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 synthetized 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 10-100 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 (10-100 s) 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. Work supported in part by NIH grants P50 GM076516 and P41 GM103540.


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Fluorescence and Nanotechnique for Active Targeting of Cancer Cells

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Significant advances in nanotechnology have been made by development of aperture-less tip enhanced fluorescence microscopy, in which so called near field effect enhances the absorption and emission rates and allows overcoming the diffraction limit. Tip Enhanced Fluorescence Spectroscopy (TEFS) involves a combination of classical scanning tunneling microscopy (STM) with optical confocal microscope where incident light is focused on the tip of metal probe enhancing electromagnetic field nearby. We applied TEFS technique for investigation of DNA-stabilized fluorescent Ag nanoclusters. DNA-stabilized Ag nanoclusters make up a class of water-soluble fluophores consisted of 1-10 silver atoms. They exhibit high chemical stability, good biocompatibility and high absorbance and quantum yield. An assembly of nanoparticles was performed in aqueous medium using silver nitrate and silver nitrate. Ag-DNA-polymer assembled structure seems to be promising in creation highly developed and bright nanoparticles containing multiple emitters therein. TEFS technique thus appears to be a powerful approach for single molecule spectroscopy and superresolution bioimaging.

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Interferometric Scattering Microscopy: A New Camera for the Nano-World

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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 these 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, image speed remains limited to a 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 nanosecond 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)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