Nanofluidics to Enhance Single Molecule DNA Imaging
Detecting Genomic Structural Variation in Humans

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simultaneously label by means of fluorescence the genetic locus and the synthesized mRNA using the EGFP-labeled MS2 coat protein [1]. Our method, previously applied to the tracking of gene arrays in cultured cells [2], has temporal resolution of 10-100ms, and additionally records the 3D position of the genetic locus by moving along a circular orbit the focused laser beam. Distinct regions of active transcription display a well defined spatial organization, coralling the denser part of the genetic locus. In most cases each region maintains a defined angle in the reference system of the orbit, and the transcriptional activities of different regions are not cross-correlated.

The fluorescence time traces of each of these regions highlight the existence of slow (10-100s) transitions between distinct intensity values, corresponding to the timescale of a single mRNA dwell on the gene or to that of a transcription burst. We observe autocorrelation of the fluorescence intensity on timescales smaller than 1s. We relate these fast fluctuations to the faster kinetics of mRNA transcription, down to individual MS2-EGFP molecules binding to the newly transcribed mRNAs.

Measurements of the size and shape of the genetic array by calculating the modulation of the first and second harmonic of the fluorescence along each orbit suggest that the gene’s decondensation is not a necessary condition for transcription to occur.


1993-Pos Board B723 Nanofluidics to Enhance Single Molecule DNA Imaging: Detecting Genomic Structural Variation in Human Cells

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Using a novel nanofluidic device, we recently showed [1] how to obtain a bird’s-eye view of genomic structure at ~1kb resolution from a single DNA molecule and used it to discover novel structural variation in an individual’s genome that is too large to be easily identified by current DNA sequencing methods - but too small to be identified by conventional microscopy of chromosomes [2]. These results highlight the role ancillary technologies (micro-/nano-fluidics) play in the application of ultrasensitive optical detectionsingle-molecule spectroscopy. Generating high-resolution images of DNA features requires that the molecules are stretched; in fluidic systems for high-throughput analysis of ultra-long DNA molecules (~10^6 bp), stretching has been limited to ~50% [3]. We achieve ~98% DNA stretching by combining two mechanisms: confinement in a nanoslit and hydrodynamic drag.

Crucially, this stretching totally suppressed longitudinal Brownian motion, enabling mapping of single molecules with maximal resolution: across overlapping fields-of-view, images can be perfectly merged without any rescaling, correction for drift, or morphing.


1994-Pos Board B724 Detection of Single Proteins Bound along DNA with Solid-State Nanopores

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Expression of genes in organisms is controlled through a number of mechanisms including epigenetic factors such as DNA-binding protein which cover cellular DNA at all times. Detection of these DNA bound protein is a challenge. Here we demonstrate, for the first time, that single DNA-bound proteins can be detected at the single-molecule level using solid-state nanopores. We show measurements that resolve single antibodies attached to DNA. We build on earlier work that demonstrated detection of small protein patches and show that single protein can be detected by increasing the measurement bandwidth while maintaining a good signal-to-noise ratio and addressing the stability of the DNA-protein complex in the high salt conditions required for measurement. We present experimental measurements carried out on several protein systems, discuss the current capabilities of this approach, and highlight several applications requiring single protein detection which are now feasible.