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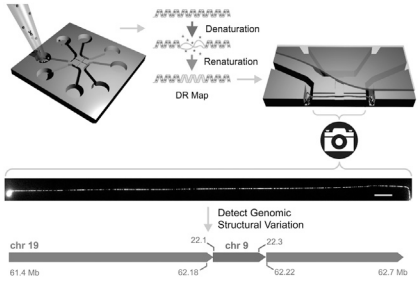
Nanofluidics to Enhance Single Molecule DNA Imaging: Detecting Genomic Structural Variation in Humans

David L.V. Bauer[1], Rodolphe M. Marie[2], Jonas N. Pedersen[2], Kristian H. Rasmussen[2], Mohammed Yusuf[1,3], Emanuela Volpi[1], Henrik Flyvbjerg[2], Anders Kristensen[2], Kalim U. Mir[1].
[1]University of Oxford, Oxford, United Kingdom, [2]Technical University of Denmark, Kongens Lyngby, Denmark, [3]London Centre for Nanotechnology, University College London, London, United Kingdom.

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.



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Detection of Single Proteins Bound along DNA with Solid-State Nanopores

Calin Plesa, Justus W. Ruitenberg, Menno J. Witteveen, Cees Dekker.
Delft University of Technology, Delft, Netherlands.

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.

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

Ivan L. Volkov, Victoriia V. Karpenko, Alexey I. Kononov.
Saint-Petersburg State University, Saint-Petersburg, Russian Federation.

Significant advances in nanoimaging have been made by development of aperture-less tip enhanced fluorescent microscopy, in which so called near field effect enhances the absorption and emission rates and allows overcoming the diffraction limit. Tip Enhanced Fluorescent Spectroscopy (TEFS) involves a combination of classical scanning tunneling microscope (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 fluorophores consisted of 1-10 silver atoms. They exhibit high chemical stability, good biocompatibility and high absorbance and quantum yield. An assembled structure of compact globule with silver clusters inside was obtained using calf thymus DNA condensed by synthetic polycation (poly)allylamine. Compact fluorescent globules 100 nm in size containing 1-2 silver clusters were visualized by TEFS technique. Fourfold enhanced fluorescent signal was obtained and fluorescent spectra of single clusters were registered. Such Ag-DNA-polymer assembled structure seems to be promising in creation highly stable 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

Philipp Kukura.
Physical and Theoretical Chemistry Laboratory, University of Oxford, Oxford, United Kingdom.

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

Tao Huang[1], Ta-Chau Chang[2], Hung-Wen Li[1].
[1]Department of Chemistry, National Taiwan University, Taipei City, Taiwan, [2]Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei City, Taiwan.

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