JOINT MEETING 2009

A forum for applications of optical tweezers and related technologies in life sciences

OPTICAL TWEEZERS IN LIFE SCIENCES

A forum for applications in scanning probe microscopy and related technologies in life sciences

SPM IN LIFE SCIENCES

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8th International Symposium on
SPM in Life Sciences
A forum for applications in scanning probe microscopy
and related technologies in life sciences

2nd International Symposium on
Optical Tweezers in Life Sciences
A forum for applications of optical tweezers
and related technologies in life sciences

Location
Umspannwerk Ost
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10243 Berlin, Germany

About NanoBioVIEWS™
NanoBioVIEWS™ is the name of a new series of international meetings initiated by JPK Instruments to
further the scientific knowledge exchange on instrumentation and applications of nanotechnology in
the life sciences.

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Program
09:00 am  Registration
10:00 am  Opening
10:10 am  A Workbench for Functional Macromolecules and Their Complexes
          Prof. Jürgen P. Rabe  –  Humboldt University at Berlin
10:40 am  Exploring the Nano-Mechanical Structure of Viral Capsids Using Force Spectroscopy
          Dr. Gijs J. L. Wuite  –  VU University Amsterdam
11:10 am  Coffee Break
11:40 am  What You Can Learn From Force Spectroscopy on a Tetrameric Ion Channel
          Patrick Bosshart  –  Basel University
12:10 pm  Cell Mechanics From DNA Conformational Dynamics to Lipid Membrane Mechanical Properties
          Dr. Sonia Antoranz-Contera  –  Oxford University
12:40 pm  Understanding Cantilever Dynamics in Amplitude Modulation AFM: A Path to High Resolution Imaging and Compositional Mapping of Complex Biological Systems
          Dr. Neil H. Thomson  –  University of Leeds
01:10 pm  Lunch Break
02:30 pm  Force Probing the Molecular Mechanics of Cell Rounding
          Prof. Daniel J. Müller  –  TU Dresden
03:10 pm  Molecular Recognition and Adhesion in Innate Immunity
          Prof. Deborah Leckband  –  University of Illinois
03:40 pm  Coffee Break
04:20 pm  The Combination of AFM With Advanced Light Microscopy – From Liquids to Cells
          Dr. Chris Wright  –  Swansea University
04:50 pm  Integrated Use of AFM in Cell Biology and Biophysics: Probing Matrix Elasticity for Stem Cells and Protein Structure for Mechanism
          Prof Dennis E. Discher  –  University of Pennsylvania
05:20 pm  Poster Session
07:30 pm  End
08:00 pm  Conference Dinner at Umspannwerk Ost Theatre & SPM Poster Award
How to Get an AFM Tip into a Living Cell?
Prof. J. K. Hörber – University of Bristol

Confined Brownian Motion Studied by Optical Trapping Interferometry
Dr. Sylvia Jeney – EPFL Lausanne

Coffee Break and Poster Session

Controlling DNA in Nanopore Using Optical Tweezers
Dr. Ulrich F. Keyser – University of Cambridge

Non-Processive Molecular Motors on a Leash: a Novel Single-Molecule, Microsecond Resolution Force Clamp
Dr. Marco Capitanio – LENS, Florence

Probing Single Biosystem Dynamics using Optical Tweezers and Spectroscopy
Dr. Satish Rao – ICFO Barcelona

Lunch Break

Tracking the Idiosyncratic Steps of Kinesin Family Members With Optical Tweezers
Prof. Christoph F. Schmidt – University of Göttingen

Novel Light Field Shaping for Optical Manipulation
Prof. Kishan Dholakia – University of St. Andrews

Coffee Break and Poster Session

Controlling Biological Motors in Living Cells
Prof. Berenike Maier – University of Münster

Unravelling the Organization of Chromatin
Dr. Remus Th. Dame – Leiden University

Optical Tweezers Poster Award and Closing

End
Talks
10:10 am – A Workbench for Functional Macromolecules and Their Complexes

N. Severin, H. Liang, W. Zhuang, J. Barner, J.P. Rabe
Institut für Physik der Humboldt-Universität zu Berlin
www.polymerphysics.de

Supramolecular structures of single functional macromolecules are a key to understand function in natural molecular systems, as well as to develop novel artificial functional systems. In order to investigate and control the conformations of single macromolecules and their complexes a "Molecular Workbench" [1] has been developed, consisting of the basal plane of highly oriented pyrolytic graphite (HOPG), coated with a layer of flat lying amphiphiles that controls the interaction between the substrate and adsorbed macromolecules.

A scanning force microscope operated in tapping or contact modes is used to both image and manipulate the macromolecules, in order to correlate their structure with mechanical properties, and to assemble macromolecular systems that would not form spontaneously. We report on recent progress on structure formation and mechanical properties, particularly on self-sorting of metallo-supramolecular coordination polyelectrolyte complexes on surfaces [2], self-folding and synthesis with of single dendronized polymers [3,4], supramolecular polymers of oligopeptide functionalized polydiacetylenes [5,6], multiblock copolymers of DNA and polyethylene glycol [7], and self-stretching and time dependent rupture of single ds-DNA on the molecular workbench.

10:40 am — Exploring the Nano-Mechanical Structure of Viral Capsids Using Force Spectroscopy

Gijs J.L. Wuite
VU University Amsterdam, NL

Protective shells of viruses are regular, self-assembled, nanometer-sized containers which are minimalistic in design, but combine passive and active functions. Bacteriophage capsids are capable of carrying a highly compacted genome under considerable pressure. We used Atomic Force Microscopy (AFM) to image and probe the mechanical properties of various capsids. We found that bacteriophage capsids are tough like hard plastic, while displaying surprising elasticity. In contrast the capsid of the plant virus CCMV is very soft. Interestingly this capsid doesn't store its genome under pressure. We also observe that the elastic response to local indentation is linear over a large force range. This response, however, varies across the surface and is correlated to the shell protein organization. As a result we can resolve the hexameric and pentameric protein organisation on the shell surface. Repeated pushing on viral shells cracks this structure, weakening, but not necessarily changing its elastic response. Characterization of the breakage lines gives information about the arrangement and the local interactions of the protein subunits in the shell.
11:40 am – What You Can Learn From Force Spectroscopy on a Tetrameric Ion Channel

Patrick Bosshart\textsuperscript{1,2,3}, Simon Kemmerling\textsuperscript{1,2}, Patrick Frederix\textsuperscript{1,2} and Andreas Engel\textsuperscript{1,2,4}

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Tetrameric potassium channels are highly specific nanopores, which mediate the flux of potassium ions across the hydrophobic core of lipid bilayers. Here we present the mechanical characterization by single molecule force spectroscopy (SMFS) \cite{1} of the cyclic nucleotide modulated potassium channel from \textit{Mesorhizobium loti}, mlCNG \cite{2-4}. The monomer of this protein consists of six transmembrane helices (S1-S6), two of which contribute to the channel pore (S5 and S6), and a C-terminal cytosolic domain that binds cyclic nucleotides, e.g. cAMP. Atomic force microscopy (AFM) and transmission electron microscopy (TEM) studies revealed that the proteins are arranged in an upside-down orientation when reconstituted into an artificial lipid bilayer. This is the preferential case for mechanical unfolding from the terminus of membrane proteins having an even number of membrane spanning elements. Furthermore, TEM analysis on two-dimensional crystals and single particles of mlCNG \cite{3} showed the four-fold symmetry of the channel, which agrees with the published X-ray structure of the transmembrane part \cite{4}.

A semi-automated SMFS procedure \cite{5} was used to unfold the reconstituted channel from its C-terminal end. The unfolding of several channel constructs allowed locating the unfolding barriers in the protein. SMFS data revealed that many molecular interactions in the pore region (S5, pore loop, S6) are sequentially broken. This indicates that various unfolding barriers stabilize the three-dimensional arrangement of this protein region, which is an essential part of an ion channel. Consecutive unfolding of two mlCNG monomers from the same tetramer resulted in a significant change in the observed force peaks in the pore region, which was ascribed to the loss of local interaction.
To investigate this observation in more detail two identical monomers were concatenated to form a tetramer made of a dimer of these concatemers. This allowed pulling two subunits out of the same tetramer in a single step. Such experiments have shown that the unfolding of the first monomer mainly affects mechanical properties of the pore region of the second, while having minor effects on the remaining part of the protein (S1-S4). The molecular assembly of the concatenated monomers within the tetramer (“dimer of a dimer”) was assessed by cystein scanning mutagenesis. These experiments have shown that the loops that connect the subunits do not cross, which is a prerequisite for successful SMFS experiments. Furthermore, single particle analysis of detergent solubilized proteins was used demonstrate that the concatenated construct had the similar dimensions than the native mlCNG.

12:10 pm – Cell Mechanics From DNA Conformational Dynamics to Lipid Membrane Mechanical Properties

Sonia Contera

Biological Physics CONTERA LAB
Oxford University, UK

We use AFM imaging, force spectroscopy, high-speed AFM and mechanical tests to characterize and quantify different physical aspects of the cell. From the role of topology and mechanics in the functional dynamics of DNA (in collaboration with Dr Sonia Trigueros) to local and global phenomena in membranes. We investigate the role of mechanical properties and electrostatics of membranes in saline solutions at different levels of complexity: from the local interactions of individual membrane protein alpha-helices to global membrane mechanical and dynamic properties. We aim at producing a comprehensive physical picture of the cell membrane where individual membrane protein activity is linked to global membrane functions through clustering and cooperativity.
Amplitude modulation AFM (AM AFM) has long been used as the method of choice for imaging biological samples. This is due to two main reasons: firstly, elimination of damaging shearing interactions between the AFM tip and the surface, and secondly, the requirement to image in ambient conditions or liquid has tended to restrict cantilevers to those with relatively low quality factors cantilevers, as compared to UHV operation where frequency modulation (FM) is usually used. Since the amplitude response of the cantilever to changing interactions between the tip and sample is slow, the cantilever is rarely in a steady-state either during imaging or during approach/retract curves. This means that to understand image formation and extract the maximum amount of information from AM AFM images, requires an in depth insight into cantilever dynamics for given situations, i.e. regions of AFM operational parameter space and/or different environmental conditions.

