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 ECFP-labeled MS2 coat protein [1]. Our method, previously applied to the tracking of gene arrays in cultured cells [2], has a temporal resolution of 10-100 ms, 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, corolling 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 (10-1000s) 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 1 s. 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.

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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 ~1 kb 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 detection-singles molecule spectroscopy. Generating high-resolution images of DNA features requires that the molecules are stretched; in fluidic systems for high-throughput analysis of single-molecule systems [2].

Genomic Structural Variation in Humans

We use an optical tweezers platform to study the folding and unfolding pathway of individual molecules containing single-stranded DNA human telomeric G-quadruplex (G4) sequence, (TTAGGG)4, in the presence of 150 mM Na+ solution, these DNA molecules are folded into G-quadruplex structure based on the Hoogsteen basepairing. When forces were applied to unfold the G4-containing DNA molecules, most of the unfolding traces show one or

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