Submicrometer Fluidic Channels for Studying Cancer Gene Expression

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Abstract
Gene expression is a dynamic process involving messenger ribonucleic acid (mRNA) quantities that can vary from tens to millions. In this work, single molecule quantification [1, 2] of a sample’s genetic content is performed in a fluidic channel with submicrometer (500 nm by 250 nm) cross-sectional dimensions. Molecular beacon (MB) probes are utilized to increase robustness and selectivity in detection via a selective change in conformation and fluorescence intensity in the presence of the complement DNA or mRNA sequence [3, 4]. The MBs used target genetic content associated with regulation and detoxification of reactive oxygen molecules that can lead to increased breast cancer risk. MB probes were designed and provided through collaboration with Prof. Weihong Tan at University of Florida.

Summary of Research
Fluidic channels are constructed in a fused silica substrate using conventional microfabrication techniques—a single layer of photolithography followed by reactive ion etch. Fluid reservoirs access the channels by through-wafer ports and the final device is assembled with a direct wafer bond technique. The process allows for rapid prototyping of many fluid channel arrays on a 100 mm substrate.

To quantify a sample’s genetic content, the single-stranded-DNA (ssDNA) or mRNA is hybridized to a MB and driven electrokinetically through the transparent fluidic channel. Using a focused laser spot to excite fluorescence in the channel cross-section, a subfemtoliter-sized focal volume is formed that contains only one DNA/MB hybrid molecule at a time (Figure 1). This combination of flow control and single molecule confinement extends the limit of detection to an arbitrarily low concentration, restricted only by the tradeoff in data collection time and the experimenter’s requirement for statistical significance in describing the sample population. In this work, detection is performed at the 100 pM level and molecules were counted at a rate of approximately 800 per minute using a driving potential of 100 volts across the fluid channel.

Fluorescence changes in ssDNA/MB hybrids, at both the 100 nM and at the 100 pM concentration regime, were studied using a bulk solution and a single molecule spectroscopy (SMS) measurement, respectively (Figures 2, 3). Both measurements exhibit a 2-3x fluorescence intensity change upon hybridization—demonstrating that MB fluorescence changes can be observed at the single molecule level and are in agreement with ensemble measurements.

The utility of SMS to study hybridization is further demonstrated when the amount of excess target ssDNA is varied. By comparison of ssDNA and MB mixtures at both 10:1 and 1:1 ratios, this measurement technique illustrates a shift in favorable binding conditions measured through a reduced fluorescence enhancement (Figure 4).

This result supports SMS as a tool for investigating binding kinetics and molecular probe specificity.

References
Figure 1: Submicrometer fluid channel and formation of the inspection volume.

Figure 2: Bulk measurement of MBs and ssDNA/MB hybrids.

Figure 3: SMS measurement of MBs and ssDNA/MB hybrids.

Figure 4: Variation in excess target ssDNA impact hybrid population’s fluorescence.