Patterning of Biomolecules Using Dip Pen Nanolithography

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Introduction:

Kinesin and dynein are biomolecular motors in eukaryotic cells that transport intracellular cargoes along the cytoskeletal networks of microtubules in opposite directions. Interestingly, these proteins bind to the same cargo and are widely believed to be responsible for the bidirectional transport of cargoes [1, 2]. While the basic working principles of individual bimolecular motors are now understood in some detail, how multiple motors interact in the cells remains entirely unclear.

This work's goal was to precisely pattern motors using Dip Pen Nanolithography (DPN) such that their interactions can be studied *in vitro*. NanoInk's DPN 5000 was used to carry out the patterning process. First, however, another protein, bovine serum albumin (BSA), a less costly and readily available protein, was patterned to develop the proper methodology of patterning. Subsequently, the approach developed for BSA will be translated to patterning the motors. Patterned motors will be examined for ATPase activity and microtubule gliding to ensure functionality in *in vitro* motility assays.

Experimental Procedure:

First, fluorescently labeled bovine serum albumin (BSA), a less costly and readily available protein, was patterned to develop the proper methodology of patterning. Subsequently, the approach developed for BSA will be translated to patterning the motors. BSA is in solution of concentration 2.62 g/L. A 1:10 mixture was composed of it and a mixture of 40% glycerol and 60% Brinkley reassociation buffer (BRB 80). We used an A-tip type probe from Nanoink, a probe with a single cantilever tip, and made a ten-dot, five-second dwell time per dot pattern. Using the fluorescing of the fluorescently-labeled BSA, these patterns were viewed using an Olympus BX-51 fluorescence microscope.

To properly examine the proper protocol for patterning, tip loading, bleeding, and the use of different substrates were examined. We experimented with loading the tip by using ink wells provided by NanoInk, pipetting the ink directly atop the tip, and manually guiding the tip into a pipetted droplet. After we determined which loading method to use, we examined whether the tip must be pre-bled by patterning multiple patterns under known working conditions established from ink that NanoInk provided and examined results under the microscope. Finally, we prepared clean cover glasses differently ensuring the substrate was solely glass. The substrates were prepared as follows: O_2 plasma treated surface, 30 second hydroflouric acid (HF) bathed surface, supercritical CO_2 sprayed surface, and a surface with the following recipe: 30 second HF bath, eight-minute de-ionized rocker bath, two-minute annealing at 300°C, Omnicoat® spun on surface for 10 seconds at 500 rpm and 20 seconds at 3000 rpm, one-minute annealing at 300°C, SU-8 5 spun under same conditions with a three-minute annealing time at 65°C, two-minute SU-8 developer bath, and finally an isopropanol rinse of the substrate.

We examined the preferred surface by patterning on the substrates and examining the surface hydrophobicity using a goniometer from Ramé Hart Instruments Co.

Results and Conclusions:

When placed under the fluorescence microscope, ink was not visible from either the ink well or pipetting method. We then tried manually guiding the tip into the ink and found an abundant amount of ink on the tip (shown in Figure 1). We also found that sonicating the tip in acetone helped the

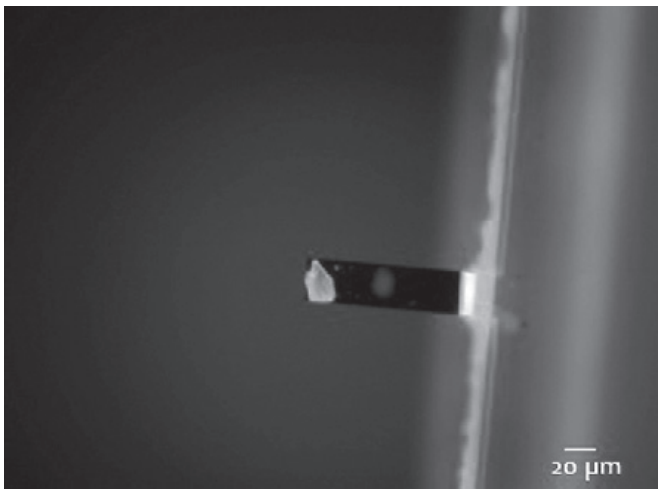

Figure 1: Microscopic image of fluorescent ink atop tip when tip is manually guided.