This presentation will focus on two application areas as exemplars of how understanding the cantilever dynamics can dramatically improve the use of AM AFM:

1) Imaging of single molecule DNA samples in air
2) Higher harmonic imaging of complex surfaces in aqueous liquids.

1) Single molecule DNA imaged in ambient conditions

A decade or more of theory and modeling of cantilever dynamics has now yielded a fundamental basis for understanding image contrast formation in AM AFM in air [1]. Despite these efforts, experimental studies are few and far between and the connection between experiment and theory has not often been made. It is important to emphasize that the models may over-simplify the complexity of the experiments but they largely reproduce the general behaviour. I will present recent work using double-stranded DNA on mica as a test system to show the connection between modeling and experiments [2].

This work highlights the effect on resolution of the cantilever oscillating in the High or Low amplitude states (Figure 1). Relationships between amplitude states, phase-shift, force regimes and tip-sample contact times will be discussed (Figure 2). Counter-intuitive results, such as the emergence of noise in certain regions of parameter space can be rationalized through the stochastic nature of the cantilever bi-stability. Understanding of the occurrence of bi-stability
in AM AFM and control of the cantilever dynamics leads to higher resolution imaging on a consistent basis including the helical pitch of DNA.

Figure 1: Imaging of double-stranded DNA using AM AFM in air. When imaging in the Low amplitude state (a), the half-width of the DNA is 14.7 nm and in the High state (b) it is 5.9nm, in this particular case.

Figure 2: Numerical simulations of tip-sample distance relationships for a) amplitude, b) phase, c) average force and d) normalized tip-sample contact time per cycle obtained at resonance for three values of cantilever free amplitude. The cantilever parameters are f0=300 kHz, Q=500, k=40N/m and the sample parameters are those typical of an intermediately stiff sample (E=1.5GPa).

2) Higher harmonic imaging in aqueous liquids
Recent developments in AM AFM include multi-frequency methods where higher eigenmodes or higher harmonics of the cantilever are excited, either through transient interaction with the sample during every cycle of intermittent contact or by direct external excitation. In liquids, higher harmonics of the driving frequency are transiently excited in the AFM cantilever, where the quality factor is typically close to unity. Higher harmonic imaging under liquids has the potential to reveal information about surface properties more directly than the fundamental amplitude and phase signals, while topographical coupling appears to be reduced. To date, very few studies have been carried out on biological samples while the tip-sample interactions affecting excitation of these higher frequencies could be better understood.
Here we used collagen fibrils physisorbed on silicon and bacteria (Staphylococcus aureus, NCTC 8532) trapped individually in polycarbonate track-etched filter membranes [3] as test systems to elucidate factors that influence the second harmonic signal [Figure 3]. Higher harmonic excitation was reduced as the AFM tip moved from the hard background supports onto the softer biological structures. Sufficient signal-to-noise was available in the second harmonic to produce images of the bacterial cell surfaces and collagen fibrils. There was a broad correlation between second harmonic amplitude and the elasticity of the surfaces, and there appeared to be more detailed contrast on the biological structures. Analysis of correlations between signals indicated that the second harmonic signal is not strongly correlated with topography (Z-piezo) [4]. Comparison of the second harmonic signal on collagen fibrils with mechanical measurements, taken using force-volume imaging, indicated that there is a negative correlation between the plastic work done on the fibrils during indentation in contact mode and the second harmonic signal in amplitude modulation. This suggests that work done in deforming the sample is one of the mechanisms by which energy is dissipated from the AFM tip to produce contrast in a higher harmonic images.

References

Figure 3: Individual Staph. aureus bacterium trapped in an specifically etched filter membrane. 3D-rendering of the second harmonic amplitude (false colours) painted onto the Z-piezo signal. Image size: 1.4 x 1.4 μm.
2:30 pm — Force Probing the Molecular Mechanics of Cell Rounding

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During mitosis tissue culture cells undergo a dramatic shape change, from essentially flat to nearly spherical. The forces and mechanisms that drive this shape change remain unexplained. Here we use assays based on atomic force microscopy to measure the height and rounding force of single mitotic cells. We show that under our conditions, human cells exert forces approaching 100 nN when they round up. The force depends not only on the actomyosin cortex but also on trans-membrane ion gradients. In further experiments we demonstrate which membrane proteins are coupled to and regulated by the actomyosin cortex to establish a hydrostatic pressure that rounds up the cell. By using single-molecule force spectroscopy we look inside these individual membrane proteins to quantify interactions and mechanisms they are functionally regulated. Based on these results we introduce an advanced model of cell rounding in which a hydrostatic outward pressure, and contractile actomyosin cortex forces govern shape.
3:10 pm — Molecular Recognition and Adhesion in Innate Immunity

Deborah E. Leckband

University of Illinois, USA

Innate immunity is the first line of defense against pathogen infection prior to the slower response of the adaptive immune system. DC-SIGN is a member of a class of C-type lectins that recognizes high mannose sugars on pathogen surfaces. It mediates cell-pathogen adhesion and subsequent pathogen internalization and processing for antigen presentation. It is also the primary target for HIV binding to and subsequent internalization by oligodendrocytes in the immune system. A structurally related protein, DC-SIGNR is also a target of viral infections, and structural variants correlate with different viral infection rates. These investigations used molecular force measurements to establish how protein architecture and ligand presentation contributes to high avidity pathogen binding by dendritic cells in the immune system.

Surface force apparatus and surface plasmon resonance measurements were used to investigate how ligand presentation and protein architecture affects the capacity for DC-SIGN (and DC-SIGNR) to recognize glycan structures on cell surfaces. Surface force apparatus measurements directly quantified both the range and magnitude of the forces between membrane-bound, oriented DC-SIGN (or DC-SIGNR) and glycan presenting bilayer membranes. The distance resolution achieved in surface force apparatus revealed that ligand docking induces a conformational change that enhances pathogen adhesion. Measurements also demonstrated that adhesion depends on both the distribution of carbohydrate ligands on the target surface and the ligand mobility. Finally, we determined how naturally occurring variations in the length of the DC-SIGNR neck region impacts recognition. These molecular level measurements thus defined key mechanisms governing pathogen recognition in innate immunity and established design rules for therapeutic agents to block viral infectivity.

4:20 pm — The Combination of AFM With Advanced Light Microscopy – From Liquids to Cells

Dr. Chris Wright
Swansea University, UK

n/a
4:50 – Integrated Use of AFM in Cell Biology and Biophysics: Probing Matrix Elasticity for Stem Cells and Protein Structure for Mechanism

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Cells generate force and are also exposed to external forces, but whether molecular rearrangements occur or not has not been clear. Do proteins within cells unfold under force and change in tertiary structure, and/or do the proteins dissociate from each other with changes in quaternary structure due to stress? We have developed a suite of nano-mechanical and chemical approaches to address these questions. Coupled AFM nano-mechano-chemical schemes with purified proteins [1] establish a general methodology for cell studies, and the strong temperature dependence of biomolecular transitions must be appreciated – not only for the special challenges it presents at the single molecule scale [2]. The nanomechanical probing has been further used to characterize the compliance of substrates that cells adhere to and apply stresses to (in proportion to substrate compliance) [3], and this fact together with the entire set of experimental/computational methods and ideas can be extended to help identify – within living cells – proteins and their sites that indeed unfold and dissociate under stress [4].

References

Figure 1: Force-induced changes in protein structure are hypothesized to expose novel binding sites for ligands. This example of a molecular dynamics simulation shows that Cysteine1167 in β-spectrin exposes 0 Å² surface area (of 224 Å²) until forced extension exposes Cysteine’s thiol group for reaction.
Conventional scanning probe microscopes with their mechanically attached tips have serious geometrical restriction to investigate 3-D structures. These restrictions allow only investigations on reasonably flat and well-oriented surfaces. To investigate complicated topologies, especially of living cells, the tip has to be controlled by other means. One possible alternative is the focus of a laser beam, which can be used to manipulate micro- to nanometer-sized particles in liquids, as Ashkin demonstrated in 1986, the same year the first AFM was built. The trapping potential of a laser focus obeys in first approximation Hooke’s law and, therefore, with an appropriate 3-D detection, the position of the probe with respect to the focus can be used like the bending of a cantilever to measure forces acting on the particle. The force range accessible for such a microscope complements a conventional AFM at the lower force range providing freedom for measurements and manipulations even on internal structures of cells and inside many other optical transparent structures in solutions.

The Photonic Force Microscope (PFM), as we called it, can be used like an AFM for imaging and to do force spectroscopy with glass or latex spheres as tips. The 3-D detection system that we developed is the essential step from just an optical trap to a force microscope. With the nanometer spatial and microsecond time resolution possible, it enables the use of the thermally driven position fluctuations of the sphere to characterize its interaction with the environment. E.g. in the case of a sphere tethered by a single molecule to a surface, the thermal fluctuation measurements can be transformed into 3-D energy profiles using Boltzmann’s equation. Energy profiles and their changes are both accessible with a resolution of one tenth of the thermal energy. From such profiles, force versus extension or stiffness versus extension profiles can be calculated along arbitrary paths to characterize the mechanical properties of the molecular structure. In a similar way, surface potentials for different types of interactions can be mapped in a solutions.

