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Dip Pen Nanolithography

Seema Jadhav*, Pournima Morey, Manisha Karpe, Vilasrao Kadam

1. Bharati Vidyapeeth's College of Pharmacy, University of Mumbai, Sector-8, C.B.D., Belapur, Navi Mumbai- 400614, Maharashtra, India

ABSTRACT

Nanolithography is the art and science of etching, writing, or printing at the nanoscopic level, in which the dimensions of characters are on the order of nanometers. The direct physical interactions between the atomic-force microscopy (AFM) tip and the sample allow local surface modification, allowing use of the AFM tip for scanning-probe lithography. Dip-pen nanolithography (DPN) differs conceptually from other scanning-probe lithography in that, rather than delivering energy to the surface, DPN directly delivers materials to the surface from an ink-coated AFM tip in a molecular printing process. DPN is a scanning probe nano patterning technique in which an atomic force microscope (AFM) tip is used to deliver molecules to a surface via a solvent meniscus, which naturally forms in the ambient atmosphere. This direct-write technique offers high resolution patterning for a number of molecular inks on a variety of substrates. DPN can be used to pattern nanostructure arrays in a massively parallel fashion. Indeed, one and two dimensional arrays of probes with numbers up to 55,000 have been already developed and proved successful in the DPN process. The use of small sample amounts in DPN should be particularly attractive to biologists for significantly lowering limits of detection of target molecules.

Keywords: Nanolithography, atomic force microscopy, dip pen nanolithography, nano patterning, applications

*Corresponding Author Email: smjadhav226@gmail.com

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INTRODUCTION

The emerging field of nanotechnology offers possibilities for studying fundamental chemical and physical principles at nanometer size scales, while also providing research avenues to new platform technologies such as nanodevices and nanosensors.[1-3] One current challenge in the nanotechnology area is the fabrication and interconnection of nanodevices on semiconducting surfaces such as a silicon wafer. Nanomaterials are defined as those whose characteristic length scale lies in 100 nanometers, which have some special properties different from individual atoms (molecules) and from bulk structures. Nanomaterials have potential applications in chemistry, physics, biology, and materials science. Therefore, nanostructured materials have attracted significant interest in recent years. Many methods have been developed to fabricate micro- to nanostructures. Lithographic methods are at the heart of modern-day microfabrication, nanotechnology, and molecular electronics. Conventional lithographic methods rely on patterning of a resistive film, followed by a chemical etch of the substrate. Nanolithography is the art and science of etching, writing, or printing at the nanoscopic level, in which the dimensions of characters are on the order of nanometers. Recently, there has been considerable interest in developing lithographic methods for patterning nanostructures because of their scientific importance and potential technological applications in integrated molecular electronics, high density information storage devices, and ultrahigh sensitive biosensors.[3] Conventional nanolithography techniques, includes optical and electron beam lithography which has either fundamental limitations with respect to complex procedure or unsuitable to handle a large variety of organic and biological molecules that are indispensable building blocks in nanotechnology. To these regards, alternative nanolithography techniques, such as nano-imprint lithography and scanning probe microscope (SPM)-based lithography, have been developed over the past twenty years.[4] In particular; SPM-based approaches offer both ultrahigh resolution and in situ imaging capabilities. Scanning probe methods are becoming more prevalent for investigations of surface chemistry because of the dual capabilities for obtaining physical measurements and structural information with unprecedented sensitivity. Scanning probe microscopy imaging modes have been used for the study of chemical and biochemical reactions and for investigation of tip-surface interactions, chemical structures, and material properties at the molecular level. The capabilities for studying and controlling processes at the nanoscale with SPM are emerging as valuable assets in both fundamental and applied research. Scanning probe instruments not only provide a means for characterizing samples with unprecedented spatial

resolution, but they can also be applied for nanoscale measurements of surface properties and as a nanolithography tool for constructing designed surface arrangements of molecules.[5-7] Fundamental understanding of the interactions of surface reactions provides essential information for developing workable applications for nanotechnology. Scientific developments using SPM are providing a foundation for new technologies in areas such as molecular electronics, materials engineering, medical diagnostics and drug discovery. However, most of these methods are indirect or surface "destructive" approaches in which a passivating layer as template for surface modification through etching, oxidation and shaving or grafting were required.[8] In addition, these methods typically suffered from low throughput due to inherent limitations associated with the instrument.[8] Scanning-probe technologies have unique potential for directly characterizing and functionalizing interfaces with biological materials at ultrahigh resolution because of their ability to function in humid air and aqueous environments.[9,10] For example, this ability permits direct observation in real time of dynamic processes in reconstituted biomolecular systems with molecular resolution in situ. Examples include the direct observation of DNA supercoiling, [11] DNA–protein interactions, and structure and dynamics of lipid membranes. Furthermore, the direct physical interactions between the atomic-force microscopy (AFM) tip and the sample allow local surface modification, allowing use of the AFM tip for scanning-probe lithography.[12] A variety of mechanisms can be used to structure the surfaces with an AFM tip, for example local application of mechanical, electrical, or thermal energy. Dip-pen nanolithography (DPN) differs conceptually from other scanning-probe lithography in that, rather than delivering energy to the surface, DPN directly delivers materials to the surface from an ink-coated AFM tip in a molecular printing process.[13-15] The standard modes of atomic force microscopy operation are merely means of characterizing a sample. With the invention of Dip pen nanolithography system (DPN) [14, 16-20,] the unique advantage is that it offers a direct-draw method for delivering various molecular species onto a single surface in one experiment.

