Development of a Non-Contact DNA Spotting Method for Biosensors

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Abstract:

Small, finely spaced magnetoresistive biosensors need to be individually functionalized with various DNA probes. Due to the small pitch of different functionalization spots, the functionalization chemistry is best applied to the biosensors with a precise robotic applicator. We currently have a robotic system that will perform DNA spotting with a hard needle. The present problem is that contact is needed to deposit the DNA onto the targeted sensor. The physical contact damages the sensor's surface which degrades its functionality.

By developing a non-contact approach, damage to the sensor will be avoided. Entirely contactless deposition of a DNA-containing fluid droplet is more difficult to achieve with a high degree of accuracy than hard-contact printing. Therefore we modified the current robotic system with new spotting pins and new software parameters that allow for DNA spotting with zero to minimal contact with the senor surface.

Time restrictions made testing of the final system unfeasible. But once tested, the sensor can be examined using a scanning electron microscope and/or tapping mode atomic force microscope to assess spot sizes, spotting uniformity, and the reduction of any damage to the sensor. If successful, the spotting technique can be carried over to a larger-scale production, allowing for cheaper and more standardized biochips.

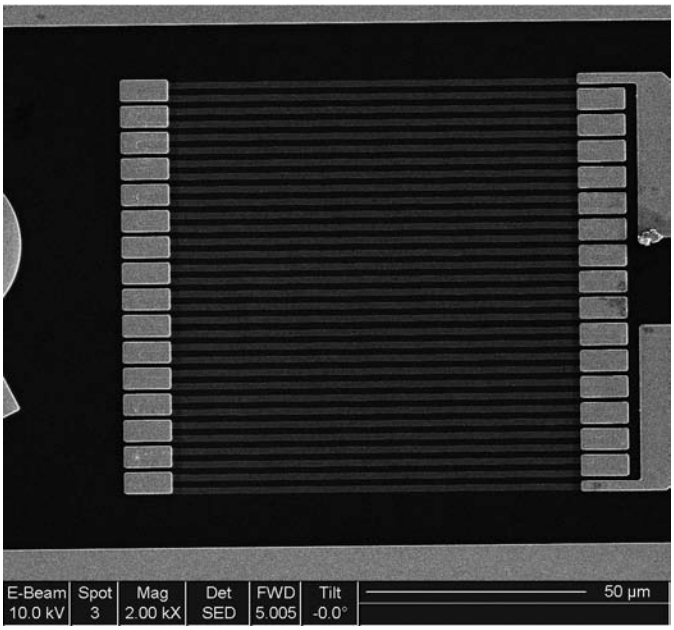

Figure 1: An individual sensor in the system. The sensor is 90 µm² and one of our spotting targets.

Introduction:

The biosensor system that is being developed will be used as a high-sensitivity DNA detection and identification system [1]. To ensure that the device functions correctly, it is important to individually functionalize each sensor with various DNA probes. Figure 1 shows an individual sensor. Currently, a microarray robotic system is being used to spot DNA onto the sensor. The microarray robot uses split pin spotting technology that requires physical contact to dispense fluid onto the intended substrate. Figure 2 shows a magnified view of the spotting process [2].

The goal of this project is to develop a method that will precisely spot DNA onto the surface of the sensor without the physical contact which damages the surface of the sensor.

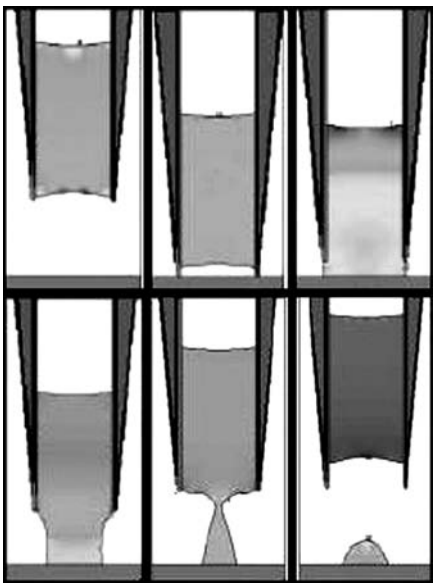

Figure 2: A magnified view of the tip for split pin spotting. Because the meniscus of the fluid is within the orifice opening, contact is needed to dispense the fluid.