The PFM proofed from the very beginning to be a powerful tool to study at the nano-meter scale lipid vesicles as well as the plasma membrane of intact cells. In the latter case, the diffusion of membrane components and their interactions can be observed over minutes with the instrument’s high spatial and time resolution. PFM measurements on intact cells for the
first time gave protein diffusion coefficients comparable to values reported for artificial lipid 
films. These experiments demonstrated that only on the nanometer scale the properties of the 
lipid component dominate the diffusion within the membrane and proofed for the first time the 
physical existence of membrane rafts. The possibility to characterize also larger membrane 
aggregates and their diffusion opened up new ways to study membrane functions. 
Furthermore, the technique also provides information about membrane elasticity and the 
interactions of membrane components with the cytoskeleton.

The development of a field called nano-optics during recent years and the knowledge acquired 
about the interaction of light with nano-structures provides now the great opportunity for a 
major step in the development of the PFM that will extend the sensing capabilities of the 
particle acting as a tip. Depending on size and shape, metal nano-structures have Plasmon 
resonances at distinct frequencies. With a tunable laser adjusted to this resonance the light 
scattering cross-section increases significantly allowing the detection of 5-50 nm beads with 
our detection system. Furthermore, metal particles can be used as chemical sensors using the 
surface enhanced Raman (SERS) effect, and they can behave like “nano-lenses”. Due to the 
electric field enhancement at their surface fluorophores close by are excited with a much 
enhanced but quickly with distance decaying probability. This gives the same advantage of 
very low background fluorescence inside a cell, as total internal reflection fluorescence 
(TIRF) excitation close to a surface, and allows to determine where and when certain 
fluorescent labeled molecules come close to the particle. With pulsed laser sources, also a 
two-photon excitation scheme is possible, having the advantage of a much less damaging 
infrared-light excitation-wavelength. The introduction of such metal nano-sensors as tips in a 
PFM creates a tool for future cell-biology studies on regulation and transport processes within 
living cells providing the information of where and when molecular interactions take place.
The dynamic behavior of a single colloidal particle in water confined by an optical trap and a plain surface is investigated at time scales where the inertia of the surrounding fluid plays a significant role. A weak optical trap with interferometric position detection allows monitoring a single micron-sized sphere with a spatial resolution better than 1 nm and a temporal resolution on the order of microseconds.

First, we quantify the influence of the confinement created by the harmonic potential of the optical trap on the particle's velocity autocorrelation, mean-square displacement and power spectral density. We find that they are in excellent agreement with the theory for a Brownian particle in a harmonic potential that accounts for hydrodynamic memory effects, which states that the transition from ballistic to diffusive motion is delayed to significantly longer times than predicted by the standard Langevin equation. This delay is a consequence of the inertia of the fluid, introducing a backflow on the particle’s fluctuations. At longer times the motion of the particle is dominated by the trapping potential.

By identifying the time below which the particle doesn’t “feel” the potential, we can exclude the existence of free diffusive motion as usually assumed in common optical trapping experiments.

Second, the particle is brought close to a hard surface and we observe how the subtle interplay of surface confinement and hydrodynamic backflow changes the decay of the particle's velocity autocorrelations from a slow $t^{-3/2}$ to a much faster power-law $t^{-5/2}$.

These findings show that the temporal resolution of Optical Trapping Interferometry can be extended down to time scales where the nature of the fluid influences diffusion, bringing the long discussed idea of using a Brownian particle as a local reporter of the dynamics of complex biological fluids one step further.
10:40 am – Controlling DNA in Nanopore Using Optical Tweezers

Ulrich F. Keyser
University of Cambridge, GB

DNA translocation through solid-state nanopores is a relatively new single-molecule technique. It allows for easy and true label-free detection of DNA molecules in solution by simple ionic current measurements.

A control of the translocation velocity would be beneficial for detecting (single) proteins bound to the DNA or for structural investigations. In this talk we will discuss how this can be achieved by combining optical tweezers with solid-state nanopores. We will show that one can control and stop the translocation of DNA in a nanopore and discuss the nature of the forces on the molecule.

This technique enables new insights into processes like DNA electrophoresis. We will also discuss future directions of this technique.
Myosin II is the motor protein that drives muscle contraction through cyclical interactions with an actin filament. In each cycle a single ATP molecule is split and a filament displacement (or working stroke) is generated. The working stroke produced by a single myosin head has been previously measured in isolated myosin molecules, but the effects of the high loads acting on the myosin molecule during muscle contraction could not be investigated. In fact, current single molecule techniques apply force with a delay of few milliseconds after actin-myosin binding, when the working stroke of skeletal muscle myosin has already been completed.

Here, we developed a novel single molecule technique in which a constant force is continuously applied to the actin filament, so that the delay between myosin binding and force application is abolished. This method is capable of resolving the development of the myosin working stroke under different loads with a very high time resolution and detecting events as short as 100 μs due to a very high signal-to-noise ratio.

We found that under loads in the range 1 to 10 pN myosin can follow two distinct pathways in its interaction with actin: a population of very fast events (240 ± 23 μs) in which myosin detaches from actin before producing any movement (the duration of these events does not depend on ATP concentration), and a second population of events where myosin steps and remains bound to actin for a longer time. For low forces (|F| < 2 pN) the lifetime of this second population of events linearly decreases with ATP concentration in the range 5-50 μM. The mean amplitude of the myosin working stroke is found to be smaller at increasing loads and vanishes at the isometric force (5.7 ± 0.6 pN). On the other hand, the rise time of the working stroke becomes longer as the force increases.
11:40 am – Probing Single Biosystem Dynamics Using Optical Tweezers and Spectroscopy

Satish Rao
ICFO, Barcelona, ES

The use of optical tweezers in biology is well established. Due to their intrinsic ability to trap micron sized objects in a liquid suspension, there is a natural application to trapping single cells, such as yeast cells or red blood cells, which are in this size range. Additionally, whole new fields of biophysics have developed where single biomolecules, through attachment to trappable micron sized dielectric handles, can be manipulated and studied. Optical tweezers provides one with the ability to hold a single biosystem, apply and measure forces from picoNewton to femtoNewton scales.

However, it is well known that biosystems, such as cells, DNA, and proteins, function through an interplay between chemical and mechanical properties. The chemical changes of a protein or the internal effects of a cell are often not accessible in a measure of mechanical properties alone. Alternatively, Raman spectroscopy is sensitive to such internal changes, both structural and chemical, but is not an active probing method. The goal of this work is to obtain a complete picture of these mechanochemical relationships by combining the force probing of optical tweezers with Raman spectroscopy.

The Raman tweezers setup utilizes separate beams for the optical trapping and Raman excitation. For the former, a 1064 nm beam is passed through an interferometer with movable mirrors in order to create two independently controlled traps at the sample. Cells or biomolecules are then trapped at each end thus stopping the inherent rotation that would occur in a single trap. A 785 nm beam is passed through the center of the object and the backscattered light is collected to a spectrometer for the Raman scattering detection.

In the first experiment, we demonstrate a static method for monitoring the chemical processes inside a cell. We trapped a single yeast cell with the 785 nm beam that also excited the Raman scattering, and monitored the growth of the cell until budding. Distinct Raman bands that are indicative to the presence of proteins and lipids appear whose intensities are tracked over time. The trends of the intensities follow a similar path for the known growth cycle. Thus, the Raman bands of the cell can be used to observe the growth cycle of the cell.

The second experiment demonstrates the dynamic method where mechanical forces are applied to a cell and their response is observed using Raman spectroscopy. In this instance, we trapped a single red blood cell at atmospheric conditions. The cell was stretched by moving one of the 1064 nm beam traps while the backscattered light from the 785 nm beam was collected. A depiction of the configuration is given in figure 1 along with a video camera image of the relaxed and stretched states of the cell.
The main Raman bands that appear are those of the heme groups that are the oxygen carriers of the hemoglobin protein. Oxygenated and deoxygenated hemoglobin have distinct Raman spectra that are well established. A Raman spectrum was recorded for the cell in a relaxed state and then another while the cell was held in an extended or stretched state. The stretched state was about 40% bigger than the normal size of the cell which corresponded to a range of about 10 pN of force. The results show that when a red blood cell had high oxygen content in the relaxed state, indicated through its Raman spectrum, the hemoglobin experienced a transition to a deoxygenated state when the cell was stretched. This was determined by observing Raman spectra that agreed with previous studies of artificially oxygenated and deoxygenated red blood cells. The increased deformation enhances interaction of hemoglobin with the membrane and with neighboring hemoglobin. For the former, more acidifying and O$_2$ / CO$_2$ exchange occur at the membrane thus increasing the probability of oxygen dissociation. For the later, increase contact between hemoglobin proteins constrains the movement of the heme groups which is essential for stabilizing the bound oxygen.

This idea is taken one step further by repeating the previous measurement but collecting the separate polarized components of the Raman scattering; polarization parallel and perpendicular to the excitation beam, in order to obtain depolarization ratios of certain bands. The results show that when the red blood cell is stretched, the hemoglobin proteins become more packed together and seem to orient themselves in an ordered way. This suggests that the deformation of the cell not only affects the oxygenated state of the hemoglobin, but may order the proteins as well, thus possibly playing a role in intracellular oxygen transport.

