**INTRODUCTION**

The recognition properties of biomolecules provide a powerful and versatile framework for the ‘bottom-up’ fabrication of functional materials including nanoscale molecular electronic and photonic devices. One approach to constructing such materials is to introduce patterned biochemical functionality on an underlying substrate in order to template the assembly of higher-order structures. This approach demands patterning methods that offer nanoscale resolution, biocompatibility, and high registration capabilities. We have demonstrated two different methods for using direct-write DPN to generate nanoscale patterns of oligonucleotides on both metallic and insulating substrates.

**METHOD**

Direct-write DPN involves the transfer of a molecular ink directly from a coated scanning probe microscope tip to a substrate in ambient environment at biologically compatible conditions of temperature, pressure, and humidity. The DPN process has been used to pattern inks ranging from small molecules to peptides, proteins, and oligonucleotides on surfaces ranging from gold to oxides of Si and GaAs, suggesting that it can be extended to a wide range of biological molecules and applications. No resists are needed. Biomolecules can be placed exactly (and only) where desired, eliminating cross-contamination.

**Automated.** NanoInk has developed a custom software package for performing DPN, including ink calibration, multiple ink alignment, and design and implementation of complex patterns.

**Dip Pen Nanolithography (DPN) for Biomolecules**

- **Bio-friendly.** DPN utilizes ink transport from a coated scanning probe microscope tip to a substrate in ambient environment at biologically compatible conditions of temperature, pressure, and humidity.
- **High resolution.** By optimizing particular ink/substrate combinations feature sizes as small as 12 nm with spatial resolution of ~ 5 nm have been generated.
- **Chemically general.** The DPN process has been used to pattern inks ranging from small molecules to peptides, proteins, and oligonucleotides on surfaces ranging from gold to oxides of Si and GaAs, suggesting that it can be extended to a wide range of biological molecules and applications.
- **Direct write.** No resists are needed. Biomolecules can be placed exactly (and only) where desired, eliminating cross-contamination.
- **Automated.** NanoInk has developed a custom software package for performing DPN, including ink calibration, multiple ink alignment, and design and implementation of complex patterns.

**Figure 1.** Scheme of two different chemistries of attachment of oligonucleotides to substrates. (A) Acrylamide-modified oligonucleotides bound to SiOx. (B) Alkanethiol-modified oligonucleotides bound to gold substrates.
coat tips with aqueous buffered or unbuffered DNA solutions produced only very sporadic, uncontrollable results. DNA feature size was controlled through tip-sample dwell time in a similar manner to systems (e.g., alkanethiol-gold) examined elsewhere. In addition, DNA deposition was also controllable through relative humidity.9

IMAGING AND DETECTION OF DNA NANOSTRUCTURES

The ability to pattern biomolecules on the nanoscale opens up opportunities for investigation of new detection strategies for bioassays that are potentially more selective and sensitive than optical-based systems such as conventional fluorescence imaging. To demonstrate fluorescence detection of DPN deposited oligonucleotide spots on silicon oxide, we prepared a single-stranded complementary oligonucleotide labeled with a green fluorescent dye and then exposed it to the patterned surface under hybridization conditions. The labeled strand hybridized only to the complementary oligonucleotides, as shown in (Fig. 2A) and did not bind unselectively to the surrounding area or to non-complementary patterns. A similar test was performed using oligonucleotide modified gold nanoparticles as probes for the surface patterns. In these experiments, rather than fluorescence, light scattered by the nanoparticles was imaged with an optical microscope. As shown in (Fig. 2B) this effect results in a dramatic contrast between patterned and unpatterned areas.

Optical methods of detection are useful for detecting features larger than 500 nm. For smaller structures, it is necessary to devise other imaging techniques that are compatible with features as small as 100 nm and below. The scanning probe microscope is a useful tool for detecting topography changes associated with the binding of a complementary probe or analyte. We used tapping mode atomic force microscopy to image a 2-component DNA nanopattern after binding of different sized complementary gold nanoparticle-DNA probe conjugates. As seen in (Fig. 3) the pattern spots are resolved and individual sequences are easily distinguished by the height of the 2 different particles bound to the patterns.

REFERENCES


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