

Applications of dip-pen nanolithography

The ability to tailor the chemical composition and structure of a surface at the sub-100-nm length scale is important for studying topics ranging from molecular electronics to materials assembly, and for investigating biological recognition at the single biomolecule level. Dip-pen nanolithography (DPN) is a scanning probe microscopy-based nanofabrication technique that uniquely combines direct-write soft-matter compatibility with the high resolution and registry of atomic force microscopy (AFM), which makes it a powerful tool for depositing soft and hard materials, in the form of stable and functional architectures, on a variety of surfaces. The technology is accessible to any researcher who can operate an AFM instrument and is now used by more than 200 laboratories throughout the world. This article introduces DPN and reviews the rapid growth of the field of DPN-enabled research and applications over the past several years.

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Developing the ability to control the shape, size, and chemical composition of structures on the 1–100 nm length scale is an exciting challenge because it opens new research possibilities in a number of fields ranging from electronics to medical diagnostics. There are currently a wide variety of techniques for generating nanoscale architectures. Among these, scanning probe microscope-based lithographies (SPLs) offer both ultrahigh resolution and *in situ* imaging capabilities, and as such, these techniques are actively used as nanofabrication tools^{1–3}. In some cases, scanning probe methods have even enabled researchers to manipulate matter at the individual atom or molecule level⁴. However, most SPL techniques rely on the elimination or modification (through oxidation, etching, shaving, or grafting) of a passivating layer and subsequent adsorption of the patterning material from solution^{2,3,5,6}. These indirect, often surface-‘destructive’ approaches have a fundamental limitation with respect to the types and number of materials that can be patterned within a nano- or microscopic field of view. In addition, indirect SPL methods are limited in throughput because of difficulties associated with parallelization³.

Dip-pen nanolithography (DPN)^{7–9} is a novel scanning probe-based tool that uses an ‘ink’-coated AFM tip to pattern a surface (Fig. 1). Unlike the other SPL methods, DPN is a direct-write ‘constructive’ lithographic tool that allows soft and hard materials to be printed from scanning probe tips onto a surface with high registration and sub-50-nm resolution. Importantly, there is no premodification of the surface through energy delivery prior to the constructive and additive ink delivery event. Because of its direct-write capability and soft-matter compatibility, DPN can be used to deposit multiple compounds, sequentially or in parallel, precisely

and exclusively where they are needed. There is no need to expose the patterning substrate to harsh conditions such as ultraviolet, ion- or electron-beam irradiation, or even non-polar solvents, thus avoiding cross-contamination or damage. These capabilities make DPN a unique and highly desirable tool for depositing biological and other soft matter on a variety of surfaces.

The first demonstration of DPN was carried out with alkanethiol molecules⁷. These molecules could be delivered to a Au surface with 15 nm resolution, and the deposited structures were stable, crystalline and indistinguishable from self-assembled monolayers (SAMs) grown in bulk solutions^{11,12}. These results disproved a 1995 claim that alkanethiol molecules could not be deposited onto a gold surface using a scanning probe tip¹³ and resulted in the invention of DPN⁷. During subsequent years DPN has been developed to pattern a wide variety of inks, including small organic molecules^{7,11,14–21}, polymers^{22–27}, DNA^{28,29}, proteins^{30–33}, peptides^{34–36}, colloidal nanoparticles³⁷, metal ions^{38,39} and sols^{40,41}. The range of patterning substrates has also been expanded to include many insulating, semiconducting and metallic substrates^{17,20,24,42}. Many of these early developments have been reviewed by Ginger *et al.*⁸ and will not be discussed here.

Because of the unique capabilities of DPN, many research groups are using this tool to address important scientific questions inherent to nanoscale structures, as well as making fundamental contributions to the development of the DPN technique. Research efforts have ranged from theoretical^{43–50} and experimental^{51–59} studies elucidating the basic mechanisms of molecular diffusion and transport in DPN, to the development of novel methods of ink delivery such as thermal DPN (tDPN) of metals¹⁰ and the introduction of active and multifunctional tips^{60–62}.

This review will highlight the latest developments in the DPN technique with an emphasis on the unique advantages of DPN as an enabling research tool. The review is divided into three sections: (1) DPN for fabricating biological nanoarrays, (2) templated hierarchical assembly of materials, and (3) extending the capabilities of DPN.

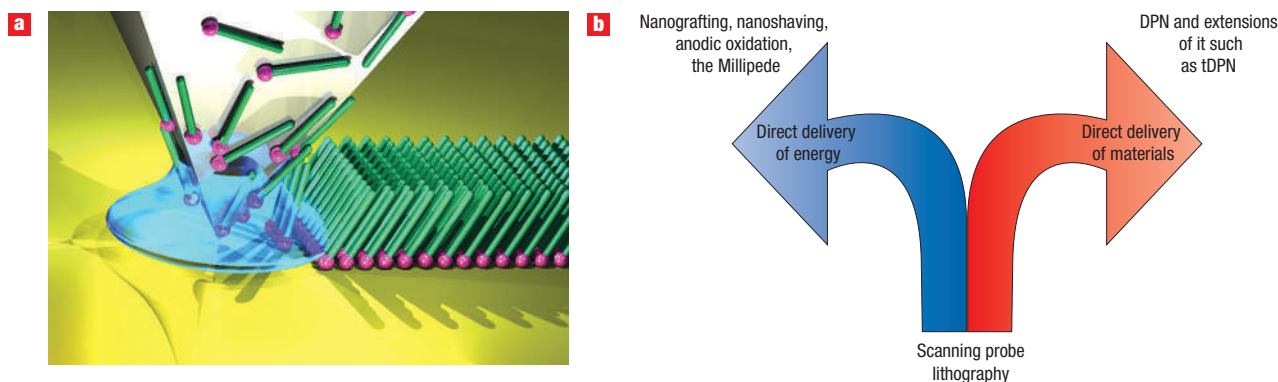


Figure 1 Dip-pen nanolithography (DPN). **a**, Schematic of DPN. An alkanethiol ink-coated AFM tip is brought into contact with a gold substrate. Molecules diffuse from the tip to the surface through or over a water meniscus and assemble in the wake of the tip path to form a stable nanostructure. **b**, Diagram showing the fundamental difference between the different types of scanning probe lithography processes. All systems developed prior to DPN involved the delivery of energy to a surface to chemically or physically modify it. For molecular printing technologies, the necessity to do this prior to an ink adsorption event presents daunting challenges in terms of parallelization and multiplexing (the use of multiple inks simultaneously). DPN involves the direct delivery of the ink to a surface, so one or more inks can be confined to different tips and many chemically pristine nanostructures can be made simultaneously. Variants of DPN such as thermal DPN¹⁰ use energy to facilitate ink flow rather than to chemically or physically change the underlying substrate.

