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Fabrication of Luminescent Nanostructures and Polymer Nanowires Using Dip-Pen Nanolithography

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ABSTRACT

We used a combination of dip-pen nanolithography and scanning optical confocal microscopy to fabricate and visualize luminescent nanoscale patterns of various materials on glass substrates. We show that this method can be used successfully to push the limits of dip-pen nanolithography down to controlled deposition of single molecules. We also demonstrate that this method is able to create and visualize protein patterns on surfaces. Finally, we show that our method can be used to fabricate polymer nanowires of controlled size using conductive polymers. We also discuss the factors that influence the size of these nanowires.

The ability to fabricate arbitrary structures at small length scales is a key element for creating functional nanoscale devices that will fulfill the promise of nanotechnology. Specifically, the task of creating nanoscale optoelectronic devices will require robust methods for controlled deposition of optically functional materials on surfaces. Popular strategies for such deposition include conventional lithography, soft lithography,[1] and molecular self-assembly.[2] However, most of these methods either break down or are poorly controlled when the feature sizes get down to nanometer scale. Dip-pen nanolithography[3,4] uses an "inked" atomic force microscope (AFM) tip to deposit the ink material directly onto the surface in an arbitrary configuration, which is ideally suited for fabricating surface patterns in the laboratory setting. Recently, dip-pen nanolithography has been used to fabricate patterns of chemical functionality,[5] dendrimers,[6] and raised gold wires.[7]

Ultimately, the limit to the feature size in DPN is determined by the probe geometry, water meniscus properties, and the chemical nature of the ink.[3,8] Currently, dip-pen nanolithography is restricted to creating patterns that AFM can visualize either by topographic imaging or by lateral force imaging. Therefore, to reveal the pattern we need to deposit material on the surface in the amount that is sufficient to produce the contrast in the AFM image. Thus, even if dip-pen nanolithography is capable of scaling down the amount and the surface concentration of the deposited material, the resulting patterns cannot be visualized by traditional AFM imaging.

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In this letter we show that we can combine dip-pen nanolithography with single molecule optical detection to create and visualize nanoscale patterns of luminescent materials down to single-molecule deposition levels. We then demonstrate that the technique can be used to create protein patterns. Finally, as a first step toward creating optoelectronic devices we use dip-pen nanolithography to fabricate light-emitting polymer nanowires of predetermined size.

Controlled Deposition of Single Molecules. Conventional dip-pen nanolithography with alkanethiol inks relies on the formation of a chemical bond between the ink and the substrate to facilitate the ink transfer from the tip to the sample surface. However, recent works demonstrated that even weaker interactions such as van der Waals interactions are often sufficient to facilitate the transfer of matter from the tip to the sample.[6,7] We have used dilute solutions of Rhodamine 6G (R6G) dye as the writing "ink" to create luminescent nanoscale patterns. A drop of the 10^{-7}M R6G solution in methanol was absorbed onto a thin paper membrane. We then gently lowered the AFM tip into contact with the paper for 30−60 s using a custom-built translation stage under the optical microscope. Lithography was performed immediately after the "inking". R6G has strong affinity for negatively charged glass surfaces; therefore, we did not use any surface treatments to promote the transfer of the "ink". All patterns were fabricated at ambient humidity. Both fabrication and subsequent imaging of the patterns were carried out using a custom-built instrument (Figure 1) that combines atomic force microscopy and scanning confocal microscopy functionalities.[9]

