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Soft lithography – a collection of techniques based on printing, molding, and embossing with an elastomeric stamp – provides a top-down approach to patterning that is complementary to methods relying on photolithography. It has emerged as a technology useful for a number of applications (so far, predominantly outside of silicon-based microelectronics) that include cell biology, microfluidics, lab-on-a-chip systems, microelectromechanical systems, and flexible electronics/photonics. Soft lithography provides access to 3-D and non-planar structures, tolerates a wide variety of materials, generates well-defined and controllable surface chemistries, and is generally compatible with biological applications. It is also low in cost, experimentally convenient, and accessible to molecular scientists. This protocol describes three of the commonly used soft lithographic techniques: *i*) microcontact printing of alkanethiols and proteins on gold-coated and glass substrates; *ii*) replica molding for fabrication of microfluidic devices in poly(dimethyl siloxane), and of polyurethane or epoxy nanostructures; and *iii*) solvent-assisted micromolding of poly(methyl methacrylate) nanostructures.

INTRODUCTION

The strategy of "smaller brings new capability" has begun to change the world of biotechnology as it has transformed microelectronics[1]. Successful applications of small systems include microarrays for high-speed DNA sequencing[2]; microfluidic devices for performing polymerase chain reaction (PCR)[3]; lab-on-a-chip (LOC) systems for synthesis and analysis of peptide and oligonucleotide libraries[4]; and microchips for drug screening[5] and for investigation of single cells[6]. As the workhorse of microfabrication, photolithography[7] has played an important role in most of these applications, including the fabrication of DNA arrays in the late 1980's[8]. This technique, however, has a number of limitations for applications related to biological systems. First of all, photolithography is an intrinsically expensive process because the equipment used has been developed for the highly demanding processes required for fabrication of microelectronic devices. The capital investment required to build a clean room, and the need to exclude organic and organometallic materials from such a facility, makes photolithography less than accessible to most chemists, biochemists, and biologists. Thus, photolithography is most successful when applied to a limited set of materials. Users wishing to study "dirty" organic systems are usually excluded from an electronis-qualified clean-room facility. Secondly, photolithography is often carried out by projecting a pattern on a photomask onto a photoresist film. Although photomasks are commercially available, the time and cost involved in the fabrication of such masks present a significant barrier to the use of photolithography in rapid, inexpensive prototyping of test patterns and devices. Thirdly, photolithography provides little or no control over surface chemistry, and it is not applicable to curved or non-planar substrates.

Soft lithography[9] represents a conceptually different approach to rapid prototyping of various types of both micro- and nanoscale structures and devices on planar, curved, flexible and soft

substrates especially when low cost is required. A large number of patterning techniques – microcontact printing (μCP)[10], replica molding (REM)[11], microtransfer molding (μTM)[12], micromolding in capillary (MIMIC)[13], solvent-assisted micromolding (SAMIM)[14], phase-shifting edge lithography[15], nanotransfer printing (nTP)[16], decal transfer lithography (DTL)[17], and nanoskiving[18] – form the basis of soft lithography; all of them are essentially based on the printing, molding, and embossing with an elastomeric stamp. New variants like dip-pen nanolithography (DPN) have also emerged[19-21]. All these techniques share one thing in common: the use of organic materials and polymers – "soft matter", in the language of physicists.

Soft lithography offers an attractive route to microscale structures and systems needed for applications in biotechnology, and most of them exceed the traditional scope defined by classic photolithography. For example, μCP offers the ability to study the role of spatial signaling in cell biology by exquisitely controlling the molecular structure of a surface in contact with cells by using patterned self-assembled monolayers (SAMs)[22]. In this case, one can create well-defined regions with "cell-friendly" (protein-covered) and "cell-unfriendly" (polyethylene glycol-terminated) SAMs to pattern endothelial cells on a surface and then examine how these cells respond to lateral confinement, and to release from confinement[23]. Furthermore, μCP has been successfully used to print precise patterns of axon guidance molecules, which can then serve as templates for growing chick retinal ganglion cell axons[24]. Alternatively, REM provides a new method for fabricating microfluidic devices using poly(dimethyl siloxane) (PDMS)[25], and this method is proving to be particularly suitable for a variety of biomedical applications. Compared with the inorganic materials such as silicon and glass that are used in microelectronics and optics, and which have been explored but largely discarded for biologically directed microfluidics, PDMS offers a number of attractive features[26]: *i*) It has a shear modulus of 0.25

MPa and a Young's modulus of roughly 0.5 MPa (characteristic of a moderate stiff elastomer). This elastomeric character allows it to conform to a surface and achieve atomic-level contact, a feature that is useful in forming and in sealing microfluidic systems. *ii*) It is non-toxic, and readily available from commercial sources at acceptable prices ($80/kg). *iii*) It is optically transparent down to about 300 nm. *iv*) It is intrinsically hydrophobic ($\theta_a^{H_2O} \sim 110$), but its surface can be modified by brief exposure to an oxygen plasma to become hydrophilic ($\theta_a^{H_2O} \sim 10$). *v*) It can adhere and seal reversibly or, after oxidation, irreversibly to many types of substrates.[25,27] The ease of production associated with PDMS microfluidic systems will become greater as the focus of microfluidics shifts from demonstration of components and devices to development of fully functional systems[28-31].

Soft lithography also provides a set of attractive tools for nanofabrication. "Nanofabrication" refers to a process that makes functional structures with at least one lateral dimension smaller than 100 nm. REM and SAMIM are two techniques based on molding and embossing with an elastomeric stamp. Both can produce nanoscale structures in a variety of soft materials, in parallel, over large areas (ca. 3 cm^2 with ca. 100-nm features), and at low cost. When combined with selective etching and lift-off, these nanostructures have been transferred into metals, and then employed as substrates for biosensing. The methods of detection have included optical diffraction[32], surface plasmon resonance (SPR)[33], surface-enhanced Raman scattering (SERS)[34], and electrochemistry[35].

Figure 1 outlines the procedure generally used for soft lithography; this procedure can be divided into four major steps: *i*) design of the pattern; *ii*) fabrication of the mask and then the master; *iii*) fabrication of the PDMS stamp; and *iv*) fabrication of micro- and nanostructures with the stamp by printing, molding, embossing, decal transfer, phase-shifting edge lithography, and

other procedures. The fabrication of patterned copies using the PDMS stamp is what is usually referred to as "soft lithography"[9]; the overall process – from design of the pattern to fabrication of functional structures is sometimes (when the convenience of the method for rapid generation of functional structures is the feature emphasized) referred to as "rapid prototyping"[36].