DPN FOR FABRICATING BIOLOGICAL NANOARRAYS

Nanoarrays of biological molecules are fundamentally exciting for a number of reasons. First, nanoarrays can hold 10^4 – 10^5 more features than conventional microarrays and, as a result, a larger number of targets can be screened more rapidly in an individual experiment. Second, the total area occupied by a fixed number of targets can be dramatically reduced, which translates into vastly decreased sample volumes and allows for detection of a smaller number of target molecules for a given analyte concentration. This can lead to significantly lower limits of detection (orders of magnitude from a copy number standpoint) than could otherwise be achieved with microarray technology, however, one of the drawbacks of the nanoarray is that it lowers the analyte concentration range that can be probed because device saturation occurs over a more narrow range with small feature sizes, so one must choose applications of the nanoarray where this is not a major concern. Third, nanoarrays can be used to address important fundamental questions pertaining to biomolecular recognition, since biorecognition is inherently a nano- rather than a micro- or macroscopic phenomenon.

Because of these important advantages, nanoarrays of biological molecules have the potential to become a revolutionary new platform for performing biological studies. Using DPN, such nanoarrays can be routinely fabricated, although massive multiplexing capabilities are yet to be demonstrated. Notably, a variety of biological molecules, including DNA^{28,29}, peptides^{34–36}, proteins^{30–33,63}, viruses^{64–66} and bacteria⁶⁷ have been patterned using direct-write or indirect adsorption approaches.

These DPN-enabled nanoarrays have also been used for biological studies. For example, Lee *et al.* used nanoarrays of the anti-p24 antibody to screen for the human immunodeficiency HIV-1 virus (HIV-1) p24 antigen in serum samples⁶³. In this work, the antibody nanoarrays were fabricated using DPN-patterned 16-mercaptohexadecanoic acid (MHA) dot features as templates for antibody immobilization. On capturing p24, the anti-p24 features increased in height by 2.3 ± 0.6 nm (measured with AFM). This height increase can be further amplified by sandwiching the captured p24 protein with anti-p24-functionalized gold

nanoparticle probes (Fig. 2). Importantly, this report demonstrates that nanoarray-based assays can exceed the detection limit of conventional enzyme-linked immunosorbent assays by orders of magnitude. In this work the antibody nanoarrays are fabricated using DPN-patterned MHA dot features as a template for antibody immobilization.

In contrast to the MHA-directed immobilization in the previous example, Kang *et al.* used DPN to directly print integrin $\alpha_v\beta_3$ nanoarrays³³. These nanoarrays provide a platform for investigating the molecular interaction between integrin $\alpha_v\beta_3$ and vitronectin cell adhesion protein. With protein recognition and binding, AFM topography measurements indicated a 30 ± 5 nm height increase. Using bovine serum albumin nanoarrays as a control, it was further confirmed that the patterned integrin proteins retain their biological selectivity after surface immobilization³³.

As illustrated in the previous examples, biomolecular DPN-based publications have yet to show sub-40-nm spatial resolution, and consequently, controlling the orientation or position of individual proteins is just beyond the reach of DPN. However, the critical dimension of viruses is at the ~20–200 nm length scale, and DPN serves as an ideal tool to investigate single virus particle control. As a step towards developing virus particle immobilization chemistry, both De Yoreo⁶⁴ and Smith *et al.*⁶⁵ have immobilized collections of cowpea mosaic virus (CPMV) particles onto DPN-generated chemical templates. Although those approaches use different chemical attachment schemes, both require the genetic modification of the CPMV virus capsid with thiol groups, and neither were able to demonstrate single-particle control. Recently, Vega *et al.* reported that individual tobacco mosaic viruses (TMV), which have an anisotropic tube shape (300×18 nm), can be immobilized by nano-affinity templates⁶⁶. By optimizing the dimensions of the DPN-generated template and using a metal-ion-based coupling approach, they showed that single TMV particles could be isolated and oriented on MHA templates with rectangular feature dimensions of 350×100 nm spaced 1 μ m apart. This is the first step towards using DPN to create specifically tailored surfaces, by positioning individual biological structures, in order to investigate biorecognition and virus-cell infectivity processes.

