track/substrate: we have combined single molecule nanometer-precision fluorescence imaging and trapping (FIAT). The performance of this apparatus was tested on a DNA molecule labeled with a single quantum dot and assembled within a microfluidic laminar flow cell between double optically trapped microspheres, in a suspended ''dumbbell'' configuration. In general this assay allows the control of the mechanical conditions of a biological track (actin, microtubules, nucleic acids), while simultaneously monitoring, by fluorescence, translocation (and, possibly, biochemical state) of a molecular motor on the track, without any requirement of surface immobilization. Accordingly, we are now interested on the application of these techniques in the study of transcriptional and translational apparatus, still not fully elucidated at the single-molecule level.

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Measurement Of The Non-conservative Force Generated By Optical Tweezers

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Optical tweezers have been widely used by biophysicists to measure forces in molecular processes on the single molecule level, such as the force generated by a motor molecular or the force required to unfold RNA. In these and similar force measurements, the usual assumption is that the force applied to a particle inside the tweezers is proportional to the displacement of the particle away from the trapping center, which would imply that the force field is conservative. However, the Gaussian beam model has indicated that the force field generated by optical tweezers is actually non-conservative, yet no experiments have measured or accounted for this effect. We introduce an experimental method that can measure the force field in optical tweezers with high precision without any assumptions about the functional form of the force field. The force field is determined by analyzing the Brownian motion of a trapped particle. We successfully measure the 3D force field with 10 nm resolution for a particle in the Rayleigh regime. The results can be well-approximated with the Gaussian beam model for small displacements, and the non-conservative effect becomes more prominent as the trapped particle is pulled farther away from the trapping center. The energy put into the system along different paths can be directly calculated using the force field. The assumption that Hooke's law applies to optical tweezers neglects the non-conservative component of the force field and can introduce a systematic error when measuring the force.

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An Optical Torque Wrench For Studying Kinesin Dynamics

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We constructed an optical tweezers instrument capable of exerting torque and measuring the angular motions of small trapped particles, based on the rotation of linear polarization of the trapping laser beam. To change polarization, we employed an electro-optic modulator (EOM), which allows for a much simpler setup than a previous design (La Porta, A. and Wang, M.D. 2004. Optical torque wrench: angular trapping, rotation, and torque detection of quartz microparticles. *Phys. Rev. Lett.* 19:190801). Torque is monitored by measuring the difference between circularly left-handed and right-handed components of the transmitted beam: constant torque is implemented by feeding this angular signal back into a custom-designed electronic servo loop. The limited dynamic range of the EOM ($\pm 180^\circ$) is extended by monitoring the drive signal with a microcontroller, which triggers a switch to flip the output polarization by $\pm 180^\circ$ once a pre-set threshold is reached (within 10 μs). These features enable us to maintain constant torque over unlimited rotations at high bandwidth (~100 kHz). In addition, we developed optically birefringent, non-spherical particles suitable for this instrument using nanofabrication techniques. The polarization-sensitive method employed by the apparatus precludes the use of Wollaston prisms to perform differential interference contrast (DIC) imaging. However, by exploiting conventional video-enhancement techniques (including background subtraction, contrast enhancement, and frame averaging), we report that individual microtubules (~25 nm in diameter) can be visualized without DIC optics at ~5 frames per second. Altogether, our instrument allows for the simultaneous application of force and torque to the study of macromolecules of interest. We are presently extending our previous studies (Gutierrez-Medina, B., et al. 2339-Pos. Torsional properties of kinesin. *Biophys. J.* 2008. 94:2339-Pos) on the torsional properties of the molecular motor kinesin to investigate the effect of torque on its stepwise motion.

1470-Pos Board B314

A Novel Method For Investigating The Azimuthal Rotation Of Molecular Motors Utilizing Dielectrophoresis And Optical Tweezers

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Molecular motors are studied *in vitro* to understand their Biophysics and Cell Biology. Most mechanical studies of myosin and other molecular motors have utilized surface-immobilized motors or filaments, which impact their range of motion. One way of avoiding surface immobilization of the filament and motor is to suspend filaments from fixed supports, giving the motor or the motor-coated cargo unimpeded freedom of motion about its track (Ali et al., *Nat Struct Biol.* 9:464, 2002). We used dielectrophoresis at 4-12 V, 2 MHz to stretch and suspend actin filaments across a 2 x 7 um^2 trench etched between two gold electrodes patterned on a glass slide. Optical tweezers were used to bring a myosin-coated bead into close proximity to a pre-selected, suspended actin filament, facilitating bead attachment to the filament in motility buffer. Using defocused images, the bead's three-dimensional position was tracked as a function of time to obtain its trajectory on the actin. Experiments were carried out with myosin V and myosin X. Both motor proteins followed left-handed helical paths with 1.5 - 2 um pitch. Variants of this technique will enable types of higher complexity found in cells to be addressed with *in vitro* experiments. We thank Drs. Mitsuo Ikebe and Osamu Sato for the gift of myosin X and the Nano/Bio Interface Center (NSF NSEC DMR-0425780) and the NIH (grant AR26846) for support.

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A High-Resolution Magnetic Tweezer for the Single-Molecule Study of DNA-Protein Interactions

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The magnetic tweezer is a powerful, simple tool to stretch single DNA molecules that tether a magnetic bead to a glass surface. The response of a tethered DNA molecule to interactions with proteins is measured by optical tracking of the bead. Traditionally, bead position is measured by analyzing the bead's diffraction pattern when viewed in a transmitted-light geometry. This approach is straightforward to use, and gives an intrinsic resolution (i.e. ignoring the bead's thermal fluctuations) in measured DNA length of ~ 2nm at 60Hz [1]. This resolution is acceptable when the bead's thermal fluctuations are large, but it disallows measurement of sub-nanometer DNA length changes in the high-force regime, where the fluctuations are reduced below the intrinsic resolution. To obtain sub-nanometer resolution in a magnetic tweezer, we have adapted Reflection Interference Contrast Microscopy (RICM), in which objective-side illumination creates an interference pattern between rays reflecting from the glass and bead surfaces: the interference fringes from RICM vary with bead height nearly twenty-fold faster than diffraction fringes. We have improved the intensity and contrast of the RICM interferogram by fabricating thin films on the bead and glass that optimize the optical properties of those surfaces. We have also removed effects of thermal drift from the system by implementing feedback control of the focal position through piezo-driven motion of the objective [2]. Using the RICM-based method, the intrinsic resolution is improved to 0.12nm at 60Hz, and is stable over one hour. We demonstrate the correct calibration of this method using the force-induced unfolding of DNA hairpins, and we present preliminary data on RICM-measured DNA-protein interactions.

1. N. Ribeck & O.A. Saleh, *Rev. Sci. Instrum.* **79**, 094301 (2008).
2. K. Kim & O.A. Saleh, *Appl. Opt.* **47**, 2070 (2008).

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Viscous Drag Torque on a Rotating Nanofabricated Cylinder Near an Infinite Plane Boundary

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Microrheological and single molecule biological measurements often involve the use of a microscopic probe particle near a surface. On such systems a precise understanding of the hydrodynamic interactions between the particle and the surface is required. Recently nanofabricated nearly cylindrical quartz particles have been used as ideal trapping particles for an angular optical trap. Here the rotational viscous drag torque imposed on a nanofabricated quartz cylinder near a surface is measured with the angular optical trap. The deviation of torque from the Stokes drag relation in solution is measured as a function of distance from the plane surface of a microscope cover glass. The surface effect is found to be significant for distances on the order of the characteristic dimension of the cylinder. These findings will allow for more accurate and quantitative torsional measurements of angular trapping systems.