Finally, we applied this concept to DNA molecules. Due to their low scattering cross section, we utilize surface enhanced Raman scattering (SERS) in order obtain measurable Raman spectra of DNA. Silver colloids, 70 nm in diameter, are passively attached to a DNA molecule with micron sized dielectric beads at each end. The beads are trapped and the DNA is moved until the characteristic Raman signal is observed, thus indicating that the DNA is in the focus of the excitation beam. The results show Raman bands that agree with previous studies on DNA solutions which demonstrate our ability to detect single DNA molecules using SERS.
We have been using optical tweezers in various geometries to measure the motions of kinesin motor proteins from different motor families in single-molecule assays. Dimeric Kinesin 1 motors are processive and can be well tracked with “single-bead assays” which involve trapping a micron-sized bead with one individual motor protein attached to it. One can deduce step size, motor speed and stall forces from these assays. Kinesin 5 motors are tetrameric with motor heads on both ends of a stalk. The one we studied, Eg5, is also processive, but shows intriguing regulation features. The C-terminal Kinesin 14 motors have the opposite directionality from all other kinesins and are not processive. For these motors we have set up a “three-bead assay”, suspending a microtubule with two optical traps over a third bead carrying the motor. In this way one can observe individual power strokes of the motor.
3:10 pm – Novel Light Field Shaping for Optical Manipulation

Kishan Dholakia
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Advanced photonics using novel holographic beam shaping has proved to be a powerful and emergent area in biophotonics. Light may be used in various guises. A prime example is optical micromanipulation. This is a powerful non-contact technique where micrometre sized particles can be grabbed, moved and generally manipulated solely with light. Optical tweezers is the most popular way to implement these forces using a single tightly focused light beam. They have forged an important bridge between physics, chemistry and biology. In recent years there has been a proliferation of activity in this area, fuelled, in part, by the recognition that we need to advance the “optical toolkit”. This essentially means creating more elaborate 2D and 3D light patterns (beam shaping) that can create an optical landscape. Particle and cellular motion on such a landscape will enhancing our ability to move and sort particles and importantly, create 2D and 3D arrays of particles [1].

Advanced beam shaping and optimal focusing may also be considered useful for the topic of cell transfection combined with optical tweezers. Here we consider the cell membrane which represents the outer extremity of all eukaryotic cells. In mammals, this is a thin (5nm) bi-layer film of lipids, embedded with various protein molecules at interspersed locations. Under normal circumstances, the lipid nature of the cell membrane acts as an impermeable barrier to the passage of most water-soluble molecules. Thus, the selective introduction of therapeutic agents to the inside of dysfunctional or diseased cells remains problematic. Methods for puncturing the cell membrane without causing any collateral damage have been devised and importantly, this includes laser-assisted techniques particularly using multi-photon processes. Bessel modes can be used for “focus-free” photoporation (see fig.1) and offer new opportunities for the field [2]. More advanced fields such as Airy modes are also of interest [3] This talk will cover both optical trapping, manipulation and cell transfection of gold nanoparticles using advanced beam shaping. Importantly we will discuss how to obtain optimal Bessel modes with uniform intensity and in situ wavefront correction for optical beam focusing using spatial light modulation.

Figure 1. (a) The Bessel beam “focus” is positioned on the cell plane. (b) Upon successful transfection, the cells express the red fluorescent protein and fluoresce red (adapted from reference 2)

Biological molecular motors are the basic elements that generate directed movement in living cells. Nanotechnological tools enable the characterization of the physical output of individual molecular motors such as force generation, energy transduction, and directional switching. However, the application of these tools to characterize the physical output and the regulation of molecular motors in the context of their natural environment has been limited.

Our research group is interested in regulation and function of force generation by type IV pili and in the related DNA import machine \textit{in vivo}. Type IV pili are major bacterial virulence factors supporting adhesion, surface motility and gene transfer. The polymeric pilus fiber is a highly dynamic molecular machine that switches between elongation and retraction.

We used laser tweezers to investigate the dynamics and force generation of individual pili of \textit{Neisseria gonorrhoeae}. In particular, we have shown that the type IV pilus motor can switch between different modes in both speed and direction and that force tunes the probability of assuming the individual states. High force generation exceeding 100pN was a conserved property of pilus systems. Even during infection of epithelial host cells, high forces were detected. The type IV pilus is therefore an ideal model system for understanding generation of high molecular forces and their regulation \textit{in vivo}. 
The bacterial genome is folded and compacted into a body referred to as the nucleoid due to the activity of nucleoid-associated proteins (NAP’s). These proteins generally bind to DNA with low sequence specificity and/or complex binding mechanisms. These properties make it hard to address their architectural effects on DNA using classical biochemical bulk approaches. Single-molecule visualization and micromanipulation techniques such as atomic force microscopy and optical tweezers, however, permit us to study these proteins in great detail (when bound at non-specific sites) and to determine their modes of binding, kinetics and structural stability. This in vitro knowledge and information from in vivo experiments combines into a detailed picture of the overall organization of the nucleoid.
Poster Session
P1 – Tracing Carbon Nanotube Uptake and Transport Inside Living Cells With Combined Fluorescence and Atomic Force Microscopy

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Carbon nanotubes (CNTs) are considered to be promising for biomedical applications as their nano-size gives them access into various cellular compartments including the nucleus. They could be used for biosensing or act as multi-functional carrier systems for therapy and diagnosis at the cellular level. Functionalization of CNTs with biomolecules facilitates internalization into the cell. However, the exact uptake mechanism remains a controversial issue as it may well depend on cell type, bio-functionalization scheme, size of the nanotube and other factors. Cellular uptake of CNTs is commonly addressed with fluorescence microscopy but a direct and label free detection of CNT uptake is challenging. At present, atomic force microscopy (AFM) offers a unique solution to study biological specimens under (near-) physiological conditions without the need for rigorous sample preparation or labelling. We expect significant bio-physical insights into the delivery process and transport mechanism of CNTs in cells by employing the AFM in combination with fluorescence microscopy to study the internalization of differently bio-functionalized CNTs into living cells.

Here we present AFM as a useful tool for a simple and direct assessment of CNT surface bio-functionalization [1]. We further show high-resolution topographic AFM images of functionalized single walled carbon nanotubes (SWNT) and double walled carbon nanotubes (DWNT) immobilized on various relevant biological membranes, including nuclear membranes and cell surfaces. [2] Present experiments combine fluorescence and AFM imaging as well as simultaneous topographical and recognition (TREC) imaging to localize binding of functionalized carbon nanotubes and nanoparticles on different substrates. These preliminary studies will facilitate the investigation of the binding of CNTs to the cell and their possible internalization in a time resolved manner. We also show first results of studies addressing cellular uptake of fluorescently labelled CNTs, where we were able to trace individual carbon nanotubes internalized into living cells.
A) Biotin-functionalized CNT on a dense layer of avidin.
B) RNA-coated CNTs on nuclear membrane. Dashed circle: individual nuclear pore complex.
C) RNA coated MWNT on cell membrane. (zoom-in)


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The integration of carbon nanotubes (CNTs) with biological species has brought advantages for the development of specific (bio-)chemical sensors with enhanced properties and high performance. In this study, we describe the incorporation of single-walled carbon nanotubes (SWNTs) by using the layer-by-layer (LbL) technique on an electrolyte-insulator-semiconductor (EIS) field-effect sensor. The influence of the LbL film morphology on the sensor signal as well as its feasibility of applying as penicillin biosensor is presented.

Field-effect sensors have been one of the most attractive approaches for the development of (bio-)chemical sensors [1]. CNTs are a potential material for issues with respect to biosensing, due to their electronic properties in conjunction with their size and high surface area [1]. The assembly of CNTs for a sensing device in the form of an ultrathin film can be produced with the LbL technique that offers fine control over film thickness and architecture. The nanostructured film was prepared by alternating layers of SWNTs with layers of the polyamidoamine (PAMAM) dendrimer. The LbL film structure was analyzed by means of atomic force microscopy (AFM) for different amount of bilayers. With increasing quantity of bilayers the formation of more uniform and densely packed films was observed which was confirmed by the film’s roughness and thickness [2].

To investigate potential benefits of the LbL films for biosensing applications, the enzyme penicillinase was adsorptively immobilized atop of the modified sensors. This incorporation of penicillinase yielded to sensors with high sensitivity to penicillin G. As an effect of the films porosity the H\textsuperscript{+}-ions resulting from the enzymatic reaction could easily penetrate through the film which led to an increased sensitivity and stability. At the same time, the presence of the LbL film caused a much more efficient adsorption and stable attachment of penicillinase on the sensor surface in contrast to an unmodified sensor surface.

References

Acknowledgements
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P3 – Changing of Cellular Mechanics During Aging

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Aging is closely correlated with drastic changes on the cellular level. Many details about aging from the molecular side of view are known, for example about changes in gene expression or the reduction of telomeres protecting DNA. How the cytoskeleton changes its structure and function and how aging alters the mechanical properties of the cell is still under investigation.

For our experiments fibroblasts from human donors differing in age were chosen. Their age lies in the range between 10 and 67 years. Initial experiments demonstrated an age-specific difference in mechano-sensitivity if cyclic stretched. Senescent cells orient faster perpendicular towards the stretching direction than cells from young donors. To correlate that age-specific difference in mechano-sensitivity to mechanical cell properties the Young-modulus of the cells was measured using atomic force microscopy (AFM). Large cell areas (100x100µm\textsuperscript{2}) were mapped with 5x5µm per pixel resolution to get an insight of the local cell properties. At each point a force-distant curve was acquired and the Young-modulus was calculated via Hertz fit.

A significant difference for the Young-Modulus values of young and senescent cells was found. To affirm the data given an actin assay was performed via G-actin / F-actin assay kit (Cytoskeleton). Here the total amounts of G- and F-actin were determined, indicating that senescent cells have a decreased total amount of actin. The ratio G/F actin seems to be independently from age. Further experiments were then performed with siRNA transfected cells. The siRNA was used to decrease the amount of actin aiming for the value for senescent cells. Using this method it was shown that the elasticity of the transfected cells lays in the same range as the elasticity of senescent cells.

