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, 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 (10-100s) 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.

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

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453

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Single DNA-Shelled Silver Nanoclusters Probed by Tip Enhanced Fluorescence Spectroscopy

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The primary goal of optical microscopy is to visualise and thereby understand microscopic structure and dynamics. Dramatic developments over the past decades have enabled routine studies down to the single molecule level and structural observations far beyond the limits defined by the diffraction limit through the use of fluorescence as a contrast mechanism. Despite its many advantages, one of the fundamental limitations of fluorescence detection is the frequency with which photons can be emitted and thus detected. As a consequence, although images and even movies of single molecules have become commonplace, imaging speed remains limited to few to tens of frames per second by the quantum nature of single emitters. The result is a considerable gap between the rate at which dynamics can be recorded and the underlying speed of motion on the nanoscale.

Here, we introduce an alternative approach to optical microscopy that relies on the ultra-efficient detection of light scattering, rather than fluorescence, called interferometric scattering microscopy (iSCAT). We show that iSCAT is capable of following the motion of nanoscopic labels comparable in size to semiconductor quantum dots with nm accuracy down to the microsecond regime, the relevant timescale for a majority of nanoscopic dynamics. Thereby, we are able to address a surprising variety of fundamental questions in molecular biophysics ranging from the mechanical properties of DNA, the mechanism of molecular motor processivity and anomalous diffusion in bilayer membranes and its possible origins. We also demonstrate the potential of iSCAT for label-free, all-optical biosensing and imaging at the single protein level.

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Effect of G-Quadruplex Stabilizing Compound on the Folding and Unfolding Pathway of Human Telomeric DNA

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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)n. 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