Critical Reviews in Biotechnology, 26:237–259, 2006

ISSN: 0738-8551 print / 1549-7801 online
DOI: 10.1080/07388550600978358

informa
healthcare

Bio-Microarray Fabrication Techniques–A Review

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ABSTRACT Microarrays with biomolecules (e.g., DNA and proteins), cells, and tissues immobilized on solid substrates are important tools for biological research, including genomics, proteomics, and cell analysis. In this paper, the current state of microarray fabrication is reviewed. According to spot formation techniques, methods are categorized as "contact printing" and "non-contact printing." Contact printing is a widely used technology, comprising methods such as contact pin printing and microstamping. These methods have many advantages, including reproducibility of printed spots and facile maintenance, as well as drawbacks, including low-throughput fabrication of arrays. Non-contact printing techniques are newer and more varied, comprising photochemistry-based methods, laser writing, electrospray deposition, and inkjet technologies. These technologies emerged from other applications and have the potential to increase microarray fabrication throughput; however, there are several challenges in applying them to microarray fabrication, including interference from satellite drops and biomolecule denaturization.

KEYWORDS DNA microarray, contact pins, contact printing, inkjet, microstamps, non-contact printing, photolithography, protein microarray

1. INTRODUCTION

Deoxyribonucleic acid (DNA) and protein microarrays, also called biochip microarrays, have accelerated the process of understanding gene and protein function in living organisms. Microarrays provide molecular signatures for cells, tissues and disease states that can be used for disease diagnosis, prediction, prevention, and drug discovery. A microarray is a two-dimensional arrangement of specific biological probes (e.g., DNA or protein molecules) deposited in an addressable fashion on a glass slide or other substrates (e.g., polymer-coated glass, plastics, nitrocellulose). The size of the glass slide is usually one by three inches, with thousands of isolated biological probes ranging from 50 to 300 μm in diameter arrayed on the surface.

DNA microarrays are widely used to measure gene expression levels following the outlined procedure. A DNA or ribonucleic acid (RNA) sample, representing

the pool of expressed genes isolated from biological sources, is first amplified and labeled with a fluorescent dye. The sample is then hybridized with a DNA microarray to produce double stranded molecular structures made up of probes attached to the microarray chip surface and their complimentary targets present in the solution. To obtain information about the target solution, the chip is then rinsed to remove non-specifically bound target molecules, and evaluated using a laser scanner. The resulting fluorescent image reveals positions of probes where hybridization was successful, and therefore identifies targets present in the original solutions. The intensity of each spot is indicative of the relative expression of that particular gene; thus, in a single experiment, it is possible to simultaneously analyze an entire genome. DNA microarrays can also be used for genotyping or detecting subtle sequence variations, which may be used for disease diagnosis, evaluation, and drug development. In these applications, DNA microarrays must be versatile in sequence design and easy to fabricate in a reproducible manner.

While DNA microarrays are effective in measuring the levels of mRNA expressed in a cell, they cannot directly measure the amounts of the proteins that are transcripted. This has led to the development of protein microarrays for screening protein-protein or protein-ligand (DNA, lipid, drugs) interactions, which can be applied to evaluating and diagnosing disease susceptibility and progression, and for discovering potential therapeutic targets faster and more accurately.[51,52] Protein microarrays use similar concepts and principles for DNA microarrays, though physicochemical differences between DNA and protein necessitate different sample handling practices. For example, to maintain function and binding capacity, proteins must maintain their fragile three-dimensional structures throughout the fabrication process. Therefore, printing and immobilization of proteins on a substrate requires much more care than DNA. In contrast to nucleic acid molecules, which by nature form highly specific interactions with complementary molecules, protein molecules do not, in general, have such specific interactions. For this reason, most protein microarrays are created from a small subset of proteins and antibodies that form specific strong interactions with target molecules. Another challenge for protein microarray fabrication is sample preparation. Isolation, purification, and synthesis of proteins are more difficult and expensive than for nucleic acids. Finally, and perhaps most importantly, proteins cannot be easily amplified like nucleic acids, using polymerase chain reaction.[90] Despite these drawbacks, protein microarrays have shown promise for parallel analyses of protein functions and in genomic research.

In addition to DNA and protein microarrays, biomicroarrays also include cell microarrays[17,80,90] and tissue microarrays.[34,60] Cell microarrays combine well-established methods for cellular investigation with the high-throughput screening capabilities of microarrays. They can be used in drug screening, *in vitro* toxicology testing and in functional genomic studies. Tissue microarrays, formed by embedding biopsies of donor tissue blocks on a substrate, are used for phenotypic analyses (e.g., immunohistochemistry). By monitoring the expression of molecular markers in these arrays, many pathological characteristics can be determined (stage and progression of the disease).

Although there are several general reviews of biochip microarrays,[14,25,67] we focus here on the fabrication (or printing) techniques used to form two-dimensional arrays of probe molecules (DNA and proteins). Microarray fabrication is inherently a biological fluid dispensing process.[56] Nanoliter drops of biological solutions are deposited in an addressable arrangement of spots on a substrate surface. The spots must be homogeneous and dense, yet spatially discrete.

Table 1 summarizes the types of printing techniques and presents the organization of this review. Printing methods can be broadly categorized into contact and non-contact printing methods. During contact printing, a printing device comes in physical contact with the substrate while depositing biological samples. In contrast, non-contact printing involves no physical contact between the device and the substrate (e.g., photolithography, ink-jet printing, and laser writing). Each of these array fabrication techniques can be sub-classified as serial or parallel. In serial deposition, serially repeated movements of the printing device limit fabrication throughput. Hence, parallel deposition techniques are a better choice for large-scale fabrication; however, most of the parallel techniques for depositing biomolecules are newer and thus, less developed than serial techniques.[59]

All microarray fabrication techniques target the same objective: efficient deposition of uniform, dense arrays of small droplets of probe molecules. In addition, a technique should minimize cost and the required volume of solution, while preventing solution contamination

TABLE 1 Microarray printing techniques

Contact		Non-Contact	
Serial	Parallel	Serial	Parallel
Solid/Split Contact Pins	Microstamps	Dynamic Controlled Pins	Photochemical Printing Electro-Printing
Nano-Tips		Laser Writing	Inkjet/Nozzle Printing Electrospray Deposition

and biomolecular damage. This review analyzes the capabilities and limitations of conventional and emerging technologies for microarray fabrication.

2. CONTACT PRINTING TECHNIQUES

Contact printing methods are used to form arrays by means of direct contact between the printing device and the substrate. As shown in Table 1, contact printing technologies employ solid pins, split pins, nano-tips, and microstamps. One of the first approaches used for microarray fabrication was contact printing with a single pin, which evolved into methods relying on an array of pins. While pin printing is a serial deposition method, microstamps are used for depositing a large number of proteins or DNA molecules in a parallel fashion. Nano-tip printing is the most recent technology based on Scanning Probe Microscopy (SPM) and yields arrays with submicron spots.

2.1 Pin Printing

Pin printing is a widely used technique for microarray fabrication. Accurate quantitative analysis of printed microarrays is only possible if spot uniformity (i.e., spot-to-spot size and shape repeatability) and positional accuracy are achieved.[88] Spot uniformity is primarily determined by the sample viscosity, pin contact area, pin surface properties, substrate surface properties, and substrate planarity. Additional factors include pin velocity, the precision of robotic controls, and environmental control of humidity, temperature, and contamination. A high pin velocity (>2 cm/s) can induce high inertial forces that drive large sample volumes out of the pin, making the size of spots very large.[72] However, inertia typically does not play a large role in pin printing. Pin printing is governed by the surface tension of the solution, and the wettability of the solution on the substrate. Maintaining a high, stable humidity prevents the sample from evaporating from the wells and pin channels. Temperature affects the sample viscosity and therefore, the dispensed volume. Contamination and dust must be controlled if high-quality arrays are to be produced with minimal risk of split pin clogging.

2.1.1 Pin Design

The first microarrays, developed in Pat Brown's lab at Stanford University, were fabricated by contact printing with pins.[74,77,86] This technique continues to be widely used for most non-commercial microarray fabrication. A key feature of this method is the pin design. Figure 1 depicts designs that are currently used for microarray contact printing. The simplest design (Figure 1(a)) is a solid pin (i.e., no slit). The first solid pins had convex tips while the solid pins used today have flat tips with a precisely controlled diameter or concave tips for more efficient printing.[47] Other designs (Figure 1(b)–(e)) incorporate splits (or "gaps") with various shapes, and diameters ranging from 60 μm to 200 μm. The most complex design (Figure 1(e))[86] has a screw to adjust the gap distance. Pins are more difficult to manufacture as the

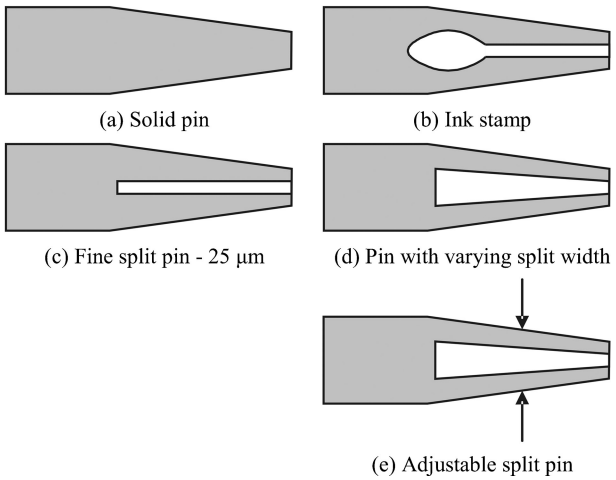


FIGURE 1 Printing pins. (a) Schematic of solid pin. (b) Schematic of ink-stamp. (c, d, e) Schematics of different split pins.

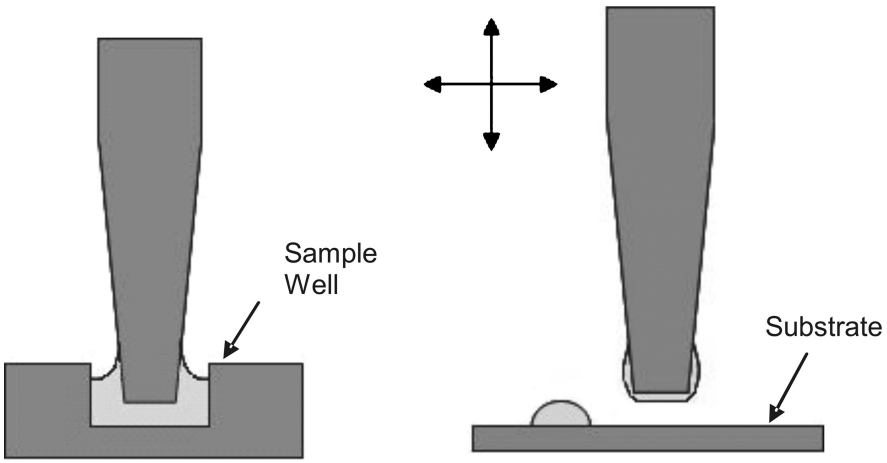


FIGURE 2 **Solid pin printing process. (a) Solid pin is loaded with solution from well plate by capillary force action. (b) Spot printing with a single load.**

pin diameter shrinks. The most commonly used contact printing tips used today are "Ink stamps," developed by TeleChem International, Inc., Sunnyvale, CA.[82]

2.1.1.1 Solid pins

The simplest method of microarray printing is solid pin contact printing. In this method, a solid pin is dipped into a reservoir to load the sample on the tip of the pin (Figure 2(a)). The pin then touches to the substrate surface to deposit the sample (Figure 2(b)). A single sample load is usually sufficient for printing a few spots. If more than a few spots are needed, the pin must be repeatedly moved between the array and the microplate, which slows the process considerably. Therefore, solid pins are usually used for low-density arrays. However, there are a number of key advantages to this system. For highly viscous solutions, which may clog small orifices, solid pin deposition is the only effective method.[89] Additionally, solid pins can be cleaned more easily than other printing methods. The simple design enables robust and reliable printing.

While solid pin printing is a relatively simple method, the construction of solid pins is not trivial. They are commonly manufactured from metals, such as stainless steel, tungsten, and titanium. There are a number of fabrication limitations when using conventional mechanical machining techniques. During machining, a pin experiences stresses that leave it vulnerable to corrosion and deformation.[89] More importantly, mechanical fabrication processes are not capable of forming uniform surfaces, which are essential for uniform drop printing. Machining creates burrs, grind marks, and polish lines that have a significant effect on the pin surface area and adhesive properties. In addition, these processes often leave residual surface impurities and contaminants. Compared to conventional mechanical machining, an alternative, electrochemical micromachining, allows for greater control of tip shape and surface characteristics.[89]

Much of the innovation in solid pin designs has been to develop different tip shapes to improve spot uniformity. For example, Weibel[89] used electrochemical techniques to develop several different tip shapes and surface textures. A particularly interesting design had a very smooth, hydrophobic surface along the side, with an etched, hydrophilic surface on the tip (Figure 3). The spot volume was controlled by texturing the pin differently along the length of the pin. Ito *et al.*[29] developed a complex tip shape capable of forming uniform spots of various diameters. The design features a notched cross in the pin tip, which acts as a small cavity to hold the droplet. After trying a number of shapes, a *w*-shaped notched tip was found to form the most consistent spots. While this design appears as an improvement, reliability was not discussed. It is likely that these pins may be fragile and are susceptible to damage after repeated use.