ATOMIC FORCE MICROSCOPY

Atomic force microscopy (AFM) was developed in 1986, following the invention of scanning tunneling microscopy (STM). STM utilizes tunneling current between a conductive tip and a sample to determine surface properties of conductive or semi-conductive materials. Scanning probe microscopy (SPM), such as scanning tunneling microscopy (STM) and atomic force microscopy (AFM) can visualizes surfaces with atomic resolution. Taking advantage of the sharpness of the tips, SPM can also be used for nanofabrication, offering the advantages of

nanometer resolution and flexibility in the generation of patterns.[21,22] However, only conductive or semi-conductive materials are applicable to STM, because STM utilizes the tunneling current between a conductive tip and a sample; while various materials, such as insulators, semiconductors and conductors can be visualized and nano patterned with AFM, because AFM utilizes the Vander Waals interaction between an AFM tip and a sample. Therefore, AFM has a wider application than STM. AFM can provide the surface properties in physics, chemistry, biology, biochemistry, engineering, technology and other disciplines. AFM can also be used to physically or chemically modify surfaces at nanometer scale and perform nano manipulation down to the level of individual molecules or atoms. In less than two decades, AFM has become a very important, widely used and versatile surface technique. As an important surface visualization technique, AFM was advanced from atomic resolution to molecular recognition. AFM has achieved atomic resolution on a variety of materials, and obtained molecular and submolecular resolution on biological systems. AFM can provide much more information other than visualizing surface properties. AFM was used to study surface electrochemical reaction. Chemically modified AFM tip allows various properties of the sample surface to be measured. A nano mechanical array of AFM cantilevers was used as biosensor to realize biomolecular recognition. AFM was also used to determine weak force such as biomolecular interaction. In recent years, AFM-based nanotechnologies were developed rapidly, making AFM a promising technique for nanofabrication. AFM-based nanofabrication technologies such as nano manipulation, force lithography, nano grafting, nanooxidation and dip-pen nanolithography, were developed to physically or chemically modify surfaces and perform manipulation at nanometer scale. Nanostructures composed of various materials (e.g., metal, semiconductor, polymer, biomolecules, organic materials and inorganic salts) were fabricated, which have applications in nanoelectronics, bioanalysis, biosensors, actuators and high-density data storage devices.[22] An atomic force microscope contains the following main parts: a sharp tip, a flexible cantilever, a high-resolution scanner, and a sensitive deflection sensor. In principle, a sharp tip scans over the sample surface in near-field, the Vander Waals interaction between the tip and the sample is utilized to determine the surface properties. When the separation between the tip and the sample surface is large, the attractive force is small and changes little with the variation in the tip-sample separation; therefore the attractive force can be neglected. The force cannot be neglected when the separation is less than hundreds of angstroms. AFM utilizes the relationship of force versus separation to provide surface properties. AFM is generally operated in two modes. The first mode is constant force mode. In this mode, the electronic feedback is switched

on, the piezoelectric scanner can respond to any changes in force that is detected, and alter the tip-sample separation to restore the force to a pre-determined value. Figure 1 is a schematic diagram of a scanned sample AFM in constant force mode. The separation between the tip and the sample is kept as a constant by the feedback loop. Therefore, as the tip moves across the sample surface, the information about surface properties is recorded. The second mode is constant height mode with the electronic feedback switched off. It is particularly useful for imaging very flat samples at high resolution.

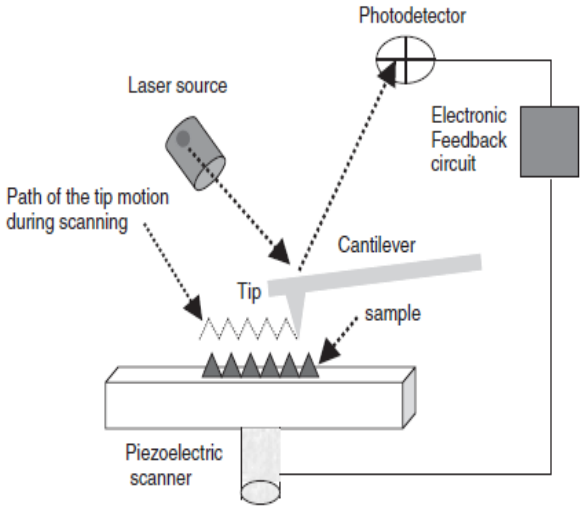

Figure 1: Schematic diagram of a scanned sample AFM

DIP PEN NANOLITHOGRAPHY

Dip-pen nanolithography (DPN) is an atomic force microscope (AFM) based lithography technique. The DPN process was first developed by Professor **Chad Mirkin** at the Northwestern University Nanotechnology Institute for depositing thin organic films in patterns with feature sizes of around 10 nm (about 20 times better than the best optical lithography). Coating an Atomic Force Microscope (AFM) tip with an ink, the scientists are able to deposit well defined lines of the ink in a manner similar to a traditional ink pen. **NanoInk** has created a dedicated DPN Writer system, NSCRIPTOR™, as a fully-integrated hardware and software system that is optimized for the DPN process. DPN is a direct-write technique, so materials of interest can be placed exactly (and only) where desired, without the use of a mask. Among sub-50nm techniques—such as e-beam lithography—DPN is the only one that can directly deposit molecules under ambient conditions.[23,24] In addition, because NanoInk's nanolithography platform—the NSCRIPTOR ™—is based on SPM technology, it is inherently capable of both pattern fabrication and immediate verification of the result by imaging. DPN techniques apply to

