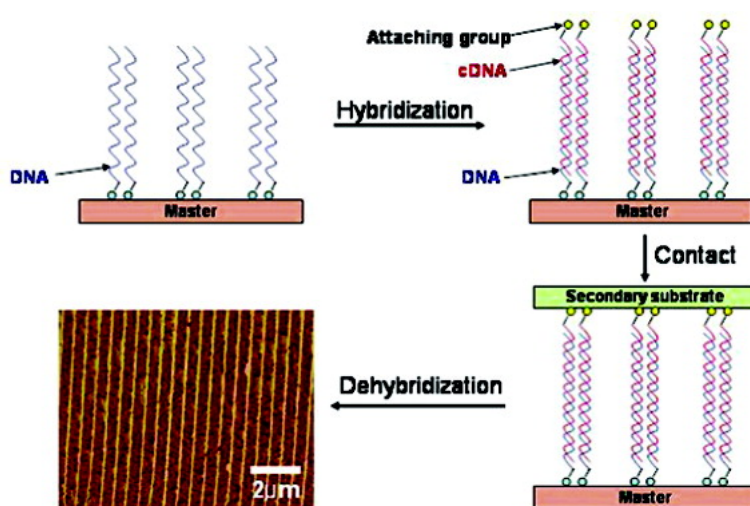


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High Resolution Printing of DNA Feature on Poly(methyl methacrylate) Substrates Using Supramolecular Nano-Stamping

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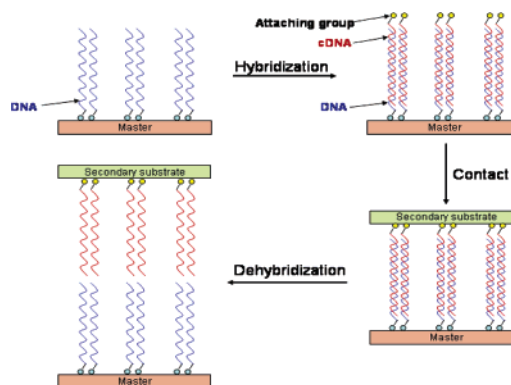
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In recent years, a large number of devices based on organic and biological materials have been developed.^{1–3} To scale-up the production of these systems to industrially acceptable standards, there is a need to develop soft-material stamping approaches with the needed resolution, complexity, and versatility. A promising technique is micro-contact printing (μ CP).⁴ Unfortunately, μ CP can print only one type of molecule at the time, and it is limited in resolution by the molecules' lateral diffusion. Supramolecular interactions have been used to improve μ CP's pattern stability^{5,6} and to print biomolecules.^{7,8} We have recently developed a DNA-based stamping method (supramolecular nano-stamping, SuNS) that has superior resolution and can print multiple molecules at the same time.⁹ A similar technique was independently developed by Crooks and co-workers.¹⁰ Here we show that SuNS can be used to efficiently print DNA features on a polymeric substrate with a 40 nm point resolution and a coverage that exceeds $100 \mu\text{m}^2$.

In SuNS, the master is a substrate containing a patterned single-stranded DNA (ssDNA) monolayer. A copy is obtained in three steps. First, the master is immersed into a solution containing DNA molecules complementary (cDNA) to the ones on the master and 5' modified with groups that can form bonds with a target surface. After hybridization, a secondary substrate is placed on top of the master to allow for cDNA/surface bonds formation. Finally, heating induces the two attached substrates to separate by DNA dehybridization (Scheme 1). Initially, we used gold–thiol chemistry to prove the SuNS concept.⁹ Here we show that another binding chemistry can be used to print from gold masters to poly(methyl methacrylate) (PMMA) substrates and vice versa. PMMA is a convenient material, both as a final substrate or as a master, because it is optically transparent (being an amorphous polymer), insulating, and has decent mechanical properties. It is often used as an inexpensive substitute for glass, thus it could be a good candidate as a bioarray substrate. Also, its surface is rigid at room temperature, but at slightly higher temperatures, it can become soft enough to conform to nonflat masters' surfaces (temperature of glass transition, $T_g = 100 \text{ }^\circ\text{C}$).