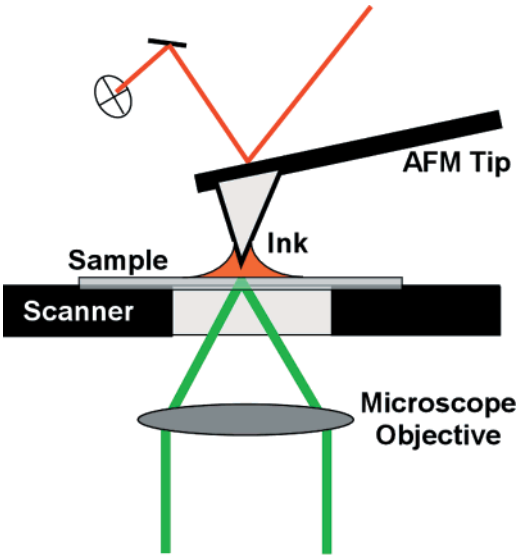

Figure 1. Schematic of the experimental setup used for dip-pen nanolithography. The instrument combines atomic force microscopy and scanning confocal microscopy functionalities. A Bioscope AFM head (Digital Instruments, Santa Barbara, CA) is mounted on a Nikon Eclipse 300 inverted microscope equipped with a custom-built stage that incorporates a closed-loop piezo scanner (PolytecPI, Germany). The scanning stage is used in both the lithography procedure and confocal imaging. The fluorescence excited by a 514 nm line of Ar−Ion laser (Coherent Innova-70) is collected with 100 × 1.4 NA lens, passed through a 50 μm pinhole to reject stray light and then detected by an avalanche photodiode (Perkin-Elmer Optoelectronics, Santa Clara, CA). Excitation light was rejected by a long-pass filter (Omega Optical, VT).

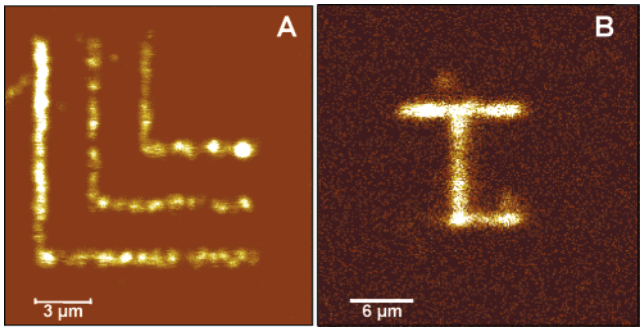

Figure 2. (A) Scanning confocal microscopy image of a Rhodamine 6G patterned in a series of lines on glass surface. The pattern was fabricated using Ultralever contact AFM probes (Park Scientific Instruments). Probe translation speed was 0.2 μm/s. (B) Scanning confocal microscopy image of a HCG antibody pattern fabricated on glass. Probe translation speed was 0.2 μm/s. Each line in the pattern was overwritten 10 times to maximize the protein surface density.

Figure 2A shows a fluorescent confocal microscopy image of a pattern of R6G deposited on glass. Several features of this image are noteworthy. First, the apparent width of the lines in the image does not reflect the actual line width of the deposited dye. Rather, image line width is determined by the size of the confocal microscope probing beam (in our case ~600 nm). Second, several features in the optical image do not show a continuous line, but rather are composed of a number of spots. Moreover, these spots showed features that are characteristic of a single molecule emission pattern, such as a striped pattern, and abrupt emission cessation that is indicative of a dye molecule going in and out of dark states and finally photobleaching.[10] Likewise, as we continued to image our pattern, it rapidly degraded from photobleaching. From these observations we concluded that our pattern was composed of a number of isolated single molecules deposited in a straight line by the AFM tip. Notably, we did not observe any evidence of the pattern in normal or lateral force AFM images. Therefore, the amount of the material deposited on the surface was not sufficient to cause a height change or an appreciable surface free energy change. This observation is also consistent with a pattern that is composed of only a number of single molecules. It is impossible to say how well the single molecules are aligned within the pattern line since the optical image does not provide nearly enough resolution to answer this question. However, we can speculate that the dye molecules are confined laterally at least within the typical line width attainable with alkanethiol dip-pen nanolithography. Such width is ~30 nm in our case, but other groups have reported the patterns with line widths as small as 15 nm.[3] However, this limit may simply reflect the fact that lateral force microscopy cannot visualize thiol lines smaller than 15 nm, even if they can be fabricated by dip-pen nanolithography. Changing the concentration of the dye solution used for inking along with careful adjustment of ambient humidity can control the average spacing between the molecules along the written line. For our sample we estimated this spacing to be 566 ± 210 nm. We expect that further improvements in the deposition process such as smaller probes, rigorous humidity control, and careful choice of the writing ink will result in smaller line widths and more regular deposition patterns. These improvements will permit better alignment of single molecules on the sample for studies of the proximity effects in single molecule interactions.