a variety of major scientific fields, such as creating protein nano-arrays in the growing field of proteomics, making templates for nano-crystal growth in biotechnology and optics research, depositing material onto semiconductor substrates for the electronics industry, and doing magnetic particle deposition for storage and sensor technology. During the contact mode operation under ambient laboratory conditions, a water meniscus naturally forms between the ink coated probe tip and the substrate. The ink moves on the substrate by capillary transport through the meniscus. One key issue of successful DPN patterning is choosing an ink and substrate with an appropriate chemical affinity. This causes the ink molecules to chemosorb onto the substrate. Proper binding self- regulates the diffusion of ink onto and across the substrate, thus controlling the resulting feature size and resolution. There are several key experimental parameters that affect feature resolution, including humidity, temperature, and the pen's drawing speed. So even in its most basic form-drawing with a single pen and a single ink-DPN can be a complex experiment.[14,16-20] Dip-pen nanolithography (DPN) is a great scanning probe nanopatterning technique in which an atomic force microscope (AFM) tip is used to deliver molecules to a surface via a solvent meniscus, which naturally forms in the ambient atmosphere. This direct-write technique offers high resolution patterning for a number of molecular inks on a variety of substrates.[25-29] One of the most important attributes of DPN is that patterns of multiple molecular inks can be formed or aligned on the same substrate since the same device is used to image and write a pattern. The success of DPN relies on two factors: a spatially narrow *deposition* of ink molecules from the tip and *self-assembly* of the ink molecules functionalized to chemisorb to the substrate and to form a compact monolayer on the surface.

Features of DPN system [16-20]

Nano-scale pattern design

Environmental and scanning probe preparation

Inking and re- inking

Ink calibration

Drawing/plotting

Microscale and nanoscale alignment

Inspection of the DPN patterns

The DPN method is a truly unique lithography tool that combines the high resolution of e-beam lithography with the ability to pattern non-traditional materials (such as biomolecules) of micro-contact printing, the ease of use and automation of a computer printer and the promise of

increasingly high-throughput as DPN Pen Systems are developed to include tens and even hundreds of thousands of individually-controlled pens. DPN built structures and patterns are:

Smaller than those produced virtually any other way,

Cheaper to produce than any other technique,

Built under the supervision of individuals with only hours of training,

Viewable literally during fabrication,

Incorporate building materials that other techniques cannot use, and

Allows for the use of multiple materials simultaneously.

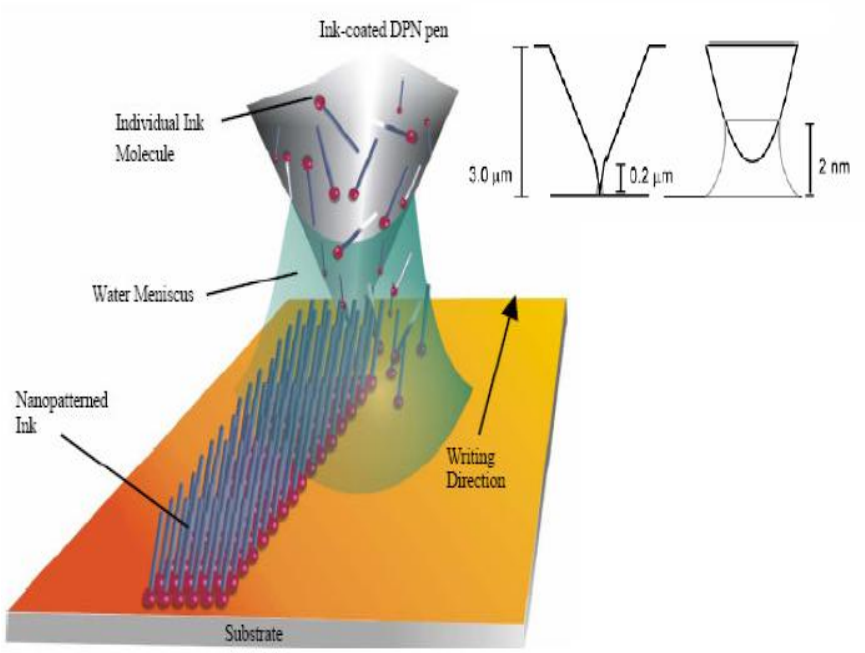

Figure 2: Schematic representation of DPN process

DPN ink- substrate

(Table 1: Overview of the various DPN ink-substrate combinations that have been reported)

Table 1: Overview of the various DPN ink-substrate combinations that have been reported

Ink	Substrate	Notes
Alkylthiols e.g. ODT and MHA	Au	15 nm resolution with sharp tips on single crystal surfaces, < 50nm on polycrystalline surfaces
Ferrocenylthiols	Au	Redox active nanostructures
Silazanes	SiO_x	Patterning on oxides
Proteins	Au, SiO_x	Both direct write and indirect assembly
DNA	Au, SiO_x	Sensitive to humidity and tip-silanization conditions

