

Multiplexed Protein Arrays

Introduction

As a new tool in the increasingly important field of proteomics, there is significant interest in being able to deposit patterns of multiple proteins with nanometer precision at defined locations on a planar surface. Protein arrays have been demonstrated to be useful for protein detection and cell micropatterning studies in applications ranging from diagnostics to tissue engineering to basic research. NanoInk has developed novel, powerful Dip Pen Nanolithography[®] (DPN[®]) instrumentation and consumables capable of creating high-quality protein arrays at nanometer to micron scales¹. Compared to conventional protein microarrays, smaller feature sizes will have the benefit of drastically reduced sample size requirements, potentially higher detection sensitivity, improved ability to interrogate sub-cellular features, and better compatibility with lab-on-a-chip technologies.

Multiplexed Protein Array Principles

To print protein arrays, NanoInk's platform uses "pens" and a carrier solution to uniformly pattern multiple proteins onto the substrate. Three DPN instrument systems are available to fit a wide variety of array deposition needs, and each can be used with a custom designed 1D array of M-type cantilever "pens" to simultaneously print a multitude of proteins. Since NanoInk's deposition process minimizes shear forces that can disrupt biological function and maintains the proteins in a hydrated state, the arrayed proteins are more likely to maintain their functionality.

NanoInk has developed a proprietary carrier solution that has been used to successfully print a variety of different antibodies. The desired antibody and the carrier solution are simply mixed together and then deposited onto the surface.

To load adjacent "pens" with different proteins, and to subsequently print multiplex protein nanoarrays, NanoInk has developed "Inkwells" consisting of numerous reservoirs to load the proteins. A system of microfluidic channels, specifically engineered to transport liquids, is fed by the reservoirs. The microfluidic channels are designed to match the 1D

cantilever pen array geometry so that each cantilever can be loaded with a different protein. Figure 1 shows a fluorescent micrograph of the microfluidic channels on NanoInk's M-type "Inkwell", filled with 4 different antibodies, each tagged with a unique fluorescent molecule. Up to 12 individual proteins can be loaded into the M-type Inkwell.

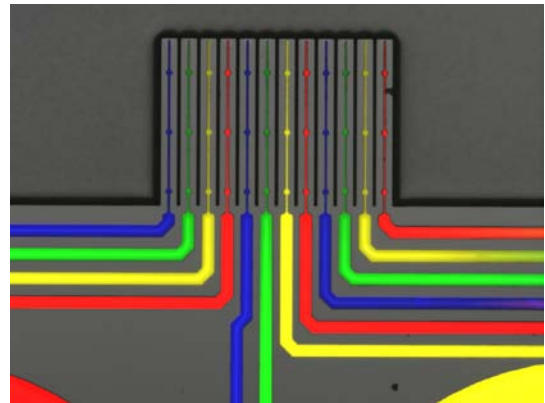


Figure 1. NanoInk's M-type Inkwell filled alternately with 4 fluorescently tagged proteins: Blue: donkey anti-sheep IgG (H+L) Alexa Fluor[®] 350, Green: chicken anti-goat IgG (H+L) Alexa Fluor[®] 488, Yellow: donkey anti-mouse IgG (H+L) Alexa Fluor[®] 546, Red: chicken anti-rabbit IgG (H+L) Alexa Fluor[®] 647.

Direct Printing of Antibodies

To demonstrate the feasibility of simultaneously arraying multiple proteins, an M-type 12-cantilever pen array was loaded by dipping into the microfluidic channels (Figure 1) containing four different antibodies. The loaded pens were then used to print the protein array (shown in Figure 2) on a silicon substrate. After deposition, the array was imaged using a Zeiss Axio Imager Z1m fluorescent microscope equipped with a 20x objective and a Zeiss AxioCam MRm camera.

In addition to silicon substrates, NanoInk's system has been proven to successfully deposit protein nanoarrays on virtually any planar surface used in proteomic and tissue engineering applications.

NanoInk's proprietary software enables flexible patterning of any geometric shape on any surface, provided the pattern conforms to the instrument stage dimensions. The pattern shown in Figure 2 represents merely one example of the different configurations possible.