In conclusion, our results suggest that actin expression decreases with aging and thus softening of the cells takes place. This may also explain the faster mechano-response of the older cells since a smaller amount of actin needs to be reorganised.
P4 – Mimicking the Cellular Environment: Effects of Elastic Nanopatterned Substrates on Integrin-Mediated Cellular Interactions

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The design of the extracellular matrix (ECM), e.g. compliance and biochemical functionality, governs a wide range of cellular properties and functions. Its influence on cell behavior such as spreading, migration and adhesion is still not conclusively evaluated by biophysical means. Therefore, an artificial substrate system, according to the biophysical and biochemical properties of the extracellular matrix in connective tissues, has been developed. The Young’s moduli E₃ of poly(ethylene glycol)-diacyrlate (PEG-DA) based hydrogel substrates span more than four orders of magnitude (0.6 kPa < E₃ < 6 MPa). Since PEG-DA substrates are protein repellent, they were decorated by quasi hexagonally ordered, extended gold nanoparticle arrays, manufactured by block copolymer micellar nanolithography (BCMN). To provide bioactivity in terms of cell adhesion c(RGDfK) peptide, which is specific for αᵥβ₃ integrins, was immobilized on the nanoparticles. The interparticle spacing and, hence, spacing of integrin binding sites ΔL could be precisely tuned, independently of the substrate rigidity, between 20 nm and 160 nm. This system was used to investigate the behavior of fibroblasts as a function of changes within two-dimensional parameters space (ΔL; E₃). To this end, cell spreading area and cell-substrate interaction forces were determined by phase contrast microscopy and single cell force spectroscopy (SCFS), respectively.

First, the effect of variation of ligand spacing on cellular behavior was investigated on hard substrates (E₃>100 kPa). We could demonstrate a strong increase in detachment force and spreading area on substrates featuring low ligand spacing. Than, substrate compliance was tuned whereas the ligand spacing was kept at approximately 50 nm. This reveals a significant decrease in spreading area and detachment force on soft substrates (E₃<8 kPa).

Additionally, both environmental parameters were varied simultaneously. Results from these experiments were determined as a function of hydrogel stiffness and integrin ligand distance. They revealed two tactile set points, thresholds in cellular sensing behavior, at E₃ ≈ 8 kPa and ΔL ≈ 70 nm, after 6, 12, and 24 hours of adhesion, respectively. Moreover, according to the hierarchical phase model in cellular behavior, elasticity was identified to be the dominant parameter in cellular sensing processes.
**P5 – High Speed AFM Characterization of the Dynamic of Synthetic Proteo-Nucleic Complexes**

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Synthetic proteo-nucleic structures (PDNA) encompassing a ss-DNA sequence covalently attached to a cytochrome b5-derived protein domain through a synthetic linker were designed. PDNAs can bind to natural or supported membranes through an histidine-tag-nickel-NTA modified phospholipids. Alternatively the structures can be attached, while keeping free lateral diffusion capabilities, by interaction of the protein his-tag with a substrate supported field of nickel ions. Floating individual tiles can be in turn self-assembled together in the presence of half-complementary ss-DNA, to form surface constrained chains of protein domains linked by ds-stranded DNA segments.

A combination of experimental (single molecule confocal microscopy, surface plasmon resonance imagery, static atomic force microscopy) and numeric simulation approaches were used to characterize factors controlling self-assembly on the surface. Dynamic of interaction between PDNA assemblies was investigated using high speed AFM in solution and evidenced unusual modes of lateral diffusion of the structures on mica surface and transient formation of linear and cycling complexes involving base pairing between very short DNA stretches. Dynamic AFM thus constitutes a very promising tool for the characterization of the time resolved aspects of the self-assembly of biomimetic structures.
**P6 – Cell Adhesion on Micro-Nanostructured Surfaces**

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**Motivation:**
Many aspects of cellular adhesion to the surrounding, the extracellular matrix (ECM), are of great interest as they control and influence various fundamental cell actions such as motility, differentiation, proliferation and apoptosis and hence, even the function of an organism may depend on this complex and highly regulated process.

A better understanding of the contact formation procedure and the demands to the ECM for a successful adhesion would provide opportunities to externally control cellular response. Research concerning a broad variety of biological processes including embryonic development, assembly of tissues and the nervous system, inflammation and wound healing, tumor metastasis, viral and bacterial infections requires controlled cellular adhesion. To gain more insights into the cellular adhesion process surfaces with defined adhesion properties in terms of bioactivity are important tools.

**Project:**
This work uses gold nanoparticles arranged in a quasi hexagonal pattern in the nanometer regime where each gold dot serves as a single anchor point for the cell’s integrins, the receptors mediating the connection between cell and environment. The distance of the binding sites can precisely be defined and a quantification of available binding sites is possible. Furthermore, a micro structuring of the gold particles allows for varying the global density of these integrin binding sites without changing the spacing between those. Such patterns can be used to investigate whether an effect, in this case the cellular adhesion, is distance or density dependent.

Former experiments have shown that adhesive interparticle distances of more than 73 nm reduce cell spreading as well as cell attachment and almost prevent the formation of focal adhesion and actin stress fibers.

To verify if this finding is due to insufficient available binding sites or only to an existing critical binding site distance which may prevent clustering of adhesive sites, the quality of cell adhesion is investigated on different patterns by optical microscopy and Single Cell Force Spectroscopy (SCFS). The optical techniques are utilized to analyze cell spreading and circularity, both are parameters by which the quality of adhesion can be quantified. SCFS is performed by means of Atomic Force Microscopy. A biofunctionalized tipless cantilever is used for detaching adherent cells from the surface to quantify their adhesion strength.

The present results propose that a successful adhesion process depends mainly on the distance of available anchor points. The overall density, thus the number of available binding sites is significantly less important for strong and stable cellular adhesive clusters than their spacing.
P7 – Interaction of Immunogenic Chlorinated Ovalbumin With Macrophages Receptors Studied by Force Spectroscopy

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Hypochlorite (HOCl/OCl–), a product of activated neutrophils, significantly contributes to protein oxidation which occurs at a site of inflammation. Proteins modified by chlorination change their biological activity such as enzymatic activity, resistance to proteolytic cleavage and immunogenecity. While enhanced immunogenecity of chlorinated ovalbumin (OVA-Cl) was already reported its mechanism is still not clear.

In this study we investigated the influence of chlorination of OVA on its recognition (endocytosis) by macrophages – the potential antigen presenting cells (APC). Using AFM-based Force Spectroscopy the strength of interactions of native and chlorinated OVA with a membrane of macrophages was quantified. Proteins were immobilized on gold-coated AFM tips using aqueous solutions of sulfonate derivative of a thiol and the proteins in 100:1 molar ration. Formation of negatively charged monolayer on the tip dramatically limited its nonspecific interactions with the surface of macrophages. Surface dilution of the proteins ensured the presence of only single or a few macromolecules at the tip surface contact. In such systems so-called adhesion probability (AP) was the measure of the interactions between the proteins and macrophages surface receptors even the measured forces varied significantly for sets of measurements.

The system with native nonimmunogenic OVA showed only negligible AP while application of OVA-Cl gave rise to very high AP (up to 85%), which vary with the contact time of the tip on the macrophage and the loading velocity. Incubation of the cells in OVA-Cl lead to significant reduction of AP that indicates blocking of the receptors by the chlorinated protein. The developed approach based on force spectroscopy measurements was used to study specific interactions between OVA-Cl and macrophages. The method may be widely used for the studies on other ligand-receptor interactions in biological poorly defined systems characterized by large variations of the measured forces.

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P8 – Nanoelectromechanics of Yeast Cells

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Mosbacher et al. [1] shown that applying of oscillating electric field generates mechanical oscillations of the cell membrane. Pelling et al. [2] demonstrated a local nanomechanical motion of the cell wall of yeast Saccharomyces cerevisiae under physiological conditions using AFM. Considering electrically polar cytoskeleton subunits (e.g. heterodimer of tubulin), we suggest this mechanical oscillations could in reverse generate oscillating electric field in the immediate vicinity of the cell.

We partially reproduced the Pelling’s experiment using cold sensitive cells Saccharomyces cerevisiae tub2 – 401 401(strain CUY67 Mata tub2-401 ura3-52 ade2-101 (20)). We have found principal peak of the oscillations at ~ 850 kHz at 25 °C.

We performed primary calculations of proposed oscillations of electric field. An experimental device was also designed to investigate these electrical oscillations; however, it seems to provide only some threshold conditions for observation of such weak electric field. To provide precise measurement, we suggest an AFM cantilever with integrated electrical sensor and preamplifier, which can measure both electrical and mechanical oscillations of the cell’s surface.

Proposed connection between oscillations may reveal a novel aspect of the cell physiology – the cellular nanoelectromechanics.

A prerequisite for an understanding of the mechanisms of nerve regeneration and neuronal development is the understanding of the principles of force generation in growth cones – the mechanical and chemical sensor of a neuron. In our working group we try to approach this issue from a variety of different aspects, combining experimental and theoretical studies of actin networks, membrane fluctuations and focal adhesion sites. Two other aspects which will be presented here are the direct measurement of forces exerted by the cells, and the neuron’s response to mechanical and chemical stimuli. After we succeeded in measuring stall forces and forces of the retrograde actin flow and the cell body with an SFM in fast-moving fish keratocyte cells we want to transpose this method to growth cones. The SFM cantilever is kept at constant height and force while the growth cone is growing against the bead which is glued at the tip, and the lateral deflection is recorded. Because forces and velocities of neurons are very small and the resulting experiment times are long, the SFM setup requires additional stabilization. We want to use a dual-beam optical trap setup to monitor potential drift of sample and cantilever to ensure a stable position of the cantilever, with respect to the sample, over very long times.

Another facet is chemo- and durotaxis of neurons. We use soft materials with locally modifies Young’s modulus, measured with SFM, and local protein coatings to induce highly polarized cell growth. The aim is to learn how such a stimulus is reflected on the molecular level and how this knowledge can be transferred to whole-cell behavior, e.g. cell differentiation, preconceiving that cells are highly adaptable systems. First results of experiments with such materials modified by a new treatment technique are presented here, along with the description of the stabilized SFM setup.