NanoInk demonstrates mercaptohexadecanoic acid (MHA) lines on gold with 14 nm line widths- All experiments were performed using the NSCRIPTOR system, which includes an environmental chamber capable of controlling temperature (from -2°C below room temperature

to 10°C above room temperature) and relative humidity (RH) (0–75% RH) through a real-time feedback loop. Environmental conditions were kept constant (± 0.1°C, and ± 0.5% RH) throughout all experiments. The NanoInk E-Chamber software permits environmental stabilization while being able to avoid associated noise by turning off the heater, fan, and nebulizer during writing and imaging. Oxide sharpened DPN pens were provided by the NanoInk MEMS facility (Campbell, CA). All of the tips were then coated with the alkanethiol MHA by dipping into a 5 mM solution of MHA in acetonitrile. Three different Au substrates were used: mica-peeled (and therefore clean until the time of writing), evaporated, and sputtered. The evaporated and sputtered samples were stored under argon in methanol-cleaned eppendorf tubes. DPN experimentation was automated by NanoInk's InkCAD^{TM} software. InkCAD controls when the MHA-coated tip is in contact with the surface, and how long it dwells (creating dots) or how fast it moves (creating lines). Further, the ink diffusion process was calibrated according to a routine called InkCal^{TM}. Many studies [14, 16,30-32] have demonstrated that alkanethiol feature sizes depend on dwell time or tip speed. InkCal automates the process of drawing dots and lines at a range of dwell times and tip speeds, and then guides the imaging of features, the measurement of feature sizes, and finally plots the data to obtain a diffusion coefficient (C) expressed in $\mu m^2/s$. In addition, it has been previously noted [30] that the diffusion behaves differently during the deposition of dots as opposed to lines. With dots, there is a static diffusion scenario in which ink diffuses from a source, and then continues to diffuse as the self assembled monolayer (SAM) forms outward from the tip. With lines, however, there is a dynamic scenario in which ink at the leading edge of motion is always seeing fresh attachment sites on the gold, and ink on the trailing edge is still diffusing over itself. As such, the InkCAD software accounts for this by allowing independent calibration of dots and lines, each were having its own ink model and diffusion constant.

Thermal DPN [33,34]

The steady shrinking of integrated circuits demands constant innovation. Though ever greater resolution remains the foremost goal, many new abilities are also needed—the reduction of toxic by-products ("green chemistry"), integration of heterogeneous materials (e.g., organic and inorganic) into a single structure, and the production of just a few integrated circuits at low cost. A new lithographic approach, thermal Dip Pen Nanolithography (tDPN), is developing to both achieve greater resolution and address many of these secondary needs.[34,35] Although tDPN can create nanoscale structures, in principle it works as simply as a soldering iron. The heart of the device is a custom-fabricated heatable atomic force microscope (AFM) tip, coated with a

material (i.e., the "solder" or "ink") that is solid at room temperature. When melted, the ink flows from the tip onto the surface (Figure 3). The use of meltable inks has many benefits. Since the ink's fluidity is controlled by the tip temperature, writing may be turned on or off and the deposition rate easily varied. Secondly, one can write new layers on top of previously deposited—now solid—layers to create complicated three-dimensional structures. Finally, tDPN can be performed in vacuum, making it compatible with conventional semiconductor device fabrication. The technique is used for **polymer deposition**. Because tDPN requires only that the polymer melt before it decomposes, a wide range of polymers may be used. To date, an insulating polymer, Mylar, and two conducting polymers, poly [2-methoxy-5-(2'-ethylhexyloxy)-p-phenylene vinylene] (MEH-PPV) and PDDT, have all been successfully deposited.

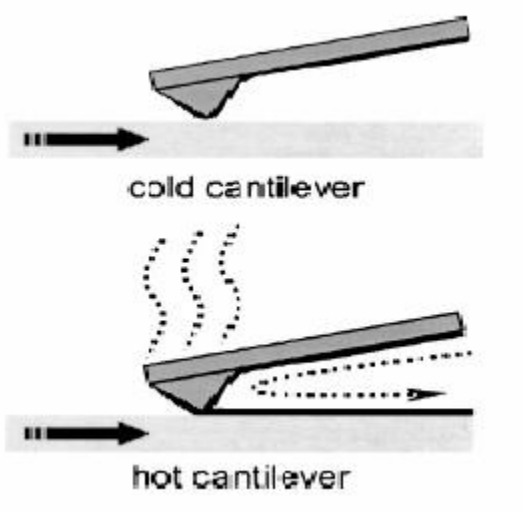

Figure 3: Scheme of Thermal Dip Pen Nanolithography.

When the cantilever is cool, the ink is solid and does not flow. When the cantilever is heated, the ink melts and flows from the tip onto the surface. Moving the tip writes the ink pattern.

An important aspect of tDPN is that ink can be deposited with sufficient thermal energy to organize into well-formed monolayers before solidifying. The coated tip was heated and scanned over a rectangular area to deposit a polymer film, precisely a single molecular layer thick onto the SiO_2 substrate. A second pass added a second monolayer without disturbing the first. The large temperature range of the cantilevers, up to 1000°C, allows many different inks to be used.

