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Biosensing enhancement using passive mixing structures for microarray-based sensors

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ABSTRACT

The combination of microarray technologies with microfluidic sample delivery and real-time detection methods has the capability to simultaneously monitor 10–1000 s of biomolecular interactions in a single experiment. Despite the benefits that microfluidic systems provide, they typically operate in the laminar flow regime under mass transfer limitations, where large analyte depletion layers act as a resistance to analyte capture. By locally stirring the fluid and delivering fresh analyte to the capture spot, the use of passive mixing structures in a microarray environment can reduce the negative effects of these depletion layers and enhance the sensor performance. Despite their large potential, little attention has been given to the integration of these mixing structures in microarray sensing environments. In this study, we use passive mixing structures to enhance the mass transfer of analyte to a capture spot within a microfluidic flow cell. Using numerical methods, different structure shapes and heights were evaluated as means to increase local fluid velocities, and in turn, rates of mass transfer to a capture spot. These results were verified experimentally via the real-time detection of 20-mer ssDNA for an array of microspots. Both numerical and experimental results showed that a passive mixing structure situated directly over the capture spot can significantly enhance the binding rate of analyte to the sensing surface. Moreover, we show that these structures can be used to enhance mass transfer in experiments regarding an array of capture spots. The results of this study can be applied to any experimental system using microfluidic sample delivery methods for microarray detection techniques.

1. Introduction

A microarray is a two-dimensional set of specific biological capture probes deposited in an orderly grid of spots on a flat substrate, typically a glass slide. Each microarray spot contains one type of capture probe, and is immobilized to a region that is tens or hundreds of microns in diameter, herein referred to as a capture spot (Wu et al., 2012). Multiplexing capabilities are produced by spatially encoding the array, where each location serves as a reporter for a specific analyte (Situma et al., 2006). Microarrayers, the instruments used for the deposition of sample probes, typically operate with a positional resolution from 2.5 up to 5 μm, where the size of the spot can be varied through a selection of contact surface tips from around 30 to above 600 μm in diameter. To maximize the performance of the detection assays, it is important to increase the rate of mass transfer to the capture spot; an increase in the amount of captured analyte within a set assay time will lead to an assay with higher sensitivity. Microarray-based assays were initially carried out in a simple manner via the immersion of the microarray substrate into a target solution (Ekins and Chu, 1991). The sensitivity of these static assays, measured as the diffusive analyte flux to the center of the capture spot, has shown to be inversely proportional to the capture spot diameter (Dandy et al., 2007; Ekins, 1998). In this static regime the flux remains proportional to the analyte diffusivity, and mass transfer rates remain low for assays involving larger biomolecules having inherently low diffusivities.

Further increases in analyte mass transfer rates to a capture spot can be obtained through the addition of fluid convection to the system. The use of a shaking plate to stir fluid over a capture spot has been shown to provide dramatic increases in assay sensitivity (Kusnezow et al., 2006; Saviranta et al., 2004). To further exploit the use of convective fluid transport in the array sensors, many researchers have turned to the use of microfluidic

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continuous flow cells. These microfluidic flow cells provide several benefits to array technologies, including a reduction of reagent consumption, reduced footprints, and decreases in the characteristic time for diffusive mixing and surface reactions.

The use of microarray technologies in a microfluidic format has been used in multiple instruments that can simultaneously sense thousands of different samples per square centimeter in a flow chamber (Campbell and Kim, 2007; Eddings et al., 2009; Homola et al., 2005; Piliarik et al., 2010; Piliarik and Homola, 2008). The ability to simultaneously investigate large numbers of targets has been effectively applied in genomics (DNA microarrays) and proteomics (protein microarrays) on a regular basis. These experiments are useful in many areas, including the identification of biomarker catalogs for diagnosis and prognosis, to relate physiological states to gene or protein expression patterns, and to study the progression of diseases as well as cellular response to stimuli (Malic et al., 2011; Trevino et al., 2007).

Current microfluidic-based microarray technologies use simple rectangular microfluidic flow cells, where there is no change in the microchannel cross-section over the length of the array. However, these benefits are also tied to the disadvantages of laminar flow, present due to the small characteristic lengths and fluid velocities encountered with microfluidics. For microarray systems under mass transfer limitations (where rates of analyte capture are much greater than the rate of analyte transport to the sensor), the lack of turbulence can lead to large analyte depletion layers that act as a resistance to analyte capture and detection. These depletion layers can also affect other similar capture spots situated directly downstream, which are subjected to lower concentrations of analyte. Several authors have taken steps to mitigate the effects of these depletion layers with the use of more complex pumping techniques. Adey et al. (2002) used an air bladder to pump fluid back and forth across a microarray chamber to improve hybridization rates. Furthermore, several authors have used alternating pairs of syringe pumps to periodically alter the flow direction within a microfluidic chamber, where the resulting flow profile serves to improve the performance of the microarray assay (Hertzsch et al., 2007; McQuain et al., 2004; Raynal et al., 2007). It should be noted, however, that although periodic changes in the microfluidic flow direction can serve to enhance the microarray performance, they do not modify the flow field to mitigate the presence of any analyte depletion layers. In this area there remains an opportunity for sensor improvement from the implementation of microfabrication techniques to modify the fluid flow profile only in the vicinity of each spot, while still allowing for the use of a simple, single source fluid delivery technique. Specifically, the use of passive mixing structures above each spot can serve to increase local rates of mass-transfer to each spot, thereby increasing the sensitivity of the assay.