MATERIALS

REAGENTS

- Photoresists. There are two different types of photoresists: positive tone and negative tone. For positive-tone resists, the region exposed to light becomes soluble in a developing solution. The negative-tone resists work in the complementary sense – the region exposed to light becomes insoluble in a developing solution. As examples of two commonly used photoresists, positive AZ resists cover the range of thickness from 0.5 to 50 μm (http://www.microchemicals.com/photoresist/thin_positive_photoresist.html) and negative SU-8 2000 resists can be used with thickness ranging from 10 and 100 μm (http://www.microchem.com/products/su_eight.htm). Positive-tone resists are better for routine use.
- Electron-beam resists. Poly(methyl methacrylate) (PMMA) is the most popular, positive-tone resist (http://www.microchem.com/products/pmma.htm). It has a resolution close to 20 nm in lateral dimensions and conveniently accessible thickness (vertical dimension) in the range of 100-1000 nm. SU-8 2005 and 2002 are two negative-tone resists. (http://www.microchem.com/products/su_eight.htm), with resolution of 100 nm for lateral dimensions and a range of accessible thickness of 100-500 nm.
- Tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (TFOCS, United Chemical

Technology, http://www.unitedchem.com/about.htm, cat. no. 6H-9283) !CAUTION Corrosive and toxic

- (7-8% Vinylmethylsiloxane) (dimethylsiloxane) copolymer (Gelest, www.gelest.com, cat. no. 6I-9467)
- 1,3,5,7- Tetramethylcyclotetrasiloxane (Gelest, cat. no. 4E-4963)
- Platinum divinyltetramethyldisiloxane (Gelest, cat. no. 6H-92)
- (25-30% Methylhydrosiloxane) (dimethylsiloxane) copolymer (Gelest, cat. no. 6D-8641) PAUSE POINT Store this copolymer in a refrigerator
- Sylgard 184 silicone elastomer base and Sylgard 184 elastomer curing agent (Dow Corning, http://www.dowcorning.com/)
- Hexadecanethiol (HDT, >99%, Aldrich, cat. no. 2917-26-2)
- Green- and red-labeled chicken anti-goat IgG antibodies (Alexa Fluor 488 and Alexa Fluor 594, Invitrogen, http://probes.invitrogen.com/)
- Bovine serum albumin (BSA, Faction V, Calbiochem, http://www.merckbiosciences.com/g.asp?f=CBC/home.html)
- UV-curable polyurethane (NOA-73, Norland Products, http://www.norlandprod.com/)
- Thermally curable epoxy (TRA BOND BA F113, TRA-CON, www.tra-con.com)
- Ferricyanide etching solution for thin films of gold[37]. $K_2S_2O_3$ (0.1 M), $K_3Fe(CN)_6$ (0.01 M), $K_4Fe(CN)_6$ (0.001 M), and KOH (1 M). !CAUTION The ferricyanide compound is sensitive to light, and the solution must be freshly prepared each time and used within 6 hours. The cyanide group can be released as hydrogen cyanide (HCN, highly toxic!) when ferricyanide is mixed with a strong acid. The etching solution should be treated as hazardous waste and

disposed according to the EH&S regulations.

- Ferricyanide etching solution for thin films of silver[37]. $K_2S_2O_3$ (0.1 M), $K_3Fe(CN)_6$ (0.01 M), and $K_4Fe(CN)_6$ (0.001 M). !CAUTION see the etching solution for gold.
- Acetone (Aldrich, cat. no. 67-64-1)
- Ethanol (Aldrich, cat. no. 64-17-5)

EQUIPMENT

- Wet chemical bench (for handling chemicals and solvents)
- Spin coater (for spin coating resists as thin films). Spin coating is a procedure used to apply a thin film of a polymer to a flat substrate. In a typical procedure, an excess of the polymer solution is dispensed onto a substrate, which is then spun at a high speed to spread the solution by centrifugal force. The solvent quickly evaporates, leaving behind a uniform thin, flat film of the polymer on the entire surface of the substrate. One can conveniently control the thickness of the film by adjusting the concentration of the polymer solution and/or the rotational speed for the substrate.
- UV mask aligner (for photolithography). This is a device used in a photolithography process to align a wafer (coated with a thin layer of a photoresist) with a photomask and then expose the resist film to an ultraviolet light source. A vacuum is drawn between the mask and the wafer to achieve an intimate contact between them.
- Electron-beam writer (for e-beam lithography, see the section on master fabrication)
- Dessicator (for surface modification)
- Vacuum line (for surface modification and removal of bubbles)

- Hotplates (for baking resist films)
- Nitrogen gas line (for drying stamps and substrates)
- Oxygen plasma cleaner (for surface modification and removal of residual polymer film)
- Thermal or electron-beam evaporator (for preparing supported thin films of gold or silver). An evaporator is a vacuum system used to deposit various metals as thin films on substrates. Metal evaporation is usually achieved by Joule-heating or using a focused electron beam to melt the metal and the vaporized atoms and clusters are deposited on the substrate. One can continuously monitor the film thickness using a quartz balance and terminate the deposition process at any point by closing a shutter on the metal source.
- Optical microscope (for characterizing patterns on masters and stamps at the microscale)
- Scanning electron microscope (SEM, for characterizing patterns on masters and stamps at both micro- and nanoscale)
- Atomic force microscope (AFM, for characterizing patterns on masters and stamps at both micro- and nanoscale)

PROCEDURE

The key element of soft lithography is an elastomeric stamp with patterns as relief structures on its surface (Figure 1). The stamp is fabricated by casting a liquid prepolymer against a master whose surface has been patterned with complementary structures. The master is often prepared by conventional photolithography or e-beam lithography (EBL), but other relief structures such as diffraction gratings and metal grids for electron microscopy can also be useful for certain simple patterns[38]. Photolithography is a classic technique for patterning structures with feature

sizes larger than 1 μm. The primary advantage of EBL over photolithography is its capacity to generate structures (20-30 nm in lateral dimensions) much smaller than those available through photolithography (1-2 μm), which is mainly limited by optical diffraction. EBL is, however, a serial process: it is generally much slower than photolithography, more expensive, and subject to much longer waits for access to facilities. Both photolithography and EBL start from design and creation of the desired pattern as a 2-D vector graphics – that is, use of geometrical primitives such as points, lines, curves, and polygons to represent the pattern.

Design of the pattern

1| One can use a number of computer-aided design (CAD) software to design the pattern. Adobe Freehand and Illustrator (http://www.adobe.com) are two vector-based drawing programs well-suited for quick design of a pattern with feature sizes down to 1 μm. They are widely accessible, easy to learn, and particularly useful for fabricating photomasks on transparency films. Autodesk AutoCAD (http://usa.autodesk.com) and DesignCAD (www.designcad.com) are two advanced tools with more capabilities and are commonly used by a commercial supplier to design the pattern of a chrome mask – a quartz plate patterned with opaque chrome on its surface. EBL also requires the use of such advanced drawing tools.

PAUSE POINT When using Freehand or Illustrator, set the canvas to the size of the printed page so the features in the pattern will not change their dimensions during the printing process.

Fabrication of the photomask

2| High-resolution printing provides a simple and convenient method for fabricating photomasks

on transparency films[36]. The pattern can be created using Freehand or Illustrator and saved as an EPS file, which is compatible with most of the conventional printers or writers. Typically, a high-resolution commercial printer (5,060 dpi, with a dot size of 5 μm) can generate lines with acceptable edge resolution as thin as 20 μm; this size range is appropriate for most microfluidic applications. Such capabilities are often available from the publication departments of universities or large companies, or from local commercial printing shops. CAD ART services in California offer 20,000 dpi printouts (www.outputcity.com). The rapid turnaround available for these printers (often less than a day) is convenient when relatively large sizes (> 20 μm) are needed and substantially less expensive than chrome masks. For higher resolutions, chrome photomasks are required, which are available from commercial suppliers like Photo Sciences (http://www.photo-sciences.com). For users in the United States, services that prepare chrome masks are accessible through the NSF-supported National Nanotechnology Infrastructure Network (NNIN, www.nnin.org).