A unique modification of the solid pin design is the pin and ring spotter.[47] In this design, a large capillary tube is used to load and hold the sample. During deposition, a solid pin is passed through the solution in the capillary, piercing the lower meniscus, to contact the substrate, depositing a drop. This design is capable of efficient spotting, because the solid pin does not have

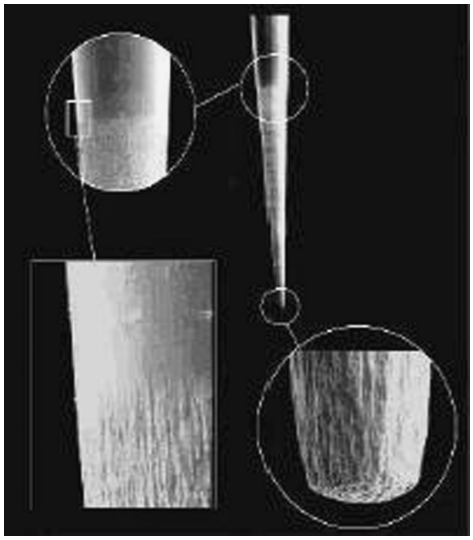


FIGURE 3 **Scanning Electron Microscope (SEM) pictures of solid pin etched textures. "Zonal texturing" increases surface area, which affects spot size and density without changing pin size.[89]**

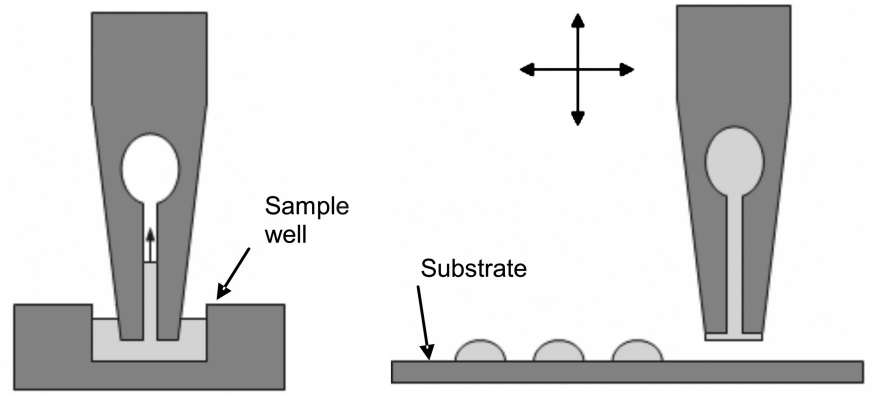
to be cleaned and reloaded between each application. Mace *et al.*[47] also demonstrated that this printing device can produce droplets with consistent diameters. Unfortunately, this system requires significant modification of conventional microarray robotic systems, which limits its utility for widespread immediate use.

2.1.1.2 Split pins

Split pins are the most commonly used printing devices in DNA microarray production. The main advantage of this method over using solid pins is the ability to print many spots serially without having to reload sample, providing higher throughput. Figure 4 shows the microarray printing process using split pins. First, the pins are wetted with biological fluid in wells of a microplate. During this process, the sample is loaded into a fine microchannel in each pin (10 to 100 μm in diameter) by capillary forces. As surface tension forces dominate over inertial forces at the microscale, the gravity effect is negligible. When the pin touches a substrate surface, picoliter-nanoliter volumes of fluid are transferred onto the surface, depending on materials, shapes, and diameters of the pins. To overcome surface tension and drive the fluid onto the tip during deposition, the pin is accelerated and then decelerated as it strikes the surface. Ink stamps,[82] a variation of split pins, do not require tapping force (i.e., contact with the substrate) since the sample solution is already at the pin tip. However, a small tapping force is usually applied to compensate for uneven substrate surfaces and to ensure sample-substrate contact.

When a pin is used to print multiple solutions, it must be washed and cleaned to avoid cross contamination. The load-print-wash process is repeated until the desired number of biological spots is printed on the substrate surface. It has been shown that as substrate surfaces are more hydrophilic, spot size increases by nearly 50% and spot geometry transitions from square to round.[50] The increase in spot size is also proportional to the log of viscosity of the printing solution.[50]

During spot deposition, the tapping force can cause the pin tip to deform; thus, the choice of pin materials is important. Split pins are more prone to deformation than solid pins due to the tip structure. Split pins are commonly constructed from stainless steel, tungsten, or titanium[89] by Electric Discharge Machining (EDM). Differences between pin materials have



(a) Pin loading (b) Solution printing

FIGURE 4 **Split pin printing process. (a) Split pin (ink stamp) is loaded with solution from well plate by capillary force action. (b) Multiple spot printing with a single load.**

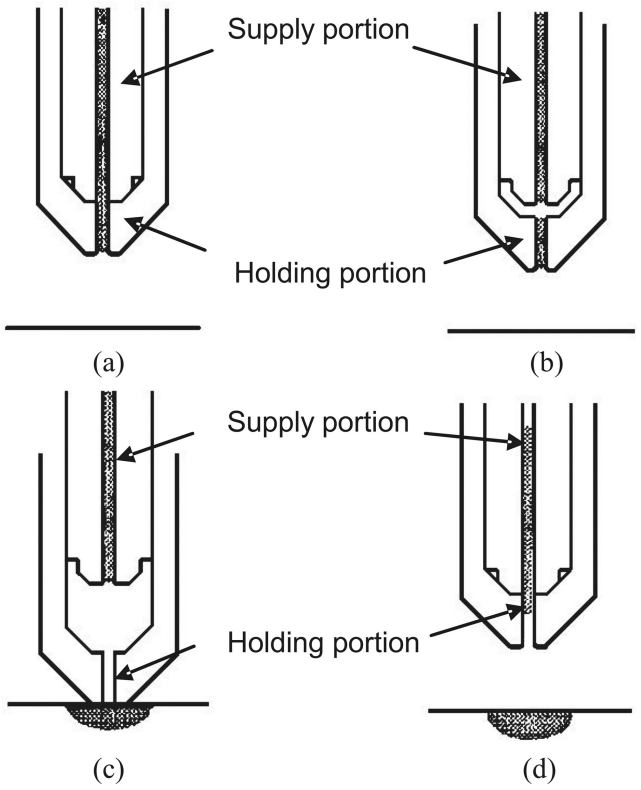


FIGURE 5 **Two-part printing system comprising a pin with a supply section and a holding section. (a) Two parts touch, such that the holding portion is filled with solution from the supply portion. (b) Two parts separate. (c) Pin delivers solution from the holding portion by physical contact. (d) Supply portion delivers new solution load to the holding portion by capillary force action. Adapted from Ito and Tachibana.[28]**

been reported.[86] Titanium is more difficult to machine, which leads to poor slot geometry fidelity, while tungsten carbide is susceptible to shattering, especially at the tip. Ceramics are also used for pin manufacturing. Ceramics provide robustness so that the tip is less susceptible to damage caused by tapping forces. Rose *et al.*[73] patented a split pin printing system consisting of a ceramic tip coated with a hydrophobic film. George *et al.*[18] reported increased consistency in spot morphology printed by ceramic microcapillaries when compared to spots printed by standard stainless steel split pins.

In addition to tip deformation, another shortcoming of split pin technology is clogging by dust particles or contaminants, making it unsuitable for printing high viscosity solutions, such as protein solutions. Wider pin tips are preferable for this technique because smaller tips are more susceptible to clogging. However, as reported,[86] wide slits suffer from spot size irreproducibility. Tip size is often a compromise between spotting accuracy and susceptibility to clogging.

Ito and Tachibana[28] developed a variation on the split pin design, shown in Figure 5. The split pin spots reproducible volumes onto a water-absorbing substrate, such as a nylon membrane. The pin consists of two parts, a supply portion and holding portion. These parts can slide relative to each other so that the supply portion can deliver a small volume of solution to the holding portion by capillary force when the two parts are in contact. Upon separation, the holding part is brought into contact with the substrate, to deliver a sample droplet. Because the volume of the delivered droplet is determined by the size of the holding-portion channel, spot volume is much more consistent than for conventional split pin designs. This design is developed for printing onto a water-absorbing substrate, making it of questionable value for printing onto standard glass slides.

An interesting method for printing biomolecule samples, which resembles split pin printing, was developed by Sheehan *et al.*[78] The Radiograph® drafting pen can deposit spots with diameters ranging from 100 μm to 600 μm. In this system, an array of pens is mounted on a translation stage, enabling simultaneous deposition of tens of spots of DNA solution onto the substrate. As shown in Figure 6, this design features an integrated chamber that can contain significant amounts of solution compared to other pin types. A fine metal wire extends from the tip to the chamber. When the wire is pressed, a gap is formed such that fluid can flow to the tip. This device is simple to implement and relatively inexpensive. Additionally, the spotting solution can be stored in the pen for multiple uses.

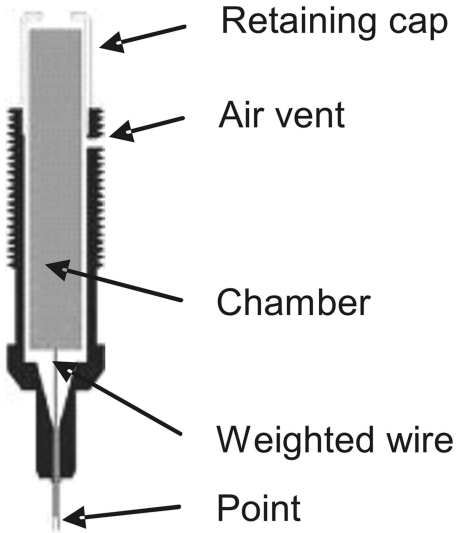


FIGURE 6 **Schematic of Radiograph® drafting pen.[78]**

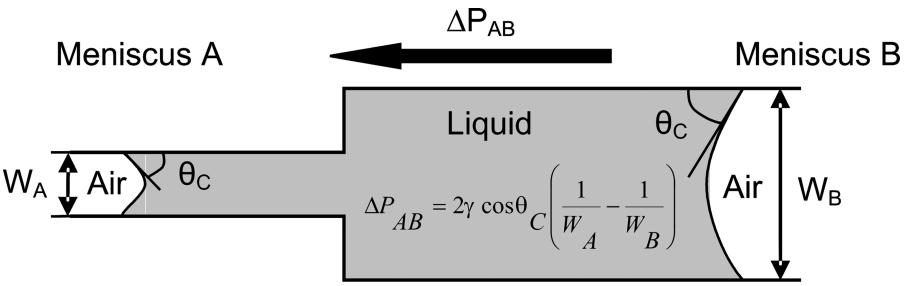


FIGURE 7 Pin with loaded sample. Pressure difference in smaller and bigger menisci drives printing solution towards the pin tip. Adapted from Tsai and Kim.[83]

2.1.1.3 Silicon pins

Recently, silicon pins have been developed as an alternative to conventional metal pins. Silicon presents several advantages over metal, including reproducibility, parallel fabrication, smaller features, and robust material properties. Micromachined silicon pins reduce costs and allow for greater dimensional tolerances and smaller features. For example, silicon channels can be made much narrower than 50 μm, a typical limit for metal pins. These smaller channels produce smaller droplets, which in turn increase spot density and throughput. In addition to dimensional advantages, silicon has outstanding material properties. It is much harder than most metals and is less susceptible to deformation and wearing. Silicon pin arrays are also much lighter than conventional pin arrays. The lighter weight causes less force on the pin tips and the printing surface for improved performance and durability.

Despite the advantages of silicon over conventional materials, it also has several notable disadvantages. Although the mechanical strength of silicon is useful in resisting deformation, these pins occasionally cause fractures in substrate coating, yielding undesirable, doughnut-shaped spots.[50] Furthermore, pin clogging, pin sliding (i.e., movement in the horizontal direction) and the need for preprinting are problematic for pins formed from silicon and metal alike. For these reasons, micromachined silicon pin technology has not yet been widely adopted. However, silicon shows promise for printing systems relying on disposable, low-cost devices.

Tsai and Kim[83] developed a silicon split pin to address the issues of microarray accuracy and repeatability. Using these silicon spotting pins, spots were printed with diameters as small as 16 μm. Additionally, each pin was able to load 1 μl of DNA solution, which doubles the volume of a conventional pin load. The pins were tested in a commercial microarray printing system and $\pm$ 3 μm consistency in spot size was achieved. The pin channel, shown in Figure 7, consisted of two parts with different widths (40 μm and 420 μm), providing constant pressure differences and sample driving forces. The pins were fabricated by deep reactive ion etching (DRIE) on silicon-on-insulator (SOI) substrates and wet release with buffered hydrofluoric acid (Figure 8). The use of SOI allows for a very thin tip while retaining the handle bulk layer for pin handling.

Belaubre *et al.*[7] and Leichle *et al.*[38] developed silicon pins with improved sample loading by means of electrowetting (i.e., electrical enhancement of the surface wettability). Leichle *et al.*[38] fabricated pins with metallic electrodes using SOI wafers. Pins were microfabricated by DRIE. Conductive electrodes were either deposited by electron beam evaporation or formed by silicon

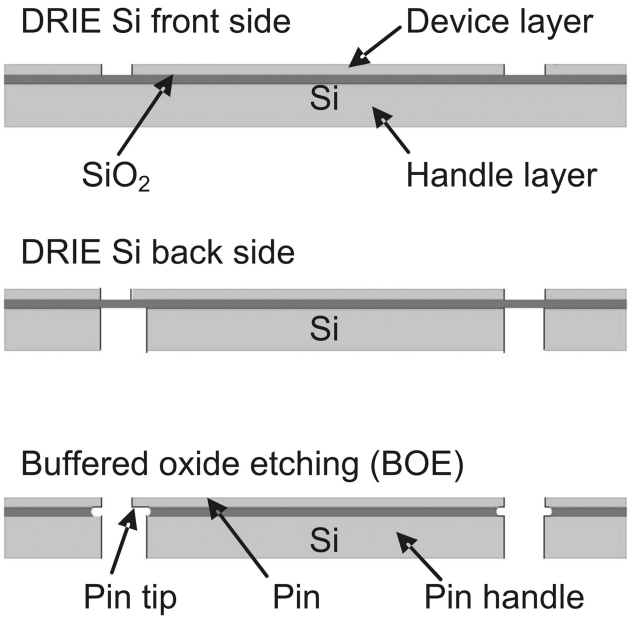


FIGURE 8 Fabrication process for a shallow-channel silicon printing pin. Adapted from Tsai and Kim.[83]

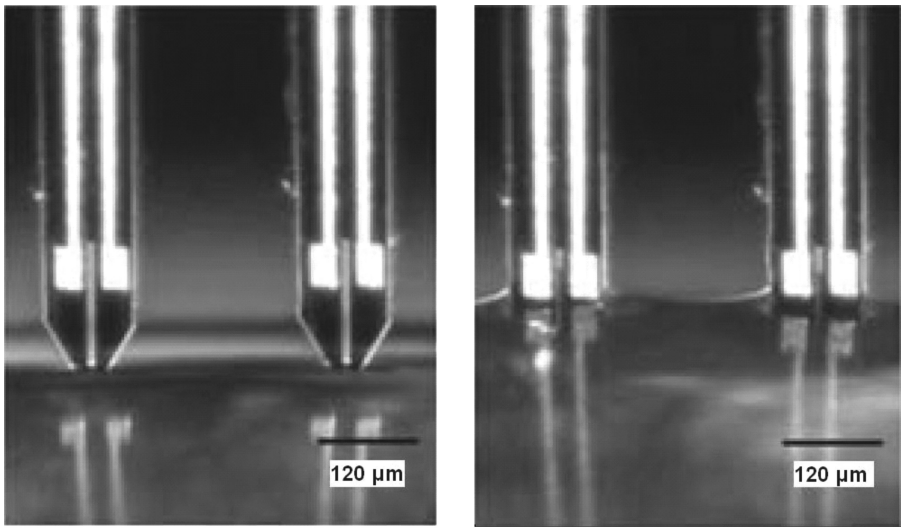


FIGURE 9 **Loading of doped Si pins: (a) No voltage is applied. (b) After applying 30 V.[38]**

doping with phosphorous. Metallic or doped electrodes were formed on the channel walls of 5 μm × 5 μm cantilevers. When an electrical potential was applied between the electrodes and the liquid, the liquid level in the channel rose (Figure 9). Applied voltages ranged from 4 V to 30 V for sample loading. Spots of 10 μm[38] and 30 μm[7] were produced by contact deposition.