Figure 1 NG 108-15 cell extending on the soft stripes of a polyacryl amide gel (left) although there is no surface protein coating (right, fluorescent stain of laminin)

Figure 2 Schematics of force measurement with dual-beam optical trap stabilizing sample (left bead) and cantilever (right bead)
P10 – Single Molecule Force Measurements Delineate Co-Solute and Interfacial Effects on Biopolymer Adhesion

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The adhesion of polypeptides and proteins onto interfaces is important for a wide variety of systems from the adhesion onto vessels during their production process over functional coatings to protein-membrane interactions in single cells. We use an atomic force microscopy based single molecule method and various biopolymers to determine the adhesion strength [1,2] and the location of polypeptides at interfaces.

We observe a linear dependence of the adhesion force on the concentration of three selected salts and a Hofmeister series both for anions and cations [3]. Surprisingly, the support (solid, liquid or gas) does hardly influence the adhesion in aqueous environment. These findings allow us to propose the location of the biopolymer at the interface and a compensation mechanism between dispersive and hydration forces.

Publications:

Influence of Hofmeister Salts on the adhesion of spider silk proteins onto hydrophobic substrates: an AFM-based single molecule study

Peptide adsorption on a hydrophobic surface results from an interplay of solvation, surface, and intrapeptide forces

Single molecule force measurements delineate salt, pH and surface effects on biopolymer adhesion
A fundamental step in cell migration is the advancement of the cell’s leading edge. It is generally accepted that this motion is driven by actin polymerization against the plasma membrane but this has not been directly measured.

Here we present precise force measurements using a newly established SFM-technique combined with high resolution imaging and lamellipodium feature tracking analysis. Our AFM-based technique uses the vertical and lateral deflection of a modified cantilever and allows direct measurements of the forces exerted by the cell. Interference reflection microscopy allows us to observe the cell during locomotion beyond the cantilever. We measure the maximum forces which are generated at the leading edge of the lamellipodium, retrograde forces within the lamellipodium, and the cell body. Through selective manipulation of molecular components by addition of different drugs, such as Jasplakinolide, Cytochalasin D, and ML-7 the measured forces and velocity changes can be compared. Our studies provide a unique dynamic force map giving the magnitude and direction of the intracellular forces in fish keratocytes and reveal the molecular origin of the forces.

We resolve that the force generating mechanism at the leading edge is indeed actin polymerization, and we directly measured a force attributed to the retrograde flow within the lamella, which critically demonstrates that the protrusion forces are decoupled from the cell body and are generated exclusively at the leading edge.

Fig. 1 SFM-measurement of a migrating cell. The lateral deflection (red) reflects the forces the cell is pushing the cantilever, while moving beyond it (black line: height signal). Sketch of the experiment shown below.
Recent developments in sequencing methods aim towards single-molecule sequencing techniques, but most of those methods require some kind of labeling which might influence the biochemical behavior of the sample. Our approach applies tip-enhanced Raman spectroscopy (TERS), a method that combines a conventional Raman setup with an atomic force microscope (AFM), so that vibrational spectroscopic measurements with a high lateral resolution can be conducted.

For TERS experiments the AFM tip is coated with silver to enhance the intrinsically weak Raman signal. This tip is then positioned in the focus of the laser and only the sample moved underneath. TERS spectra were collected on several points on single strands of calf thymus DNA. Figure 1 shows a typical example for a TERS spectrum of DNA and a reference spectrum on a sample free spot, which has been recorded to prove that the probe itself is not contaminated.

Due to the size of the interaction area of the TERS tip and the DNA strand, the respective TERS spectra contain spectral contributions of approximately 30-60 bases. Our results show that although the Raman scattering cross section of the four nucleobases differs remarkably, specific bands of all bases can be determined in the spectra.

This is a further step towards a direct and label-free Raman sequencing method.

Fig. 1: TERS spectrum of a single strand of calf thymus DNA (black) and reference spectrum (red).
P13 – Scanning Force Microscopy of Reassembled Collagen Fibrils and Natural Fibrils in Cortical Human Bone

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Bone is a nanocomposite of proteins and minerals. At the molecular length scale the soft organic matrix (type I collagen) is reinforced by a stiff inorganic component (hydroxylapatite). Our study is focused on cortical human bone that is mechanically grinded, polished, and chemically etched prior to imaging with tapping mode scanning force microscopy (SFM). For comparison we study collagen fibrils reassembled from purified collagen isolated from bovine hide. In both specimens we find individual collagen fibrils with the typical D-band showing a periodicity of 67 nm. Measurements in moist air led to a controlled swelling of the purified collagen fibrils. The swelling was found to be completely reversible upon reduction of the ambient humidity. In contrast, the bone samples display no swelling under the same conditions. Additionally, the indentation of the SFM tip into collagen fibrils as function of the amplitude set-point was probed during swelling. We also performed bimodal SFM measurements; here the amplitude and the phase of the second flexural eigenmode of the cantilever were used for imaging while the amplitude of the first eigenmode was used as feedback signal. We will compare the results obtained on reassembled and natural collagen fibrils embedded in cortical human bone.
P14 – Micromechanics of Thin Films of Elastomeric Polypropylene

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Elastomeric polypropylene consists of lamellar crystals embedded in an amorphous matrix. The arrangement, distribution, and connectivity of these crystals are important factors which determine the mechanical properties of the polymer. A typical morphology of the material consists of long mother lamellae with short branches of epitaxially grown daughter lamellae. The angle formed between mother and daughter lamellae is 80°[1-4]. These epitaxial branches are very rigid connections and do not change on straining up to strains of 40%. The mechanical stability of these branches is similar to that of the lamellae themselves.

We developed a sample preparation technique and a micro stretching device, that allow imaging the deformations of individual crystalline lamellae at increasing and decreasing degrees of strain of ~ 1 µm thick films with scanning force microscopy (SFM) [1a]). Besides of observing the changes in shape, orientation, and morphology of crystalline regions we are able to measure forces during stepwise stretching and relaxing by an integrated silicon force sensor.

During a strain experiment of an elastomeric polypropylene with 12% crystallinity polymerized by metallocene catalysis we observed an unexpected expansion perpendicular to strain direction of some epitaxially grown crystalline lamellae on the 100-nm-scale, similar to auxeticity [5-7].

P15 – Single Molecule Studies of Bacterial DNA Remodelling Proteins

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In bacteria, the remodelling of DNA by proteins (often termed nucleoid-associated proteins (NAPs)), plays a major role in the compaction, replication and ultimately the expression of genetic material within cells. Whilst the NAPs from *E. coli* (a model Gram negative bacteria) have been widely studied (1, 2) and their interactions largely understood (3-5), our understanding of comparative proteins in other organisms including those in *B. Subtilis* (a model Gram negative bacteria) remains unclear. Recently, primosomal proteins, DnaD and DnaB from *B. subtilis* have been shown partly by AFM to exhibit novel and opposing DNA remodelling activities (6-8) that are essential to initial DNA replication. The C-domain of DnaD (Cd) appears to bind both supercoiled and linear DNA to form open scaffolds, whereas DnaB laterally compacts the DNA (8, 9). The complexity and putative roles of DnaD and DnaB has limited their detailed study in conventional biological assays; resulting in studies of their interactions with DNA using biophysical techniques such as AFM imaging and single molecule force measurements. Currently, the effects of DNA remodelling upon DnaB binding are being studied by single molecule force pulling experiments. Also, biological cloning techniques are being used to obtain hybrid constructs of DnaD and DnaB by domain swapping to better understand the precise roles of these individual proteins. Further work aims to provide fundamental insights into the remodelling of DNA by such proteins and improve our understanding of processes involved in Gram positive bacterial replication with the hope to identifying new antibacterial drug targets to combat medically relevant Gram positive bacterial infections.

Single molecule force spectroscopy using the Atomic Force Microscope (AFM) can yield important information on the behaviour of single molecules to force. An example of this is the mechanical unfolding of proteins using the AFM in a constant velocity setup. By unfolding a concatenated protein at differing velocities a dynamic force spectra can be built up which allows reconstruction of the energy landscape that the protein traverses during unfolding. This procedure has lead to new insights into the determinants of mechanical strength. However, certain factors limit the information that can be gathered by this technique.

In a constant velocity experiment a protein is tethered between a substrate and the AFM tip, and the deflection of the cantilever is used as a measure of the force. The use of a deflecting cantilever is non-ideal for a number of reasons. Firstly, due to the deflecting cantilever the rate at which force is loaded onto protein is non-uniform and the maximum loading rate is limited by the soft AFM cantilever. This limits the dynamic range over which dynamic force spectra can be measured. Secondly, after unfolding of a protein the deflected cantilever recoils, during this time little information can be gained about the dynamics of the protein. Thirdly, the thermal fluctuations of soft cantilevers limit the force resolution.

To circumvent these issues a force sensor has been developed that does not rely on the deflection of the cantilever. Instead a secondary laser is used to control the deflection of a cantilever. When the secondary laser is controlled by a feedback loop the deflection of the cantilever can be ‘locked’, and the laser power required to keep the cantilever locked now becomes a measure of the force acting on the lever. This setup has been applied to constant velocity experiments using Protein L and has revealed features in the energy landscape not detectable in conventional experiments. Additionally new features attributable to refolding intermediates are observed immediately after unfolding.
P17 – Determination of Physical Properties of a Single Peptide by AFM Experiments

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By stretching a polymer in solution using single molecule techniques it is possible to infer about its physical properties. In particular, AFM stretching experiments allow for a full characterization of the elasto-mechanical properties of the sample under study, taking into account both statical and dynamical regimes [1]. In the presented work, single molecule AFM force spectroscopy experiments have been used to determine mechanical properties of a polymer obtained starting from the Exon 28 (Ex28) of the human elastin gene. Elastin is a protein with important mechanical properties and, in particular, it shows quasi ideal elastic behavior associated to the presence of many hydrophobic unstructured domains (such as Ex28) into the protein structure. A typical result of an AFM length clamp stretching experiment is a force versus distance curve directly showing the response of the polymer to the external constant velocity stretching, from the tip-sample contact point up to the rupture of the bond between the probe and the sample (resulting in a steep peak). This experiment, performed in liquid environment, can give information on the mechanical properties of the molecule. The main contribution in the force exerted by the polymer on the AFM tip is due to the presence of a thermal bath driving the system towards a maximum entropy configuration. This entropic-only chain behaviour [2] can be described, switching to a mathematical description, in terms of the worm-like chain (WLC) model [3]. The Ex28 coded polymer has been used as a starting point to obtain bio-materials with specialized elasto-mechanical functions. In particular, a mutated polypeptide based on the EX28 sequence has been synthesisized (named EX28K) with the aim of obtaining a new polymer with the same mechanical and physical properties of the native molecule but with increased aggregation properties, induced by a cross-linking reaction. AFM stretching experiments have been used to verify the mechanical properties of the engineered proteins at a single molecule level. The obtained results allowed us not only to answer this question, but also to give some insight into the first aggregation steps of the polymer towards the formation of reticulated structures.