Fabrication of the master

Photolithography (Step 4) and e-beam lithography (Step 5) are two commonly used techniques for generating the relief structures on the master. Although the elastomeric character is essential for a conformal contact between the stamp and substrate required for contact printing, transfer molding, and decal transfer, it can become a source of technical problems (e.g., lateral collapse of narrowly spaced features, or pairing, and sagging) in some designs[39]. As illustrated in Figure 2, if the aspect ratio (H/L) of the raised region is too large, the stress originating from gravity, adhesion, and capillary force may cause the relief structures to collapse. Alternatively, if the aspect ratio H/D is too small, the recessed region will not be able to retain its structure during

stamping. These problems could be minimized by designing patterns having the aspect ratios between 0.5 and 5 for H/L, and >0.05 for H/D, respectively. The use of thin PDMS stamps mounted on rigid supports[40], or designs having "posts" to prevent collapse[41], also provide solutions to these problems in some cases.

3| Determine the relief height (H in Figure 2) on the master by optimizing the aspect ratios – H/L and H/D – to prevent pairing and sagging. This information serves as a guide to select the range of thickness for the photoresist or e-beam resist film.

4| Photolithography is often conducted in a clean room. In a typical procedure, a thin layer of a photoresist is deposited on a silicon wafer by spin-coating and then exposed to UV light through the photomask. The exposed region becomes either more (for positive tone) or less (for negative tone) soluble in a developing solution. In either case, the pattern on the photomask is faithfully transferred into the photoresist film. Depending on the photoresist and aligner (the device used to control the exposure of photoresist film to UV light), one has to follow the specific protocols that have been optimized for spin-coating, UV exposure, and film developing. For details, refer to the resource at http://www.cnf.cornell.edu/cnf_processes.html or the information offered by the commercial supplier of the photoresist. Every user of a clean room needs to go through an extensive training session before he/she can access the facility.

PAUSE POINT Clean rooms are largely developed and used by the semiconductor industry to manufacture microelectronic chips. In a clean room, air is filtered to exclude dust particles that can interfere with the production of chips, and both temperature and humidity are controlled to ensure stable performance for the materials. In academia, a clean room is usually located in a

facility associated with an engineering program. For users in the United States, access to such a facility is available through the NNIN.

5| E-beam lithography is required when the pattern on a master contains structures smaller than 1 μm in lateral dimensions. EBL works differently from photolithography as no mask is required and the pattern is directly written as latent images in an e-beam resist film by focusing a beam of electrons at the resist and moving across the surface. An SEM system or a dedicated electron beam writer is needed, and the resist can be PMMA (positive tone) or SU-8 (negative tone). After developing, the latent images become permanent relief structures in the resist film. EBL can be performed outside a clean room and the typical procedure includes *i*) design of the pattern using AutoCAD or DesignCAD programs; *ii*) cleaning of substrate; *iii*) spin-coating of e-beam resist; *iv*) pre-baking of resist; *v*) e-beam writing using an e-beam writer or a typical SEM equipped with nanometer pattern generation system (NPGS) capability; and *vi*) development of resist; and *vii*) post-baking of resist. The user also needs extensive training. For details, refer to the EBL resource at http://www.cnf.cornell.edu/cnf_processes.html.

PAUSE POINT Access to EBL and related tools is also available at a number of multi-user facilities incorporated in the NNIN. EBL is a process that requires expensive equipment and an experienced operator.

Surface modification of the master

6| To make the surface of the master "non-stick" and avoid adhesion to the PDMS, place the master in a Petri dish and then in a dessicator, together with a smaller Petri dish containing a few drops of TFOCS (Figure 3). Connect the dessicator to the vacuum line of the fume hood and

evacuate the air from the dessicator – this process allows TFOCS to vaporize and deposit as a monolayer on the master through siloxane bonding. Allow 30 min for deposition with applied vacuum and continuous flow of TFOCS from dessicator to vacuum line. Upon completion, turn off vacuum, vent the dessicator to air in the fume hood, remove the master, and test the hydrophobicity of the master with a drop of water. If the modification by TFOCS is successful, the water drop should not spread on the surface of the master. Before moving to the next step, completely remove any water that may result from the hydrophobicity test in a nitrogen stream.

CAUTION Make sure to place the dessicator in the fume hood as TFOCS is corrosive and toxic.

PAUSE POINT Do not clean the master with any organic solvent such as ethanol, acetone, or isopropanol alcohol (IPA), because these conventional solvents can dissolve (at least, partially) the patterned relief structures of the resist on the master. If necessary, it is safe to rinse the master with deionized water, followed by complete drying in a nitrogen stream.

CRITICAL STEP The TFOCS vapor should transform the surface of a master from hydrophilic to hydrophobic; this modification prevents the cured PDMS from adhering to the master. Step 6 is required for any newly fabricated master. Once the modification is done, the master can be used to fabricate many copies (at least 50) of PDMS stamps without additional surface treatment.

Fabrication of the elastomer stamp

The mechanical properties of an elastomeric stamp are critical to its ability to transfer a pattern with high fidelity. In principle, any elastomer can be used to cast the stamp although most work has focused on the silicone rubber or cross-linked PDMS. Among various types of commercial PDMS, Sylgard 184 from Dow Corning has been most commonly used for fabrication of stamps with feature sizes larger than 500 nm. Composite stamps consisting of two layers – a flexible

layer (Sylgard 184) supported by a hard layer (*h*-PDMS) – can extend the capabilities of soft lithography down to the 50-100 nm regime[40]. Figure 4 outlines a procedure for fabricating Sylgard 184 and *h*-/184 PDMS composite stamps from masters with relatively low relief structures.

Fabrication of Sylgard 184 PDMS stamp

Figure 5 shows photographs of four major stages involved in the fabrication of a PDMS stamp from Sylgard 184. Sylgard 184 is a thermally curing elastomer, which comes as a two-component kit consisting of a base part and a curing agent part.

7| Take a common plastic cup and fill it with ten parts of base and one part of curing agent by weight. Weighing can be carried out on a Mettler Toledo balance (BD202). When a Petri dish of 75 mm in diameter is used (see Step 9), one needs to weigh 70 grams of base and 7 grams of curing agent in order to obtain PDMS stamps of 10 mm thick. The mass ratio can be controlled to adjust the softness of cured elastomer – for example, 5:1 for stiffer PDMS and 20:1 for softer PDMS.

PAUSE POINT It is not necessary to weigh the base and curing agent with accuracy greater than 5% as a small variation in the amount of either component will not affect the performance of resultant PDMS stamps.

8| Use a plastic spoon or a few sticks of wood Q-tips (Cotton Tip Applicator 6" Q-Tips) to thoroughly mix the base and curing agent for at least 3 min. Air bubbles are generated in the

mixing process but they can be easily removed in the next step.

CRITICAL STEP Make sure the two components are completely mixed. Incomplete mixing may affect the curing behavior, homogeneity, and mechanical properties of the resultant PDMS.

9| Place the master on a Petri dish and pour the mixed PDMS liquid (with trapped bubbles) to the desired thickness. Remove the bubbles by placing the Petri dish in a dessicator and connect to the vacuum line of a fume hood for 15 min or until most of the bubbles are gone.