Protein Patterns. One of the promising potential applications of nanolithography is fabrication of ultra high density protein patterns for use in "biochips".[11] Some of the current approaches use soft lithography[11] or "nanografting"[12] to create reactive sites in a self-assembled monolayer. Proteins then attach to these reactive sites via a chemical reaction. Both of these methods are complex multistage processes and use chemical attachment, which is always complicated by the problem of nonspecific adsorption. Dip-pen nanolithography provides a simple and straightforward way for creating protein patterns since features can be directly written onto the surface. Significantly, this method avoids the complications associated with nonspecific binding. If the protein is tagged by a specific fluorophore, then the pattern can be easily identified and verified using optical imaging. To demonstrate the feasibility of this approach we fabricated patterns of human chorionic gonadotropin (HCG) antibody tagged with tetramethylrhodamine (TMR) dye on the glass surface (Figure 2B). The glass surface was pretreated with 3-glycidoxypropyltrimethoxysilane to introduce the epoxy groups that facilitate protein adhesion to the surface. Reliable fabrication of protein chips will require the ability to deposit proteins at high surface concentration. To demonstrate this capability, we used a high concentration of the protein "ink"

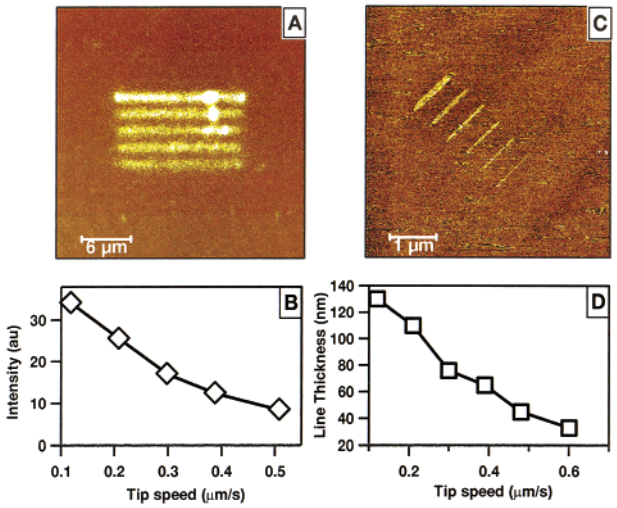

Figure 3. (A) Scanning confocal microscopy image of a series of MEH−PPV polymer nanowires fabricated on glass surface. (B) Plot of average fluorescence intensity of a polymer wires in Figure 3A versus probe translation speed used to fabricate the wires. (C) Lateral force image of a series of lines written on gold surface using mercaptohexadecanoic acid solution as ink. (D) Plot of average line thickness for the alkanethiol lines on the Figure 3C versus probe translation speed used to fabricate these lines.

($\sim 2 \times 10^{-5}$M (1 mg/mL) solution in PBS buffer) for fabrication of the pattern in Figure 2B. In addition, each line in the pattern was overwritten 10 times to enhance the surface concentration of the protein. The lines in the optical image on the Figure 2B appear continuous and no sufficient photobleaching was observed during scanning. The absence of single molecule behavior suggests that we have achieved high protein density in our lines.

In addition, optical detection of a written pattern provides a straightforward way to generalize the process for creating patterns that incorporate many different proteins. Such patterns can be successfully fabricated and imaged by using different protein inks tagged with different fluorophores in a manner analogous to dip-pen nanolithography with multiple inks.[5]