P18 – Investigation of the pH-Dependent Hydrolysis in Covalent Mechanochemistry

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Chemical bonds control the strength properties of materials and are therefore of fundamental importance in many practical applications. The scission of individual chemical bonds is a thermally activated process following Arrhenius kinetics, and like in any other chemical reaction, the activation energy has to be provided, e.g. mechanically, in order to overcome barriers in the potential energy surface.

In the present study, the strength of individual bond ruptures was investigated as a function of the force-loading rate $\frac{df}{dt}$ at acidic and nearly neutral pH using an atomic force microscope (AFM) in the dynamic force spectroscopy mode. Therefore, individual carboxymethylated amylose polymers were anchored between an amino-functionalized silicon-nitride AFM tip and an amino-functionalized glass substrate and the tension was continuously increased until the weakest link between AFM tip and substrate surface failed.

To extract the dynamics of these single bond rupture events, as well as the structural parameters of the underlying binding potential, i.e., the bond dissociation energy $D_e$, the maximum rupture force $f_{\text{max}}$, and the force-dependent Arrhenius $A$-factor, the results were analyzed with a theoretical model based on Arrhenius kinetics combined with a Morse potential as an analytic representation of the binding potential.

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References

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P19 – Force Spectroscopy on Model Membranes to Predict the Mechanical Properties of Biological Cell Membranes

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Mechanical properties of biomembranes define several biological processes, for example osmotic shrinkage and swelling of cell volume or cell membrane deformation during endo- and exocytosis or cell migration. Therefore the determination of these properties is highly relevant to understand the above described cell processes. Using atomic force microscopy (AFM) membrane elasticity (Young modulus), bending stiffness or area compression modulus can be determined gently pressing a cantilever onto the membrane surface and simultaneously recording the restoring force vs. deflection (Fig.1). In the literature numerous models are known to interpret these force distance curves to calculate the mechanical parameters of the membrane. Here, we used a model where shallow spherical shells deform under point loads [1], [2], [3]. As shells spherically closed phospholipid bilayers (unilamellar vesicles) coated by surface proteins were used. These serve for adequate cell membrane model systems based on their composition, structure and size. Moreover, they are filled with buffer instead of biological polymers and yield information exclusively on the mechanical properties of the membrane and not on the whole cell. The fact that our results are in good agreement with other values from the literature demonstrates that AFM is an appropriate technique to study mechanical biomembrane properties. Simultaneously, protein coated unilamellar vesicles proved to be a suitable model system for cell membranes from the mechanical point of view.

Fig.1 Scheme of the AFM set-up with vesicle as sample.

P20 – TERS for Labelfree Cell Diagnostic

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In order to investigate cell-cell interactions or interactions between drugs and cell surfaces, information on the cell membrane composition is essential. It is known that cell surface glycoproteins are acting as cell-specific identifiers for cell-cell interactions. Those macromolecules are often important integral membrane proteins, where they play a role as a receptor for active ingredients and second messengers.

A common method to identify membrane proteins is antibody labeling. Depending on the nature of the markers it is possible to use fluorescence or Raman spectroscopy as an analytical method. Especially silver and gold-labeled antibodies turned out to be very interesting as they can be used to increase the sensitivity of Raman labels via a plasmon enhancement¹. However, the lateral resolution capability with respect to the location of specific protein arrangements of this method is limited. A limitation of labeling with antibodies is the selectivity of the marker. Different and specific markers must be chosen for each protein of interest.

To provide spectroscopic information with high spatial resolution tip-enhanced Raman scattering (TERS) was chosen². The combination of an atomic force microscope (AFM) with a Raman microscope simultaneously provides information on the topography and the molecular structure of a sample with high sensitivity.

We present TERS measurements on colon cancer cells (cell line HT29, fixed with 2% formaldehyde) and demonstrate the distinction of different membrane proteins. In particular, an area of 90x90 nm was analyzed. Within this area spectra were recorded on a square grid with a spacing of 10 nm. The TER spectra were processed using multivariate data analysis like principle component analysis and cluster analysis.

Based on the clustering, a band assignment of the mean spectrum of each cluster was done. As expected all the TERS bands can be attributed to proteins or lipids, the known components of the cell membrane. By correlating the band assignment and the cluster analysis the location of distinct cell membrane components can be shown.

We demonstrate that the combination of high lateral resolution and specificity of TERS potentially allows a direct characterization of single membrane proteins.

References
The Working Group Nanoforce Metrology for Tactile Sensors of PTB is engaged in determining the probing forces of stylus instruments and the stiffness of AFM cantilevers. In recent years different types of micro force sensors based on piezoresistive and electrostatic principles have been developed. The motivation for the development of force sensors in the micro- and sub-micro-Newton force range was micro-components with very low hardness. These lead to scratches and considerable measurement uncertainties when measured with stylus instruments with probing forces in the mN range. By reducing the probing forces it was, therefore, possible for the first time to measure soft surfaces non-destructively.

PTB has developed transfer standards to calibrate the stiffness of AFM cantilevers and the probing forces of stylus instruments.

A piezoresistive cantilever-type standard for the calibration of stiffness and force has been developed (Fig. 1). The cantilevers are manufactured by the Institute for Semiconductor Technology of Braunschweig Technical University. Metrological investigations have been carried out at PTB since 2002. For a detailed description of the calibration setup and the calibration procedure, see Doering et al. at the IMEKO Conference 2002.

Furthermore, an electrostatic comb-drive MEMS actuator with a multi-folded spring system has been developed for the measurement of AFM cantilever stiffness and probing force (Fig. 2). Details are published in TM 06/2009 by Gao et al. In the article the principle of measurement, the design, the manufacture, and the assembly of the MEMS as well as the first test results and the performance parameters achieved are described.

PTB is able to calibrate the sensors described in the nano- and -in future- also in the pico-Newton range. The sensors and the force and stiffness calibration set-ups are described in the poster.
Proteins are involved in all processes which are necessary for living, e.g. they are responsible for growth and cleavage of cells, which makes them an interesting topic for science and medical application. Small GTPases, like Ras, are GTP-binding proteins involved in cellular processes, e.g. vesicle transport, cell cycles, nuclear import and signal transduction. GTPases act as molecular switches, which cycle between a GDP-bound inactive and a GTP-bound active state regulated through different factors. In oncogenic mutants of Ras this regulation is disturbed. Most small GTPases are associated with membranes within the cell. The association with the lipid membrane is achieved by posttranslational added lipid anchors at N- or C- terminus, mostly containing farnesyl residues.

In this study our first AFM-studies on solid supported lipid-bilayers with bound proteins are shown. Considering the protein Ras, so far most investigations were done without membrane. We want to study the protein in its natural environment bound to a lipid-bilayer, in order to get a more detailed view of the function of GTPases within the cell. Furthermore we want to observe the interaction between GTPases and its interacting proteins. Therefore the aim of the project is to combine spectroscopic methods like trFTIR-Spectroscopy with imaging methods like Atomic Force Microscopy and Confocal Raman Microscopy. By means of time-resolved Fourier-transform infrared (trFTIR) spectroscopy detailed information on reaction mechanisms of proteins can be gained[1]. Especially the method of Attenuated Total Reflection-FTIR-Spectroscopy (ATR-FTIR) enables measurement of proteins in a more in vivo like environment, e.g. Ras bound to a lipid-bilayer[2]. However, ATR-FTIR can only detect the overall changes on the surface without spatial resolution. In order to attain the space-resolved information, e.g. on the distribution of the proteins on a membrane, this method can be combined with Atomic Force Microscopy (AFM), which allows the characterization of the three-dimensional topography and the physical properties of a surface. A further step is possible with Tip Enhanced Raman Scattering (TERS)[3], a technique where the AFM tip provides the surface enhanced effect and molecular information on nanometre scale can be obtained.

Figure 1: Scheme for the combination of time-resolved Fourier-transform Infrared Spectroscopy (trFTIR) with space-resolved methods, like Atomic Force Microscopy (AFM) and Tip Enhanced Raman Scattering (TERS)

P23 – Analysis of Pyridine Coordination Compounds Using Single Molecule Force Spectroscopy

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Multivalency is a fundamental effect in supramolecular Chemistry (e.g. recognition in devices), Nanotechnology (e.g. self organization) or Biochemistry (e.g. transduction of signals) \([1]\). It can increase binding constants disproportionately high and thereby shift balances strongly to complex side. Thus it is important to gain a deeper knowledge on multivalent interactions. Single Molecule Force Spectroscopy (SMFS) is a direct measurement of forces \([2]\) and an therefore an ideal tool to study multivalency on the molecular level.