Electrochemical DPN: Polymer Nanowires

Conducting polymers are already of widespread interest for applications ranging from electronic devices to mechanical actuators.[35-37] Current photolithography, microcontact printing, template synthesis, and scanning electrochemical microlithography techniques afford conducting polymer

microstructures, but these techniques possess significant limitations for patterning structures of <100 nm dimensions.[38-41] The capability to direct-write and pattern polymeric materials with interesting electronic and electro-optical properties at the nanoscale creates a number of opportunities since a large variety of monomers/polymers are available. Here, Electrochemical Dip-Pen Nanolithography (E-DPN) is used to fabricate **polythiophene nanostructures** on semiconducting and insulating surfaces in the sub-100 nm regime. Electrochemical Dip-Pen Nanolithography is a new AFM lithography technique.[43] E-DPN, like other DPN techniques, relies on spontaneous condensation to facilitate transport of material from the AFM tip to the surface.[14,24,42,43-44] It involves a chemical/physical process, such as covalent bonding or an electrochemical reaction, and then immobilization of the material on the surface. Since the reaction occurs at the AFM tip, material deposition localizes on the patterns traced by the tip. Using DPN techniques, nanostructures composed of organic[14,44], semiconducting[42], or metallic materials[42,43]are easily obtained of controlled and well-defined nanometer shape and size. 3,4-ethylenedioxythiophene (EDOT) is electrochemically polymerized at the AFM tip/substrate interface to create poly-EDOT nanowires. Poly-EDOT is a well-known conducting polymer possessing interesting electrical and electrooptic properties, and of interest for antistatic, electrostatic, and conducting coatings as well as for light emitting diodes. In a typical experiment on a Nanoscope IIIa AFM (Digital Instruments), a highly doped tapping mode AFM tip (Silicon-MDT, dopant concentration 1017 atoms/cm^3) is first coated with monomer by immersion in a solution of 1:1 v/v EDOT/$CHCl_3$ and dried. A clean, silicon (111) wafer with native oxide is used as the substrate. To pattern nanostructures, a negative bias voltage is applied between the AFM tip and the surface, the setpoint voltage is reduced to 15% of the original value to reduce the tip-surface separation, and the tip is translated across the surface in a preprogrammed pattern. The applied voltage electrochemically polymerizes the monomer, resulting in tip-defined deposition of poly-EDOT on the substrate. Polymeric nanostructure morphology is dependent on the humidity, applied voltage, and translation speed of the tip. The applied voltage depends on the thickness of the oxide layer on top of the Si wafer; patterning on native oxide requires between -9 and -15 V, but higher voltages are required for thicker oxides. Since polymerization occurs at a negative voltage, oxidation of the underlying silicon surface may compete with polymerization. To confirm that the deposited nanostructures are composed of poly-EDOT rather than locally oxidized silicon, a number of experiments were performed. First, the polymer nanostructures are resistant to HF etching, whereas HF removes SiO_2 features. The polymer nanostructures can be removed by ultrasonication in non-aqueous solvents such as $CHCl_3$, unlike

SiO_2 features. In addition, the deposited nanostructures act as nucleation sites for gold nanoparticle formation. Specifically, in situ reduction of $HAuCl_4$ in the presence of an oxidized Si wafer surface containing a poly-EDOT nanostructure affords a higher density of Au nanoparticles on the poly-EDOT nanostructure than on the SiO_2 surface. As expected, thiophene has a higher propensity for binding gold than oxidized silicon, and has been previously used to aid gold nanoparticle formation.[45] Finally, the nanostructures created by E-DPN of EDOT are not resistant to strong chemical oxidation. Well-defined poly-EDOT nanostructures of less than 100 nm dimensions are created on semiconducting and insulating surfaces with E-PDN. This technique is intrinsically site-specific and may be used for laboratory scale fabrication of complicated structures using multiple "inks". Importantly, with E-DPN the current repertoire of building blocks expands to now include monomers used to prepare polymers with tailored electronic and electro-optic properties. In conjunction with other micro- and nanofabrication strategies, E-DPN is likely to facilitate the design and development of novel devices for the electronics, defense, pharmaceutical, and biotechnological industries.

Conventional DPN applications use a single AFM probe and, as a result, are relatively slow and serial. The process can be accelerated by a factor of *n* if an array of *n* identical pens is used. Ideally, the tip-to-tip spacing between probes should be minimized. The first attempt at multiprobe patterning used a one-dimensional array of undiced commercial probes. However, the array spacing (~1.5mm per probe) was far from optimal. The focus is to develop high-density, one-dimensional, passive and active probe arrays that satisfy the need for true high density pattern generation. Passive probes were first developed, followed by active probe arrays.

Passive DPN probes

Passive probes with small tip-to-spacing have been produced from silicon nitride and silicon.[46,47] The silicon nitride probes are 375µm long (base to writing tip), 50µm wide, 600nm thick, and have a tip-to-tip spacing of 100µm. The spacing is small enough to allow the patterns of adjacent tips to touch when using a 100µm AFM scanner. The probes have an analytically estimated spring constant of 0.018 N/m. The silicon probes are 15µm wide and 5µm thick. The tip-to-tip spacing is 310µm. The suspension is folded to increase the beam's effective length and lower its spring constant to an FEA estimated value of 0.315 N/m. Both designs have been used for topographic imaging and lithography with line widths down to 60nm. The probe tips are first formed by anisotropicly etching the surface of a <100> silicon wafer, followed by oxidation sharpening.[48] For silicon nitride probes, the wafer is coated with low pressure chemical vapor deposited (LPCVD) silicon nitride and the beams are etched out using reactive ion etching. The

beams are then released by anisotropic wet etching from the front side of the wafer. For silicon devices, the beams are etched out of anion implanted boron etch stop layer and the remaining silicon is removed by anisotropic wet etching.