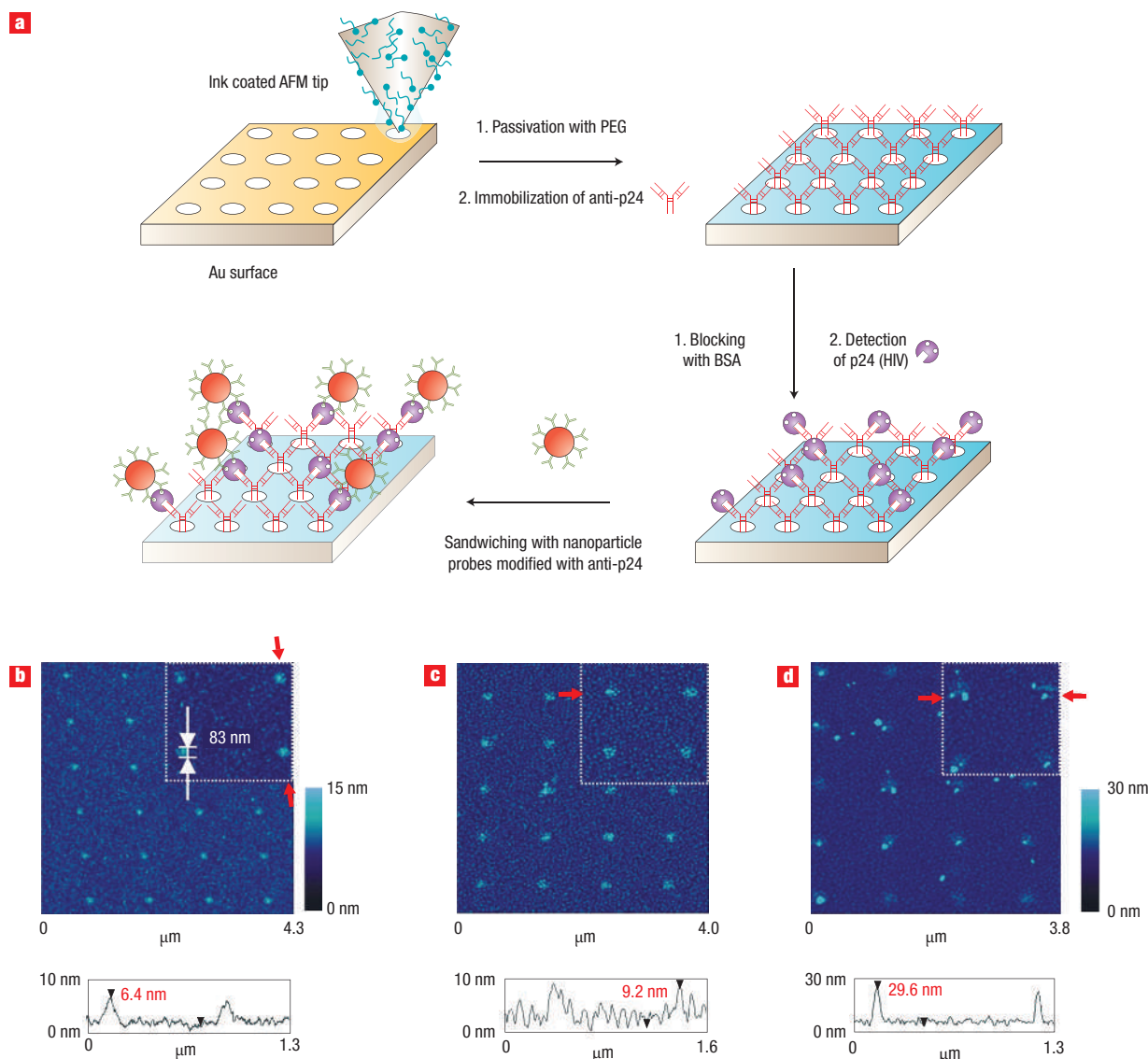


Figure 2 Detection of HIV-1 p24 antigen using a nanoarray of anti-p24 antibody. **a**, DPN-generated nanoarrays of MHA are used to immobilize anti-p24 antibodies on a Au substrate. The bare Au regions were passivated with 11-mercaptopundecyl-tri(ethylene glycol) (PEG), and nonspecific protein adsorption was minimized by blocking with bovine serum albumin (BSA). The presence of HIV-1 p24 in patient plasma is probed by measuring the height profile of the anti-p24 antibody array. The height increase signal as a result of specific antigen–antibody binding is further amplified by a nanoparticle sandwich assay. **b–d**, AFM images that demonstrate the detection of HIV-1 p24 antigen. **b**, Anti-p24 IgG protein nanoarray. Topography trace of adsorbed anti-p24 IgG on MHA. **c**, After p24 binding to anti-p24 IgG, a height increase for the IgG features is observed. **d**, p24 detection after amplification with anti-p24 IgG coated gold nanoparticles (20 nm). Reproduced with permission from ref. 63. Copyright (2004) ACS.

A variety of enzymes, patterned using a DPN-based approach, have been used to direct localized surface reactions. Enzymes are attractive inks because of the range, high yield and specificity of catalysed reactions that can be performed under very mild conditions. Chilkoti and co-workers first demonstrated that DPN can be used to deliver DNase I (a nonspecific endonuclease that digests double-stranded DNA into nucleotide fragments) onto a DNA modified Au substrate⁶⁸. By measuring the height of the patterned regions before and after activation of the DNase I enzyme with Mg^{2+} , they demonstrated that the enzyme was biologically active and that it could catalyse the digestion of a DNA monolayer. Another approach for using enzymes in nanoscale patterning is the application of the enzyme to chemically patterned regions of a substrate to initiate specific reactions. For example, Kaplan *et al.*

demonstrated that horseradish peroxidase (HRP) can be used to polymerize the aromatic ring of 4-aminothiophenol and tyrosine ethyl ester hydrochloride to form conducting wires⁶⁹. In a recent report⁷⁰, they further showed that HRP could be used to polymerize caffeic acid nanostructures that were deposited onto an amino-terminated monolayer. Interestingly, surface-immobilized enzymes can be used to generate metallic structures using the byproducts of catalysis. For example, Willner and co-workers demonstrated that glucose oxidase (GOx) and alkaline phosphatase (AlkPh) can be used to selectively reduce Au and Ag metal salts to form metallic wires⁷¹. Here DPN was used to generate the biologically active lines of both GOx and AlkPh in the same nanoscopic field of view.

The above examples provide a glimpse into the effectiveness of DPN for generating biological nanoarrays. Most of these

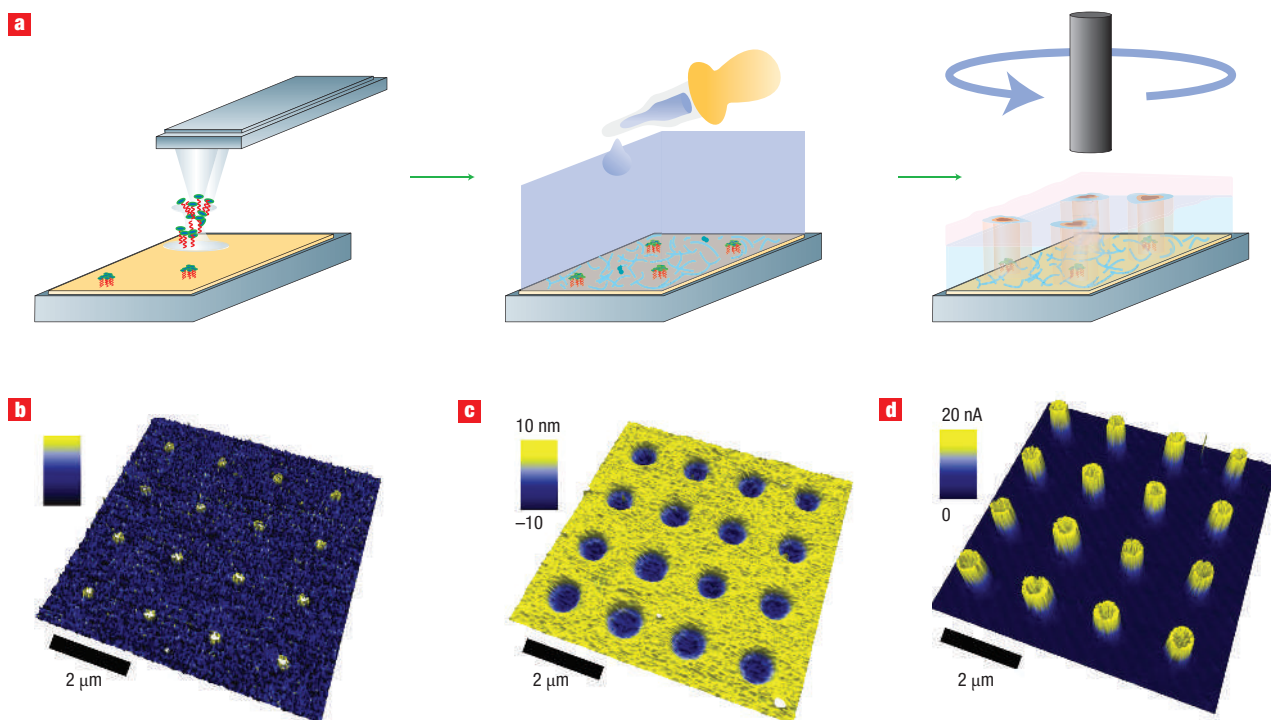


Figure 3 Monolayer templates defined by DPN are used to screen phase separation in polymer blend films. **a**, A scheme depicting the approach. DPN is used to generate nanoarrays of MHA onto an Au substrate and then a 20–60-nm-thick polymer blend of P3HT/PS is spin-coated onto this patterned surface. The resulting film morphology is subsequently characterized by AFM and optical microscopy. **b**, AFM lateral force microscopy image of MHA dot template. **c**, AFM height image (10 nm z-scale). **d**, Conductive AFM image (20 nA z-scale) of the P3HT/PS film showing distinct domains in the deposited polymer blend on top of the MHA patterns. Recessed areas with high conductivity correspond to the semiconducting P3HT-rich domains. Reproduced with permission from ref. 72. Copyright (2005) ACS.

