AGC CCG AAG TTG CGT - 3'
18B	5' - ATC TCA CCT GTG CAC TGC - 3'
18B'	5' - GCA GTG CAC AGG TGA GAT - 3'
50A	5' - TCC CAA AGA ACA GTG GTG GCT CAA GCT ACG GCC CCT CAT GAA AAT CCT GG - 3'
50A'	5' - CCA GGA TTT TCA TGA GGG GCC GTA GCT TGA GCC ACC ACT GTT CTT TGG GA - 3'

50B	5' - CCC TCA GCC CTC GCC ATC ATG GAA AAT GCC AAT GTT CTG GCC CGT TAT GC - 3'
50B'	5' - GCA TAA CGG GCC AGA ACA TTG GCA TTT TCC ATG ATG GCG AGG GCT GAG GG - 3'

All oligonucleotides were 5' modified with groups, such as hexyl-thiol (**HS** HS-$(CH_2)_6$-), hexyl-amine (**H_2N**-, H_2N -$(CH_2)_6$-) or acrylic phosphoramidite (**Acrydite^{TM}**-,). In the following sections these molecules will be called with the group name or acronym followed by the sequence code name, such as **HS-18A** for hexyl-thiolated **18A**.

Thiolated DNA were actually purchased as disulfides (and a thiol protecting group), e.g. **HS-18A** was purchased as **DMTO-$(CH_2)_5$-S—S-$(CH_2)_6$-18A (DMT = dimethoxytrityl).** Dithiolated oligonucleotides were reduced by dissolving ~8 nmoles of DNA in 1 ml of a 0.1M phosphate buffer (pH 7) aqueous solution containing solid phase DTT, followed by filtering the solution through a syringe filter (pore size : 0.2μm). To reach the targeted ionic strength, 1M potassium phosphate buffer solution (pH 3.8) was added to the filtered solution to reach a 4-5 μM DNA concentration unless specified. In ordered to favour hybridization, complementary DNA molecules (*the sequences whose name contains a prime*), were processed using , 1M NaCl in TE buffer (10mM Tris buffer pH 7.2 and 1mM EDTA) instead of 1M potassium phosphates; the final concentration of cDNA used for hybridization is 1-2 μM DNA. Amine terminated DNA or acrydite terminated DNA was used without any additional treatment.

Thermally evaporated gold (111) on freshly cleaved mica substrates were purchased from Molecular Imaging, AZ, and used as received; or they were prepared in UHV chamber by using electron beam evaporation in laboratory. In order to improve the adhesion between the gold thin film and its underlying substrate sometimes we transferred the gold layer from mica to a glass slide coated with a thermal curing epoxy. The procedure followed is described in the reference (Gupta, P.; Loos, K.; Korniakov, A.; Spagnoli, C.; Cowman, M.; Ulman, A. *Angew. Chem.-Int. Edit.* **2004**, *43*, 520-523)

11.2. Printing on gold substrates : Chapter 3 and Chapter 4

11.2.1. Sample preparation

Homogenous DNA monolayer on Gold. Gold-on-mica substrates were immersed in a DNA solution (4-5 μM in 0.5 M Potassium Phosphate buffer) for at least 72 hr. The substrates were cleaned with water, immersed in a 1mM 6-mercapto-1-hexanol (MCH) aqueous solution for 1 hr to minimize nonspecific adsorption of single-stranded DNA, rinsed, and nitrogen dried.

UV Lithography pattern. The grating pattern with 450nm pitch was fabricated on silicon oxide substrates with UV lithography. The grating was then coated, by e-beam deposition,

with 5 nm of chromium as adhesion layer followed by 5 nm of gold. The substrate was immersed in a 4μM solution of **HS-18A** solution for 72 hours and cleaned with water.

Interference Lithography pattern. The silicon grating was fabricated starting with a technique called Achromatic Interference Lithography *[T.A. Savas, M.L. Schattenburg, J.M. Carter, and Henry I. Smith, J. Vac. Sci. Technol. B 14 4167 (1996).]*. 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. The substrate was immersed in a 4μM solution of **HS-18B**, or **HS-50A** solution for 72 hours and cleaned with water.

Dip Pen NanoLithography Pattern. A 40 μm x 40 μm box of octadecane-thiol was written on a gold on mica substrate following a published procedure. The substrate was then immersed in a 4μM solution of **HS-18B** solution for 72 hours. The substrate was cleaned with water.