Parallel Synthesis Technologies (PST), Inc., Santa Clara, CA, developed a silicon pin and a print head for reduced pin sliding.[63] As shown in Figure 10, this system features a collimator with 5 μm clearance for pin alignment. The pins are held in place in the printing head by elastomeric foam, which controls the linear force at vertical deflections and brings the pins to their original position after deflection. In contrast to the designs described above,[7,38,83] the PST pins are formed from regular silicon wafers rather than more costly SOI wafers. The pins are formed by etching silicon at the tip to the desired pin thickness. With a minimum thickness of 50 μm,[63] these pins are thicker than those formed using SOI wafers.

2.1.2 Pin Printing System

Figure 11 shows an example of a microarray printing system. Pins are carried by a pin head as it transfers samples from the microplate to the substrate. The pins float under their own weight and are free to move in the vertical direction in the pin head when in contact with the

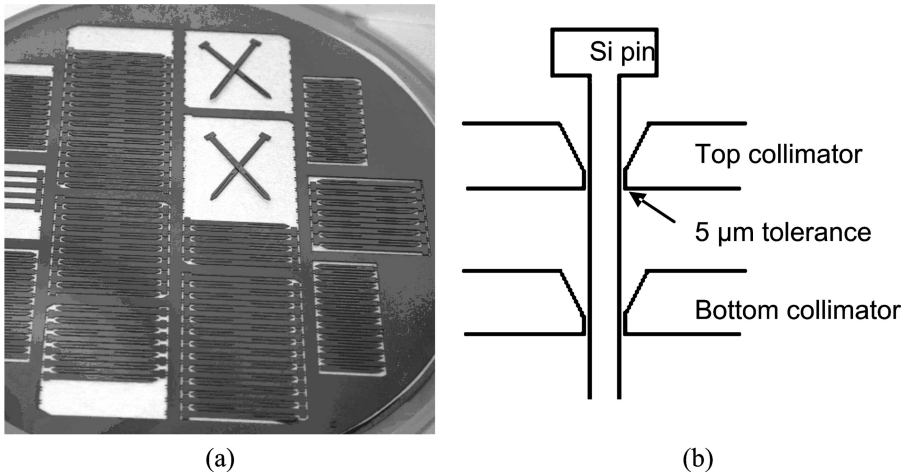


FIGURE 10 **(a) Parallel Synthesis Technologies pins fabricated on a 100 mm (4") silicon wafer by deep reactive ion etching (DRIE).[63] (b) Collimation plates and silicon pin. The top side of the collimator is wet etched to facilitate loading of the pin and the bottom side of the collimator is shaped by DRIE to provide 5 µm tolerance between the pin shaft and the collimator.[63]**

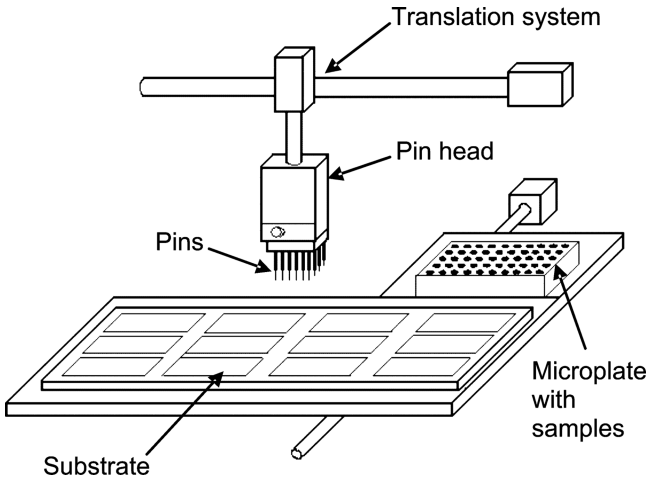


FIGURE 11 **System for microarrray printing. Print head mounted on translation system carries printing devices (pins) from the source microplate to the substrate, where it deposits sample. Adapted from Ito and Tachibana.[28]**

substrate. There are usually 16, 64, or 96 pins in one pin head.[72] The usual pin spacing of 4.5 mm or 9 mm is determined by the commercial microplate configuration–394 or 96 wells, respectively. Microarrays do not exactly map the source plate; in fact, spot distance on microarrays is much smaller than the distance between the source plate wells. A typical density of spots with 75-360 μm diameters is 400-10000 spots/cm^2. As described, the pins are dipped into the wells, and the solution is held by surface tension on the outside of solid pins or driven into the slit/channel in split pins by capillary forces. Dipping split pins in a larger sample volume results in spot inconsistency in the first few spots due to the draining of the excess sample solution from the pin sidewalls; therefore, preprinting is necessary until uniform printing is achieved.[72]

The typical substrates for microarray printing are microscope slides. Prior to spotting, the slides are treated with poly-lysine, amino silanes or amino-reactive silanes that enable DNA or protein to bind to the surface and prevent the sample from being washed away during the hybridization process. In addition, the dispensed sample spot spreads less if the surface is hydrophobic, allowing higher array densities. Other materials, such as polymer-coated glass or plastics, are also used as substrates for microarray printing.

Accurate quantitative analysis of printed microarrays is only possible if spot uniformity (i.e., the spot size and shape is consistent and reproducible) and positional accuracy are achieved.[88] As discussed above, spot uniformity is affected by the sample viscosity, pin contact area, pin surface properties, substrate surface properties, and substrate planarity. Additional factors that define spot sizes are pin velocity, the precision of robotic controls, environmental control of humidity, temperature, and contamination. A high pin velocity (>2 cm/s) can induce high inertia forces that drive large sample volumes out of the pin, making the size of spots very large.[72] Setting a high, stable humidity level prevents the sample from evaporating from the wells and pin channels. Temperature affects sample viscosity and therefore, dispensed volume. Contamination and dust must be controlled if high-quality arrays are to be produced with minimal risk of split pin clogging.

Pin printing is probably the most popular method for microarray fabrication. However, the primary reason for the development of other printing methods is the tedious, time-consuming nature of pin printing. Rose[72] reported that typical printing time for an Arrayit ChipMaker^{TM} 2 microprinting system (TeleChem International, Inc., Sunnyvale, CA), including loading, preprinting, printing, and washing of pins, was 0.2 h for a 384-well microplate using 32 pins, and 6.4 h for a 384-well microplate using a single pin. These inherent inefficiencies have spurred the development of other spotting methods.

2.2 Microstamping

Pin printing is an inherently serial technique in which a single pin or groups of pins are iteratively loaded for spotting. An alternative to pin printing is microstamping. With microstamps, hundreds of spots are printed in parallel, enabling high-throughput microarray fabrication. The microstamping process, depicted in Figure 12, is simple and inexpensive and can be readily conducted in a laboratory. A sample is first adsorbed on the patterned surface of a stamp and then transferred to a substrate by physical contact. In order to obtain good contact, microstamps are generally made from elastomeric materials, such as poly (dimethylsiloxane) (PDMS), which conform to surface roughness under an applied load.

Microcontact printing with elastomeric stamps, along with other soft lithographic microfabrication techniques, was developed by Whitesides' group[92,95] and first demonstrated for self-assembled monolayers (SAMs) of alkanethiols on gold.[35] Elastomeric stamps are manufactured by a micromolding technique that requires only a single photolithography step (Figure 13).

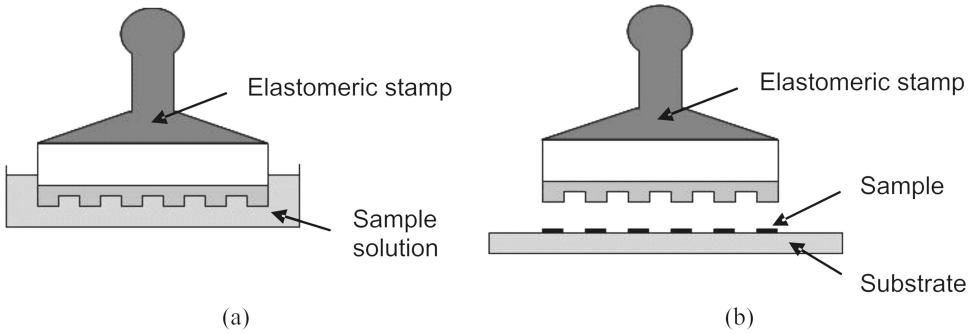


FIGURE 12 **Microstamping process. (a) Stamp inking in a sample solution well. (b) Transferring sample to the substrate by physical contact between the elastomeric stamp and the substrate.**

Mold masters are fabricated by photolithography to define a pattern of the stamp. Then, an uncured liquid elastomer (e.g., PDMS) is cast on the master. After curing, the stamp is released from the master. This process enables reproducible, low-cost batch production, resulting in inexpensive and disposable microstamps. Using disposable stamps minimizes the problems of sample carry-over, cross-contamination, and the time-consuming cleaning processes that are required for pin printing. Hydrogels have also been used as a stamp material for protein patterning.[49]

One limitation of elastomeric microstamp fabrication procedure is the necessity of using photolithography and cleanroom facilities to form the stamp molds. In response to this challenge, Xia *et al.*[91] developed several non-photolithographic methods for elastomeric stamp fabrication. One such method, shown in Figure 14, uses polystyrene spherical beads for casting PDMS stamps. Polystyrene spheres suspended in a solvent are deposited on a flat silicon substrate in a thin layer. They form a hexagonal densely packed configuration as a result of laminar flow and attractive capillary forces. After the solvent evaporates, the bead assembly is used as a mold master for making PDMS stamps.

Microstamping has the capacity to form arrays with very high resolution (e.g., inter-spot spacing of

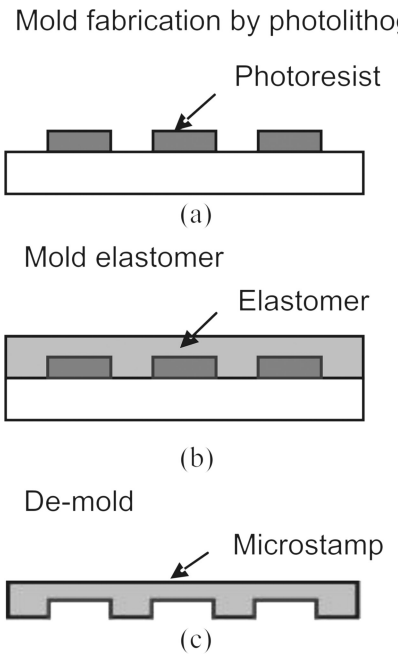


FIGURE 13 **Microstamp fabrication. The master is fabricated by photolithography to define the feature of the stamp. The liquid elastomer is then cast on the master to produced designed patterns. After the curing process, the stamp is released from the master.[84]**

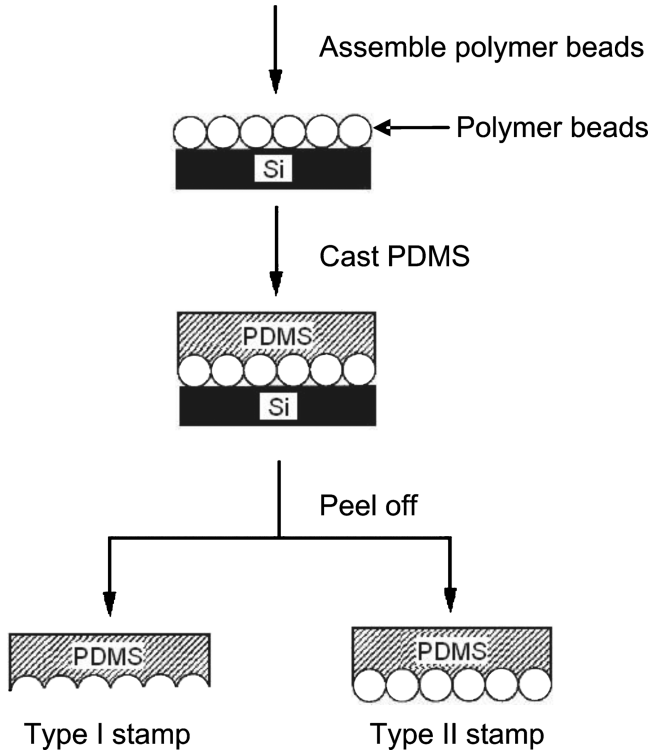


FIGURE 14 **Casting PDMS stamps from polystyrene microspheres assembled on a flat surface.[91]**

100 nm).[8,70] The low elastic modulus of the commonly used PDMS (e.g., Sylgard 184, Dow Corning) limits the production of arrays with spots smaller than 100 nm.[61] Stamp tips smaller than 100 nm buckle and deform under stamping forces. Based on the design by Schmid and Michel,[75] Odom *et al.*[61] developed stamps capable of forming even smaller spots using two layer devices. These stamps were formed from a stiff layer made of a "hard" PDMS and a flexible layer made of conventional PDMS.