10| Turn off vacuum, vent dessicator, and place the Petri dish containing both master and PDMS in an oven set to 60 ^{o}C and cure it for 12 h. The PDMS can also be cured at room temperature, but it may take up to 48 h. Figure 5A shows a photograph of the cured PDMS.

11| Break the side of the Petri dish with a plier and separate the block of PDMS/master from the Petri dish carefully. Use a scalpel or razor blade to remove the thin layer of PDMS on the backside of the master. Peel the PDMS stamp off the master carefully (Figure 5B). If the stamp contains periodic structures with lateral dimensions in the range of 1-10 μm, one can quickly inspect the quality of the stamp by viewing the diffraction color from an angle (Figure 5C). With the patterned side facing up, use a razor blade or scalpel to cut the PDMS block into stamps with the desired dimensions for different applications (Figure 5D).

CRITICAL STEP Use a <111> rather than <100> silicon wafer to fabricate the master. Otherwise, the master can be easily broken during the preparation of a PDMS stamp.

PAUSE POINT One can typically use a master to fabricate 50 or more PDMS stamps. Keep the master in a covered Petri dish or other containers to avoid contamination of its surface by dust

particles.

Fabrication of *h*-/184 PDMS composite stamp

"Hard" PDMS or *h*-PDMS is based on vinyl and hydrosilane end-linked polymers with a higher modulus (~9 N/mm^2) than Sylgard 184 PDMS (~2 N/mm^2). A typical composite stamp is composed of two layers – a stiff layer (30-40 μm thick *h*-PDMS) supported by a flexible layer (3-5 mm thick Sylgard 184 PDMS). Note that composite stamps were developed to deal with masters containing relief structures <500 nm in dimensions. Such masters are typically fabricated using EBL, and they are usually smaller in area (1 cm^2) than those fabricated using photolithography (>40 cm^2)

12| Follow Steps 7 to prepare a mixture of 50 grams of Sylgard 184 base and 5 grams of curing agent.

13| Use a stick of wood Q-tip to mix 3.4 gram of (7-8% vinylmethylsiloxane)(dimethylsiloxane) copolymer with two drops of 1,3,5,7- tetramethylcyclotetrasiloxane and one drop of platinum divinyltetramethyldisiloxane in a Petri dish. This amount of material is adequate to coat a master with a surface area of 1 cm^2.

14| Remove bubbles by placing the Petri dish in a dessicator and connect to the vacuum line of a fume hood for 5 min or until most of the bubbles have disappeared. Turn off the vacuum, vent the dessicator, and take out the Petri dish.

15| While the bubbles are being removed in the previous step, adjust the parameters of a spin-coater to 500 rpm for 5 sec, followed by 1,000 rpm for 40 sec.

CRITICAL STEP It is important to set up the spin-coater to a ready-to-run condition before you proceed to Step 16.

16| Add 1 gram of (25-30% methylhydrosiloxane)(dimethylsiloxane) copolymer to the mixture in Step 14 and mix quickly.

CRITICAL STEP The new mixture will solidify within 5 min, giving very little time to remove the bubbles as described in Step 14. Proceed to Step 17 immediately and the bubbles can be removed during the spin-coating process.

17| Load a master to the spin-coater, pour the mixture prepared in Step 16 onto the master, and turn on spinning immediately.

CRITICAL STEP If the mixture prepared in Step 16 has solidified, it is necessary to prepare a new mixture by repeating Steps 13-16.

18| Place the coated master in a Petri dish and bake in an oven at 60 ^{o}C for 10 min.

19| Pour the mixture of Sylgard 184 prepared in Step 12 onto the *h*-PDMS layer, followed by Steps 9-11.

Microcontact printing (μCP)

Figure 6 shows photographs of three major steps involved in microcontact printing (μCP), by

which an "ink" – typically an alkanethoil (a compound containing an -SH group) – is transferred from PDMS to a surface defined by a thin film of gold or silver supported on a silicon wafer or glass slide. Conformal, molecular/atomic-level contact between a PDMS stamp and the surface of a substrate is the key to successful transfer of ink molecules from stamp to substrate. Microcontact printing is widely used in printing alkanethiols on thin films of gold[10], silver[42], palladium[43], and platinum[44], and to a smaller extent (and with more difficulties) of alkylsiloxanes on silicon/silicon dioxide or glass[45]. SAMs formed on these surfaces during a printing process. SAMs are ordered molecular assemblies that form spontaneously via chemisorption of functionalized long-chain molecules on the surface of appropriate substrates[22]. The surface properties of a SAM can be controlled by modifying the end group (the functional group distal to the surface); a major use of μCP is to pattern organic groups useful in molecular recognition of biomolecules or of adhesive proteins for use in the cell biology[46]. Microcontact printing has been used to pattern arrays of proteins on silicon or glass substrates[47]; μCP (using a stamp fabricated in gels rather than PDMS) can also be used to print bacterial[48] or mammalian cells[49] directly.

Microcontact printing of hexadecanethiol

20| Prepare gold or silver substrates using a thermal (or e-beam) evaporator that is often available in a conventional microfabrication facility.

21| Prepare a HDT solution in ethanol (~2 mM).

22| Apply the solution of HDT (with a cotton Q-tip) as a thin film (the amount transferred is not critical, since the ethanol will evaporate, and the HDT can dissolve in the PDMS) to the PDMS

stamp and dry the stamp in a stream of nitrogen for ~30 sec.

CRITICAL POINT To handle the stamp easily with fingers, the thickness of the stamp should be set to the range of 0.5 to 1.0 cm.

23| Bring the PDMS stamp into contact with the gold or silver surface for ~10 sec (Figure 6, left and middle).

CRITICAL STEP Avoid trapping air bubbles between the stamp and the substrate by initiating physical contact between these two surfaces from the edge with an angle.

24| Remove the PDMS stamp from the surface vertically without dragging (Figure 6, right).

CRITICAL POINT If needed for other experiments, one can fill the bare regions of the surface with a second SAM simply by dipping the printed substrate into an ethanol solution (~2 mM) of another alkanethiol terminated in a different end group for a period of 1-2 min.

25| Immerse the substrate patterned with HDT monolayers in a ferricyanide ething solution to selectively remove the unprotected region of gold or silver under magnetic stirring (300 rpm) at room temperature[37]. For silver films of 20 nm thick, the etching time is around 7 min. Figure 7 (top) shows SEM image of a typical example – an array of silver disks 2.5 μm in diameter and separated by 2.5 μm.

Microcontact printing of proteins

26| Prepare an IgG solution with a concentration in the range of 50 μg/mL to100 μg/mL in phosphate-buffered saline (PBS).

27| Immerse a PDMS stamp into the IgG solution, incubate for 45 min at room temperature, and then remove it.

28| Rinse the PDMS with PBS and DI water three times and dry the stamp under a stream of nitrogen gas.

29| Follow Steps 23 and 24. Figure 7 (middle) shows an optical fluorescence micrograph of a typical example – an array of green-labeled IgG dots of 10 μm in size. The protein was probably denatured to an uncharacterized extent.

CRITICAL POINT Use a fresh stamp each time to achieve well-resolved patterns. When having problems to print certain proteins other than IgG directly, one can use a BSA solution in PBS (100 μg/mL) as an "ink" to print patterned BSA on a glass substrate, followed by filling the bare regions of the surface with a solution of the second protein. There may be some exchange and/or mixing between the second protein and the printed BSA.