nanoarrays consist of many features made of the same protein and this platform has allowed researchers to investigate biological recognition processes and is designed to manipulate structures such as viruses at the single particle level. It is worth noting that arrays have been fabricated that contain features made of different proteins^{30,31}, but applications for them are just beginning to be explored. These combined capabilities will be critical in shedding light on the roles of cooperativity and multivalency in viral infection studies, which are typically missed because of population averaging. The micro- and nano-environment of a cell plays a critical role in determining its fate and many of its functions. By interfacing cells with patterned substrates that precisely present biologically active proteins and extracellular-matrix components we can take a step towards mimicking aspects of the environment encountered by cells. As such, DPN is positioned to make a big impact in the study of important processes ranging from cell–cell adhesion to the mechanisms of cell migration. Nonetheless, one of the biggest challenges in performing experiments with live cells is the inherent heterogeneity found from sample to sample. Subsequently, the throughput limitation of single-pen DPN becomes a bottleneck in such experiments. The recent advent of massively parallel capabilities (which will be discussed later) addresses this limitation, and opens the door to performing a statistically significant number of cell-surface experiments in an accelerated fashion.

TEMPLATED HIERARCHICAL ASSEMBLY OF MATERIALS

DPN offers a rapid method of generating chemical templates with tailored chemical composition and structure, which provides

a powerful way of investigating nanoscale phenomena in fields ranging from crystallization to phase separation, and from magnetic information storage to nanoscale electronics. Surfaces can be directly modified to present almost any chemically stable functional group with sub-50-nm lateral resolution. What is particularly exciting here is that combinatorial libraries containing a range of pattern sizes, shapes, and chemical compositions can be systematically varied and routinely produced to probe physical and chemical phenomena at the micro- to nanometre length scales.

For example, Ginger *et al.* used DPN as a rapid prototyping tool to generate and study nanoscale structures in thin composite polymer films⁷². They demonstrated that DPN provides a means to study heterogeneous nucleation on surfaces by screening size-dependent interactions between polymer films and surface features. The study was performed by spin-coating 20–60-nm-thick polymer blends of poly-3-hexylthiophene/polystyrene (P3HT/PS) onto MHA dot nucleation sites generated by DPN with sizes that were systematically varied from 50 to 750 nm (Fig. 3). The domain size of the P3HT phase was found to correspond to the size of the MHA nucleation site as well as the relative ratio of P3HT/PS. In another example, Ivanisevic and co-workers investigated the patterning of polyelectrolytes on SiO_x surfaces^{22,73}. The assembly of polymers on templated patterns can be performed over large areas using parallel DPN. We recently demonstrated this capability by investigating the assembly of polyelectrolyte multilayer organic thin films on an array of 67,000 MHA dot features (200 nm diameter)⁷⁴.

DPN-defined chemical templates of various sizes, shapes, and compositions have been used to screen the directed assembly of

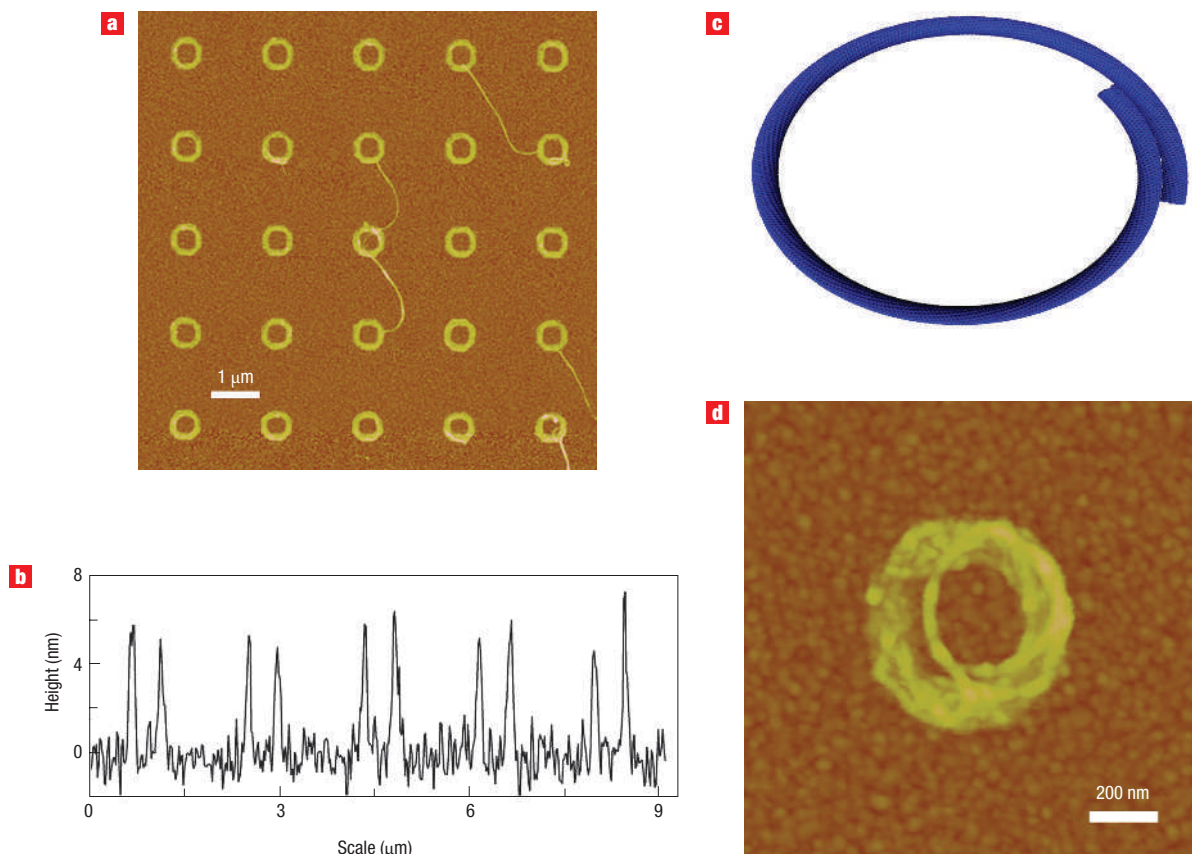


Figure 4 SWNTs assembled into rings on chemical templates. AFM tapping mode topographic images (**a**) and height profiles (**b**) of SWNT rings in a 5 x 5 array are shown. Height profile indicates that multiple SWNTs stack to form rings with relatively uniform thicknesses. Note that some SWNTs are not constrained within the ring templates and instead bridge multiple ring sites. **c**, A molecular model of a coiled SWNT. **d**, A zoom-in view of one SWNT ring. Reproduced with permission from ref. 76. Copyright (2006) National Academy of Sciences USA.