Some disadvantages for microstamping are related to the sample volumes transferred. In microstamping, the amount of sample transferred from the stamp to the substrate is not well controlled and depends on both surface and sample properties. Additionally, for the same amount of printed sample, microstamping requires larger initial sample volumes, as only a small amount of solution in a well is adsorbed onto the stamp surface, and only a small fraction of the adsorbed solution is transferred from the stamp to the substrate, due to strong non-specific adsorption to the hydrophobic stamp material. Likewise, if microstamps are to be reused, the washing process is more tedious than for pins because of non-specific adsorption.[59]

Microstamping techniques for biomolecules can be categorized into indirect stamping[40,49,69,80] and the more recently developed direct stamping.[13,30,41,43,70] Both categories utilize similar tools but different stamping procedures. In indirect stamping, the SAM is first patterned on the substrate, which is then exposed to the sample solution. In direct microstamping, samples (DNA, proteins) are simply transferred from an inked stamp to the substrate in a single step. Regardless of direct or indirect stamping, the first step in microstamping is "inking" sample onto the stamp surface.

2.2.1 Inking

The first step in microstamping is to "ink" a stamp with the sample solution or SAM solution. The inking process is important because it significantly affects spot size and accuracy. Since PDMS is hydrophobic, the inking process begins with hydrophilic treatment of the stamp surface so that inking solutions can adhere. This treatment is typically accomplished by coating the stamps with a thin aluminum film[41] or by exposure to oxygen plasma.[36] For example, Lin *et al.*[41] treated the tip surface with a 1 μm thin aluminum film to make the tip hydrophilic while maintaining hydrophobic side surfaces. In this way, the spot size of the protein solution was reproducible since each of the stamps collected an equal amount of sample solution. The reported average protein spot size was 350 μm by 350 μm with a variation within 10%.

Once the stamp surface is treated to be hydrophilic, the stamp is inked by simply dipping the stamp into the sample well. Lin *et al.*[41] showed that the sample well chip can be designed to ink sections of a stamp with different proteins. Alternatively, injecting devices (e.g., piezoneedles[36]) have been used to deliver equal amounts of DNA solution to stamp sites. Such metered inking ensures good control of spot size and requires robotically controlled equipment. The capability of microstamping to yield multiple arrays from a single sample load has also been demonstrated.[36]

Martin *et al.*[49] introduced hydrogel polymer poly(6-acryloyl-β-*O*-methylgalactopyranoside-*co*-methylene-bis-acrylamide) as a stamp material for protein microarray fabrication. The stamp consisted of a narrow capillary with hydrogel inserted at the end of the capillary. An antibody solution was loaded into the dry polymer through a 40 μm diameter feedline, hydrating the polymer. The swollen polymer containing protein was then stamped onto the substrate surface for about 2 sec leaving circular spots of antibody. The authors used the stamp for serial microarray fabrication, suggesting it can be assembled into an array of stamps for parallel stamping.

Contact inking was used by Libioulle *et al.*[40] to form accurate SAM patterns of alkanethiols on gold. Spots smaller than 500 nm in diameter are difficult to achieve due to the diffusion of ink molecules away from the contact region. The design[40] was developed to overcome this diffusion problem by transferring sample from an impregnated, flat PDMS pad to a PDMS stamp by simply bringing them in contact, as depicted in Figure 15. In this manner, only the contact zones of a stamp are inked, resulting in high-resolution stamping. The amount of ink transferred from the pad to the stamp was controlled by changing the concentration of the impregnating thiol solution. This method was originally proposed for indirect stamping, but should also be applicable to direct stamping.

Lin *et al.*[43] developed a novel inking method by loading the sample solution into wells fabricated on the back of a PDMS stamp. This microstamp chip, illustrated in Figure 16, contains sample wells from which different DNA solutions are delivered to the microstamp tips. Prior to loading samples, the stamp surfaces were treated

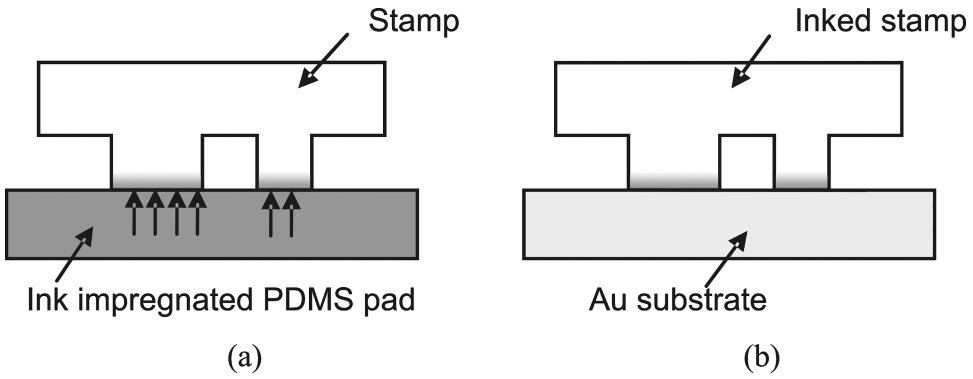


FIGURE 15 **(a) Contact inking of the patterned stamp.[40] A flat inker pad is impregnated by immersion in a dilute solution of thiols. The patterned stamp is brought in contact with inker pad and consequently inked only at the contact zones. (b) Contact printing of thiols via contact between the inked stamp and Au substrate.**

in oxygen-plasma to make them hydrophilic. A single cylindrical stamp had a height of 100 μm, an inner diameter of 50 μm, and an outer diameter of 200 μm. Under an approximately 1.4 N uniformly distributed force, spots of 210 μm in diameter were deposited, which is only 5% larger than the size of the stamp tips.

2.2.2 Direct Stamping

In direct stamping, the stamp is inked with biological sample, and then brought into physical contact with the substrate. A low-magnitude load is usually applied for a few seconds to ensure the stamp surface conforms to the substrate. Then the stamp is removed from the substrate leaving sample spots behind. Lin *et al.*[43] identified stamp removal speed from the substrate and sample contact angle as key parameters in achieving uniform shape and volume of printed spots, along with surface roughness and the gap between the stamp tips and the substrate. Numerical simulations[42,43] revealed that the transfer process and physics of the stamping process rely on the liquid and gas phase fluid dynamics.

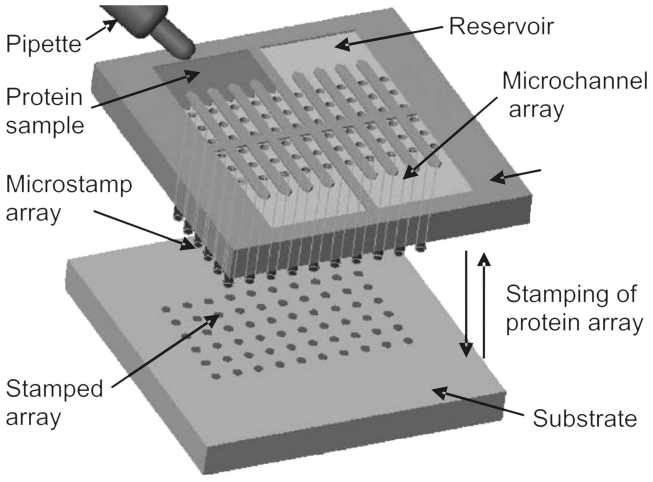


FIGURE 16 **Microstamping system: the microstamp consists of discrete wells, which can be filled with different samples. Samples are directed by surface tension to the tips of the microstamps through the embedded microchannels. When brought into contact with the substrate (Bio-Assay Chip) for less than 1 min, the microstamp prints sample arrays.[43]**

The applied contact pressure is another important parameter for stamping spot uniformity, but has not been reported in the literature. The deformation of the PDMS stamp under applied pressure can cause significant spot size increase and shape deformation, which is exacerbated when combined with substantial substrate roughness. More importantly, the dependence of the minimum load needed for uniform contact needs to be evaluated as a function of substrate roughness and the minimum time needed for contact.

Besides controlling the spot size, control of solution concentration is also required for quantitative fluorescence readings. Renault *et al.*[70] demonstrated that careful control of the concentration of the protein solutions to be inked onto the stamp surface enables accurate control of the concentration of protein molecules in the stamped spots. As an extreme example, the authors printed single antibody molecules by reducing the microstamp feature diameters below 100 nm and by diluting the molecules in the ink.

Direct stamping can also be implemented by means of microfluidic channels,[30] a method first introduced for stamping and curing polymers.[31] A patterned PDMS device with an array of channels is temporarily bound to the substrate and a bio-sample solution fills the channels by capillary forces (Figure 17(a)). The PDMS stamp is then peeled off, leaving behind a pattern on the substrate defined by the channel geometry. This process is not limited to single bio-sample patterning. Different samples can be

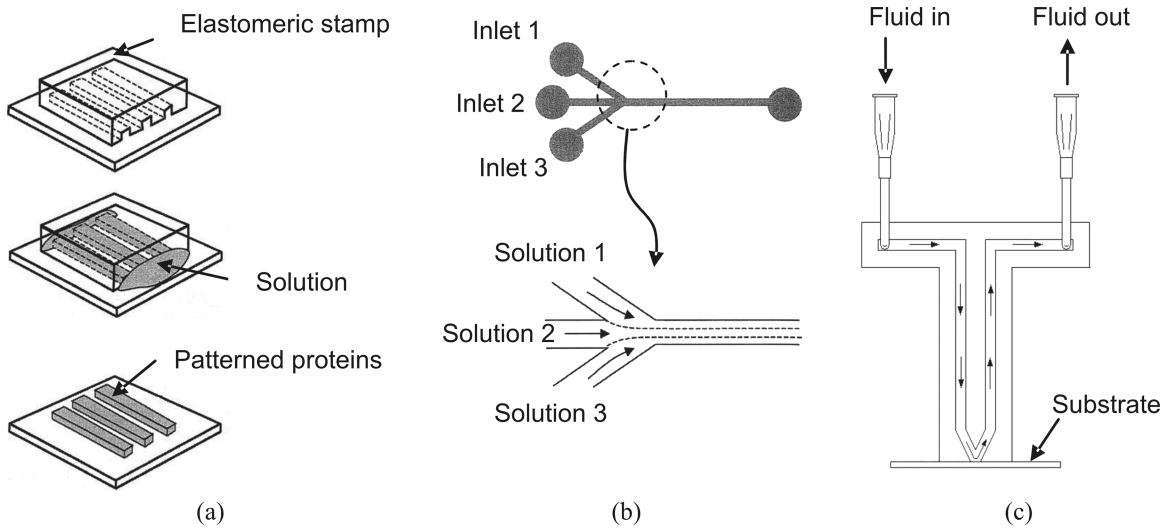


FIGURE 17 **Microfluidic channels for protein patterning. (a) Elastomeric stamps are fitted against substrate and used as capillaries. Protein solutions are loaded into the capillaries by capillary action, allowing selective contact with the substrate. Several different proteins can be registered into designated regions.[30] (b) Different protein solutions are introduced in the same channel by laminar flow.[30] (c) PDMS microfluidic stamping device with inlet and outlet ports for sample supply. Stamp is pressed against the substrate and solution flows through the channel and prints a spot.[13]**

introduced into designated channels simultaneously. Microchannels can also be designed to allow flow of different solutions in the same channel, as Figure 17(b) illustrates, because flow is laminar (i.e., no advective mixing) inside the microchannels.

The PDMS microfluidic deposition device illustrated in Figure 17(c),[13] has a microchannel with inlet and outlet ports to hold solutions to be stamped. When the stamp is pressed against a substrate, the solution flows through the channel and prints a spot on the substrate. This flow deposition system enables printing of highly concentrated samples by allowing desired molecules to bond to the substrate while washing away unwanted materials.

2.2.3 Indirect Stamping

In indirect stamping, the stamp is inked with chemical groups or SAMs rather than biological samples to be deposited.[3,40,49,69,80] Patterned SAMs are generally formed by printing alkanethiol on a gold substrate,[35] which is typically followed by coating the rest of the exposed surface with other SAMs to prevent nonspecific sample binding, as shown in Figure 18. Active chemical groups can also be introduced onto a patterned stamp surface, instead of the substrate, to selectively bind molecules from the ink (i.e., a sample solution).[69] By activating individual stamp tips with different chemical groups, various microarray patterns can be achieved.