To covalently bind DNA to a PMMA surface, we reacted amine-terminated DNA with aldehyde surface-functionalized PMMA¹¹ (Supporting Information). The overall procedure used to stamp on PMMA is similar to the one used to stamp on gold,⁹ and it is described in detail in the Supporting Information. Molecules forming a monolayer through a thiol–gold bond have always had some lateral mobility, and when in solution, they are in a dynamic equilibrium between an adsorbed and a solvated form. Here, more

Scheme 1



irreversible bonds allow for the suppression of these phenomena and should lead to more stable patterns.¹²

A series of test patterns made of gold (over a chromium adhesion layer) were fabricated on a silicon substrate using electron-beam lithography on a relatively large area ($2 \times 2 \text{ mm}^2$). The substrate was placed in a $5 \mu\text{M}$ 5'-hexylthiol-modified DNA (HS-A) solution for 5 days. It was cleaned with deionized water and placed in another $1 \mu\text{M}$ solution containing 5'-hexylamine-modified cDNA ($\text{H}_2\text{N}-\text{A}'$) for 3 h. After rinsing with deionized water, it was placed onto an aldehyde-modified PMMA substrate. Slight pressure ($\sim 2 \times 10^3 \text{ Pa}$) was applied just by gently placing a microscope glass slide on top of the two substrates. To obtain optimal contact, the coupled substrates were placed in an oven for 20 min at $75 \text{ }^\circ\text{C}$. We believe (given the quality of our printing results) that, at this temperature, the slightly softened PMMA surface is able to conform to the master's intrinsic roughness and to protuberances, such as dust. Also, temperature accelerates the rate of DNA–PMMA bond formation. The coupled substrates (in a Petri dish) were placed in an oven, kept at $90 \text{ }^\circ\text{C}$, for $\sim 30 \text{ min}$; 2 mL of dehybridization buffer solution was dropped in the container. Most of the times, the two substrates spontaneously separated when the container was gently shaken. The printed patterns were imaged using Tapping Mode Atomic Force Microscopy (TM-AFM); typical results are shown in Figure 1.

Parallel lines (100 nm in thickness) with a 500 nm pitch were printed with almost no defect over a $100 \mu\text{m}^2$ area (Figure 1a). Substantially better coverage was achieved, compared to our previous results on gold.⁹ Also, by printing a series of wires of decreasing thickness, we tested our point resolution. We found that we could reliably print down to 50 nm. These are among the best stamping results for soft and biological materials.⁴ In Supporting Information, a gallery of images is shown, different test patterns

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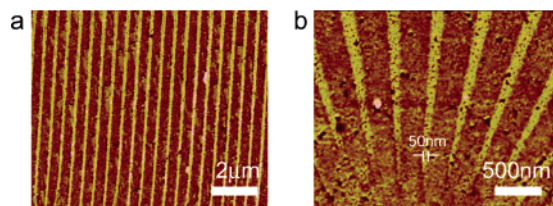


Figure 1. AFM images of DNA wires printed on a PMMA substrate. The arrows in (b) indicate the thinnest continuous part of the wire that was successfully printed. In an isolated case, we could print down to a thickness of 25 nm.

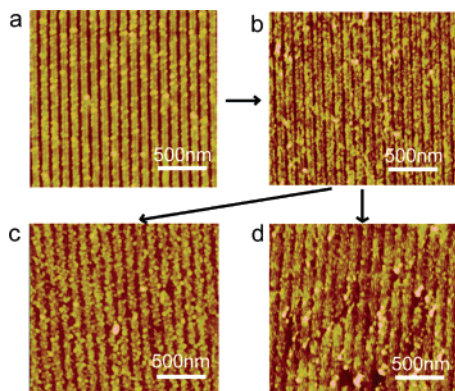


Figure 2. AFM images of DNA wires. (a) Assembled on gold coating of a series of SiO_2 parallel wires 50 nm thick. The wires become 70 nm thick after DNA assembly, probably due to assembly at the edges. (b) DNA wires printed on a PMMA substrate, the average thickness is 75 nm. (c and d) DNA wires printed on gold-on-glass substrates using the sample shown in b as a master. (c) Printed first, and (d) printed second after rehybridization.

were printed from the same master. Each image was obtained in a different area, implying large area coverage.