DNA Microarray The C3B 10k oligonucleotide microarray was designed beginning with the acquisition of the MWG 10kA human oligonucleotide library (Cat # 2190-000000, MWG), which served as the initial gene library. The C3B oligo library was printed on glycidoxy-modified (3-glycidoxypropyltrimethoxysilane; 0.1 wt/o in anhydrous ethanol for 30 min at 42°C then cured for 20 min at 110°C) 1" x 3" borosilicate glass microscope slides using a Cartesian PixSys 5500 microarrayer. Contact printing was performed under 50 %

relative humidity using eight (Chip Maker 4, Telechem) stainless steel spotting quills. Oligos were spotted at 20 ng/μl from a spotting buffer that comprised 0.75 M betaine and 1.5 X SSC.

11.2.2. Printing

Substrates prepared as described in the previous section were immersed in a solution (2 μM, 0.5 M NaCl in TE buffer) containing DNA molecules with sequences complementary to the one (or the ones) present in the master for 2-3 hours. In the case of mixture of sequences the concentration of each sequence was 2 μM. The substrate was rinsed with water but not dried. A second gold substrate was quickly placed on the master substrate so that a thin water meniscus would form in between the two substrates. At times a small mechanical force was applied to the substrates to optimize the contact. We believe that most of the contact was achieved by a capillary force mechanism. After 5-10 hrs, we often observed that the two substrates were bonded together. The coupled substrates were placed in a convection oven, set at 80 °C and 100 °C for 18mer and 50mer DNA sequences respectively. After 20 min for the substrates to be in thermally equilibrium with the oven, a 0.1M NaCl/TE buffer solution (kept at the same temperature) was added drop wise on the substrates (in the oven). The substrates spontaneously separate. All the samples were rinsed with water.

In order to improve resolution we found useful, for sample containing features smaller than 200 nm, to dry the water that remains trapped in between the two substrates, just before dehybridization step. This was achieved by placing the coupled substrates in a

desiccator. In fact, we believe that the presence of water favors uniform diffusion of DNA from the master to second substrate.

Often after printing, substrates were immersed in a 2 μM solution of DNA molecules 5' modified with fluorescent molecules (Rhodamin Green and Rhodamin Red) for 2 or 3 hours in 1 M NaCl in TE buffer solution. The samples were then thoroughly rinsed with water.

11.3. Printing on PMMA substrates : Chapter 7

11.3.1. Sample preparation

Commercial PMMA sheet was purchased from J. Freeman. Inc. (MA, USA), a distributor for Flexiglass® (Rohm, Germany). The 2.5 mm thick sheet with was cut into 5mm x 5 mm square using a die saw. The functionalization of PMMA surface was done using a mostly according to the method described in ref. 3 in chapter 7: Fixe, F., Dufva, M., Telleman, P. & Christensen, C. B. V. Functionalization of poly(methyl methacrylate) (PMMA) as a substrate for DNA microarrays. *Nucleic Acids Research* **32** (2004). After washing in isopropanol (99%) for 30 min and in Millipore water for 30 min, the cut substrates were immersed in 10% (w/v) hexamethyldiamine in 100mM borate buffer (pH 11.5) for 8 hours. They were cleaned with Millipore water twice (15 min), and immersed in 2.5% v/v glutaraldehyde in 0.1M phosphate buffer (pH 7) for 8 hours. The modified substrates were cleaned in Millipore water twice (10 min), and dried in a desiccator at room temperature.

Various test patterns were fabricated with Electron beam lithography (EB). After making a pattern, gold and chromium was deposited on the pattern followed by lift off.

The silicon grating was fabricated starting with a technique called Achromatic Interference Lithography *[T.A. Savas, M.L. Schattenburg, J.M. Carter, and Henry I. Smith, J. Vac. Sci. Technol. B 14 4167 (1996).]*. 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. The substrate was immersed in a 4μM solution of **HS-50A** solution for 5 days and cleaned with water.

Hexagonal pattern of Si_3N_4 membrane on gold substrate (MDEA pattern). The MDEA sample was generously provided ABTECH Scientific, Inc.