Indirect printing is not suitable to use when more than one solution is to be printed on the same substrate.[49] When forming multiplex microarrays, the entire surface is exposed to a series of solutions containing different molecules/cells to be patterned. Very

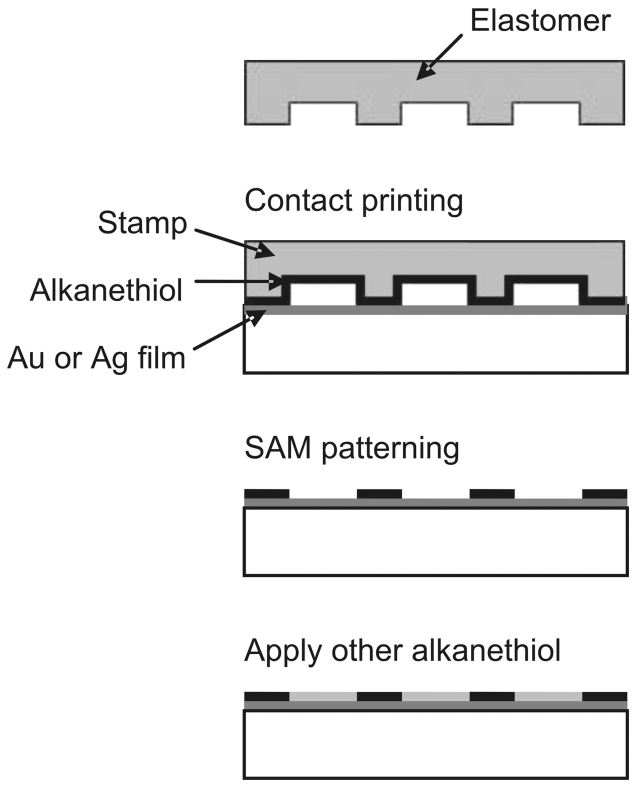


FIGURE 18 **Indirect stamp-patterning a self assembling monolayer (SAM) of alkanethiol on gold or silver substrate for sample selective binding.[84]**

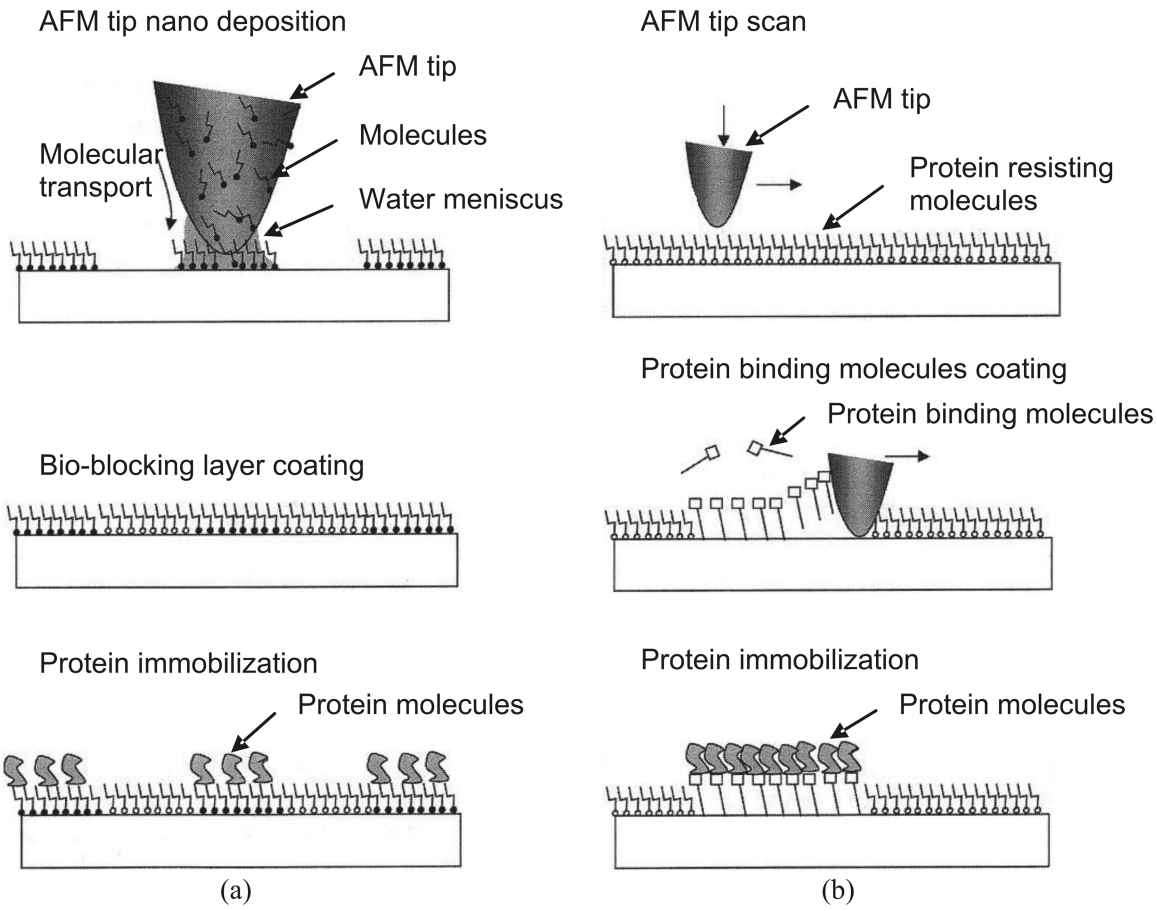


FIGURE 19 **(a) Schematic of AFM dip-pen lithography. (b) Schematic of AFM grafting.[84]**

often, undesired nonspecific molecule adsorption or cross-reaction into a previously patterned region occurs.

2.3 Nano-Tip Printing

In order to achieve a higher spot density and more complex arrays, spot sizes must be reduced. The technologies developed for printing spots at the submicron scale are based on atomic force microscopy (AFM). These methods employ an AFM nano-tip for: (1) adding a sample or its binding molecules to the substrate (Figure 19(a)), known as dip-pen lithography;[37,65] or (2) removing SAM molecules (Figure 19(b)), known as AFM grafting.[87,93] When an AFM tip is brought into contact with a substrate, the solution flows from the coated tip to the substrate or vice versa by capillary action.

In dip-pen lithography (Figure 19(a)) a sample that binds proteins or other biomolecules is transferred from the tip to the substrate creating nano-patterns. The rest of the substrate is blocked with molecules that do not bind biomolecules and the substrate is then exposed to the biomolecule solution. Lee *et al.*[37] used dip-pen lithography for printing a solution of Retronectin and demonstrated cell adhesion to the resulting submicron spots. The resolution of the printed nano-patterns depends on the tip-substrate contact time and relative humidity.[65] Piner *et al.*[65] demonstrated that substrate roughness is an important parameter. When printing 30 nm wide lines of alkanethiols on a gold substrate, they found that the lines were discontinuous and followed the substrate grain shape.

The AFM grafting method utilizes an AFM tip to remove one SAM layer (e.g., molecules that resist protein adsorption) on selected areas and simultaneously add another SAM (e.g., protein binding molecules), as illustrated in Figure 19(b). Thus, nano-patterns can be modified and improved *in situ,* without repeating the whole fabrication procedure.

AFM printing technology is a serial printing method, and is therefore slow compared to microstamping. Slow printing reduces fabrication efficiency and also limits its device functionality because sample volumes are very small and tend to dry out quickly. Furthermore,

non-specific binding becomes a serious issue because the size of spots approaches the size of protein molecules. Regardless, these techniques are useful for printing nanometer sized features in complex microarrays, where different types of molecules are placed selectively at different sites. The great advantage of AFM printing is that the same tip is used for both printing and reading (i.e., detection). By utilizing AFM reading, molecular and physical properties (e.g., height, roughness, shape) can be directly detected.[87]

3. NON-CONTACT PRINTING TECHNIQUES

Contact printing methods include a variety of techniques, but all methods ultimately involve contact between the substrate surface and a stamp or pin. In contrast, non-contact printing techniques vary considerably from photochemistry-based methods to laser writing to fluid droplet dispensing.

There are two main advantages to non-contact printing: reduced contamination and higher throughput. By keeping the printing device and the substrate separated at all times, the likelihood of contamination is greatly reduced. Hence, the need to constantly clean the printing device between uses is eliminated. Furthermore, non-contact printing methods hold the greatest potential for increasing microarray fabrication throughput. Many non-contact methods deposit solutions in parallel, allowing entire arrays to be produced simultaneously.

3.1 Photochemistry-Based Printing

Photochemistry microarray printing is based on chemical treatment of the substrate and UV light exposure through photomasks. The two main methods are photolithography[9,46,55,66] and direct photochemical patterning.[9,26,68] In photolithography, a positive photoresist layer is spin-coated onto the substrate, exposed to UV light through a photomask and then developed to form micrometer sized open regions where adhesion-promoting molecules are bound (Figure 20(a)). The substrate is then immersed in solvent to remove the remaining photoresist, and adhesion-resistant molecules are bound to the exposed glass surfaces. Direct photochemical patterning is very similar to photolithography except that it does not require a photoresist layer. A substrate is coated with photochemical molecules and exposed to UV light through a photo mask, as illustrated in Figure 20(b). UV-exposed molecules are either activated or deactivated to bind biological molecules of interest. Photochemistry-based fabrication methods are mainly applied to protein and DNA arrays although cell adhesion regions can also be fabricated in this way.[10,23,33,46] Similar to all parallel patterning methods, photochemistry features high throughput. The disadvantages include the risk of biomolecule denaturization by photoresist solvents and the difficulty in patterning different samples in a single step.[84]

Photolithographic printing is commonly used in generating DNA microarrays of *in situ* synthesized oligonucleotides.[53,64] For example, the Affymetrix Inc., Santa Clara, CA, process comprises serial light exposures through different photolithographic masks followed by the chemical synthesis of DNA bases at the exposed/activated sites. After each exposure a single DNA base is coupled to the activated sites and the process is repeated until the sequences are generated. As a serial process, this method can be time consuming for longer sequences of oligonucleotides, but it provides high-density arrays. The GeneChip® (Affymetrix Inc.), a high-density-oligonucleotide array, is generated on fused silica substrates that carry 50 to 400 replicate arrays. Each has up to 400,000 probes on a 1.6 cm^2 area.[67] A recent innovation is to use a virtual mask created on a computer and projected onto the substrate with a digital micromirror array.[39,79] Microfeatures measuring 16 μm^2 were created in this way.[79]

3.2 Electro-Printing

A few groups have been developing techniques for biomolecule microarray fabrication that utilize electrochemistry and other on-chip electric field effects.

The NanoChip® (Nanogen, San Diego, CA) utilizes the negative charges of DNA and RNA molecules to immobilize them on an array of positively charged microelectrodes.[19,24] Electrodes are coated with a streptavidin-agarose permeation layer and probes are biotinylated such that after the field is turned off, the probe molecules remain non-covalently bound to the surface. In addition, positive charge at individual test sites attracts target DNA molecules whose rapid concentration enables reduced hybridization times. The NanoChip® can have 25 to 10,000 test sites/electrodes, with dimensions of 80 μm to 30 μm, respectively. Dense and complex arrays require more sophisticated technology, with integrated complementary

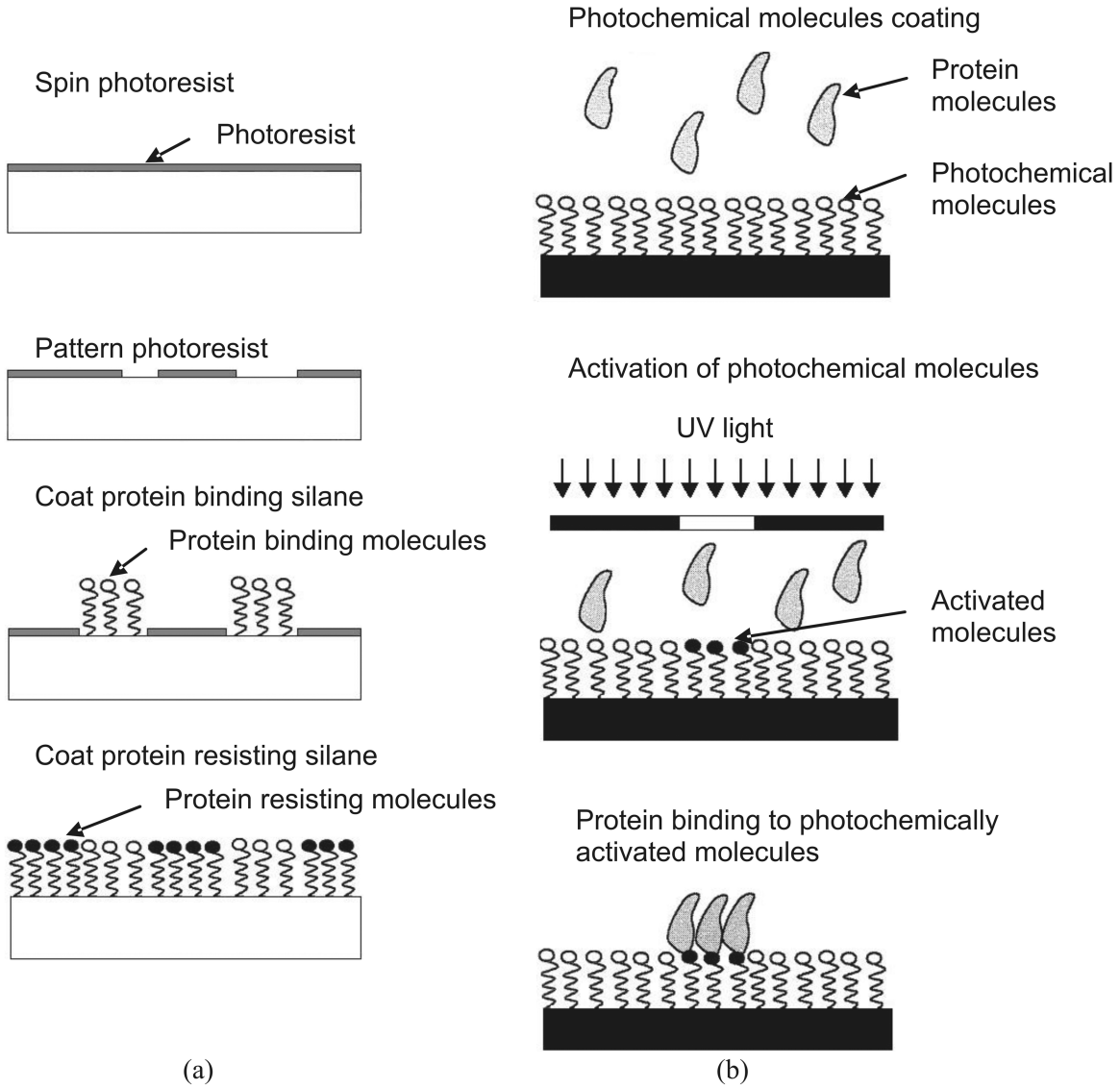


FIGURE 20 **Schematic illustrations of photochemistry based manufacturing procedures. (a) Photolithography applied to silane self-assembled monolayers (SAMs). (b) Direct photochemical protein patterning by activation of a photochemical coupling species.[84]**

metal-oxide-semiconductor field-effect transistor (CMOS) features.

Livache *et al.*[44,45] demonstrated immobilization of DNA in a microarray by electrochemical means. By sequentially activating 50 × 50 μm gold electrodes fabricated on silicon, a mixture of conductive polypyrrole (PPy) and PPy modified by a specific oligonucleotide probe is electrooxidized which results in copolymerization of a PPy film carrying covalently linked oligonucleotides immobilized onto the electrodes.