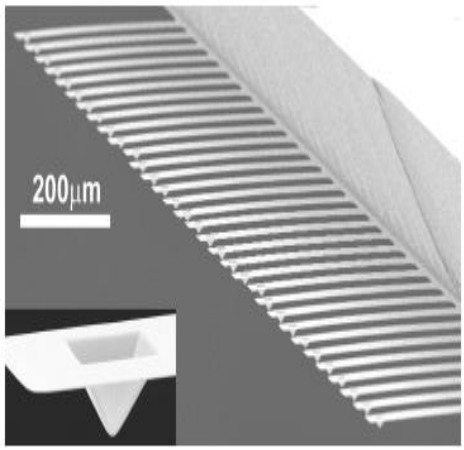

Figure 4: SEM of a passive, 32-probe silicon nitride DPN array

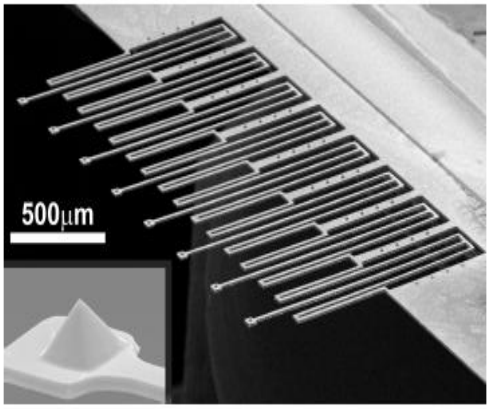

Figure 5: SEM of a passive 8-probe silicon. DPN array

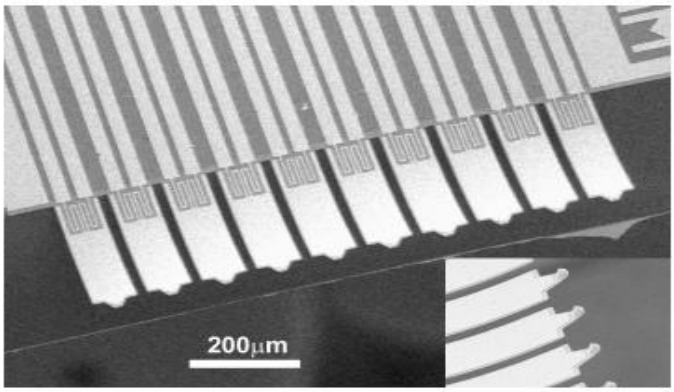

Figure 6: A 10 probe thermally actuated DPN array.

Active DPN probes

In active arrays, each probe contains an actuator capable of lifting its tip off the substrate

independently of the others. This allows all the probes to travel the same path but write different patterns. There are a number of possible actuation methods including thermal bimetallic bending, electrostatic actuation, piezoelectric actuation, etc. Example is thermal actuation which has large deflection, fabrication simplicity, and robust operation. The fabrication process for the active array is an extension of the process used to make the passive silicon nitride devices.

APPLICATIONS OF DPN

Hydrogel patterning [49-51]

Hydrogels are three-dimensional cross-linked polymer networks that have physical characteristics similar to those of natural tissue. The versatility of poly (ethylene glycol) (PEG) chemistry and the excellent biocompatibility of PEG-based hydrogels have been instrumental in hydrogel advances related to controlled material release, directed cellular function, and regenerative medicine applications. Sub-cellular scale patterned hydrogels with defined mechanical properties are valuable as scaffolds for tissue engineering work and for *in vitro* cell culture studies. Hydrogels are found in many commercial products and in even more research applications. From photonic crystal sensors to drug delivery vectors to mechanical actuators that respond to external stimuli, synthetic hydrogels are no longer restricted to biologists. The incredible diversity of function comes, in part, from the tunability of the physical properties of the polymers that compose modern hydrogels. Researchers can vary the composition, molecular weight and porosity of the polymers to fit many applications, but the true power and appeal of the hydrogel comes from the ability to alter the gel behaviour quickly and easily through the cross-linking or encapsulation of different molecules on or within the gel matrix. Here, consistent and reproducible direct deposition of hydrogel precursors at defined locations and subsequent polymerization of these precursors to form PEG-based hydrogels is explained.

Principles of PEG-Based Hydrogel Formation

Since they exhibit high degrees of hydrophilicity and biocompatability, PEG-based hydrogels have been used extensively in tissue engineering and drug delivery applications. PEG is a versatile material; it is available in a wide range of molecular weights and with various functional end groups. The mechanical and swelling properties of PEG hydrogels can be fine-tuned by altering the PEG molecule chain length or by varying the degree of cross-linking, achieved by modifying the number of acrylate groups or by adjusting the UV exposure time, respectively. Hydrogel patterns can be printed on a variety of surfaces including silicon, glass and gold. A schematic of the PEG hydrogel deposition process is shown in Figure 7. PEG-

dimethacrylate (PEGDMA) is used as the precursor material. Prior to DPN precursor patterning, a small amount of photoinitiator (~ 1% of total volume) is added to the precursor to assist in the later polymerization reaction. NanoInk's desktop nanolithography platform, the NLP 2000 System, is used to pattern the precursors. Precursors are loaded into reservoirs on the NLP 2000 System's "Inkwells". These Inkwell reservoirs feed microfluidic channels specifically engineered to transport liquids and to match NanoInk's M-type cantilever "pen" tip geometry. Precursor material then moves from the Inkwells to load each cantilever "pen" tip. The loaded tips print the desired DPN pattern, and resulting PEG-DMA features are polymerized using UV-induced cross linking to from hydrogels.

