process being studied reflect transient molecular actions that are otherwise inaccessible to traditional biochemical methods.

**826-Pos Board B626**

**Electron-Beam Fabrication of Micron-Scale Birefringent Quartz Particles for Optical Torque Wrenches**

Zhuangxiong Huang, Francesco Pedaci, Matthew J. Wiggin,
**Maarten M. van Oene**, Nynke H. Dekker.

Torque plays a fundamental role in biological processes such as transcription, replication, and repair. A number of techniques have been established for the measurement of torque in single-molecule processes, amongst them the optical torque wrench [1]. This approach relies on the incorporation of birefringent particles into optical tweezers, using control of the laser polarization to apply torque, and measurement of the polarization following trapping to measure torque.

Such birefringent particles, which are also employed in micron-scale pumps to generate flow in microfluidics [2], and in microrheology as sensors that measure the local properties of surrounding fluid [3,4], would benefit from carefully-controlled geometries. This has been demonstrated by Deufel et al. [5] and Gutierrez-Medina et al. [6] in the context of optical lithography. Here, we demonstrate the ability of fabricate such birefringent particles of controlled geometry using electron-beam writing and discuss the relative merits of this approach.

[1] La Porta, A. and M.D. Wang, Physical Review Letters **92,** 190801 (2004).
[2] Leach, J., et al., Lab on a Chip **6**(6), pp. 735–739 (2006).
[3] Bishop, A.I., et al., Physical Review Letters **92**(19), 198104 (2004).
[4] Parkin, S.J., et al., Physical Review E **76**(4): 041507 (2007).
[5] Deufel, C., et al., Nature Methods **4**(3): pp. 223–225 (2007).
[6] Gutierrez-Medina, B., et al. in Methods in Enzymology:
Single Molecule Tools, Pt B. **474**: pp. 377–404 (2010).

**827-Pos Board B627**

**Translation and Rotation Dynamics in a Magnetic-Label Biosensor**

**Leo J. van IJzendoorn**, Kim van Ommering, Alexander van Reenen, Xander J.A. Janssen, Menno W.J. Prins.

Magnetic particles are used in magnetic-label biosensors to accelerate molecular binding to the sensor surface as well as to apply stringency by magnetic forces [1]. The biochemical and physical interactions of the particles with the biosensor surface play a key role in the molecular association and dissociation processes. In this paper we quantify the translation and rotation dynamics of particles at a sensor surface, interacting with the surface by nucleic-acids or protein molecules. We apply magnetic fields to actuate the particles and investigate their dynamics with single-particle resolution.

We will present measurements on the 3-dimensional mobility of 500 nm particles that are biologically bound to a biosensor surface, recorded using evanescent field microscopy with millisecond time resolution [2]. Our data show that the position and intensity histograms scale systematically with the length of the captured nucleic-acid analyte molecules and with the magnitude of the applied magnetic field.

We also present measurements on the rotation dynamics of protein-coated particles in a rotating magnetic field [3]. We demonstrate that a controlled torque is generated by the magnetic particles, which is used to quantify the rotation behavior and torsion stiffness of proteins captured onto the sensor surface by the magnetic particles. The data show that different protein pairs have distinctly different torsion moduli.

[1] D. M. Bruls et al., Lab Chip 9 (2009) 3504.
[2] K. van Ommering et al., J. Phys. D: Appl. Phys. 43 (2010) 155501 & 385501.
[3] X.J.A. Janssen et al., submitted to the Biophysical Journal (2010).

**828-Pos Board B628**

**Simultaneous AFM Force Spectroscopy and FRET Measurements on Single Biological Molecules**

**Hui Li**, Chi-Fu Yen, Sanjeevi Sivasankar.
Department of Physics and Astronomy, Iowa State University, Ames, Iowa 50011.

Abstract
Single Molecule Fluorescence Resonance Energy Transfer (FRET) and single molecule force measurements with the Atomic Force Microscope (AFM) are two powerful techniques that have facilitated much progress in the biological sciences. However each of these techniques suffers from limitations that can be overcome by the use of a combined single molecule AFM-FRET approach. Here, we describe an instrument that successfully combines single molecule AFM with FRET to apply forces on individual biological molecules and simultaneously monitor their conformational dynamics. To validate this technique, we measured the force induced shearing of dye-labeled, double stranded DNA. Single DNA molecules were sheared and mechanical transitions corresponding to DNA rupture were correlated with changes in FRET.