In addition to pre-formed oligonucelotide capture, electrochemical means have also been used for in situ synthesis of oligonucleotides.[4,16] CombiMatrix Corp., Mukilteo, WA, has developed a chip that contains 1024 programmable electrodes on a 1 cm^2 footprint that is capable of electrochemically facilitating in situ synthesis of oligonucelotides. This microarray chip is also based on CMOS semiconductor wafer technology.

3.3 Droplet Dispensing

Droplet dispensing can be classified into three types: motion controlled pin printing, inkjet printing, and electrospray deposition (ESD).

3.3.1 Motion Controlled Non-Contact Pin Printing

Zeng *et al.*[94] proposed the use of existing contact pin printing technologies to produce microarrays without contacting the substrate. This motion controlled

non-contact pin printing method uses conventional split pins. Sample solution is loaded into a split pin by capillary forces, then the pin is accelerated towards the substrate. It is abruptly stopped before contact, driving the solution within the pin out by momentum and forming a liquid bridge between the pin and the substrate. Finally, as the pin is drawn away, the liquid bridge pinches off and a droplet is left behind on the substrate surface.

Motion controlled non-contact pin printing relies on precise control of pin acceleration, velocity, and position. The pin tip must be stopped a minimum of 2 μm from the substrate.[94] The deposited spot volume is strongly dependent on the velocity of the pin as it is retracted from the surface. According to numerical simulation, the entire process takes less than 4 ms. No experimental results were reported to confirm the feasibility of this approach.[94] This method solves the problems of pin-tapping, and is most likely compatible with existing contact printing equipment controlled with highly precise robotics. Some difficulties associated with motion controlled printing can be foreseen with the use of even smaller printing pins because surface tension effects scale as 1/L, while inertial effects scale with fluid volume (L^3), where L is length.[48]

3.3.2 Inkjet Printing

The established technique of pin printing requires expensive and sophisticated robotic systems. In order to reduce the cost of printing biomolecules, attempts have been made to use ink-jet printing technology for microarray fabrication. In most cases, commercially available printers are modified to dispense a biomolecule solution instead of ink.[1] These printers are often one or two orders of magnitude cheaper than robotic pin printing systems. The two main types of inkjet printers are thermal and piezoelectric.[1] In thermal inkjet printing, resistive heaters ($\sim$200°C) evaporate a small volume of ink which then drives a droplet of ink through a nozzle. Piezoelectric printers avoid high temperatures by using piezoelectric actuators to dispense droplets.

Inkjet printing technology is attractive as it is inexpensive and delivers small droplets with reproducible volumes. Unfortunately, there are four significant drawbacks. First, commercial printers are not designed to print on glass slides. Thus, microarrays can only be spotted onto flexible membranes such as cellulose, nylon, and nitrocellulose.[2] Although these membranes are compatible with inkjet printers, droplet smearing and contamination often occur. Second, inkjet nozzles have a tendency to produce undesirable satellite droplets that contaminate surrounding spots and thus, reduce printing resolution.[85] Third, it is difficult to completely flush printing nozzles before a new solution is loaded.[2] This problem is more serious in piezoelectric printers since the nozzle is separated from the ink reservoir and all linking channels must be flushed clean. Fourth, the droplets experience high shear rates while passing through the nozzle and impacting the substrate surface. Under these shear rates or high temperatures, there is a risk of denaturing biomolecules in the solution. Allain *et al.*[2] summarized a number of studies that demonstrated that DNA can be spotted with inkjet printers and remain intact; however, proteins are more fragile and may be more sensitive to the extreme conditions of inkjet printing. Okamoto *et al.*[62] have fabricated microarrays with bubble jet technology, and report that DNA solutions can be used without damage. Furthermore, they suggest that the elevated temperature of solutions spotted with inkjet printers may enhance DNA reaction times, and is a notable advantage.[62]

Efforts have been made to modify inkjet printers to make them more suitable for microarray printing. For example, Tseng *et al.*[85] proposed a novel thermal inkjet nozzle that eliminates satellite droplets and improves the speed and control of droplet deposition. The new device was a modification of a commercial thermal inkjet printer, with an additional resistive heater. This heater is used to form a second vapor bubble, which eliminates satellite droplets by pinching off or "tail trimming" the exiting droplet (Figure 21).

Other printing systems use existing inkjet printing technology, modified with new dispensing mechanisms to overcome the limitations of commercial inkjet printers. The most common configuration for these types of spotters is a deposition head with a large number of top loaded reservoirs that each feeds to a separate nozzle. Using this configuration, many droplets of different solutions can be dispensed simultaneously. For example, Takagi *et al.*[81] developed a dispenser having 128 separate nozzles. Each nozzle is independently actuated by an electrostatically moving membrane, which generates a pressure pulse. Gutmann *et al.*[21] used 24 separate nozzles with a common piston that provides a pressure pulse to all of the nozzles simultaneously. The common piston ensures that an identical pressure pulse is provided to all of the nozzles, but also risks contamination.[22]

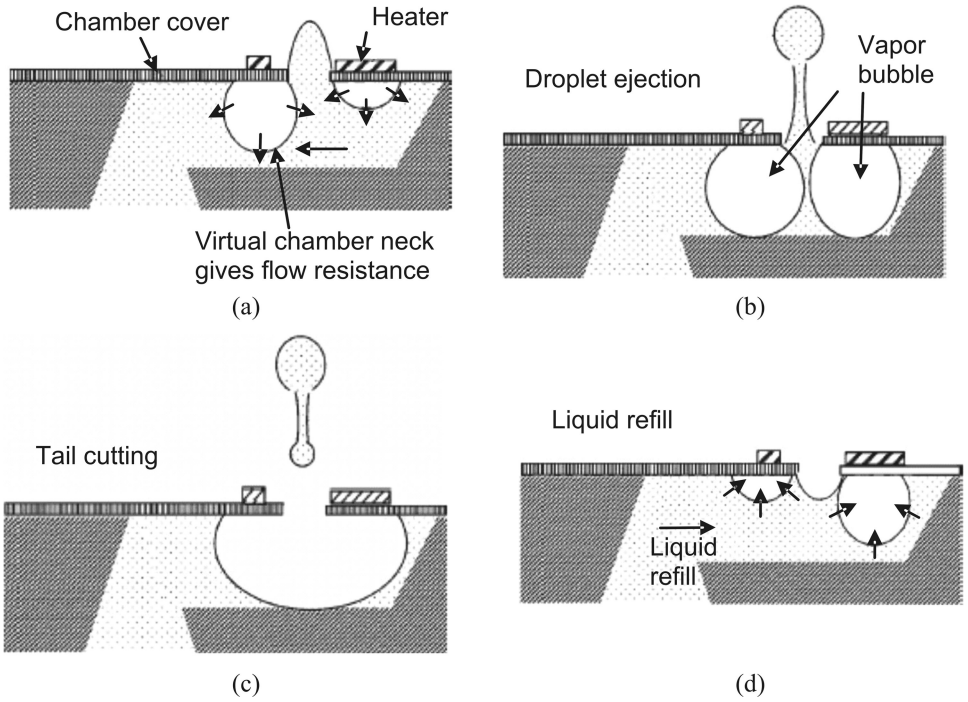


FIGURE 21 **Thermal inkjet operation schematic. Resistive heater creates a vapor bubble that pinches off the droplet and eliminates satellite droplets.[85]**

Inkjet printing has also found an application in generating microarrays of *in situ* synthesized oligonucleotides which is typically performed by photochemical means. Instead of using light to direct synthesis, jets of reagents for DNA synthesis are delivered to microscopic spots on a substrate. Different piezoelectric jets fabricated in glass, silicon or ceramics are employed to synthesize DNA arrays,[12,27] and solvents that have low volatility, high surface tension and high viscosity are preferable to prevent evaporation and mixing of adjacent sites. An alternative to prevent mixing is to create non-wetting regions between synthesis sites.[12]

3.3.3 Electrospray Deposition (ESD)

As with inkjet printing, electrospray deposition (ESD) is a technique borrowed from an existing application and applied to microarray fabrication. ESD is most commonly used to deposit thin films of polymers, semiconductive ceramics, and radioactive sources.[58] In recent years, there have been a variety of studies using this technique to deposit biological solutions.[5,6,32,54,57,58] In ESD, a dielectric mask is placed between a capillary tube containing the solution to be deposited and the substrate (Figure 22). An electrostatic field is activated between the capillary and the substrate, which drives the solution out of the capillary nozzle. The solution droplets are attracted to the substrate through the holes in the mask. The size of the droplets deposited on the surface is controlled by the size of the holes in the mask. A microarray is created by filling a series of capillaries each with a different solution to be spotted. The array is then created by spraying from one capillary, moving the substrate or mask, and then spraying from a different capillary.

ESD allows for fast and parallel fabrication of microarrays. The use of a single capillary tube for each

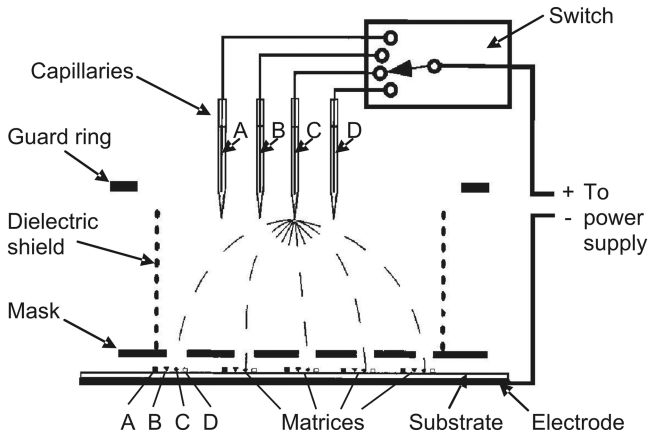


FIGURE 22 **Schematic of electrospray deposition. Biomolecule solution loaded in capillaries is dispensed through the dielectric mask onto the substrate by applying electrical field between capillaries and the substrate.[58]**

solution reduces the reservoir filling time. In addition to the high efficiency, ESD is also capable of producing very small spots. To produce spots of 2–6 μm in diameter, mask openings are about 25 μm.[58] The shortcoming of the ESD method is that the inter-spot distance (i.e., pitch) is inherently large because the mask holes are much larger than spot sizes. Typical spot spacing is 1 mm.[58] Thus, although ESD can produce microarrays quickly with uniform sized spots, spot density is poor.

In addition to limited spot density, the ESD method is also limited by significant challenges related to fluidics. The droplet distribution within an ESD spray is not uniform. The highest density of droplets occurs directly below the capillary and decreases with radial distance from the center line. Morozov and Morozova[58] investigated techniques to remedy this problem. One solution involved moving the mask and substrate to allow for uniform deposition. However, this technique still resulted in the formation of irregularly shaped spots,[58] which is an indication of either droplet splashing during impact or the deposition of satellite droplets. Fortunately, since the inter-spot spacing is large, the possibility of contamination of neighboring spots is low.

As with inkjet printing, ESD is also limited by the potential to damage biomolecules during deposition. The droplets undergo significant shear rates during expulsion from the nozzle and during impact. Additionally, the solution becomes charged when the electrostatic field is activated, and some proteins may deform under such conditions. Finally, the electric field can cause electrochemical reactions that can affect pH levels of the solution. When using ESD, it is important to select solutions that are inert under these conditions.

A less complex ESD system was developed by Moerman *et al.*[54] This system does not require a mask, relying instead on a single dispensing capillary very close to the substrate surface. Instead of producing a spray of droplets, the system dispenses each droplet individually. Droplet deposition is controlled by varying the electric field or the height of the substrate from the capillary. Unlike the approach of Morozov and Morozova,[57] this system requires serial fabrication of an array and is quite slow. It also requires precise spatial control to ensure uniform distribution and spot size.

Another non-contact printing technique similar to ESD is a Scanning Probe Microscopy (SPM) technique[11,20,22] based on scanning ion conductance microscopy. This technique allows for depositing

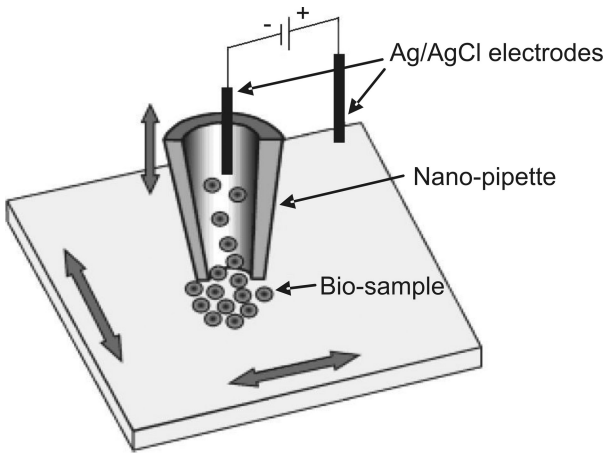


FIGURE 23 **Schematic of SPM based printing technique. A nano-pipette is filled with a 100 nM solution of DNA or protein. A voltage is applied between one Ag/AgCl electrode inside the nano-pipet and another Ag/AgCl electrode inserted into the bath of ionic solution. The ion current is used to enable fine control of molecule delivery and for the tip-surface distance control.[11]**

nano-spots by applying an electrical field pulse between the substrate and a nano-pipette that is filled with sample (Figure 23). Using 1 V pulses, sub-micron diameter spots of DNA and protein have been formed. The pipette tips typically have inner diameters of 100–150 nm and distances between the pipette tip and the surface 100–150 nm.