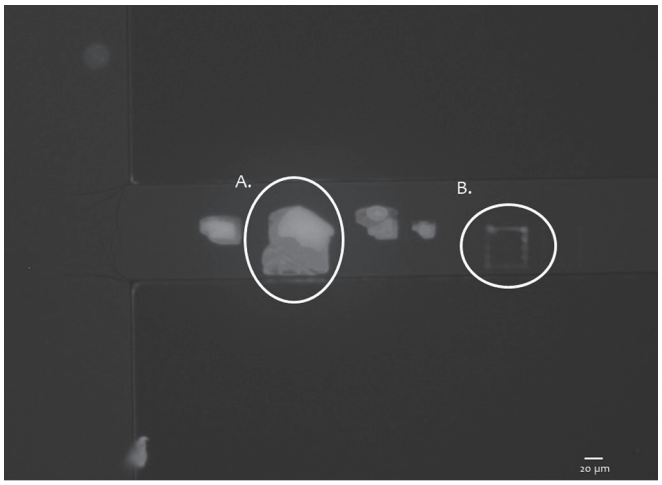

Figure 2: Microscopic image of resulting pattern when tip is not pre-bled.

tip maintain ink. To further conclude the better method, patterning was conducted under each loading method and we found that the inkwell and pipetting method produced no consistent pattern while the other method did.

Using the guided tip technique, we next examined whether the tip had to be pre-bled. The tip not pre-bled (Figure 2A) resulted in a blob of ink as opposed to a proper pattern (Figure 2B); indicating that the tip must be pre-bled so that the excess ink can be exhausted.

The most effective way to bleed the tip is to use the laser feedback on the DPN while the tip is on the substrate. If the laser feedback is not steady then there is excess ink. Also, approaching and withdrawing the tip from the surface until the laser feedback is steady is necessary to provide the most accurate patterns.

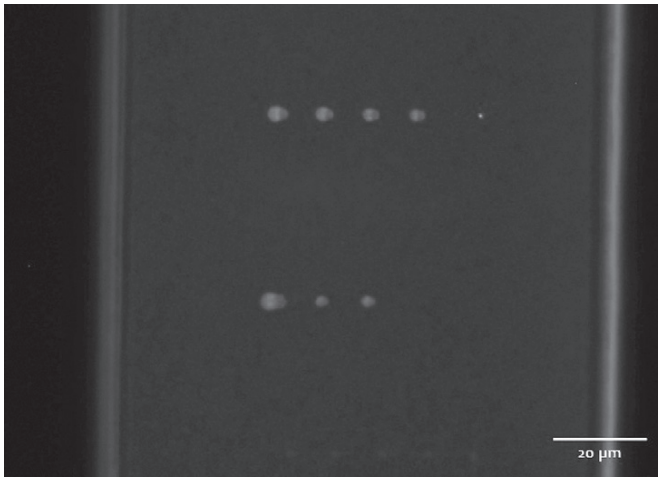

Figure 3: Microscopic image of pattern produced on SU-8 developed substrate.

Quantitative Analysis of Substrate				
Substrate	*Average*	*Standard Deviation*	*Streaking*	*Advancing Angle (°)*
O_2 Plasma Etch	7.822	1.008	Yes	0
HF Etch	3.352	0.9103	Yes	0
CO_2 Etch	2.212	1.332	No	30
SU8 Developed	1.032	0.213	No	130

Figure 4: Table depicting quantitative comparison of different substrates.

Figure 4 shows an indirect correlation between surface hydrophobicity and dot size and also shows that streaking occurs on the really hydrophilic surfaces and not on the less hydrophilic surfaces. It also shows that the SU-8 developer treated substrate produces the most consistent dot size.

It was also noticed that the constant height mode was more efficient than constant force mode of the DPN 5000 since the tip withdrew after each dwell time, thereby further preventing streaking. It was important to prevent streaking in order to ensure molecules were only present in desired locations to properly analyze the motors.

Future Work:

The method established must now be translated to the patterning of motor proteins. If patterning is successful under the same protocol, the (motor) proteins must be examined for functionality when patterned. If the motors are functional, we must develop an *in vitro* motility assays to examine the interaction between motor proteins.

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References:

[1] Hollenbeck, P. Saxton, W. The Axonal Transport of Mitochondria. Journal of Cell Science. 118. 5411-5419. 2005.

[2] Holzbaur, E et. Goldman, Y. Coordination of Molecular Motors: From *in vitro* Assays of intracellular Dynamics. Current Opinion in Cell Biology. 22. 4-13.