11.3.2. Printing

To allow a DNA monolayer on the patterns of gold-top, the substrates were immersed in a 4μM solution of **HS-50A** solution for at least 72 hours. After cleaning with water, they were immersed in a 1mM 6-mercapto-1-hexanol (MCH) aqueous solution for 1 hr to minimize nonspecific adsorption of single-stranded DNA, rinsed and dried,

For hybridization, the masters prepared as described in the previous section were immersed in a solution (1μM, 1 M NaCl in TE buffer) containing complementary DNA molecules (**H_2N-50A'**) for 3 hours followed by rinsing with 0.1M NaCl/TE buffer and

Millipore water briefly. After drying the hybridized masters, a functionalized PMMA substrate was placed on the master. A microscope glass slide was additionally placed on the top of coupled substrates to apply a slight pressure (2 X 10^3 Pa) and then the substrates were placed in the oven at the temperature of 75 °C for 20 min to obtain optimal contact followed by drying in a desiccator for overnight to remove water, a product of the bond formation reaction between cDNA and PMMA. For dehybridization, the coupled substrates were placed in the oven at the temperature of 90 °C. After 30 min, a small amount of dehybridization buffer (0.1M NaCl/TE buffer) was dropped on the substrates in a Petri dish and the container was gently shaken to induce the substrates separation. The separated substrates were rinsed with Millipore water and nitrogen dried.

After printing, the unused functional group on printed PMMA substrates was deactivated by reduction in $NaBH_4$ solution (Fixe, F., Dufva, M., Telleman, P. & Christensen, C. B. V. Functionalization of poly(methyl methacrylate) (PMMA) as a substrate for DNA microarrays. *Nucleic Acids Research* **32** (2004)). The printed substrates were washed in 0.2% sodium dodecyl sulfate aqueous solution (SDS) twice (5 min) and in Millipore water twice (5 min). The washed substrates were immersed in a sodium borohydride solution (0.08M) in 0.1M phosphate buffered saline (PBS, pH 7) and pure ethanol with 3:1 ratio (v:v) for 5 min. The reduced substrates were washed with 0.2% SDS three times for 1 min each and with Millipore water twice for 1 min each and dried in a nitrogen flux.

For 2nd generation printing, the printed AIL pattern was hybridized with 5'-thiolated DNA (1μM, **HS-50A** in 1M NaCl/TE buffer). After rinsing with 0.1M NaCl/TE buffer and DI water briefly, the printed one was dried. A gold-on-glass substrate was place on this hybridized 1st generation followed by contact optimization at the temperature of 75 °C for 20 min. The two attached substrates were separated at 90°C and cleaned with Millipore water.

In order to imprint the negative DNA pattern on MDEA sample, it was necessary to allow a longer time (for 1hr) at the same temperature (75°C) and with same pressure (2 X 10^3 Pa) for contact between the PMMA substrate and the hybridized DNA pattern on the gold layer under silicon nitride membrane with hexagonal array of holes. For analysis of printed MDEAs pattern, the printed substrate was immersed in a 1 μM solution of 5' – fluorescently modified DNA molecules (Rhodamine Green, **RhoG-50A**) for 2 or 3 hours in 1M NaCl/TE buffer solution. The sample was then rinsed with Millipore water and dried.

11.4. Printing from/to PDMS substrates : Chapter 8

11.4.1. Sample preparations

PDMS curing and functionalization Silicon elastomer base and curing agent (Sylgard 184) were thoroughly mixed with a weight ratio of 10:1. This mixture was poured into a Petri dish containing a silicon substrate with grid pattern in micron scale (20μm pitch, 10μm thickness). It was allowed to settle in the Petri dish for 1 hour to remove trapped air bubbles followed by curing at 60°C for 2 hour. After taken off from the mold, the cured PDMS substrates with pose pattern was cut into 2cm X 3cm size. For oxydization, they were placed in the chamber of oxygen cleaning system oxygen plasma was applied for 1min under vacuum of 20mmHg. The oxidized substrates were immediately exposed onto HCl vapor for 1min. They were placed in the oven where the substrates were exposed to the vapor of triethoxysilylbutyraldehyde (TESA) at the temperature of 100°C for 30min under vacuum (~ 30 mmHg).