Multiplexed Protein Arrays

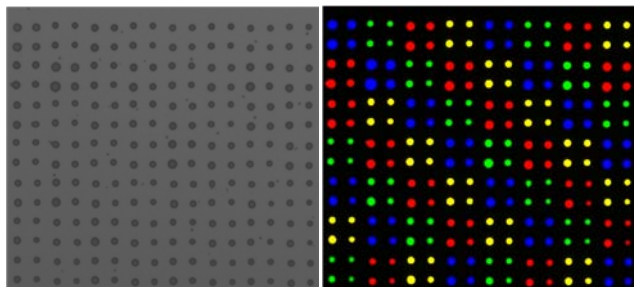


Figure 2. Printing of 4 different antibodies, each tagged with a unique fluorophore. (Left) Brightfield image at 200 x magnification. (Right) 4 channel fluorescent image.

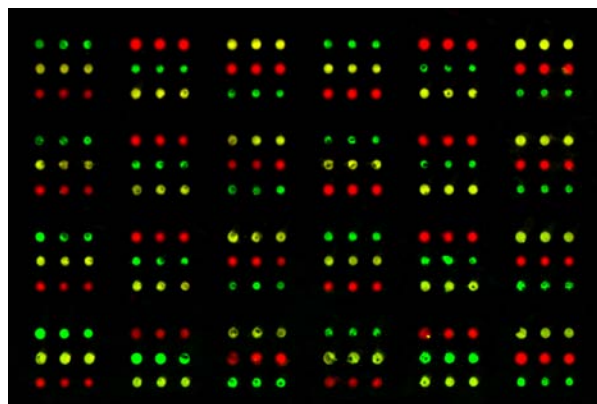


Figure 3. Fluorescence image of printing 3 normal IgG proteins and subsequent reaction with their labeled conjugates. The binding of the conjugate protein to the different IgG strains displays the reactivity of the proteins bound to the surface which maintain their selectivity.

To further demonstrate the NanoInk platform's ability to print and retain proteins on a chemically functionalized surface, goat IgG, mouse IgG and rabbit IgG were diluted with protein carrier solution to a concentration of 2 mg/ml. These antibodies were then loaded onto an M-type 12-cantilever pen array and printed on an epoxy-functionalized silicon substrate. The patterned substrate was placed in a humid container (to prevent evaporation) at room temperature for 3 hours to allow the protein to bind chemically to the epoxy surface. The remainder of the functionalized surface was backfilled with a blocking agent to eliminate background signal. Next, the substrate was incubated with a solution of the three anti-species antibodies (each tagged with a different fluorophore) for 1 hour at room temperature. The substrate was then washed and imaged using

the Zeiss Axio Imager Z1m fluorescence microscope. The data clearly demonstrates that the antibodies attached to the functionalized surface at the printed locales (Figure 3). Additionally, no background fluorescence was detected, indicating that the proteins were deposited at the intended locations and did not migrate along the surface.

Conclusion

It is clear from these results that NanoInk's platform is capable of creating high-quality, multiplex protein arrays with micron-scale features and nanometer resolution on a functionalized surface. Using NanoInk's proprietary instruments and reagents, proteomic researchers will be able to reliably and uniformly transfer proteins to a modified surface for further study. While we have presented data from experiments involving four different printed proteins, NanoInk's "Ink wells" and multiple cantilever pens can be used to array much larger numbers of unique proteins in very close proximity. This type of patterning is expected to be ideal for creating future bioassays or functionalizing sensor devices.

Reference

1. J.-W. Jang; A. Smetana; P. Stiles. Multiplexed Dip Pen Nanolithography Patterning by Simple Desktop Nanolithography Platform. *Scanning*, 31 (2010) 1-6.

NanoInk Products Used

NLP 2000 System
 DPN[®] Pen Arrays: Type M
 DPN[®] Inkwell Arrays: Type M-12MW
 DPN[®] Substrates: Silicon Dioxide

Learn more about NanoInk products and services at www.nanoink.net. Or call us at 847-679-NANO (6266).

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