Uniform PEG Hydrogel Microarrays

To demonstrate the feasibility of using DPN to create large-area hydrogel microarrays, a one-dimensional 12-pen M-type cantilever "pen" array was used to print PEG hydrogel patterns. Within five minutes, the DPN system deposited 3000 hydrogel domains, covering a total area of 0.8 × 0.6 mm^2, on a glass substrate. Features within the PEG hydrogel array had an average diameter of 1.25 µm and a pitch of 13 µm. The patterned PEG hydrogel domains exhibited uniform size distribution. Average feature size diameter was calculated for four individual "pen" tips as well as for entire arrays. The coefficient of variation calculated over the entire pattern was 16%, while the CV for individual tips varied between 6 - 16%. These hydrogels patterns were generated at 25°C and 20% RH. The diameter of the printed hydrogel domains increased to 6 µm by increasing the deposition temperature to 37°C.

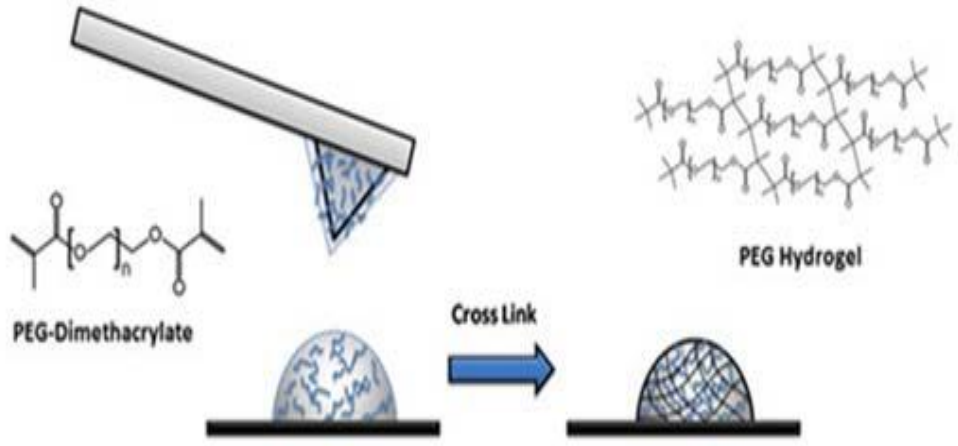

Figure 7: Schematic of the DPN PEG hydrogel printing process.

PEG Hydrogel Nanostructures

DPN can also be used to fabricate PEG hydrogel nanostructures. Submicron-sized PEG hydrogel patterns are easily generated by lowering print molecule diffusion rate. This rate decrease is

achieved by using larger-chain length PEG-DMA precursors and patterning at low humidity conditions with print times of less than 1 second per spot.

Patterns of PEG hydrogels at micron and submicron scales are easily generated using NanoInk's DPN instrument systems. Using a 1D cantilever "pen" tip array to parallel print PEG precursors, an area of 0.8 × 0.6 mm^2 can be patterned with PEG hydrogels in a relatively short period of time. Resulting PEG hydrogel large-scale microarrays exhibit uniform spot size.

Protein nanoarrays

Microarrays of biomolecules such as DNA and proteins have proven useful as high-throughput screening tools in proteomics, genomics, and the identification of new pharmaceutical compounds.[52,53] For example, DNA microarrays can be used to probe gene expression and in panel assays for research- and clinical-based diagnostics.[55] Arrays of proteins have been used to study the interactions of cells with underlying substrates.[54,55] Promising advances have been made in making DNA and protein patterns with features with nanoscopic dimensions (<200 nm).[55,56] However, except in the case of chemically modified collagen and small peptides, protein nanopatterns all have been made by indirect methods that either involve resists, or prefabricated chemical affinity templates[55] that direct the assembly of a single protein structure from solution onto a set of nanoscopic features on a surface of interest. Here, chemically modified AFM tips and dip-pen nanolithography (DPN) is used to generate two-component nanoarrays of native proteins that are biologically active [52,57] and capable of recognizing a biological complement in solution.