DNA 2D Arrays on Aldehyde functionalized substrates (glass slides, PDMS) Using a spotter or micropipette, a concentrated solution of amine terminated DNA (20μM, (**H_2N-50A or H_2N-50B)** in 0.5M potassium phosphate buffer (pH 3.8) was dropped on surface. A patterned substrate was incubated in humid chamber at 50 °C for 2 days. For post-treatment to deactivate unused aldehyde functional group, the substrate was soaked in pre-

warmed 0.1 M Tris, 50 mM ethanolamine (pH 9.0, 50°C) for 30 min followed by rinsing with Millipore water twice. Again, the substrates were washed with pre-warmed 4 x SSC 0.1% SDS (50°C) for 30 minutes followed by rinsing with Millipore water twice.

11.4.2. Printing

From PDMS to gold

DNA microarrays were hybridized with thiol terminated cDNA molecules for overnight. After cleaning in a mixture of 1x SSC buffer and 0.1% SDS followed by rinsing with Millipore water, a gold substrate was placed on the slightly wet surface of PDMS master. A gentle pressure was applied for contact. After 3 hours, the coupled substrates were placed in the oven at 75°C for dehybridization. The printed gold substrate was gently detached from the master.

From PDMS to aldehyde coated glass.

DNA microarrays were hybridized with amine terminated cDNA molecules for overnight. After cleaning in a mixture of 1x SSC buffer and 0.1% SDS followed by rinsing with Millipore water, an aldehyde functionalized glass substrate is placed on the slightly wet surface of PDMS master. A gentle pressure was applied for contact. After 3 hours, the coupled substrates were placed in the oven at 75°C for dehybridization. The printed glass substrate was gently detached from the master.

Post-printing treatment

Printed gold substrates were soaked in a 1mM mercaptohexanol solution for 1 hour. Printed glass substrates functionalized with aldehyde were soaked in pre-warmed 0.1 M Tris, 50 mM ethanolamine (pH 9.0, 50°C) for 90 min followed by rinsing with Millipore water two times. Again, the substrates were washed with pre-warmed 4 x SSC 0.1% SDS (50°C) for 30 minutes followed by rinsing with Millipore water two times.

For fluorescence analysis, the printed arrays were hybridized with 2μM fluorescence modified DNA with original sequences (**RhoR-50A**) in 1M NaCl/TE buffer solution for overnight. After cleaning in a mixture of 1x SSC buffer and 0.1% SDS followed by rinsing with Millipore water, they were analyzed with a fluorescence microscope.

11.5. Liquid Supramolecular Nano-Stamping (LiSuNS) : Chapter 9

11.5.1. Sample preparations

DNA 2-D array: Using a micropipette, a concentrated solution of thiol terminated DNA (20μM, **HS-50A**) in 0.5M potassium phosphate buffer (pH 3.8) is dropped on a gold-on-

glass slide forming a pattern. A patterned slide is incubated in humid chamber at room temperature for 2 days. To deactivate bare gold surface, the substrate is exposed to 1mM 6-merchaptohexanol for 1 hour.

10 μm X 10 μm square array: A silicon wafer coated with a commercially available photoresist (OCG 825) was exposed to UV light using an optical mask containing an array of 10 μm X 10 μm square shaped holes, followed by development the exposed regions. After evaporating Cr (5 nm, as adhesion layer) and gold (25 nm), lift-off was performed in N-Methyl-2-Pyrrolidone. A DNA monolayer was formed onto the gold features by immersing the substrate in a DNA solution (**HS-50A)** in 1 M KH_2PO_4 buffer for at least 5 days.

Resolution test pattern: Various gold patterns with different size and shape were fabricated on a silicon substrate with E-beam lithography. To introduce DNA monolayer on top gold layer, the substrate was immersed in a DNA solution (**HS-50A)** in 1 M KH_2PO_4 buffer for at least 5 days.

Line pattern (50 μm thick) composed of two different DNA: A PDMS mold containing microfluidic network composed of 6 parallel channels was placed on a gold-on-glass substrate. A solution of DNA A (**HS-50A, 80 μM**) in 1 M KH_2PO_4 buffer was injected into 3 of the channels, and a solution of DNA B (**HS-50B, 80 μM**) in 1 M KH_2PO_4 buffer was injected into the other 3 channels, so to form an alternating pattern. To allow enough time to form a DNA self assembled monolayer, the substrate was incubated in a humid chamber for at least 5 days as the PDMS mold was placed on it.