Molding and embossing for polymer nanostructures

Replica Molding (REM) consists of three steps[11]: *i*) creating a topographically patterned master; *ii*) transferring the pattern on master into PDMS by replica molding; and *iii*) transferring the pattern in the PDMS back into a replica of the original master by solidifying a liquid polymer such as UV curable polyurethane (PU), or thermally curable epoxy, against the PDMS mold. REM offers a number of advantages over traditional fabrication process at the nanoscale such as EBL: *i*) REM can produce many copies (>50) of molds, replicas, and patterned surfaces from

each master with a possible resolution at the atomic level[50]; *ii)* REM can work with a wide range of polymers other than e-beam sensitive materials*; iii)* REM allows patterning over large areas rapidly, not limited to a serial process defined by EBL; and *iv)* REM can be applied to transfer patterns to non-planar surfaces[51].

Solvent-assisted micromolding (SAMIM)[14] uses an elastomeric mold, a polymer film, and a solvent that dissolves in and softens that film to emboss pattern in the film. Typically, a PDMS mold is wetted with a liquid that is a good solvent for the polymer, and is brought into contact with the surface of the polymer. The solvent dissolves a thin layer of the polymer, and the resultant fluid comprising polymer and solvent conforms to the surface topology of the mold. While the mold is maintained in conformal contact with the substrate, the polymer solidifies as the solvent dissipates by dissolving in the PDMS and by evaporation to form relief structures with pattern complementary to that on the surface of the PDMS mold. SAMIM forms patterned structures in a polymer under ambient conditions with soft molds rather than at elevated temperatures with rigid molds. As such, the process shares characteristics with both replica molding and embossing.

REM of microfluidic devices in PDMS

30| Use photolithography to fabricate a master (Step 4), with a photomask printed on a transparency film.

31| Make a PDMS replica of the master in Sylgard 184 (Steps 7-11).

32| Place the PDMS replica (with the patterned surface facing up) and a silicon or glass substrate

in a plasma cleaner for oxidation at 200 mTorr of oxygen gas and 40 W of power for 1 min.

CRITICAL POINT This treatment generates silanol group (Si-OH) on the surface of the PDMS replica due to oxidation.[27] Surface-oxidized PDMS can seal to PDMS, glass, silicon, polystyrene, polyethylene, or silicon nitride, providing that these surfaces have also been exposed to oxygen plasma.

33| Place the side of the PDMS patterned with channels on the silicon or glass substrate to form a conformal contact.

34| Place the device in an oven at 60 ^{o}C for 1 h to strengthen the adhesive seal (probably by dehydration: Si-OH + HO-Si → Si-O-Si + H_2O).

35| Use a Harris Micro-Puncher to punch holes in PDMS that will serve as "inlets" and "outlets" for introducing and recovering fluids (e.g., samples, reagents, or buffers), respectively. Insert PE-60 polyethylene tubes into the inlets and outlets. The other end of the inlet tube is then connected to a 21-gauge syringe needle for sample injection. This procedure allows one to couple syringes and syringe pumps to microfluidic channels.

CRITICAL POINT The holes punched in PDMS should be slightly smaller than the outer diameter of the tube. When the tube is inserted, it exerts pressure on the PDMS and makes a waterproof seal. The tube can be removed and replaced with minimal effort.

REM of polyurethane and epoxy nanostructures

36| Use EBL to fabricate a master (Step 5).

37| Fabricate an *h*-/184 PDMS composite stamp (or mold, in the case of molding rather than printing) following Steps 12-19.

CRITICAL POINT To facilitate separation in Step 42, control the total thickness of the PDMS mold in the range of 0.2 to 0.5 cm.

38| Place a drop of UV-curable PU or thermally curable epoxy on a glass or silicon substrate.

CRITICAL POINT Mix the epoxy prepolymer and curing agent thoroughly and place the mixture in a dessicator to eliminate air bubbles by connecting to the vacuum line of a fume hood.

CRITICAL POINT In general, prepolymers with high molecular weights are reasonably insoluble in PDMS. However, small compounds such as monomers and curing agents tend to be soluble in PDMS, causing the cured product to stick to PDMS during the molding process.

39| Place the *h*-/184 PDMS mold on top of the drop of prepolymer (with the patterned side facing the prepolymer liquid) and press down with hand to form contact.

CRITICAL POINT If necessary, squeeze out air bubbles by tapping the PDMS mold with the flat head of tweezers.

40| For UV-curable PU, cure under UV light and at room temperature for 30 min. For epoxy, cure at room temperature for 15 min and then in an oven at 60 ^{o}C for 30 min. Peel off the PDMS mold carefully. The PU or epoxy structures should remain on the supporting substrate, provided that the adhesion between them and support is stronger than that between them and the PDMS.

CRITICAL POINT If the curing time is too long, the PU or epoxy structures will stick to the PDMS

mold and ruin the patterned surface. If it is too short, the prepolymer may have not been completely cured, which can leave behind some liquid prepolymer on both the PU replica and PDMS mold.

SAMIM of PMMA structures

41| Prepare a 3% wt. PMMA solution by diluting the commercial e-beam resist with anisole. Dispense some of this solution onto a silicon or glass substrate to cover roughly 75% of the surface, and spin-coat at 4,000 rpm for 40 sec to generate a uniform film of 100 nm thick.

42| Bake the PMMA-coated substrate on a hotplate at 180 ^{o}C for 90 sec to completely remove all anisole trapped in the PMMA film. Since anisole has a relatively high boiling point (154 ^{o}C), it tends to evaporate slowly, interfering with the molding process.

43| Apply acetone (2 μL per 1 cm^2 of area) to the patterned surface of an *h*-/184 PDMS mold and bring it into contact with the PMMA film for 5 min.

CRITICAL POINT Acetone is a good solvent for PMMA. Acetone cannot, however, spread on a PDMS surface, and will stay as a droplet on the PDMS mold until the mold is brought into contact with the PMMA film.

44| Bake the PMMA/acetone/*h*-PDMS sample on a hotplate at 60 ^{o}C for 5 min to remove acetone completely.

CRITICAL POINT The PMMA layer is dissolved by acetone upon contact with the PDMS mold and the resultant (probably gel-like) fluid comprising PMMA and acetone conforms to the

surface topography of the mold. While maintained in conformal contact with the substrate, the PMMA solidifies as acetone dissipates, forming relief structures with a pattern complementary to that on the surface of the mold.

45| Remove the PDMS stamp carefully to leave patterned PMMA nanostructures on the surface of the substrate.

CRITICAL POINT SAMIM can generate patterned relief structures over a relative large area (ca. 3 cm^2) within a short period of time (ca. 10 min). However, this process often generates a thin film of PMMA (the residual layer) between the raised features protruding from the substrate. This residual layer prevents the underlying substrate from being attacked by chemical etchants, and must be removed by O_2 plasma etching if further process such as substrate etching or deposition is involved. If necessary, proceed to Step 46 to remove residue layer of PMMA.

46| Place the sample with PMMA replica in a plasma cleaner to etch the surface for 2 min at 150 mTorr of oxygen gas and 40 W of power.

ANTICIPATED RESULTS

Figure 7 shows three typical examples of micro- and nanostructures fabricated using soft lithography: (top) an SEM image of silver disks patterned using μCP, followed by wet etching in a ferricyanide solution; (middle) a fluorescence optical micrograph of IgG dots directly printed on a glass substrate with a PDMS stamp; and (bottom) an SEM image of gold disks obtained by lift-off with the PMMA template (see the inset) fabricated using SAMIM.

