Abstract:

Rare chemical modifications to deoxyribonucleic acid (DNA) and histone proteins, building blocks of chromatin, are fundamental to the field of epigenetics. These modifications occur regularly as the result of diet [1], the presence of environmental toxins [2], and have been shown to influence the progression of cancer and other diseases [3]. We report the first demonstration of single molecule chromatin analysis at the nanoscale (SCAN) using a nanofluidic channel to perform high throughput screening of epigenetic state. Studies performed using SCAN have the potential to unlock information currently inaccessible with conventional immunoassay methods, such as chromatin immunoprecipitation (ChIP), by investigating multiple modifications simultaneously and also requiring substantially smaller quantities of input sample material.

Summary of Research:

Nanofluidic channels that confine an individual molecule are fundamental to the SCAN platform. Fluidic channels used in this work (cross-section 250 nm × 500 nm) were constructed in a fused silica substrate using conventional nanofabrication techniques—a single layer of photolithography followed by reactive ion etch (Figure 1). Fluid reservoirs access the channels by through-wafer ports and the final device is assembled with a direct wafer bond. The process allows for rapid prototyping of many fluid channel arrays on a 100 mm substrate.

We have demonstrated the ability to perform SCAN on native chromatin fragments derived from a HeLa cell line [4]. To identify fragments where both DNA and histones were bound together, a green fluorescent protein (GFP) was introduced on histone 2B and a red-stain was labeled to DNA. During the subsequent SCANS, we searched for two-colored molecules at rates exceeding 2000 molecules/min and successfully identified in-tact chromatin fragments within our device (Figure 2). To verify the linear dynamic

Figure 1: Photomicrograph of a fluid channel array. Molecules entered through the bottom microfluidic channel and were analyzed in the nanofluidic channel constriction. Scale bar is 25 µm.

Figure 2: Native chromatin fragments from a HeLa cell source were analyzed within a nanofluidic channel. Two-color, time-coincidence analysis reveals about 50% of all DNA (red stain, TOTO-3) also contains a bound H2B histone (green label, GFP), indicating detection of a complete nucleosome. The SCAN method investigated genomic material at a rate of about 10 Mbp/min.
range of detection for this technique, chromatin was extracted from mixtures of HeLa cells with and without GFP, and SCANs illustrated excellent linearity throughout all mixture proportions of HeLa-GFP cell content (Figure 3).

Our single molecule analysis was then applied to identify DNA methylation, an epigenetic mark, using a methyl-binding domain (MBD) protein. Analogous to the prior experiment, MBD was labeled with a green fluorescent dye and DNA was labeled with a red stain. Mixtures with varying proportion of methylated and unmethylated DNA clearly showed the detection of DNA methylation using the MBD probe (Figure 4) and elucidated the utility of SCAN for epigenetic analysis. Continued development of SCAN could lead to the unprecedented ability to analyze multiple epigenetic marks simultaneously and evaluate their shared or exclusive relationships.

References:

Figure 3: By adjusting the mixture of chromatin derived from HeLa cells expressing H2B-GFP and wild-type HeLa cells, we verified the authentic detection of two-color chromatin fragments within a nanofluidic channel and also showed the excellent detection linearity of SCAN.

Figure 4: The epigenetic mark, DNA methylation, was identified in mixtures of methylated and unmethylated DNA using an MBD protein. In response to increasing levels of methylated DNA in the mixture, our single molecule analysis detected an increase in two-color, bound MBD-DNA complexes that emerge above a background of detection events.