3.4 Laser Writing

Laser ablation fabrication has recently been used to produce microarrays of protein solutions[84] by means of direct and indirect spot deposition. For direct writing, a quartz disk is coated with a mixture of biological samples, glycerol and buffer.[15,71] A pulsed laser is scanned across the surface of the disk, locally evaporating small regions of the coating. The sample evaporates and releases liquid droplets that accumulate on the substrate (Figure 24). Droplets deposited in this manner are much smaller than those deposited by conventional contact printing. Ringeisen *et al.*[71] produced uniform 50 μm spots and claimed that spots less than 10 μm can be formed using this method. This method requires the use of very little sample, which is an advantage over conventional pin printing methods that often induce significant sample waste.[71]

Indirect laser writing makes use of the established micromachining process of lift-off. A laminate or photoresist is applied to a substrate and selectively removed with laser and a mask (Figure 25). A biological solution is then applied to the surface, and the remaining

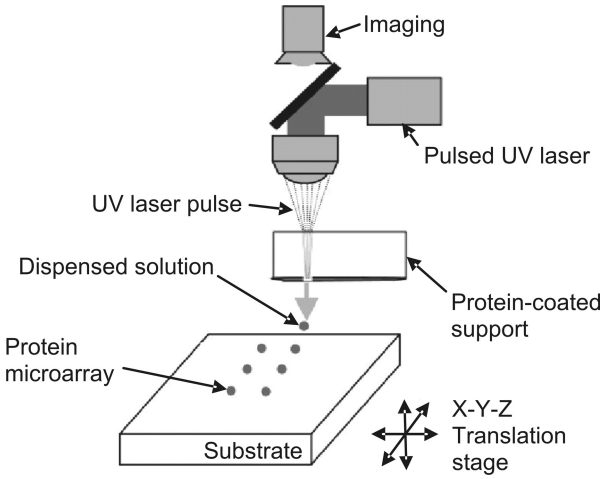


FIGURE 24 **A schematic of a system for direct laser writing of protein solutions. Droplets are deposited by evaporating the coated solution using a pulsed laser. Adapted from Ringeisen *et al.*[71]**

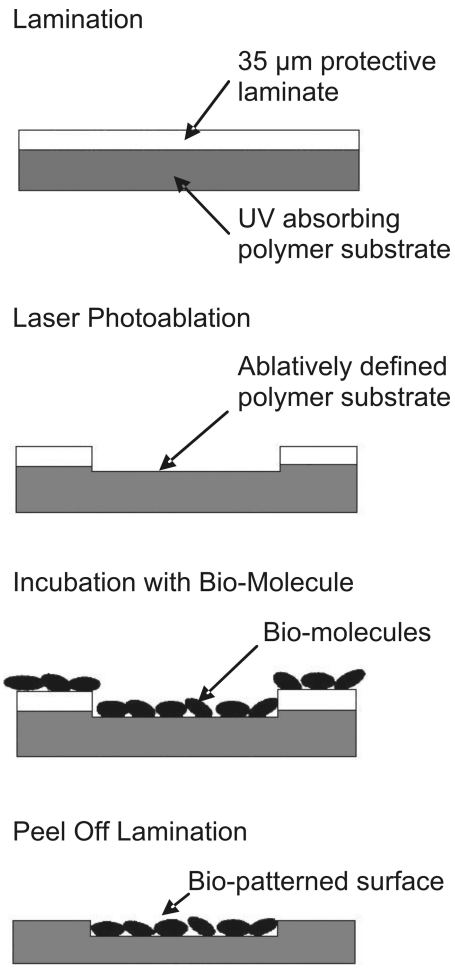


FIGURE 25 **Schematic of an indirect laser writing system. A laminate or photoresist deposited on a substrate is selectively removed with laser and a mask using lift-off technique. Adapted from Schwarz *et al.*[76]**

laminate is removed, leaving a patterned sample on the substrate[76]

4. SUMMARY

An ideal printing system should be capable of creating uniform, dense arrays of small droplets using a minimum volume of solution, while preventing solution contamination and biomolecular damage. Furthermore, the system should be inexpensive, reliable, and durable. None of the existing methods fulfill all of these criteria. Contact printing with solid and split pins is currently the most prevalent system used in industry and research laboratories. Overall, this technology imparts reproducible results with little required maintenance; however, contact printing is slow, expensive, and at times suffers from problems of contamination.

Contact pin printing is plagued by several problems that have led to the development of competing technologies. The main limitations for split pin printing include low speed, the requirement of pre-printing, pin clogging, tip deformation, and droplet uniformity. Precise control of the pin motion would eliminate the need for pin tapping to deposit fluid, thereby improving droplet uniformity and increasing pin life. Clogging is a very serious problem for split pins and can lead to poor performance and contamination.

Recent pin designs have relied on alternative materials to metals, such as silicon, ceramics, and polymers. These new materials have allowed engineers to evaluate a wide range of geometries and material characteristics. For example, silicon micromachining enables parallel construction of many devices, which greatly increases fabrication efficiency and reduces cost. Elastomeric polymers such as PDMS are used to mold microstamps that are also capable of parallel contact printing. Microstamping is an inexpensive and efficient printing technique compared to contact pin printing and is well-suited for high-throughput microarray fabrication. However, an improvement in the control of printed sample volumes is necessary for wide-spread use of this technique.

In addition to new materials and fabrication technologies, new surface treatment methods are being explored to increase control over wetting phenomena. For example, electrowetting, which enhances capillary forces by controlling the surface tension in an electrostatic field, is being used to drive fluids through nozzles. Besides microscale methods, nanoscale techniques,

based on atomic force microscopy, are being investigated for printing high density arrays at a submicron scale.

Completely different approaches are developed for non-contact printing. Of these methods, inkjet technology shows great promise for reducing the cost of microarray fabrication. Unfortunately, the printing inaccuracies that are often acceptable in document printing cause unacceptable problems for biochip microarray fabrication. Splashing and satellite droplets cause contamination and irregular spot sizes. Another method, electrospray deposition, suffers from similar drawbacks, with the additional disadvantage of negative effects of electric fields on certain types of biomolecules. Photochemical non-contact printing, which enables very efficient high-throughput microarray fabrication, can lead to biomolecular denaturization as well.

In summary, there are a variety of new technologies that hold promise for faster, more efficient, less expensive microarray fabrication. In order for competing technologies to be viable in the long term, microarray quality (e.g., spot uniformity and size) must also be evaluated alongside throughput and cost considerations. Although no single one of these new methods has yet supplanted the conventional method of contact pin printing, the continued development of new materials and micromachining techniques may soon lead to an alternative technology, making the technique of biomolecular microarray analysis even more accessible to the scientific community.

REFERENCES

[1] Allain, L. R., Askari, M., Stokes, D. L., and Vo-Dinh, T. 2001. Microarray sampling-platform fabrication using bubble-jet technology for a biochip system. *Fresenius Journal of Analytical Chemistry*. 371(2): 146–150.

[2] Allain, L. R., Stratis-Cullum, D. N., and Vo-Dinh, T. 2004. Investigation of microfabrication of biological sample arrays using piezoelectric and bubble-jet printing technologies. *Analytica Chimica Acta*. 518(1-2): 77–85.

[3] Arenkov, P., Kukhtin, A., Gemmell, A., Voloshchuk, S., Chupeeva, V., and Mirzabekov, A. 2000. Protein microchips: Use for immunoassay and enzymatic reactions. *Analytical Biochemistry*. 278(2): 123–131.

[4] Arjomand, A., and Kumar, A. 2003. An open-platform microarray system for R&D. www.currentdrugdiscovery.com.

[5] Avseenko, N. V., Morozova, T. Y., Ataullakhanov, F. I., and Morozov, V. N. 2001. Immobilization of proteins in immunochemical microarrays fabricated by electrospray deposition. *Analytical Chemistry*. 73(24): 6047–6052.

[6] Avseenko, N. V., Morozova, T. Y., Ataullakhanov, F. I., and Morozov, V. N. 2002. Immunoassay with multicomponent protein microarrays fabricated by electrospray deposition. *Analytical Chemistry*. 74(5): 927–933.

[7] Belaubre, P., Guirardel, M., Garcia, G., Pourciel, J. B., Leberre, V., Dagkessamanskaia, A., Trevisiol, E., Francois, J. M., and Bergaud, C. May 2003. Fabrication of biological microarrays using microcantilevers. *Applied Physics Letter*. 82(18): 3122–3124.

[8] Bernard, A., Delamarche, E., Schmid, H., Michel, B., Bosshard, H. R., and Biebuyck, H. 1998. Printing patterns of proteins. *Langmuir*. 14(9): 2225–2229.

[9] Blawas, A. S., and Reichert, W. M. 1998. Protein patterning. *Biomaterials*. 19(7-9): 595–609.

[10] Britland, S., Clark, P., Connolly, P., and Moores, G. 1992. Micropatterned substratum adhesiveness—a model for morphogenetic cues controlling cell behavior. *Experimental Cell Research*. 198(1): 124–129.

[11] Bruckbauer, A., Ying, L. M., Rothery, A. M., Zhou, D. J., Shevchuk, A. I., Abell, C., Korchev, Y. E., and Klenerman, D. 2002. Writing with DNA and protein using a nanopipet for controlled delivery. *Journal of the American Chemical Society*. 124(30): 8810–8811.

[12] Butler, J. H., Cronin, M., Anderson, K. M., Biddison, G. M., Chatelain, F., Cummer, M., Davi, D. J., Fisher, L., Frauendorf, A. W., Frueh, F. W., Gjerstad, C., Harper, T. F., Kernahan, S. D., Long, D. Q., Pho, M., Walker, J. A., and Brennan, T. M. 2001. In situ synthesis of oligonucleotide arrays by using surface tension. *Journal of the American Chemical Society*. 123(37): 8887–8894.

[13] Chang-Yen, D. A., Myszka, D., and Gale, B. K. 2005. A novel PDMS microfluidic spotter for fabrication of protein chips and microarrays. *Proceedings of SPIE*. 5718: 110–120.

[14] Choudhuri, S. 2004. Microarrays in biology and medicine. *Journal of Biochemical and Molecular Toxicology*. 18(4): 171–179.

[15] Chrisey, D. B., McGill, R. A., and Pique, A. 2001. Matrix assisted pulsed laser evaporation direct write. U. S. Patent 6,177–151.

[16] Egeland, R. D., and Southern, E. M. 2005. Electrochemically directed synthesis of oligonucleotides for DNA microarray fabrication. *Nucleic Acids Research*. 33(14): e125.

[17] Flaim, C. J., Chien, S., and Bhatia, S. N. 2005. An extracellular matrix microarray for probing cellular differentiation. *Nature Methods*. 2(2): 119–125.

[18] George, R. A., Woolley, J. P., and Spellman, P. T. 2001. Ceramic capillaries for use in microarray fabrication. *Genome Research*. 11(10): 1780–1783.

[19] Gilles, P. N., Wu, D. J., Foster, C. B., Dillon, P. J., and Chanock, S. J. 1999. Single nucleotide polymorphic discrimination by an electronic dot blot assay on semiconductor microchips. *Nature Biotechnology*. 17(4): 365–370.

[20] Gutmann, O., Kuehlewein, R., Reinbold, S., Niekrawietz, R., Steinert, C. P., de Heij, B., Zengerle, R., and Daub, M. 2004. A highly parallel nanoliter dispenser for microarray fabrication. *Biomedical Microdevices*. 6(2): 131–137.

[21] Gutmann, O., Niekrawietz, R., Kuehlewein, R., Steinert, C. P., de Heij, B., Zengerle, R., and Daub, M. 2004. Impact of medium properties on droplet release in a highly parallel nanoliter dispenser. *Sensors and Actuators A-Physical*. 116(2): 187–194.

[22] Gutmann, O., Kuehlewein, R., Reinbold, S., Niekrawietz, R., Steinert, C. P., de Heij, B., Zengerle, R., and Daub, M. 2005. Fast and reliable protein microarray production by a new drop-in-drop technique. *Lab on a Chip*. 5(6): 675–681.

[23] Healy, K. E., Thomas, C. H., Rezania, A., Kim, J. E., McKeown, P. J., Lom, B., and Hockberger, P. E. 1996. Kinetics of bone cell organization and mineralization on materials with patterned surface chemistry. *Biomaterials*. 17(2): 195–208.

[24] Heller, M. J., Forster, A. H., and Tu, E. 2000. Active microelectronic chip devices which utilize controlled electrophoretic fields for multiplex DNA hybridization and other genomic applications. *Electrophoresis*. 21(1): 157–164.

[25] Heller, M. J. 2002. DNA microarray technology: Devices, systems, and applications. *Annual Review of Biomedical Engineering*. 4: 129–153.