**829-Pos Board B629**

**Nanomechanical Recognition Measurements of Individual DNA Molecules Reveal Epigenetic Methylation Patterns**

Rong Zhu, Stefan Howorka, Johannes Pröll, Ferry Kienberger,
Johannes Preiner, Jan Hesse, Andreas Ebner, Vassilli Ph Pastushenko,
Hermann J. Gruber, **Peter Hinterdorfer**.

Atomic force microscopy (AFM) is a powerful tool for analyzing the shapes of individual molecules and the forces acting on them. AFM-based force spectroscopy provides insight into structural and energetic dynami of biomolecules and molecular bonds, by probing the interaction within individual biomolecules, or between a surface-bound molecule and a cantilever that carries a complementary binding partner. Here we show that an AFM cantilever with an antibody tether can measure the distances between 5-methylcytidine bases in individual DNA strands with a resolution of 4Å, thereby revealing the DNA methylation pattern, which has an important role in the epigenetic control of gene expression. The antibody is able to bind two 5-methylcytidine bases of a surface-immobilized DNA strand, and retracting the cantilever results in a unique rupture signature reflecting the spacing between two tagged bases. This approach might also allow related chemical patterns to be retrieved from biopolymers at the single-molecule level.

Published in Nature Nanotechnology, November 2010. This work was supported by the Gen-Au project 'Ultra sensitive Proteomics and Genomics' from the Austrian federal ministry for education, science and culture (R. Z., S. H., J. Prö., P. H.), the Austrian science fund project P15295 (H. J. G.), the Austria Nano-Initiative / NABIOS (R. Z.,, S. H., H. J. G., P. H.), and the European Commission grant 'Single Molecule Workstation (SMW)' No. NMP4-SE-2008-213717 (R. Z., P. H.).

**830-Pos Board B630**

**Droplet Tracking for Single Molecule Confinement**

Benjamin D. Gamari, Mark E. Arsenault, Peker Milas, Richard E. Buckman,
Christophe Wilhelm, **Lori S. Goldner**.

We report on a robust system for tracking femtoliter and smaller aqueous droplets in oil over tens of minutes with a few nanometers accuracy in a 150 Hz bandwidth. Tracking permits the droplet position to be maintained in the detection volume of a confocal microscope without need for physical or optical trapping, permitting detailed optical analysis of droplet contents. In addition, droplet tracks can be used to determine droplet size. As an alternative to surface attachment or liposomal confinement, we use droplets to confine individual biomolecules for study using single-molecule fluorescence trajectories. Back focal plane imaging onto a position sensitive detector (PSD), similar to the configuration frequently used to determine the position of a particle in an optical tweezer, is used to determine droplet position with respect to the confocal detection volume. Our tracking system, inspired in part by the work in Ref. (1), utilizes a dedicated microprocessor to implement a feedback loop between the PSD and a fast piezoelectric flexure stage. Motion of the stage keeps the particle in the confocal detection volume. Here we present data characterizing the system and also report on progress in using tracked droplets in single-molecule studies of RNA.

1. Desai, K. V., T. G. Bishop, L. Vicci, E. T. O'Brien, R. M. Taylor, and R. Superfine. 2008. Agnostic particle tracking for three-dimensional motion of cellular granules and membrane-tethered bead dynamics. Biophys. J. 94:2374–2384.

**831-Pos Board B631**

**Horizontal Magnetic Tweezers for Micromanipulation of Single DNA Protein Complexes**

**Christopher P. McAndrew**, Joseph Zischkau, Christopher Tyson,
Benjamin Buckeye, Helen DeCelles-Zwerneman, Jonathan Luke,
Patrick Mehl, Abhijit Sarkar.

We have built a novel magnetic force transducer or ''tweezer'' that can apply a wide range of pico-newton scale forces on single DNA molecules in the horizontal plane. As the pulling force is applied, the changes in DNA's end-to-end extension occur in the focus plane, eliminating the need to autofocus the objective. The resulting low-noise force extension data enables very high-resolution spatial tracking of changes to the DNA tether's extension. These data are acquired by analyzing images from DNA pulling experiments using particle tracking software that we have also developed. The DNA constructs - λ-DNA end labeled with a 3μmpolystyrene bead and a 2.8μm paramagnetic sphere- and appropriate buffer are introduced into a 400μL to 650μL closed cell. This closed cell, created using two #1 or thinner cover-slips and 1mm glass spacers, isolates our sample and limits thermal currents and evaporative losses of our buffer. Initial experiments have shown the ability to easily and repeatedly find, capture, and manipulate single molecules of end labeled DNA.