

Biosensor Functionalization

Introduction

The last two decades have seen significant advances in the development of micro electro mechanical systems (MEMS) for use as sensors. MEMS based sensors have applications in fields of science ranging from physical and chemical sensing to biological disease diagnosis. The major advantages of MEMS sensors over conventional sensors include their potential for higher sensitivity, lower cost, smaller sample size, and label-free detection. Another important distinction is that MEMS sensors can easily be multiplexed to simultaneously detect multiple analytes. MEMS technology holds promise as the next generation of high sensitivity sensors.

Microcantilevers are the most simplified MEMS based devices available for analyte sensing applications. Microcantilevers have been used successfully for physical, chemical and biological sensing work. In the field of medicine, microcantilever technology is specifically well-suited to disease screening, point mutation analysis, blood glucose monitoring, and chemical and biological warfare agent detection. Over the last few years, nanocantilevers have even been developed to achieve attogram level sensitivity.

Although micro- and nanocantilever sensors are robust, their high throughput applications have been limited due to the technical hurdles involved in functionalizing these elements. A method for easy and reliable microstructure functionalization has been needed to drive cantilever technology into the realm of practical sensing applications. To meet this need, NanoInk has developed instrumentation and associated technology for directed placement of materials at the nano- to microscale. Here, we demonstrate the deposition of proteins on microcantilevers.

Biosensor Principles

AFM cantilevers are one of the MEMS based devices most commonly used for sensing applications. Several reports have demonstrated the ability of cantilever sensors to detect miniscule quantities of biomolecules¹. Cantilevers used for this purpose are typically functionalized with a capture agent and then exposed to samples containing the target molecules. Binding of the target to the cantilever results in a change in cantilever dynamics (stress, resonance,

deflection), resulting in a measurable signal being generated. Although it is feasible to fabricate multiple cantilevers into a single sensor, methods for functionalizing cantilevers have (until now) been incapable of treating multiple cantilevers differently. NanoInk's Nano Lithography Platform (NLP 2000 System) is designed to deposit femtoliter to nanoliter amounts of molecules with high positional accuracy, making it the perfect tool for functionalizing multiple cantilevers with different molecules.



Figure 1. (Top) Brightfield live image showing the printing of 6-micron dots of fluorescently tagged IgG onto a commercially available AFM cantilever. (Bottom) Fluorescent image of the printed domains on the cantilever.

NanoInk has developed a proprietary carrier solution that has been used to successfully print a variety of

Biosensor Functionalization

different antibodies. The desired protein is simply mixed in solution with NanoInk's proprietary carrier and the solution is then printed onto the cantilever surface. NanoInk has three commercial DPN[®] instruments, along with a custom designed 1D array of cantilever pens (M-type), to simultaneously pattern multiple proteins on virtually any planar surface, including cantilevers. To load adjacent "pens" with different proteins, NanoInk has developed "Ink wells" 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. This enables printing of several different proteins in very close registration to one another, as shown in Figure 1.

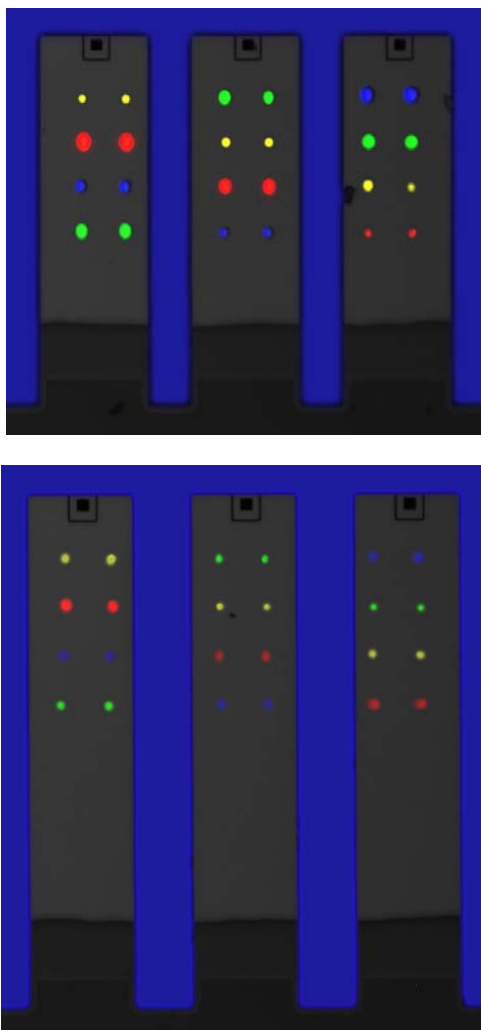


Figure 2. Four different fluorescently tagged proteins printed on custom cantilever arrays having different spring constants.

To demonstrate the versatility of NanoInk's technology, proteins were printed onto cantilevers that had different spring constants; results are presented in Figures 1 and 2. The commercial AFM cantilever shown in Figure 1 has a reported spring constant of 25-75 N/m. The custom NanoInk (E-type) cantilever array shown in Figure 2 has spring constants of 0.104 N/m (top) and 0.041 N/m (bottom). These results demonstrate that accurate protein printing can be accomplished on very delicate surfaces without damaging the cantilevers.

Conclusion

We have demonstrated that proteins can easily be printed onto the surface of cantilevers possessing a range of spring constants. Cantilevers have been reported in the literature to be sensitive biosensor devices for detection of target molecules. The ability to simultaneously place multiple proteins at several specific and addressable locations, at both the nano- and microscale, provides a practical method for functionalizing MEMS based sensing elements. This technique could finally lead to realizing the huge potential of MEMS based biosensors.

Reference

1. Yue, M.; Stachowiak, J. C.; Datar, R.; Cote, R.; Majumadar, A. Label-Free Protein Recognition Two-Dimensional Array Using Nanomechanical Sensors *Nano Letters* 2008, 8, 520.

NanoInk Products Used

NLP 2000 System
 DPN[®] Pen Arrays: Type M
 DPN[®] Pen Arrays: Type E
 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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