We successfully printed a grating pattern, composed of gold-coated 50 nm thick SiO_2 wires and 100 nm pitch (Figure 2a), onto a PMMA substrate; the result was imaged by TM-AFM (Figure 2b). The printed lines showed a somewhat increased thickness (from 70 to 75 nm). Over a $100 \mu\text{m}^2$ area, straight lines with an average height higher than the substrate's plane are visible. Multiple areas were imaged, all showed the grating pattern, albeit some with more defects. One of SuNS's main advantages is that any printed pattern can be used as a new master. To further prove this point, but also to prove that (1) PMMA substrates containing DNA features can be used as masters and that (2) there is no need to place DNA on a master with height defined features, we used the printed PMMA substrate as a master. Using HS-A, the DNA wires were successfully printed on a gold-on-glass substrate twice (see Figure 2c and d). At each printing, the wires' average thickness and irregularity increased, possibly due to heating-induced deformations of the pattern or of the PMMA surface. It should be noted that the two printed copies are identical in substrate material and DNA sequence to the original master. Additionally, it is known that imide bonds can, under certain conditions, be hydrolyzed and thus broken. This could lead to loss of patterns. We have not observed this phenomenon in the PMMA substrates used so far. In particular, the substrate described above (illustrated in Figure 2b) was in water for 16.5 h (15 h at room temperature and 1.5 h at 90°C) and did not lose DNA significantly.

A major advantage of PMMA is that, at reasonably low temperature, it can become soft. We took advantage of this to combine SuNS with nano-imprinting.¹³ The master was made on a substrate composed of a $1 \mu\text{m}$ thick Si_3N_4 membrane placed on a

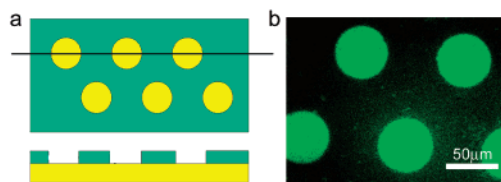


Figure 3. (a) Schematic drawing (top view and section; yellow = Au, green = Si_3N_4) of the sample used as a master to print with a combination of SuNS and nano-imprinting. The DNA monolayer was built on the gold inside the holes and transferred to a PMMA sample imprinted to have posts of the same size of the holes. (b) Confocal fluorescence microscopy image of the printed array of DNA after hybridization with fluorescently labeled complementary DNA strands.

gold surface. The membrane had a hexagonal pattern of $50 \mu\text{m}$ holes spaced $100 \mu\text{m}$. A ssDNA monolayer was assembled on the exposed gold bottom of these holes. Efficient printing was achieved by placing the PMMA substrate on the master ($\sim 2 \times 10^3$ Pa) and then heating at 75°C for 60 min. The combination of pressure, temperature, and time allows the PMMA substrate to conform to the Si_3N_4 membrane (nano-imprinting), thus forming posts that reach and attach to the cDNA on the gold surface (SuNS). The printed sample was placed in a solution containing DNA molecule of the original sequence 5'-modified with Rhodamine Green and imaged with confocal microscopy. The bright dots shown in Figure 3 are the transferred DNA monolayer.

In conclusion, the extension of SuNS to a polymeric substrate (PMMA) has been successful in terms of both resolution and coverage achieved. The use of an acrylic polymer with good mechanical, insulating, and optical properties allows one to envision applications in the stamping of sensors and DNA micro- and nanoarrays.

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Supporting Information Available: Experimental Section, scheme for PMMA functionalization, SEM of master samples, AFM images of test patterns, and complete ref 6. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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