After incubation, all masters were immersed in 1 mM 6-mercapto 1-hexanol (**MH**) water solution to minimize the unspecific DNA binding. The **MH** treated masters were washed in DI water thoroughly 3 times.

11.5.2. Printing

For hybridization, masters were immersed in a solution of DNA A' (complementary to sequence A) modified with 12 Thymine (**12T**) and a group containing acryl at the end (**Acrydite™-12T-50A'**) for at least 2 hours. The acryl group is expected to take part in the polymerization reaction of the prepolymer solution to be applied in the next step and 12T was introduced to protect the DNA molecules from the polymerization. If the master was composed of two different DNA (A and B), it was exposed to a mixture solution of DNA A' (**Acrydite™-12T-50A'**) and DNA B' (**Acrydite™-12T-50B'**). The hybridized masters were washed in 4X SSC, 2X SSC + 0.1% SDS, and 0.5X SSC consecutively followed by air dry before printing.

As a prepolymer solution, poly-dimethylsiloxane (PDMS) was used in most cases shown in this paper. Similar results were achieved when a photopolymerizable polyurethane-based prepolymer (Norland optical adhesive 63 (**NOA63™**, Norland) was used.

PDMS prepolymer solution was prepared by mixing a silicon elastomer base and a curing agent (Sylgard 184, Dow corning) with a weight ratio of 10:1. It was allowed to settle in the container used for mixing for 1 hour to remove trapped air bubbles. This mixture was poured into a Petri dish containing a hybridized master and cured at 70°C for

1.5 hour. After curing, the oven temperature was increased to 75 °C and the PDMS was detached from the master with tweezers.

When **NOA63**™ was used, a small amount of the prepolymer solution (~1mL) was dropped onto a hybridized master. In order to overflowing of the solution, the area to be printed on the master was surrounded by the barricade made of PDMS. The prepolymer covered on the master was cured for 20min by a small UV lamp (wavelength: 370 nm), used for TLC fluorescence check.

After printing, PDMS substrates were immersed in a 2 μM solution of DNA molecules 5' modified with fluorescent molecules (Rhodamine Green or Rhodamine Red) for at least 2 hours in 1 M NaCl in TE buffer solution. The samples were then thoroughly rinsed with 4X SSC, 2X SSC + 0.1% SDS, and 0.5X SSC consecutively followed by air dry.

11.6. Characterization

Tapping mode AFM Digital Instrument MultiMode Nanoscope IIIa was used. All experiments presented in the thesis were performed using Veeco Nanoprobe™ tips (Model #: RTESP; Length: 125μm, Resonance Frequency ~ 300kHz). In order to check the coverage of the DNA monolayer on the printed substrate phase images were used and analyzed using Digital Instrument software.

Scanning Electron Microscopy (SEM) SEM images shown in this thesis were obtained using a Leo, DSM 982.

Fluorescence Microscopy. Fluorescence images shown in this thesis were obtained using three different fluorescence microscopes as the following.

Confocal fluorescence microscopy images were obtained using a Zeiss Axiovert 200 provided with a Xenon lamp. The filters used for imaging DNA pattenrs modified with Rhodamine Green, in excitation and collection had maximum transmittance at 480 nm and 528 nm respectively. For Rhodamine Red, they had maxima at 555 nm and 617 nm. Quantification of the fluorescence intensity was made by integrating the signal over a 260μm x 220μm area using MetaMorph version 5.0rl software of Universal Imaging Corp.

Fluorescence microscopy images were obtained using a Zeiss Axioplan 2 with a mercury lamp. To image DNA modified with Rhodamine Green, the filters used in excitation and collection had maximum transmittance at 470 nm and 515 nm respectively. For Rhodamine Red, they had maxima at 556 nm and 590 nm. Fluorescence intensity profiles were obtained using Image J (a version of the freeware program NIH Image). When intensity profiles of two samples were compared, The average height of the fluorescence over the squares was calculated (subtracting the average background intensity) for both samples

Fluorescence Mosaic images were obtained using a Zeiss Axiovert 200 provided with a Xenon lamp. To image printed DNA 2D arrays coded with Rhodamine Red, a filter used in excitation and collection had maximum transmittance at 556 nm and 590 nm respectively. After partial images were taken, the all images were merged/stitched using a Axiovert program.