Fabrication of Polymer Nanowires. We have used our apparatus to fabricate and image nanowires of luminescent conductive polymers. These materials have long been under investigation as potential components of polymer LEDs[13,14] and fabrication of nanometer scale polymer structures is a potentially significant step for creating nano-LED device prototypes. In addition, nanowires of these polymers present an ideal system for studying photoluminescence and electron transport in organic one-dimensional materials. Polymer nanowires have previously been fabricated using template-directed synthesis[15] and stretching of polymer bridges.[16] However, these methods do not allow good control over the size and position of a nanowire or any way to assemble these nanowires in a predetermined configuration. Dip-pen nanolithography with its ability to create surface patterns is ideally suited for solving these problems. We demonstrated a controlled deposition procedure by writing a series of polymer lines on a glass surface (Figure 3A) using a 10^{-7} M solution of poly[2-methoxy-5−2′-ethylhexyl)oxy-1,4-phenylenevinylene] (MEH−PPV) in chloroform as ink. Only the following three factors limit the length of these nanowires: (1) the available range of the force microscope scanner, (2) the amount of material on the probe tip, and (3) the ability of the tip to deliver continuous ink flow. As in the two experiments that we discussed earlier, the apparent width of the structure in the image does not correspond to the real width of the nanowire; instead, it is determined by the size of the confocal microscope excitation beam. Besides the tip size, the major variable in controlling the nanowire thickness is the tip translation speed. To verify this we created a series a series of the polymer lines (Figure 3A) using different tip speed to fabricate each line. We used the fluorescence intensity as a measure of the amount of material deposited in each line, since it is impossible to infer the line width from the optical image due to probing beam size limitation. We found that the amount of material deposited in the line during nanolithography decreases with the increase in tip translation speed (Figure 3B). To compare this system to a well-characterized system, we performed a similar measurement for dip-pen nanolithography using alkanethiol ink. Figure 3C shows a series of lines written on gold substrates using 10^{-3} M mercaptohexadecanoic acid solution in ethanol as ink. We found that in this system the line thickness also increases as the probe translation speed decreases (Figure 3D). The observed trends confirm that at constant humidity the amount of material deposited on the surface is effectively controlled by the probe translation speed. Ultimately, the amount of the material deposited on the sample is determined by the kinetics of the material transfer from the tip to the sample. Quantitative description of this kinetics is beyond the scope of this paper and will be the subject of another publication.[8]

Last, as evident from the image in the Figure 3A, sometimes the tip can "skip" and leave "blotches" of material on the sample. Improved control over the tip chemical functionality and the inking process should minimize such pattern irregularities. Currently we are unable to verify the continuity of the nanowires or determine the alignment of the polymer chains on the wire. Further investigations of the electron transport properties and photoluminescent properties of these wires will provide answers to these questions and are underway in our laboratory.

In summary, we have used dip-pen nanolithography combined with scanning confocal optical microscopy to create and image luminescent surface patterns of arbitrary shape. We demonstrated that this method affords a robust and versatile way to pattern a variety of photoluminescent materials on the surface. Significantly, we showed that dip-pen nanolithography could be used to deposit increasingly small amounts of material down to single isolated molecules. We also showed that a combination of dip-pen nanolithography and optical detection could be used to create and identify protein patterns on the surface while avoiding the problem of nonspecific adsorption. Finally, we demonstrated that our method affords a direct and flexible route to creating polymer nanowires of a predetermined size and shape on surfaces.

These results open up several possibilities for fundamental and applied research. Further refinements in the control of the spacing between isolated single molecules will present an ideal model system for studying proximity effects in the interactions between isolated fluorophores. The ability to fabricate arrays of single molecules with controlled spacing will allow studies of collective behavior in such systems. Patterning optically tagged proteins could provide a direct route to fabrication of prototype "biochips" once the protein surface attachment chemistry is refined. In addition to significant reduction in the feature size, dip-pen nanolithography simplifies fabrication process since it eliminates multiple protection/deprotection steps necessary for conventional lithographic manufacturing of such chips. Finally, fabrication of conductive polymer nanowires could open up numerous possibilities for fundamental studies of electron transfer and photoconductivity in such structures. In addition, fine control over the size and shape and position of these wires will greatly facilitate their incorporation into prototype optoelectronic devices.

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Appendix

Substrate Preparation and Chemicals. Round cover glasses (Fisher Scientific) were cleaned by sonicating for 20 min in 2% solution of Helmanex cleaner (Hellma, Germany) and then rinsed with copious amounts of deionized water (Millipore). They were then sonicated for 10−20 min in deionized water, rinsed and stored under water until use. Gold films on mica substrates were purchased from Molecular Imaging and used as received. All chemicals were used as received without any further purification. Mercaptohexadecanoic acid (Aldrich) was dissolved in ethanol to 10^{-3} M concentration. Rhodamnime 6G (Molecular Probes, Eugene, OR) was dissolved in methanol to 10^{-7} M concentration. C. Hollars (LLNL) generously donated HCG antibody tagged with tetramethylrhodamine (TMR) fluorescent dye. A 1 mg/mL solution in PBS buffer of TMR-labeled HCG antibody was used for inking. Poly[2-methoxy-5−2′-ethylhexyl)oxy-1,4-phenylenevinylene] (MEH−PPV) polymer was available from previous studies.[17] Prior to inking the polymer was dissolved in chloroform to 10^{-7} M concentration.

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