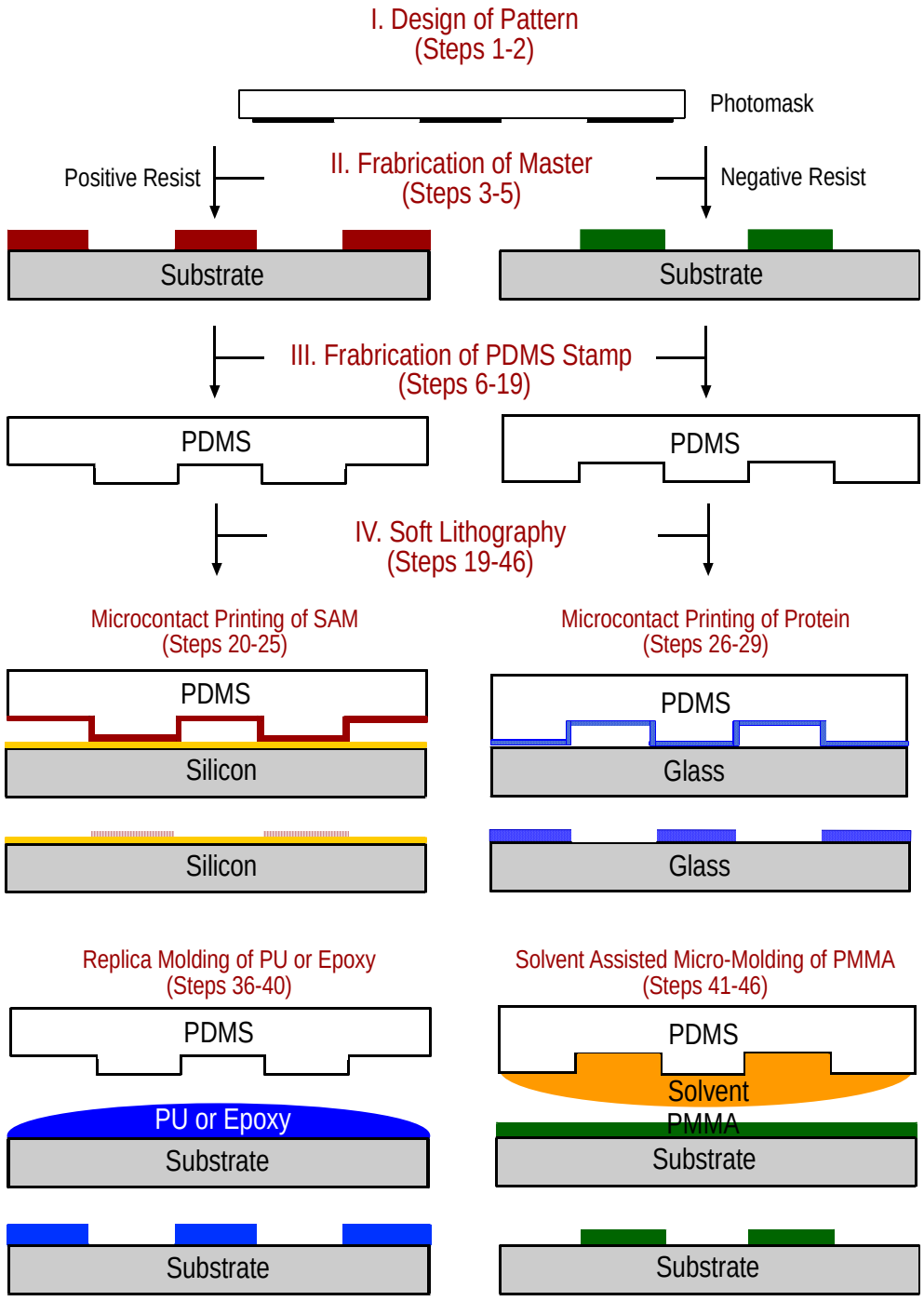

Figure 1 | Schematic illustration of four major steps involved in soft lithography and three major soft lithographic techniques.

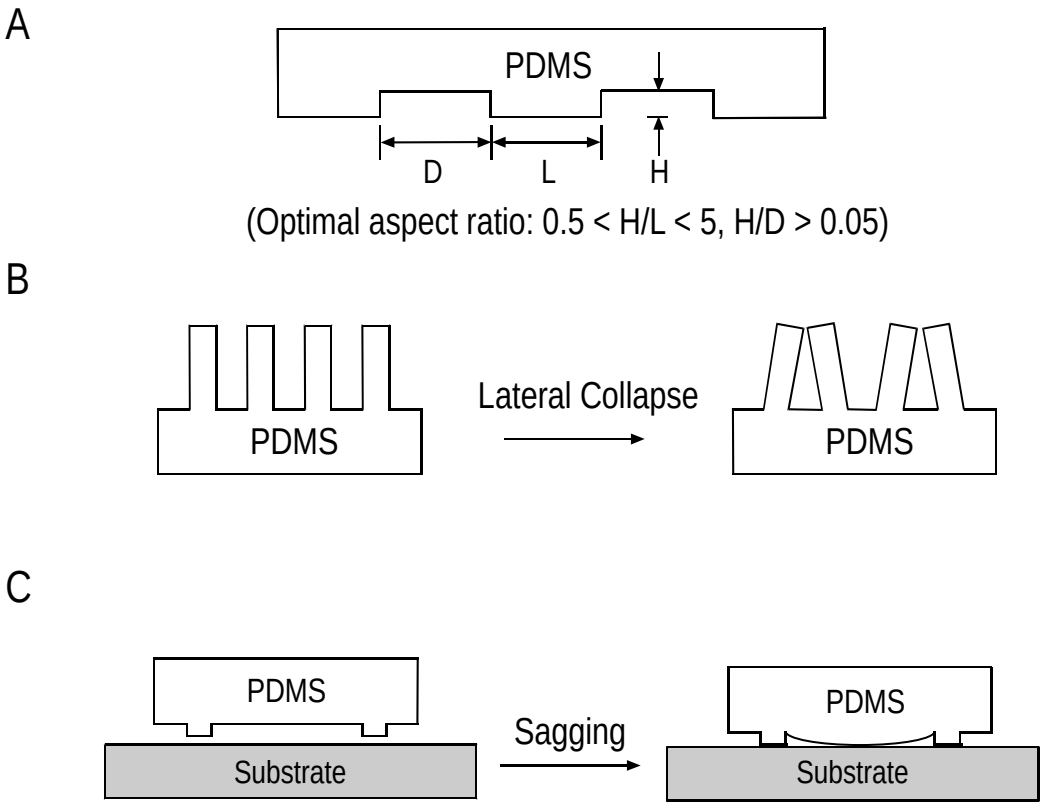

Figure 2 | Schematic illustration of two possible problems that may arise from the softness of an elastomer: (B) lateral collapse of relief structures (or commonly known as "pairing") with aspect ratios H/L>5; and (C) sagging of recessed structures with aspect ratios H/L<0.5.