single-walled carbon nanotubes (SWNTs)^{75,76} and other nanoscale building blocks^{77–80}. Hong and co-workers initially reported that SWNTs could be assembled along line features of chemical templates (such as imidazole) in a solution through electrostatic interactions between SWNTs and the chemical templates⁷⁵. In related but different work, we found that SWNTs can be assembled and manipulated using templates of MHA defined by DPN⁷⁶. In this work, unusual, and in some cases highly strained, SWNT structures, such as dots, rings, arcs and letters, could be fabricated easily from the appropriate template (Fig. 5). Such precise control of the SWNT structure arose from an interesting phenomena occurring at the boundaries between the MHA features and the passivating 1-octadecanethiol (ODT) SAM, where the SWNTs are strongly attracted to the carboxylic groups of the MHA. Interestingly, SWNTs with lengths greater than the dimensions of the MHA feature align along the boundary of the pre-generated nano-feature in order to maximize SWNT/COOH interactions and compensate for the tension arising from SWNT bending. Taking advantage of the high-resolution and rapid prototyping capabilities of DPN, we have carried out a series of comparisons between experiments and theoretical calculations to illustrate and quantify the driving forces for this directed-assembly approach. Notably, a mathematical relationship has been developed that explains the assembly process quantitatively⁷⁶. This relationship balances the geometrically weighted interactions between the

SWNTs and the two different SAMs that are required to overcome solvent–SWNT interactions and to have an effect on assembly. Because there is no specific chemical bonding required, this technique remains effective for the directed assembly of other nanoscale building blocks such as nanowires and nanoparticles. Notably, DPN-patterned negatively charged chemical templates (such as MHA) also allow one to reversibly capture positively charged inorganic V₂O₅ nanowires dispersed in a solution⁷⁷.

The examples described above highlight how DPN-generated chemical templates can be used as screening tools to investigate the bottom-up assembly of polymer composites and nanoscale building blocks. In another case, DPN was found useful in bridging the gap between top-down lithography and self-assembly by integrating DNA recognition into macroscopically addressable electrode junctions. In particular, Chung *et al.* used DPN to directly deposit oligonucleotide probes on lithographically defined electrode nanogaps²⁹. The subsequent capture of complementary DNA-functionalized gold nanoparticles in the nanogap bridges the two electrodes and switches the sensor from an electrically insulating state to an electrically conducting one, which can be used in a simple ‘on/off’ readout scheme. This work illustrates how DPN can be used to interface DNA-directed nanoparticle assembly with conventional microfabrication techniques to produce primitive tunnel-junction circuits. Indeed, many proposed micro- and nanoscale biosensing schemes could benefit from such a means of directly depositing

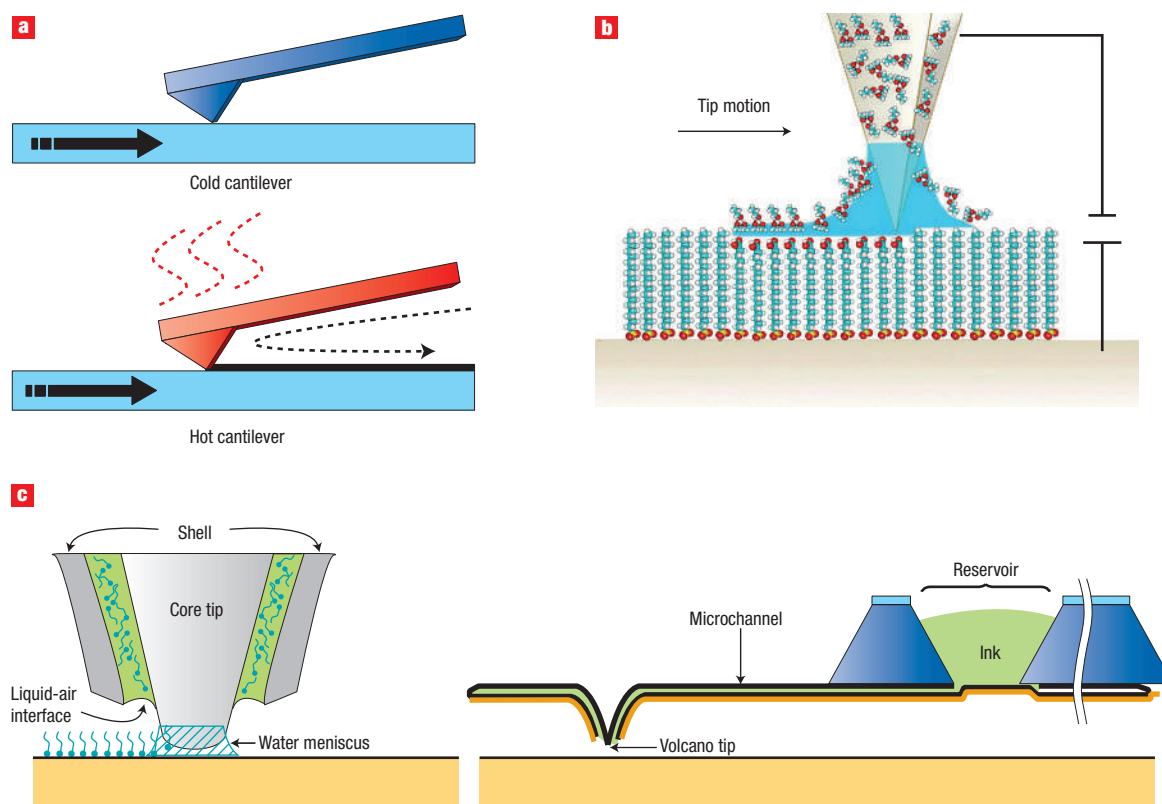


Figure 5 Examples of variants of DPN. **a**, Thermal DPN, which uses a heated AFM cantilever whose tip is coated with a solid 'ink'. When the tip is hot enough, the ink melts and flows onto the substrate. No deposition occurs when the tip is cold, so imaging without any unintended deposition is possible. **b**, Electro pen nanolithography on an octadecyltrichlorosilane (OTS)-coated surface. The terminal methyl group of the OTS is converted to a reactive COOH-terminated surface (OTSx) by applying a voltage between the conducting AFM tip and the conducting silicon substrate in a humid environment. 'Ink' molecules are delivered from the 'inked tip' to the reactive OTSx surface, forming a second layer in the same sweep. No second layer is formed on the methyl-terminated regions. **c**, Writing mechanism of the Nano Fountain Pen. Molecular ink fed from the reservoir forms a liquid-air interface at the annular aperture of the volcano tip. Molecules are transferred by diffusion from the interface to a substrate and a water meniscus is formed by capillary condensation.

ligand/receptor molecules onto specific substrate locations with the high resolution and registration offered by DPN, especially the many recent systems involving nanowire⁸¹ and nanotube⁸² arrays.

