

A microarray assay with fluorescent microparticles

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INTRODUCTION: Microarrays make it possible to study thousands of biomolecular interactions in parallel and with high-throughput. While DNA microarrays have already become a standard for the analysis of nucleic acids, protein chips are emerging as a powerful tool for applications in biological research, drug discovery, drug development and diagnostics. The demand for highly sensitive assays and further assay miniaturization requires the optimization of the detection sensitivities. The aim of this project is to develop a novel bioanalytical assay for the sensitive detection of biomolecular interactions. In this work, microarray assays were performed with fluorescent microparticles coated with capture elements. In order to decrease the background noise, the use of fluidic laminar forces for the discrimination between specific and non-specific bound particles was investigated.

METHODS: Biotinylated bovine serum albumin (BSA-biotin) was spotted onto Ta₂O₅ waveguides coated with dodecylphosphate (Zeptosens, a division of Bayer Schweiz AG, Switzerland). The substrates were blocked by dip-and-rinse with ZeptoMARK Blocking buffer (Zeptosens, Switzerland). The streptavidin coated fluorescent particles were introduced using a continuous flow. The sample was rinsed with buffer at the same flow rate. Confocal fluorescence microscopy and a ZeptoREADER based on planar waveguide technology were used for imaging.

RESULTS: Microarrays of BSA-biotin were exposed to a continuous flow of streptavidin coated beads. Different flow rates were investigated. The ratio of the bead density on the spot to the bead density in the background is shown in Fig 1. The optimal flow rate was found to be situated between 1 $\mu\text{l}/\text{min}$ and 2.5 $\mu\text{l}/\text{min}$. The lowest concentrations could only be discerned when a flow rate of 2.5 $\mu\text{l}/\text{min}$ was applied. However, further reduction led to sedimentation and thus higher background values. The bead-based approach led to a significant increase in spot signal compared to the standard assay performed with fluorescently labeled streptavidin as shown in Fig. 2. While the beads on the spot were clearly visible, the spot incubated with fluorescently labeled streptavidin could not be imaged.

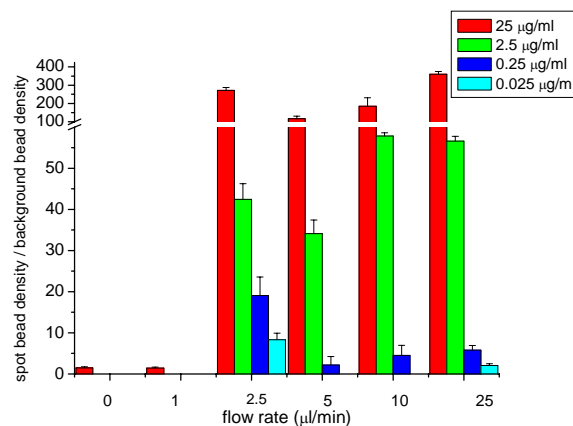


Fig. 1: Ratio of bead density on the spot and bead density in the background for four different BSA-biotin concentrations and different flow rates. The optimal flow rate was 2.5 $\mu\text{l}/\text{min}$.

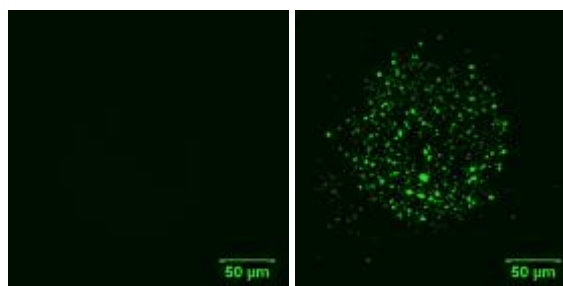


Fig. 2: Microscopy images of a BSA-biotin spot after incubation with streptavidin AlexaFluor488 (left) and streptavidin coated fluorescent beads (right). The same settings were used for both images. The bead-based assay leads to signal amplification.

CONCLUSIONS: We presented a novel approach to perform microarray assays using fluorescently labeled particles and fluidic flow discrimination. Signal amplification could be achieved when an optimized fluidic force was applied to remove non-specific bound particles.

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