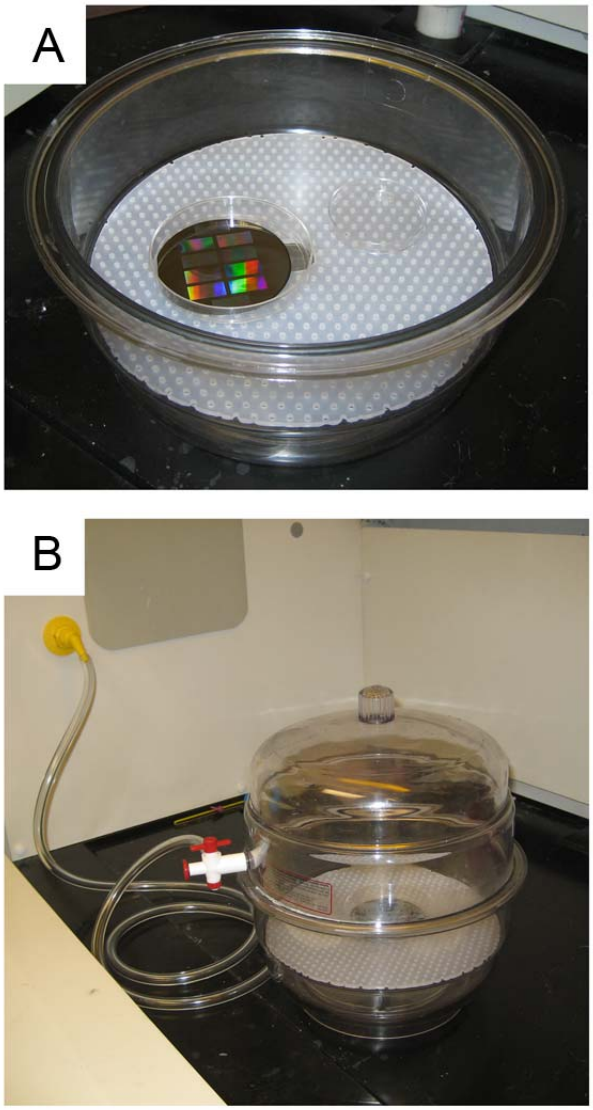

Figure 3 | Photographs of the dessicator used to treat the surface of the master with TFOCS vapor. A few drops of TFOCS liquid were placed in the small Petri dish (A) before the dessicator was capped and connected to the vacuum line (B). Because the master contained periodic arrays of lines and dots 5 μm in size, one can clearly see the diffraction colors from the surface.

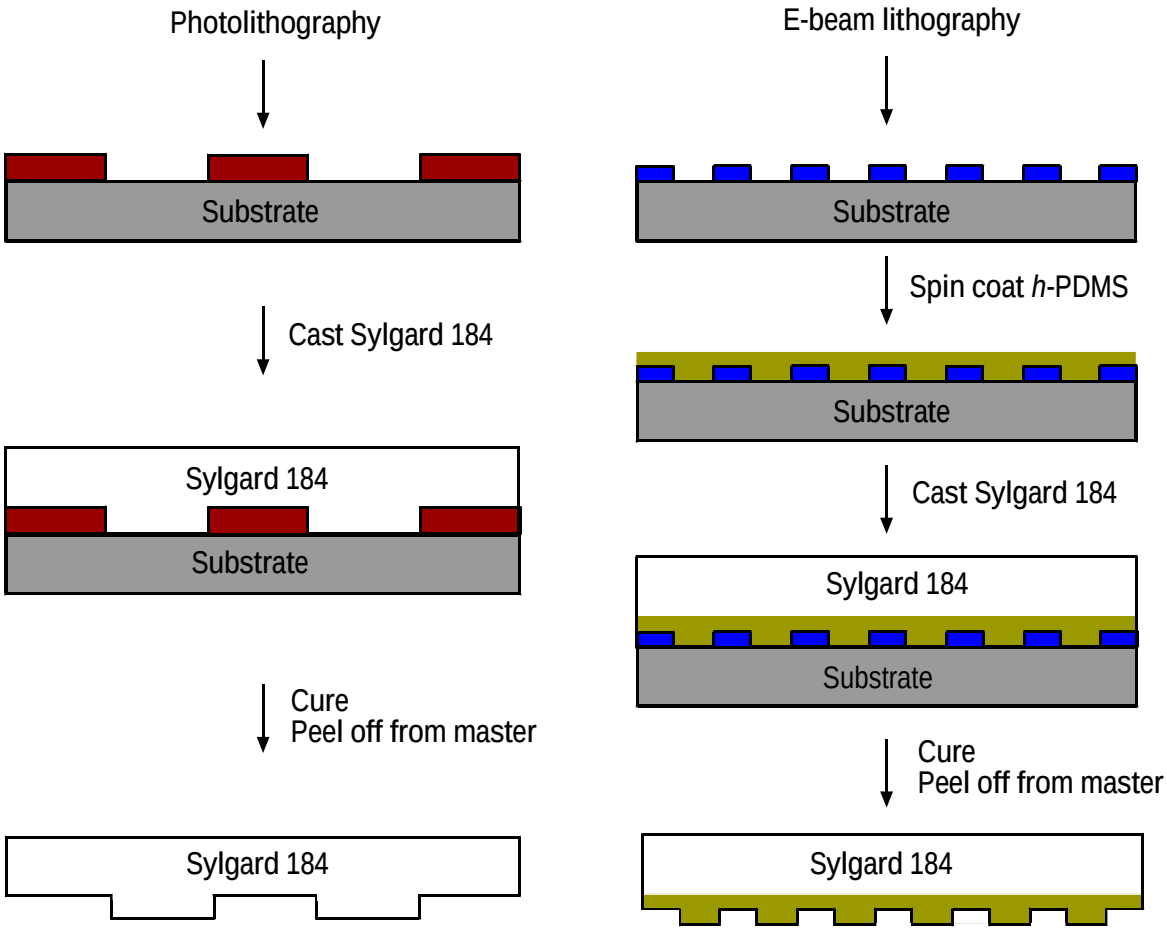

Figure 4 | Schematic illustration of the procedures for fabricating normal PDMS and *h*-/PDMS stamp, respectively. The addition of a thin layer of hard PDMS allows one to work with features <500 nm in lateral dimensions.