Another attractive feature of DPN is its ability to observe molecular processes *in situ*. For example, DPN was recently used to monitor the initiation and kinetics of biopolymer crystallization and growth on cleaved mica substrates²⁴. This work built upon previous research showing that DPN could be used as a tool to study monolayer growth processes by serially delivering adsorbate to a gold surface¹². In particular, poly-DL-lysine hydrobromide crystallized epitaxially into triangular single crystals when delivered to a freshly cleaved mica substrate during a DPN experiment²⁴. The crystallization process could be studied and kinetically controlled on the nanometre to many-micrometre length scales. Such crystallization morphology was not observed when poly-DL-lysine was deposited with macroscopic or microscopic patterning tools. It is believed that the 'tapping mode' used in the deposition process was responsible for the nanoscale crystal nucleation observed²⁴.

Phase separation behaviour has been observed in DPN experiments with binary ink mixtures. For example, when mixtures of alkanethiols (such as 3-mercaptopropanoic acid and 1-hexadecanethiol) are assembled onto a bulk Au surface at certain mixing ratios, nanoscale domains form randomly on the surface^{83,84}. However, when binary mixtures of alkanethiols are deposited

by DPN, phase-separated domains form in which the exterior predominantly comprises the more hydrophobic ink molecules and the interior comprises the hydrophilic ink⁵⁵. This process has been used to improve the resolution of the technique and realize features even smaller than those attainable with conventional DPN. One ink can be used as a sacrificial material whereas the other can be used as an active component to control subsequent template-driven assembly processes⁵⁵. Indeed, it has been shown recently that by depositing alkanethiols by DPN, it is possible to create multicomponent soft matter and solid-state structures that can be removed selectively⁸⁵.

Although most of the examples reported thus far describe chemical patterning of inks bearing simple functional groups ($-\text{COOH}$, $-\text{NH}_2$, $-\text{SH}$ and so on), it is important to note that a wide variety of chemicals and chemistry have been found to be compatible with DPN. For example, Reinhoudt and co-workers recently demonstrated that complex supramolecular host-guest chemistry could be site-specifically directed using DPN^{17,18}. In this example, a variety of adamantyl-modified guest molecules with different valencies were directly deposited onto a β -cyclodextrin SAM with sub-100-nm resolution. Because each β -cyclodextrin molecule has a specific recognition site, the SAM functions as a molecular 'printboard' that allows the formation of kinetically stable high-resolution patterns of guest molecules^{86,87}. Vansco *et al.* have

shown that amine-terminated poly(amidoamine) dendrimers can be directly deposited onto chemically activated ester SAMs⁸⁸. The use of a reactive SAM as a patterning substrate for DPN will not only widely expand the library of ink-substrate combinations, but may also allow site-specific molecular interactions and chemistries to be explored.

EXTENDING THE CAPABILITIES OF DPN

So far we have reviewed some of the exciting work where DPN has played an important role as an enabling research tool. To extend the basic capabilities of DPN beyond the scope of single silicon nitride cantilever tips, a number of research groups, including our own, are involved in further development of the technology. These efforts include both the introduction of novel variants of the DPN technique and the development of high-throughput massively parallel DPN, as outlined below.

VARIANTS OF DPN

A number of variants of DPN have emerged with the introduction of novel multifunctional tips that are more wear resistant⁸⁹, can carry more ink^{60,90–92} or can be individually actuated^{61,93,94}. In addition, advances with tips that use external heating¹⁰ or an electric field^{95–97} to drive physical or chemical transformation of the ink molecules have been reported. These innovative variations have significantly expanded the capabilities of DPN.

For example, Sheehan and co-workers demonstrated that by thermally heating a tip, continuous indium lines as thin as 80 nm can be directly deposited onto glass or silicon surfaces (tDPN, Fig. 5a)¹⁰. An advantage of this approach is that the rate of ink diffusion can be tuned by changing the tip temperature. In an earlier paper, they claimed that solid inks, such as octadecylphosphonic acid (melting point 100 °C) are immobile at room temperature, but can be deposited through the use of tDPN⁹⁸. However, we have recently shown that conventional DPN also works for octadecylphosphonic acid and other high melting point compounds, provided they have some solubility in the water meniscus⁹⁹. Indeed, solubility is one of the key factors in controlling transport rate, and therefore the commercial systems use humidity control systems to influence ink transport rates. tDPN is useful for transporting metallic and certain other materials that are solids at room temperature and do not have appreciable solubility in a carrier solvent.

By combining DPN with electrochemistry, Ocko *et al.* introduced a new surface modification approach termed electro pen nanolithography (EPN), in which a substrate is electrochemically modified and then rapidly coupled to ink material transported from the tip⁹⁶. This is related to the earlier work by Sagiv involving tip-induced local electro-oxidation of surface-immobilized molecules with vinyl groups⁶. By using the water meniscus both as an ink transport media and as an electrochemical cell for the oxidation of an octadecyltrichlorosilane SAM on a SiO_x surface, Ocko and co-workers showed that multiple chemical functionalities (such as a terminal SH or NH₂) could be generated in a one-step process. Significantly, this technique provides for relatively fast patterning (10 μm s⁻¹) and the construction of multilayered three-dimensional patterns (Fig. 5b).

To facilitate tip inking and to increase the quantity of adsorbed ink, novel tip modifications often have to be introduced. For example, AFM tips have been fabricated out of or coated with poly(dimethylsiloxane) (PDMS), which allows for increased ink loading^{90,92,100}. Another strategy, demonstrated by Espinosa *et al.* integrates microfluidic channels into the AFM cantilevers to fabricate ‘nanofountain pen’ (NFP) probe tips capable of depositing alkanethiols onto Au surfaces with

sub-50-nm resolution (Fig. 5c)^{60,91}. Such fluidic ink delivery systems ensure a constant supply of ink to the surface, and keep ink molecules solvated, which can be a critical issue in maintaining the biological activity and tertiary structure of many proteins. Both nano- and micropipettes have been used as scanning probe tips to deliver reagents directly to a substrate^{101–104}, but the implementation of microfabricated delivery channels in the context of DPN experiment should lead to a more reliable and versatile form of nanolithography.

PARALLELIZATION OF DPN

Parallelization of the DPN technique is driven by the need for a lithographic tool that is not only capable of high-resolution patterning, but also has high throughput and massive multiplexing capabilities. Such a tool, if broadly accessible, will open up many applications important to biologists and materials scientists. These include the screening of cell-surface interactions, viral infectivity at the single-particle/single-cell level, and the ability to fabricate nanoscale photonic and electronic devices^{105,106}.