[26] Hengsakul, M. and Cass, A. E. G. 1996. Protein patterning with a photoactivatable derivative of biotin. *Bioconjugate Chemistry*. 7(2): 249–254.
[27] Hughes, T. R., Mao, M., Jones, A. R., Burchard, J., Marton, M. J., Shannon, K. W., Lefkowitz, S. M., Ziman, M., Schelter, J. M., Meyer, M. R., Kobayashi, S., Davis, C., Dai, H. Y., He, Y. D. D., Stephaniants, S. B., Cavet, G., Walker, W. L., West, A., Coffey, E., Shoemaker, D. D., Stoughton, R., Blanchard, A. P., Friend, S. H., and Linsley, P. S. 2001. Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. *Nature Biotechnology*. 19(4): 342–347.
[28] Ito, S. and Tachibana, M. 2004. Spotting Pin. U. S. Patent 6,835–352 B2.
[29] Ito, S., Tachibana, M., and Yokokawa, N. 2004. Spotting Pin. U. S. Patent 6,726–883.
[30] Kane, R. S., Takayama, S., Ostuni, E., Ingber, D. E., and Whitesides, G. M. 1999. Patterning proteins and cells using soft lithography. *Biomaterials*. 20(23-24): 2363–2376.
[31] Kim, E., Xia, Y. N., and Whitesides, G. M. 1995. Polymer microstructures formed by molding in capillaries. *Nature*. 376(6541): 581–584.
[32] Kim, J. W., Yamagata, Y., Takasaki, M., Lee, B. H., Ohmori, H., and Higuchi, T. 2005. A device for fabricating protein chips by using a surface acoustic wave atomizer and electrostatic deposition. *Sensors and Actuators B-Chemical*. 107(2): 535–545.
[33] Kleinfeld, D., Kahler, K. H., and Hockberger, P. E. 1988. Controlled outgrowth of dissociated neurons on patterned substrates. *Journal of Neuroscience*. 8(11): 4098–4120.
[34] Kononen, J., Bubendorf, L., Kallioniemi, A., Barlund, M., Schraml, P., Leighton, S., Torhorst, J., Mihatsch, M. J., Sauter, G., and Kallioniemi, O. P. 1998. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nature Medicine*. 4(7): 844–847.
[35] Kumar, A., and Whitesides, G. M. 1993. Features of gold having micrometer to centimeter dimensions can be formed through a combination of stamping with an elastomeric stamp and an alkanethiol ink followed by hemical etching. *Applied Physics Letters*. 63(14): 2002–2004.
[36] Lange, S. A., Benes, V., Kern, D. P., Horber, J. K. H., and Bernard, A. 2004. Microcontact printing of DNA molecules. *Analytical Chemistry*. 76(6): 1641–1647.
[37] Lee, K. B., Park, S. J., Mirkin, C. A., Smith, J. C., and Mrksich, M. 2002. Protein nanoarrays generated by dip-pen nanolithography. *Science*. 295(5560): 1702–1705.
[38] Leichle, T., Saya, D., Belaubre, P., Pourciel, J. B., Mathieu, F., Laur, J. P., Nicu, L., and Bergaud, C. June 5-9, 2005. Liquid loading of silicon based cantilevers using electrowetting actuation for microspotting applications. pp. 135–138. 13th International Conference on Solid-State Sensors, Actuators and Microsystems, Seoul, Korea.
[39] LeProust, E., Pellois, J. P., Yu, P. L., Zhang, H., Gao, X. L., Srivannavit, O., Gulari, E., and Zhou, X. C. 2000. Digital light-directed synthesis. A microarray platform that permits rapid reaction optimization on a combinatorial basis. *Journal of Combinatorial Chemistry*. 2(4): 349–354.
[40] Libioulle, L., Bietsch, A., Schmid, H., Michel, B., and Delamarche, E. 1999. Contact-inking stamps for microcontact printing of alkanethiols on gold. *Langmuir*. 15(2): 300–304.
[41] Lin, S. C., Tseng, F. G., Huang, H. M., Huang, C. Y., and Chieng, C. C. 2001. Microsized 2D protein arrays immobilized by microstamps and micro-wells for disease diagnosis and drug screening. *Fresenius Journal of Analytical Chemistry*. 371(2): 202–208.
[42] Lin, S. C., Tseng, F. G., and Chieng, C. C. 2003. Numerical simulation of the stamping process through microchannels. *Journal of Colloid and Interface Science*. 258(1): 179–185.
[43] Lin, S. C., Tseng, F. G., Huang, H. M., Chen, Y. F., Tsai, Y. C., Ho, C. E., and Chieng, C. C. 2004. Simultaneous immobilization of protein microarrays by a micro stamper with back-filling reservoir. *Sensors and Actuators B-Chemical*. 99(1): 174–185.
[44] Livache, T., Roget, A., Dejean, E., Barthet, C., Bidan, G., and Teoule, R. 1994. Preparation of a DNA matrix via an electrochemically directed copolymerization of pyrrole and oligonucleotides bearing a pyrrole group. *Nucleic Acids Research*. 22(15): 2915–2921.
[45] Livache, T., Fouque, B., Roget, A., Marchand, J., Bidan, G., Teoule, R., and Mathis, G. 1998. Polypyrrole DNA chip on a silicon device: Example of hepatitis C virus genotyping. *Analytical Biochemistry*. 255(2): 188–194.
[46] Lom, B., Healy, K. E., and Hockberger, P. E. 1993. A versatile technique for patterning biomolecules onto glass coverslips. *Journal of Neuroscience Methods*. 50(3): 385–397.
[47] Mace Jr., M. L., Montagu, J., Rose, S. D., and McGuinness, G. M. 2000. Novel microarray printing and detection technologies. In: *Microarray Biochip Technology*. pp. 39–64. Schena, M., Ed., Eaton Publishing, Natick, MA.
[48] Madou, M. J. 2002. *Fundamentals of Microfabrication*. CRC Press, New York.
[49] Martin, B. D., Gaber, B. P., Patterson, C. H., and Turner, D. C. 1998. Direct protein microarray fabrication using a hydrogel "stamper," *Langmuir*. 14: 3971–3975.
[50] Martinsky, T., and Haje, P. 2000. Microarray Tools, Kits, Reagents, and Services. In: *Microarray Biochip Technology*. pp. 201–220. Schena, M., Ed., Eaton Publishing, Natick, MA.
[51] Martinsky, T. 2004. Protein microarray manufacturing. *PharmaGenomics*. 2: 42–46.
[52] Martinsky, T. 2005. Enabling Tools for Protein Microarrays. In: *Protein Microarrays*. pp. 441–461. Schena, M., Ed., Jones and Bartlett Publishers, Inc., Sudbury, MA.
[53] McGall, G. H., Barone, A. D., Diggelmann, M., Fodor, S. P. A., Gentalen, E., and Ngo, N. 1997. The efficiency of light-directed synthesis of DNA arrays on glass substrates. *Journal of the American Chemical Society*. 119(22): 5081–5090.
[54] Moerman, R., Frank, J., Marijnissen, J. C. M., Schalkhammer, T. G. M., and van Dedem, G. W. K. 2001. Miniaturized electrospraying as a technique for the production of microarrays of reproducible micrometer-sized protein spots. *Analytical Chemistry*. 73(10): 2183–2189.
[55] Mooney, J. F., Hunt, A. J., McIntosh, J. R., Liberko, C. A., Walba, D. M., and Rogers, C. T. 1996. Patterning of functional antibodies and other proteins by photolithography of silane monolayers. *Proceedings of the National Academy of Sciences of the United States of America*. 93(22): 12287–12291.
[56] Moore, S. K. 2001. Making chips to probe genes. *IEEE Spectrum*. 38(3): 54–60.
[57] Morozov, V., and Morozova, T. Y. 2002. Electrospray apparatus for mass fabrication of chips and libraries. U.S. Patent 6,350–609.
[58] Morozov, V. N., and Morozova, T. Y. 1999. Electrospray deposition as a method for mass fabrication of mono- and multicomponent microarrays of biological and biologically active substances. *Analytical Chemistry*. 71(15): 3110–3117.
[59] Morozov, V. N. 2005. Protein microarrays: Principles and limitations. In: *Protein Microarrays*. pp. 71–106. Schena, M., Ed., Jones and Bartlett Publishers, Inc., Sudbury, MA.
[60] Nocito, A., Kononen, J., Kallioniemi, O. P., and Sauter, G. 2001. Tissue microarrays (TMAs) for high-throughput molecular pathology research. *International Journal of Cancer*. 94(1): 1–5.
[61] Odom, T. W., Love, J. C., Wolfe, D. B., Paul, K. E., and George, M. W. 2002. Improved pattern transfer in soft lithography using composite stamps. *Langmiur*. 18: 5314–5320.
[62] Okamoto, T., Suzuki, T., and Yamamoto, N. 2000. Microarray fabrication with covalent attachment of DNA using Bubble Jet technology. *Nature Biotechnology*. 18(4): 438–441.
[63] Parallel Synthesis Technologies Inc. 2005. http://www.parallel-synthesis.com/smt-info.htm.
[64] Pease, A. C., Solas, D., Sullivan, E. J., Cronin, M. T., Holmes, C. P., and Fodor, S. P. A. 1994. Light-generated oligonucleotide arrays for rapid DNA-sequence analysis. *Proceedings of the National Academy of Sciences of the United States of America*. 91(11): 5022–5026.
[65] Piner, R. D., Zhu, J., Xu, F., Hong, S. H., and Mirkin, C. A. 1999. "Dip-pen" nanolithography. *Science*. 283(5402): 661-663.

[66] Pirrung, M. C., and Huang, C. Y. 1996. A general method for the spatially defined immobilization of biomolecules on glass surfaces using "caged" biotin. *Bioconjugate Chemistry*. 7(3): 317–321.
[67] Pirrung, M. C. 2002. How to make a DNA chip. *Angewandte Chemie-International Edition*. 41(8): 1277–1289.
[68] Pritchard, D. J., Morgan, H., and Cooper, J. M. 1995. Patterning and regeneration of surfaces with antibodies. *Analytical Chemistry*. 67(19): 3605–3607.
[69] Renault, J. P., Bernard, A., Juncker, D., Michel, B., Bosshard, H. R., and Delamarche, E. 2002. Fabricating microarrays of functional proteins using affinity contact printing. *Angewandte Chemie-International Edition*. 41(13): 2320–2323.
[70] Renault, J. P., Bernard, A., Bietsch, A., Michel, B., Bosshard, H. R., Delamarche, E., Kreiter, M., Hecht, B., and Wild, U. P. 2003. Fabricating arrays of single protein molecules on glass using microcontact printing. *Journal of Physical Chemistry B*. 107: 703–711.
[71] Ringeisen, B. R., Wu, P. K., Kim, H., Pique, A., Auyeung, R. Y. C., Young, H. D., Chrisey, D. B., and Krizman, D. B. 2002. Picoliter-scale protein microarrays by laser direct write. *Biotechnology Progress*. 18(5): 1126–1129.
[72] Rose, D. 2000. Microfluidic technologies and instrumentation for printing DNA microarrays. In: *Microarray Biochip Technology*. pp. 19-38. Schena, M., Ed., Eaton Publishing, Natick, MA.
[73] Rose, D. and Tisone, T. C. 2003. Tip design and random access array for microfluidic transfer. U. S. Patent 6,551–557 B1.
[74] Schena, M., Shalon, D., Davis, R. W., and Brown, P. O. 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science*. 270(5235): 467–470.
[75] Schmid, H. and Michel, B. 2000. Siloxane polymers for high-resolution, high-accuracy soft lithography. *Macromolecules*. 33(8): 3042–3049.
[76] Schwarz, A., Rossier, J. S., Roulet, E., Mermod, N., Roberts, M. A., and Girault, H. H. 1998. Micropatterning of biomolecules on polymer substrates. *Langmuir*. 14(19): 5526–5531.
[77] Shalon, D., Smith, S. J., and Brown, P. O. 1996. A DNA microarray system for analyzing complex DNA samples using two- color fluorescent probe hybridization. *Genome Research*. 6(7): 639–645.
[78] Sheehan, P. E., Edelstein, R. L., Tamanaha, C. R., and Whitman, L. J. 2003. A simple pen-spotting method for arraying biomolecules on solid substrates. *Biosensors and Bioelectronics*. 18: 1455–1459.
[79] Singh-Gasson, S., Green, R. D., Yue, Y. J., Nelson, C., Blattner, F., Sussman, M. R., and Cerrina, F. 1999. Maskless fabrication of light-directed oligonucleotide microarrays using a digital micromirror array. *Nature Biotechnology*. 17(10): 974–978.
[80] Singhvi, R., Kumar, A., Lopez, G. P., Stephanopoulos, G. N., Wang, D. I. C., Whitesides, G. M., and Ingber, D. E. 1994. Engineering cell-shape and function. *Science*. 264(5159): 696–698.
[81] Takagi, F., Kurosawa, R., Sawaki, D., Kamisuki, S., Takai, M., Ishihara, K., and Atobe, M. 2004. Pico liter dispenser with 128 independent nozzles for high throughput biochip fabrication. pp. 276–279. Mems 2004: 17th IEEE International Conference on Micro Electro Mechanical Systems, Technical Digest, New York, USA.
[82] TeleChem International Inc. 2005. http://arrayit.com.
[83] Tsai, J., and Kim, C. J. 2002. A silicon-micromachined pin for contact droplet printing. pp. 295–298. Second Joint EMBS/BMES Conference, Houston, TX, USA.
[84] Tseng, F.-G., Lin, S.-C., Yao, D.-J., Huang, H., and Chieng, C.-C. 2005. Technological aspects of protein microarrays and nanoarrays. In: *Protein Microarrays*. pp. 305–338. Schena, M., Ed., Jones and Bartlett Publishers, Inc., Sudbury, MA.
[85] Tseng, F. G., Kim, C. J., and Ho, C. M. 2002. A high-resolution high-frequency monolithic top-shooting microinjector free of satellite drops - Part I: Concept, design, and model. *Journal of Microelectromechanical Systems*. 11(5): 427–436.
[86] University of Stanford. 2005. http://cmgm.stanford.edu/pbrown/mguide/tips.html.
[87] Wadu-Mesthrige, K., Xu, S., Amro, N. A., and Liu, G. Y. 1999. Fabrication and imaging of nanometer-sized protein patterns. *Langmuir*. 15(25): 8580–8583.
[88] Wang, X. H., Istepanian, R. S. H., and Song, Y. H. 2003. Microarray image enhancement by denoising using stationary wavelet transform. *IEEE Transactions on Nanobiosicence*. 2: 184–189.
[89] Weibel, C. June/July 2002. The Spotting Accelerator^{TM}, customizable head assembly for advanced microarraying. *Journal of the Association for Laboratory Automation*. 7(3): 91–96.
[90] Wu, R. Z., Bailey, S. N., and Sabatini, D. M. 2002. Cell-biological applications of transfected-cell microarrays. *Trends in Cell Biology*. 12(10): 485–488.
[91] Xia, Y. N., Tien, J., Qin, D., and Whitesides, G. M. 1996. Non-photolithographic methods for fabrication of elastomeric stamps for use in microcontact printing. *Langmuir*. 12(16): 4033–4038.
[92] Xia, Y. N. and Whitesides, G. M. 1998. Soft lithography. *Angewandte Chemie-International Edition*. 37(5): 551–575.
[93] Xu, S. and Liu, G. Y. 1997. Nanometer-scale fabrication by simultaneous nanoshaving and molecular self-assembly. *Langmuir*. 13(2): 127–129.
[94] Zeng, J., Deshpande, M., Kan, H.-C., and Gilbert, J. R. 2001. A dynamic spotting method for split-pin based microarrays. pp. 143–144. Micro Total Analysis Systems (MicroTAS) Conference, Monterey, CA, USA.
[95] Zhao, X. M., Xia, Y. N., and Whitesides, G. M. 1997. Soft lithographic methods for nano-fabrication. *Journal of Materials Chemistry*. 7(7): 1069–1074.