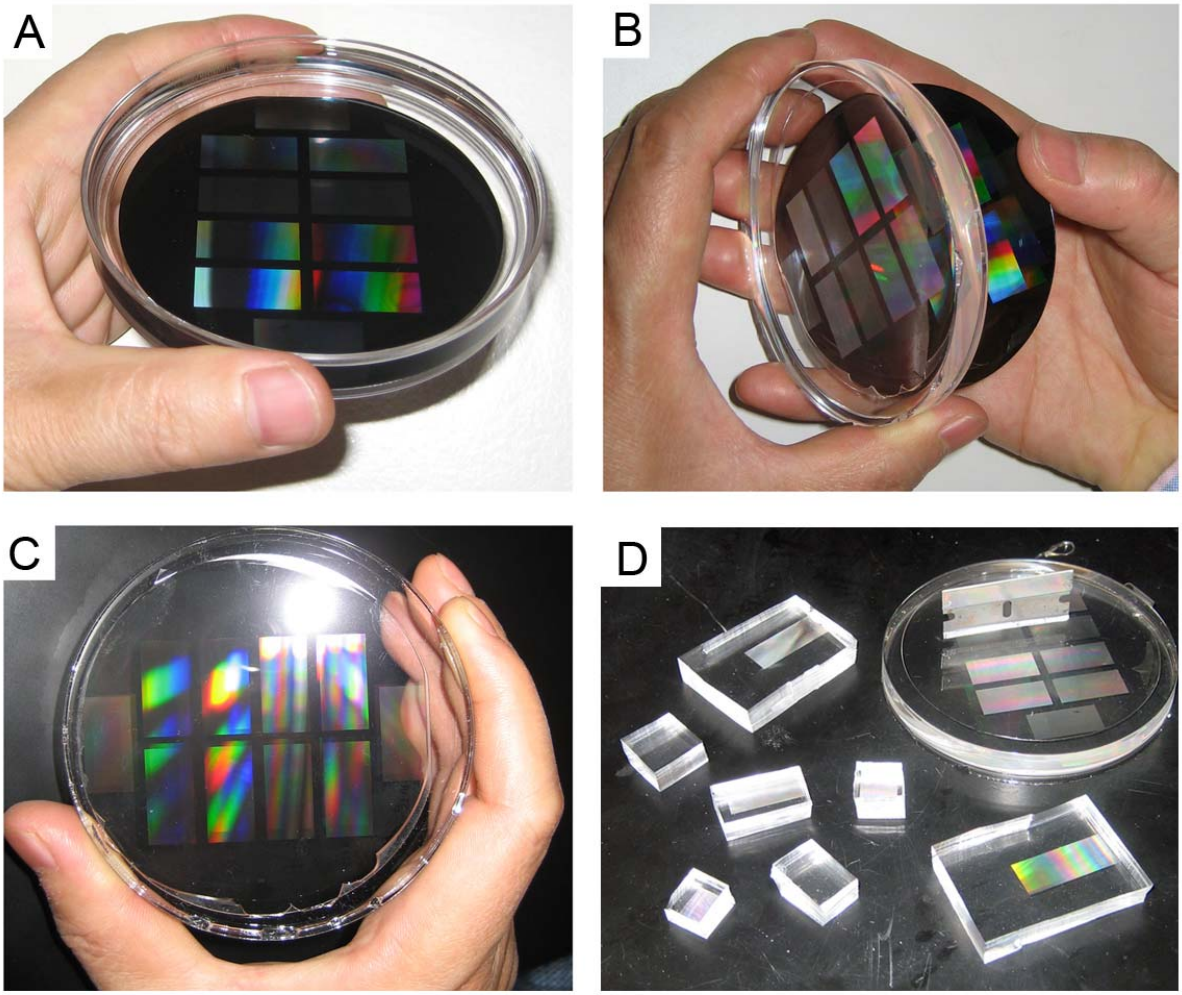

Figure 5 | Photographs of four major stages involved in the fabrication of PDMS stamps: (A) curing a liquid prepolymer to PDMS on top of the master placed on a Petri dish; (B) separating the cured PDMS block from the master; (C) viewing the diffraction colors from an oblique angle; and (D) cutting the PDMS block into smaller pieces (stamps) using a razor blade. When the pattern contains periodic structures with feature sizes in the range of 1-10 μm, one can easily see diffraction colors (as shown in A-C) from the surface of the master or stamp.

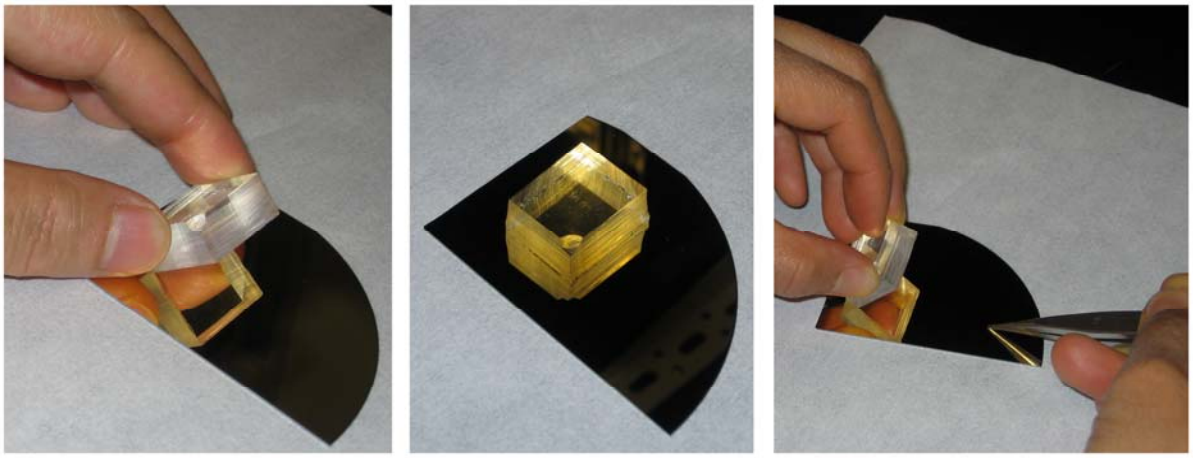

Figure 6 | Photographs of three major steps involved in μCP of alkanethiol on gold, which is evaporated as a thin film on a silicon substrate with 5-10 nm titanium (for e-beam evaporation) chromium (for e-beam or thermal evaporation) as the adhesion layer: (left) initiating contact between the PDMS stamp and the gold surface from the edge with an angle to avoid trapping air bubbles between them; (middle) leaving the stamp in contact with gold for 10 sec; and (right) separating the stamp from the gold surface.

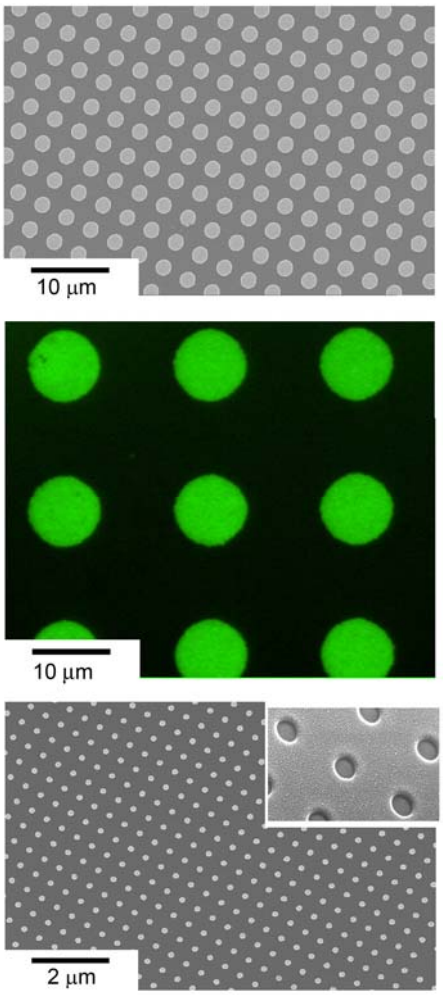

Figure 7 | (top) SEM image of an array of silver disks fabricated using μCP of HDT, followed by selective wet etching in a ferricyanide solution. (middle) Fluorescence optical micrograph of an array of IgG dots fabricated using μCP. (bottom) SEM image of an array of gold disks (100 nm in diameter) fabricated using SAMIM, followed by gold evaporation and lift-off. The inset shows SEM image of the pattern in PMMA fabricated using SAMIM.

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