Throughput limitations are a problem inherent to the field of scanning probe microscopy and lithography in general. They are arguably the single largest barrier in the development of useful production-scale techniques based upon SPLs. Several groups have sought to address this problem through the use of parallel arrays of cantilevers. Most notably, the Quate group at Stanford has fabricated both linear and two-dimensional arrays of cantilevers for both imaging and lithographic applications^{107–110}. They have successfully demonstrated that up to 50 conductive AFM tips in a linear array could be used to perform anodic oxidation of silicon surfaces^{107–110}. For the last decade, IBM has been investigating the use of two-dimensional tip arrays for ultrahigh-density data storage applications. The approach is based on heating a cantilever tip in contact with a thin polymer film and subsequently melting the polymer above its glass transition temperature to create pits¹⁰⁷. Cantilevers write data bits by heating, and data can be read by measuring the amount of heat dissipation from each tip. Arrays with as many as 32 × 32 individually addressable cantilevers, known as ‘the Millipede’, have been used^{111–115}, and up to 4,024 cantilevers have been fabricated¹¹¹. Although these early demonstrations are impressive, it is important to note that none of these parallel approaches involve molecular transport in a lithographic process (Fig. 1b). Moreover, the challenges and design constraints for making a functional array are completely different when the focus is to deliver of energy, rather than material, to an underlying substrate.

Increasing the throughput of DPN has taken on two different approaches, one with individually actuated tips and the other with passive ‘duplicating’ tips. Independent tip actuation has been previously demonstrated using piezoelectric, thermoelectric, and electrostatic mechanisms^{61,93,94}. Earlier demonstrations of active DPN pen arrays were based on thermally actuated tips⁹³. Such tips are composed of two different materials with different thermal expansion coefficients, which leads to bending when they are heated. Using the thermal actuation approach, Liu’s group and our group demonstrated the ‘on/off’ actuation and deposition of ODT with an array of ten cantilevers⁹⁴. The thermal bimetallic actuation is robust, simple and effective; however, its main limitation is the undesirable effects of heat on the ink material being deposited. To address this problem, electrostatically actuated DPN probe arrays were fabricated and used to pattern ODT⁶¹. Parallel actuation was demonstrated with an array of ten cantilevers, which were used to generate 30–40-nm ODT lines on a Au substrate. Electrostatic actuation is an attractive approach because of reduced probe heating and reduced tip-to-tip cross talk⁶¹.

Independently actuated tips afford the flexibility to generate chemically complex patterns. Alternatively, a simpler approach

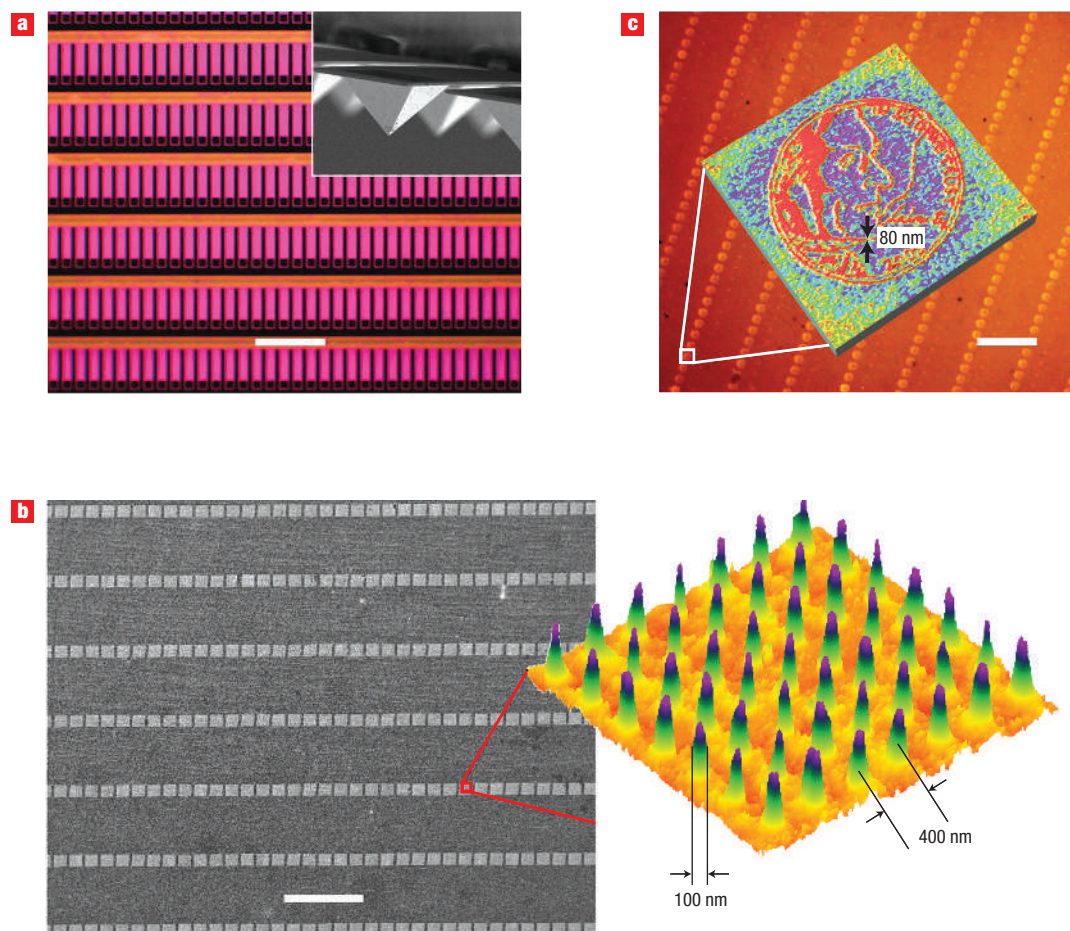


Figure 6 DPN patterning with 55,000 AFM cantilevers in parallel. **a**, Optical micrograph of a small portion of the two-dimensional array of cantilevers used for patterning. The inset shows an SEM image of the tips. **b**, SEM image of a portion of an 88,000,000 gold dot array (40×40 within each block) on an oxidized silicon substrate. On the right-hand side is a representative AFM topographical image of part of a block. **c**, Optical micrograph of a representative region of the substrate where ~55,000 sophisticated features were patterned. Each of the circular features is a miniaturized replica of the face of the 2005 US five cent coin. The coin bears a picture of Thomas Jefferson, who helped develop the polygraph, a letter duplicator that relies on an array of pens. Scale Bars: 100 μm . Reproduced with permission from ref. 119. Copyright (2006) Wiley.

to increasing the throughput of DPN is to use large arrays of passive probes^{11,116}. In collaboration with the Liu group we have used both commercial and custom-fabricated cantilevers in linear array formats to perform DPN in parallel over a distance of one centimetre^{116,117}. The resulting molecular patterns were developed in solid-state Au structures using wet chemical etching techniques and characterized using optical and electron microscopy. Notably, our group, in collaboration with NanoInk and Liu, has fabricated large two-dimensional arrays of cantilevers, which comprise as many as 1.2 million cantilevers on a 3 inch wafer⁸. Liu has also reported a novel 'spring-on-tip' design that could potentially enable higher-density tip arrays to be fabricated as the cantilever in such a design is incorporated within a pyramidal tip¹¹⁸.

