OPTIMIZATION OF A MICROFLUIDIC CHIP FOR THE BIOBARCODE ASSAY

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ABSTRACT

We demonstrate in this work the successful application of functionalized gold nanoparticles and magnetic microparticles in the development of an integrated lab-on-a-chip-system. By employing the BioBarCode Assay, we have shown that it is possible to quantitatively identify ultra-low concentrations of proteins not only in buffer solutions, but also in serum samples using a microfluidic device. Furthermore, we have developed a method using microarrayed DNA patterns inside the microfluidic channels that will make possible multiplexed detection of biomarkers in a single experiment.

Keywords: Biobarcode Assay, Multiplexing, Nanoparticles, Protein Detection

In this paper we present a microfluidic device that employs functionalized nanoparticles for ultra-sensitive detection of target analytes. The BioBarCode Assay was initially developed by the Mirkin group for ultra-sensitive detection of analytes [1]. Initial results employing the assay in a microfluidic system were reported at MicroTAS 2004 [2]. This paper includes a complete set of experimental results detecting prostate specific antigen (PSA), a recognized marker for prostate and breast cancer, in both buffer and serum. We also report preliminary data demonstrating multiplexed detection of a panel of biomarkers including protein and nucleic acid molecules. For the assay development effort, both bench-top format and microfluidic formats are being developed simultaneously.

The assay employs magnetic particles that are functionalized with monoclonal antibodies to specifically recognize a target protein. Gold nanoparticles are functionalized with polyclonal antibodies, and are also densely coated with a 40-mer “barcode” DNA sequence that is unique for every target analyte. Figure 1 shows a schematic of the microfluidic device. After binding, the sandwich assay is magnetically separated, and the barcode DNA is released. The DNA is sent through a detection area that is patterned with half of the complementary sequence. A second set of gold nanoparticles is then sent through the detection area to bind with the other end of the barcode DNA. Finally, the chip is removed, the detection area is silver stained, and the results are detected scanometrically. Figure 2 shows a fabricated device.

Experiments were conducted in triplicate at 50 fM, 5 fM, and 500 aM concentrations of prostate specific antigen in phosphate buffered solution. Using only 1 µL of sample per experiment, the concentrations correspond to approximately 30000, 3000, and 300 molecules in solution, several orders of magnitude below the current clinical detection limit. Results similar to the control experiment (no PSA) were obtained when β-Galactosidase was used in place of PSA, thus demonstrating that the detection scheme binds specifically to the target protein. Next, by spiking the target PSA protein into goat serum, we were able to demonstrate the effectiveness of our microfluidic device in
handling complex samples as shown in Figure 4. The control (no PSA) showed virtually no signal, while the 500 aM and 5 fM samples stained in agreement with previous results.

Previously, the entire detection area was patterned with a unique single stranded DNA sequence. In order to obtain multiplexing, a new approach was developed that employs a microarrayer to deposit 200 micron spots of multiple DNA sequences. Initial experiments employ two 2x2 arrays of unique DNA sequences. Alignment of the spots within the microfluidic channels is achieved by first aligning the glass slide to a spotting template followed by a second alignment of the microfluidic device to the slide using a microscope and a custom made alignment tool. The results of 3 experiments containing different combinations of DNA sequences are shown in Figure 5. The results demonstrate that a unique barcode DNA sequence will bind specifically to the patterned complementary DNA spot on the glass slide thus allowing for simultaneous detection of multiple proteins.

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![Figure 1. Schematic diagram of the microfluidic device.](image1)

![Figure 2. Microfluidic Device for carrying out the biobarc ode assay in a sequential manner controlled by valves.](image2)
Figure 3. Experimental results for PSA detection in a microfluidic device

Figure 4. Experimental results of goat serum sample with (A) no PSA added (B) 500 aM final PSA concentration, and (C) 5 fM final PSA concentration.

Figure 5. Photograph images multiplexing experiments using (A) DNA sequence 1 (B) DNA sequence 2, and (C) both sequence 1 and 2 present in solution.

REFERENCES


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