Investigation:

We started by investigating the spotting options that would be applicable for this project. We considered three main options in our decision-making. One option was the current microarray spotting system. The benefit of this machine is that it was already in-house, but the system would need to be modified to allow for non-contact spotting. A liquid handling system was another option. This machine provided a non-contact method for spotting but was limited by its spot size and spot spacing that was too large for our applications. A third alternative that we looked at was inkjet printing technology. This would provide a non-contact method for spotting, with parameters that would meet our specifications. By using this technology, we would need to incorporate it onto our current robotic system or a new machine would need to be brought into the lab, being complicated and expensive respectively.

We decided to modify the current microarray spotting system to allow for non-contact spotting. One reason that this decision was made is because a new pin design was found that would allow for the possibility of non-contact spotting which the conventional split pins did not have. Figure 3 shows the difference between the conventional tweezer and split pin designs, and the new micro spotting pins [3]. The micro spotting pins have a small amount of fluid outside of the capillary space making it possible for the fluid to be dispensed onto the substrate without physical contact between pin and sensor.

A camera system was also designed for the robotic system allowing us to see the tip of the pin and observe when it maked contact with the sensor. The camera was also used to align the sensor for the spotting process.

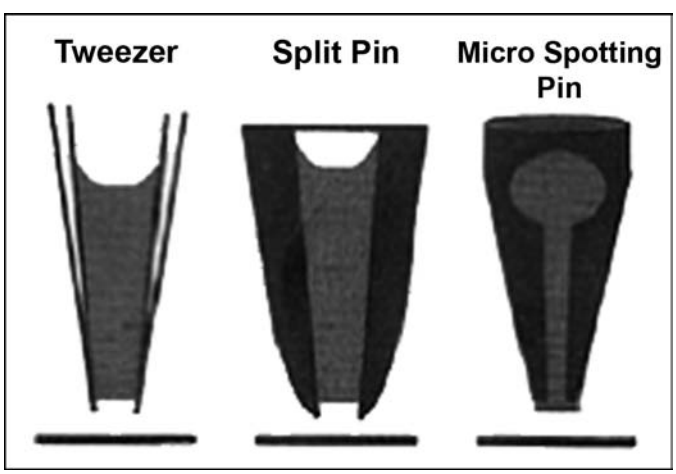

Figure 3: Different spotting pin designs. Notice the fluid outside of the orifice on the micro spotting pin and not the other designs.

We then worked on optimizing the robotic movement by using a software program that would allow us to change the robotic parameters and would show us the position error curve relative to the position of the pin. We found parameters that would minimize the movement error to 1-2.5 μm, down from ~ 20 μm for the conventional spotting set up.

Summary:

We decided to use the microarray robot for our spotting system. Several modifications were made to convert the current system, which used physical contact to spot fluid, into a system with the ability to spot fluid without damaging the sensor surface. A camera bracket, software program, new robotic parameters, and new micro spotting pins were implemented into the system to allow for a non-contact spotting method.

Future Work:

It is important to verify that this procedure is spotting the sensors without damaging the surface. Sensors will be spotted with the microarray robot, the system modifications, and the micro spotting pins. The sensors will then be examined with a scanning electron microscope and/or atomic force microscope to determine if damage has occurred to the sensor surface.

Acknowledgements:

I would like to thank Shan Wang, Nader Pourmand, and Sebastian Osterfeld for their help and assistance in this project. I would also like to thank Stanford University, the NNIN, and the NSF for the research opportunity.

References:

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[2] J. Zeng et al. "A Dynamic Spotting Method for Split-Pin Based Microarrays," Technical Proceedings of Micro Total Analysis Systems (2001).

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