A working two-dimensional pen array for DPN, however, has been demonstrated only recently, and it required several innovations to make it operate in a flexible and highly controllable manner¹¹⁹. Again, it is important to note that IBM's Millipede system^{111–115} was developed to deliver energy not material to a surface, and although potentially suitable for data storage applications, its design is not optimum for DPN because the tip feedback is based on heat dissipation, which drastically affects molecular transport⁵⁸. The

first functional two-dimensional pen array for DPN incorporates a novel pen-array architecture that involves low aspect ratio pyramidal tips and curved cantilevers. In addition, a robust but simple and straightforward gravity-driven alignment protocol is used to engage the array during writing. These innovations allow a user to simultaneously align ~55,000 cantilevers with a substrate and subsequently pattern over square centimetre areas with sub-100-nm resolution (Fig. 6). Such arrays have been used to pattern small molecules like ODT and bio-relevant structures like phospholipid membranes¹²⁰. With this approach, the throughput of DPN has been increased by more than four orders of magnitude, and as many as 450,000,000 sub-100-nm features have been patterned in less than 30 min. Remarkably, very sophisticated structures can be made using a dot matrix approach (Fig. 6c). The massive increase in throughput will undoubtedly have an impact on increasing the accessibility and lowering the cost of fabricating nanostructured materials on surfaces. Importantly, this passive 'wire-free' approach for massive parallelization of DPN has emphasized the fundamental difference between molecule-scale nanolithography and thermomechanical data storage. Indeed, for data storage, each tip must be electrically addressable, and

therefore, extremely impressive but complex systems such as IBM's Millipede are required.

For molecular-deposition based nanolithographies such as DPN, relatively simple array architectures and more user-accessible approaches are possible. However, one must design and construct systems that are compatible with the wide array of inks and substrates required for each intended application (for example, combinatorial libraries of proteins, oligonucleotides, polymers and solid-state materials). Although it is conceivable that approaches based upon the Millipede could be used for materials deposition, this has not been shown to date, and if it can be accomplished, it is likely that the range of inks one can use will be limited to structures that can withstand the thermal changes that must accompany device operation. In addition, lithographic control will have to take into account the temperature-dependent diffusion coefficients of the inks. Finally, in doing so, it is critical that the end device is not so complicated that it prohibits researchers from wanting to use it. The passive pen-array approach is an important first step toward the development of a massively multiplexed molecule-based printing technology that would be widely accessible to anyone with a modest understanding of how to operate an AFM-based instrument.

With the advent of DPN, a dichotomy has emerged in the field of scanning probe lithography (Fig. 1b). Techniques can be classified as either direct and 'constructive' or indirect and 'destructive'. Constructive processes such as DPN directly deliver molecules or materials that are to be patterned. Destructive processes such as anodic oxidation¹⁰⁸ and nanografting¹²¹ typically deliver energy to a surface that is often intended to damage the underlying structure, regardless of whether or not there is a subsequent molecular deposition or adsorption event. These functional differences limit where these techniques can be used, the degree of parallelization possible, and ultimately the types of applications that can be considered for each technique. Prior to DPN, patterning more than the field of view of a conventional AFM ($\sim 100 \times 100 \mu\text{m}$) by SPL processes involved the delivery of energy rather than materials or molecules directly to a surface, even if the intent was to eventually pattern a surface with a new chemical entity. The approach used in parallelization and ultimately application depends greatly on the modality of the patterning technique. For this reason, advances from the indirect 'destructive' approaches cannot simply be implemented in the direct-deposition 'constructive' approaches. This is painfully realized when considering that a technology like the Millipede, which was developed for polymer hole fabrication and annealing, relies on arrays with pens that maintain feedback using thermoresistive sensing and operate at $\sim 350^\circ\text{C}$. If such an approach to parallelization was applied to a technology like DPN, there would be no control over ink transport and hence feature size.

SUMMARY AND OUTLOOK

DPN is a unique and highly versatile tool that can routinely pattern a wide range of soft materials on a variety of substrates at the sub-50-nm to many-micrometre length scale. Indeed, it is the only nanofabrication tool that uniquely combines high-resolution direct-write capabilities with soft-matter compatibility. Several unconventional lithographic tools such as microcontact printing and nanoimprint lithography have been developed with the primary intention of competing with the tools used by the semiconductor industry for microelectronic applications¹. Although DPN is finding applications in certain areas that impact the semiconductor industry such as mask fabrication, inspection and repair, thus far, its unique attributes have positioned it on a different path aimed largely at exploiting the properties and applications of nanopatterned soft materials. Over the past several years, DPN has played a pivotal role as an enabling research tool

in areas as diverse as biological recognition to materials assembly. It is opening up the field of nanoarrays and allowing researchers to explore single-particle binding and positioning events.

One of the greatest limitations in nanoscience and nanotechnology involves the lack of efficient methods for macroscopically addressing and manipulating individual nanostructures within the context of an integrated device or material. This limitation is realized in the area of nanoelectronics where researchers have become quite proficient at making nanostructures with unusual and often useful properties, but they still rely on very crude methods for aligning and integrating them within the context of a more sophisticated structure. Once these structures have been assembled, in many cases (for example, in chemical and biological sensors) there is still the problem of chemically functionalizing the individual components within the integrated structure. DPN and more sophisticated variations of DPN provide a solution to this problem, especially because researchers can take advantage of the wide range of chemistries compatible with this technique. The technique becomes even more attractive when considering the recent technological advances that have moved DPN from a low throughput but custom single-pen process to one with massively parallel, high-throughput and large-area capabilities. Taken together, the advances in DPN technology point towards the development of a 'desktop fab' for the custom fabrication of biological chips, nanomaterials, combinatorial chemical templates, and tools important for the semiconductor industry.

On the hardware side, addressable tip inking is the next challenge in developing a suite of DPN-related tools. Such capabilities will enable nanoarrays with almost unlimited chemical and biochemical complexity to be created. Researchers will be able to use these structures to control materials assembly in a very sophisticated manner and open up the field of multivalency in understanding single particle (for example, large proteins, cells, viruses, and spores) surface binding events. There are many possibilities for addressing this challenge including the use of inkjet printing technology¹²² and ink wells^{123,124} to coat the tips, which are sharp on the nanoscopic scale but separated on the microscopic length scale. On the scientific side, we are just beginning to learn how to use these new technologies. When one considers that virtually every material when miniaturized to the sub-100-nm length scale has new properties, DPN becomes an extraordinarily useful tool to study the fundamental consequences of miniaturization.

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Competing Financial Interests

The authors declare that they have no competing financial interests.

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