Over the past 15 years there has been a large effort focused on enhancing the rate of mixing in microfluidic systems. These efforts can be divided into two broad categories, mixing via an active or passive manner. Active systems manipulate and mix fluid quickly (often in an adjustable manner) through a variety of mechanisms, including piezoelectric, thermal, acoustic, and magnetic methods (Hessel et al., 2005). Although these systems possess the ability to provide efficient mixing profiles, they are generally very complex and require additional equipment or materials. Furthermore, they often suffer from problems associated with heat transfer and bubble formation. In contrast, passive mixers have the ability to enhance fluid mixing without the requirement of supplementary energy while often employing a much simpler fabrication process (Beebe et al., 2002; Wu et al., 2004).

Despite the large amount of literature devoted to passive mixing techniques, there remains little information concerning the use of these mixers for the enhancement of mass transfer to a sensor surface. Liu et al. (2006) used a chaotic micromixer to mix the target solution (and remove depletion layers) entering and leaving a hybridization chamber; however, the mixer was situated outside the boundaries of the microarray, and the system required a built in peristaltic pump for operation. Several authors have utilized microfluidic mixers situated directly over the sensing chamber. Kirtland et al. (2006) used theoretical and numerical methods to show the benefits that chaotic mixing in a rectangular channel can have on mass transfer to a channel surface. In another numerical study, Forbes and Kralj (2012) examined optimal geometric designs of the staggered herringbone mixer for the enhancement of interaction between fluid streamlines and a sensing surface. Experimentally, Golden et al. (2007) demonstrated an enhancement of binding for an affinity assay in a microchannel fabricated with grooved passive mixing structures. Similarly, Foley et al. (2008) showed only modest improvements in mass-transfer using similar structures. These studies utilized sensing domains consisting of a long reactive boundary of length 1–100 mm for the capture of a single analyte. Obviously, this characteristic size is much larger than that of a typical microarray, where within the same space using a microarray there might be hundreds or thousands of reactive surfaces, each having specificity for a unique analyte. To our knowledge there exists no information on the use of passive mixing structures to enhance the mass transfer of an analyte to a reactive surface having a characteristic size similar to a traditional microarray capture spot. The use of such structures has great potential in the enhancement of these microarrays; increases in mass transfer will lead to shorter assay times, and furthermore, a reduction in the microarray limit of detection.

In this paper we explore the utilization of passive mixing structures to increase the efficiency of mass transfer of analyte to an individual capture spot. Fig. 1 shows the particular approach of this work, where passive mixing structures situated on the surface opposite of a capture spot serves to provide higher fluid velocities, and in turn, higher rates of analyte delivery. These benefits come at the expense of small increases in the viscous resistance of the channel; however, the additional pressure drops are negligible with respect to those that would be encountered by simply reducing the overall height of the channel. Furthermore, these structures act to stir the fluid for the prevention of any detrimental downstream effects, which would not be seen in a simple channel of reduced height.

Using computational methods, we examine how the shape of a several passive mixing structures affect local rates of mass transfer to a capture spot. Additionally, we examine the relationship between the positions of the passive mixing structures relative

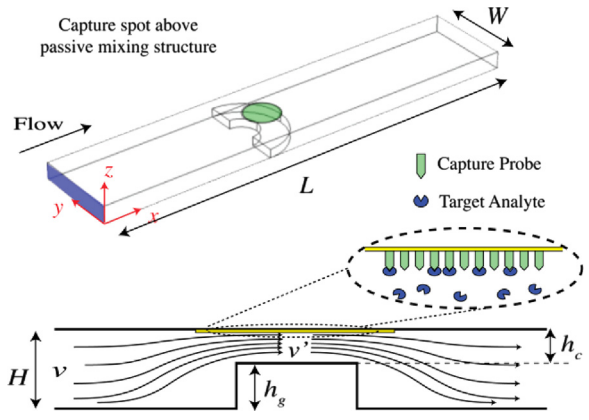

Fig. 1. Example of the work presented here: a single passive mixing structure situated beneath a capture spot serves to increase the average fluid velocity near the spot (from v to v') and increase local rates of mass transfer.