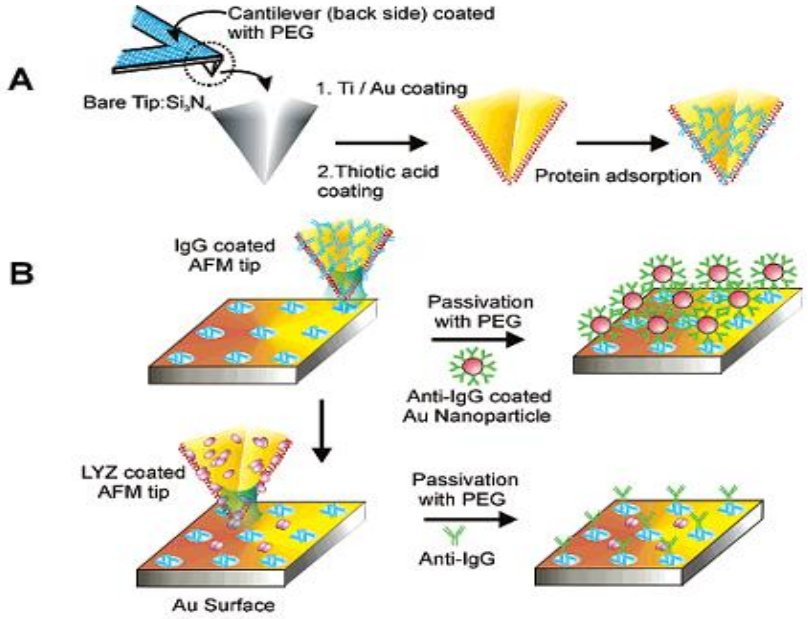

Figure 8: (A) Tip Modification Scheme; (B) Protein Patterning Schemes

To use DPN to direct-write protein nanoarrays, the surface of a conventional, commercially available AFM tip (ThermoMicroscopes sharpened Si_3N_4 Microlever A, force constant = 0.05 N/m) is chemically modified. The modification procedure involves immersing the gold coated cantilever in a 1mM ethanolic solution of a symmetric 11-mercapto undecylpenta (ethylene glycol) disulfide (PEG). This results in the formation of a monolayer of PEG that prevents adsorption of protein,[57] on the reflective Au surface of the cantilever (backside). Tips treated in this manner were rinsed with ethanol, dried, and coated with gold (7 nm, with a 3nmTi adhesion layer) on the tip side by thermal evaporation methods. The cantilevers with the gold-coated tips were immersed in 0.1 mM thiotic acid in ethanol for 1 h, rinsed with ethanol, and then dried with N_2 at room temperature. To prepare tips for DPN experiments, they were immersed in solutions of the desired protein (500 µg/mL, 10 mM phosphate-buffered saline (PBS), pH 7.1) for 1 h and then used immediately. The hydrophilic tips with the carboxylic acid-terminated SAMs facilitate protein adsorption on the tip surface.[58,59] Humidity is a critical variable, and optimum patterning results were achieved when the experiments were carried out in an environmentally controlled glovebox at a relative humidity of 80-90% at room temperature. Humidity values below 70% resulted in inconsistent transport properties. All DPN patterning was done with a ThermoMicroscopes CP AFM interfaced with DPN Write (NanoInk, Chicago, IL). Tapping mode images were taken with a Nanoscope IIIa and MultiMode microscope from Digital Instruments. Au substrates, prepared via literature methods, were chosen for two reasons. First, the interaction between the cysteine residues of proteins and the Au surface provides a strong driving force for protein adsorption. Second, they allow one to use PEG as a passivating layer in the areas not occupied by the proteins to resist nonspecific adsorption of proteins from solution. As proof-of-concept experiments, lysozyme (Lyz) and rabbit immunoglobulin-gamma (IgG) nanodot arrays were constructed in direct-write fashion, Feature size could be controlled over the range 45 nm to many micrometers by controlling the tip-substrate contact time. In general, longer contact times led to larger features, but the rate of transport is highly dependent upon protein composition. The areas surrounding the patterns were then passivated with PEG by adding a droplet of 1 mM PEG in Nanopure H_2O (18.1 MΩ) directly on the patterned area for 45 min in a sealed vessel followed by copious rinsing with Nanopure H_2O. Nanopure H_2O was used as a solvent for the PEG to minimize denaturation of protein structures in the patterned area. Organic solvents such as ethanol, which are used often with PEG, have the potential to denature the protein structures and subsequently cause them to lose their biorecognition properties. In the case of IgG, to test the biorecognition properties of the nanoarray, it was incubated in a solution

of gold nano particles (10 nm, diluted 1/10 in 10 mM PBS, obtained from Ted Pella) coated with anti-rabbit IgG for 3 h. AFM height profiles of the array before and after treatment with this solution is compared.

Other applications [60]

NanoInk markets instrumentation specifically designed for Dip Pen Nanolithography® (DPN®), a tip-based patterning technique. The primary strengths of this technology are:

- Directed placement of materials at defined locations with nanoscale precision
- Flexible "on-the-fly" pattern generation
- Multi-component patterning at micron and sub-micron scales.

CONCLUSION:

In the coming decade, the research and development community must complement its ongoing fundamental research activities in synthesis, assembly and processing by placing a stronger emphasis on the development of nano manufacturing science and engineering. Nano manufacturing (science based, reproducible, sustainable, and cost-effective) needs to be developed in conjunction with other areas such as nano biotechnology and nano medicine (e.g., diagnostics, drug delivery, and disease treatment), energy applications (e.g., conversion, storage, transmission, and efficiency), environmental fields (e.g., sensors, remediation, water purification) and educational challenges (e.g., promoting the value of nano science and nano engineering degrees; addressing lack of textbooks, emphasizing community college, undergraduate, and graduate education; and integrating partnerships with industry). DPN,a unique approach for positioning, manipulating and generating a variety of atoms, molecules, and materials with accuracy at the nanometer length scale. Although the passive- and active-pen arrays for patterning parallelization are far from perfection, the general approach that integrates knowledge and ideas across disciplinary boundaries has already evolved into a particularly useful tool to study the fundamental consequences of miniaturization. Furthermore, one has to meet the great challenge that involves the lack of efficient analytical tools for characterizing the patterned individual nanostructures on a surface or within the context of an integrated device. The integration of the advanced characterization tools and DPN should allow better understanding and prediction of the fundamental and cooperative properties of nanostructures. Further investigations are expected in this field.

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