Here we present first results of SMFS on a monovalent pyridine coordination compound in an aqueous solution of Fe(II). The pyridine was coupled to Au covered tips and Au surfaces, using thiol chemistry and Polyethylene-glycol (PEG) as spacer with well known stretching properties \([3]\). Force-distance measurements were performed at different pulling velocities to calculate molecular properties as bond lifetime, bond width and thermal force \([4]\). Our results are the basement for further analysis on multivalent pyridines. Furthermore, an astonishing repeatability of rupture events along few sawtooth pathways in force-distance space allowed us to analyze rupture events of successive bonds in series separately \([5]\).

We report for the first time the use of Atomic Force Microscopy (AFM) in discriminating between living normal human urothelial cells (HUC) and bladder tumour cells (MGH) on the basis of their ultrastructure and nanomechanical properties. AFM imaging revealed clear differences in the shapes and dimensions of MGH and HUC cells (Figure 1: Top): MGH cells had the tendency to cluster and therefore they did not spread much compared to their normal counterparts. As a consequence, the dimensions of MGH cells were found to be smaller than those of normal HUCs. We also observed clear differences in the nanomechanical properties of the two types of cells (Figure 1: Bottom), determined from AFM force spectroscopy (AFM-FS). The nanoscale maximum adhesion force ($F_{\text{max}}$) and adhesion energy were found to be higher in the MGH cells compared to normal HUC cells, showing that tumour cells are more compliant than normal cells. In addition, the Young’s (elastic) modulus ($E$) of MGH cells was consistently lower than that of their normal counterparts, indicating a higher elasticity of their cell walls. These AFM results clearly demonstrate that the ultrastructural and nanomechanical properties of bladder tumour cells differ from those of normal human urothelial cells. AFM can therefore be used as a potential powerful clinical tool for cell sorting and profiling.
P25 – Elastic Fully Three-Dimensional Microstructure Scaffolds for Cell Force Measurements

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The rhythmical contraction of the heart that drives the cardiac cycle involves the coordinated and synchronized action of a large number of cardiomyocytes. Determining the force contribution of an individual cardiomyocyte to overall heart contraction requires sensitive cell force measurement devices. To measure the contractile force of a single cell, we have produced elastic 3D cell culture scaffolds by means of direct laser writing (DLW) into a bio-compatible photoresist (Ormocomp). These 3D scaffolds contain flexible beam elements of submicron thickness which can be rhythmically deformed by single beating cardiomyocytes. To obtain a quantitative measure of the involved cellular contraction forces, the cell culture substrates were calibrated using the cantilever of an atomic force microscope as a micro-indenter. Matching cell-induced beam deflections required applying external forces of about 50 nN, indicating that cellular contraction forces are of similar magnitude. Furthermore, by adjusting the DLW write parameters, and thus the beam diameter (0.66 to 1.33 µm), the beam stiffness could be fine-tuned over a range of almost one order of magnitude (0.05 N/m – 0.4 N/m). In conclusion, we have demonstrated that DLW can be used to fabricate 3D cell culture substrates with tailored stiffness to measure a wide range of cellular contraction forces. In future, this method could be expanded to systematically investigate the influence of three-dimensionality and elasticity on other cell functions, such as the differentiation of individual cells and on tissue formation.
P26 — Atomic Force Microscopy to Characterize Membrane Resealing Skeletal Muscle Myotubes

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n/a

P27 — Spatial Measurement of Local Young Modulus by AFM Force Spectroscopy in Spherical Cells

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P1 – Optical Tweezers to Investigate Receptor/Ligand Interactions on a Single Contact Level

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The extraordinary features of optical tweezers having a nm-resolution in positioning a micron-sized colloid and an accuracy of (±50 fN) in measuring the forces acting on it, enable one to study the interaction within a single receptor/ligand-contact. To establish a model system, the interaction between Protein A from Staphylococcus aureus and Immunoglobulin G from rabbit serum (RIgG) is investigated. It is demonstrated that the rupture force depends on the loading rate. This effect is well known in the literature and the data obtained were found to be in good agreement with an already published theoretical model. By use of this model, the off-rate at zero force is determined. In a new project this method is used to investigate the specific binding of monoclonal antibodies to synthetic peptides depending on their phosphorylation pattern. Amongst others, the massive accumulation of tangles that mainly consist of hyperphosphorylated tau-proteins is characteristic for Alzheimer’s disease.
P2 – Constructing and Probing a Biomimetic Model of the Actin Cortex via Holographic Optical Tweezers

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The actin cortex, a quasi two-dimensional network of actin, plays an important role in cell stability, motility and viscoelasticity. In vivo, its characteristic properties are controlled by various actin binding proteins (ABPs), such as crosslinkers or ions. To investigate the influence of a specific crosslinker on the network’s behaviour exclusively we create and probe biomimetic models of the actin cortex. This is realized using microbeads trapped by holographic optical tweezers (HOTs) as scaffold for the actin filaments. With this technique we are able to create actin networks in arbitrary geometry and determine the forces exerted by different crosslinkers. Using a special microfluidic flowcell we have full control over the chemical environment in our experiments. The acting forces are measured by highspeed imaging, whereas simultaneous fluorescence microscopy yields information about the structure and density of the actin network. In another approach we use micropillars as framework and measure unzipping forces of crosslinked actin filaments.
P3 – Investigation of Erythrocytes Cell-Cell Adhesion Using Holographic Optical Tweezers

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In the classical model, the role of red blood cells (erythrocytes) in blood clot formation is thought to be passive. It is supposed that they get caught into a spectrin-network, generated in the clotting process just for reasons of geometrical restrictions. Additionally, it is commonly believed that there exist no adhesion forces among the cells. The main part in clot formation take activated platelets. Prostaglandin E$_2$ (PGE$_2$) and Lysophosphatidic acid (LPA) are messengers released from these activated platelets. Treating red blood cells (RBC) with LPA or PGE$_2$ leads to a Ca$^{++}$ influx into the cells. Measurements with a calcium Ionophor A23187 and LPA showed that this increased intracellular calcium level leads to an adhesion of the cells among each other. Thus, we postulate that the response of RBCs on PGE$_2$ and LPA reveal a direct and active participation of these cells in blood clot formation. In order to test this hypothesis we built up an integrated microfluidic holographic optical tweezers setup to study this cell adhesion. The microfluidic consist of two inlets: one for the red blood cells and one for the messenger. No mixture between the two fluids occurs due to the very low Reynolds numbers.

The holographic optical tweezers allow entry into the microfluidic chamber and allow to bring cells into contact with the messenger. The emerging adhesion can be verified by bringing two cells together. With this microfluidic device an adequate statistic of the behavior of red blood cells is possible and the aim of further research is to characterize the adhesion process in terms of adhesion energies and adhesion forces and to identify the underlying reasons for the adhesion.
P4 – Optical Tweezers Measurements of Threading DNA and DNA-Ligand-Complexes Through Solid-State Nanopores

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We developed a versatile and high precision 3D optical tweezers setup, capable for force measurements completely based on detection of backscattered light with minimal optical interference to measure forces in the sub-pN regime, and to manipulate single molecules. With this novel setup, single dsDNA-molecules were threaded into a solid-state nanopore by applying electrical voltage across the membrane, as the electrostatic force and the ionic current through the pore were measured. Here, individual force steps could be observed for each DNA-molecule entering the nanopore. Active pulling of a single Lambda-DNA-molecule out of the nanopore by linearly increasing the bead-membrane distance induced a distinct force signal, until the DNA was completely pulled out of the nanopore. Binding of dedicated protein ligands (peroxiredoxin, E.coli RNA-polymerase, and RecA) to dsDNA caused a significant change in the apparent electrostatic forces that are required for threading and unthreading the DNA-ligand-complex through the nanopore.
P5 – Measuring Adhesion Forces Between Influenza Virus and Living Cells

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Influenza Virus belongs to a wide range of viruses that are enclosed in a lipid envelope. The major spike protein of the viral envelope hemagglutinin (HA) binds sialic acid (SA) residues of glycoproteins on the plasma membrane of the host cells. This represents the first step of infection and requires multiple simultaneous interactions since the affinity between one single HA-SA pair is estimated to be very low (10^{-13} \text{ M}^{-1}). The attachment of influenza virus particles to living host cells was characterised on the level of single molecules using optical tweezers and atomic force spectroscopy. Unbinding events were analysed and revealed a multimodal rupture force distribution. This suggests sequential binding of multiple receptors. Treatment of the cells with neuraminidase (NA) which cleaves terminal sialic acid residues lead to a decrease of the binding probability by >50 %. This indicates a specific interaction between hemagglutinin and sialic acid during the force measurements.
Kinesin-1 is a molecular motor essential for cellular function. It transports components such as membrane-bound organelles and molecular complexes around a cell by travelling along microtubule filaments, which make up part of the cytoskeleton, while hydrolysing ATP. This motion is processive, in one direction only and is known to involve both of kinesin’s two heads. Although extensively studied by a variety of techniques over many years, the mechanism these single-molecule motors use for this efficient motion on the nanoscale is not fully understood.

In our investigations we use the Photonic Force Microscope (PFM) to trap and track a 500nm bead attached to a single kinesin motor as it interacts with a microtubule in vitro. The PFM is based on an inverted microscope equipped with a 1064 nm/500mW infrared laser used to produce an optical trap, which can capture a small dielectric particle through electromagnetic gradient forces. The motion of the particle can then be monitored in three dimensions with nanometre spatial and microsecond temporal resolution by analyzing the 3D-interference pattern produced between laser light and its component scattered by the bead with a quadrant photo-diode. Through analysis using Boltzmann’s distribution of the bead’s confined Brownian motion, the mechanical confining potential the molecular motor structure produces while connecting the bead to a fixed microtubule can be measured and related to its 3D-mechanical properties.

Using the three dimensional position trace of a bead interacting with a microtubule through a kinesin motor at low laser intensities, we infer information about the molecular motor’s position and mechanical properties as it processes along a microtubule. By characterising different conformational states of the kinesin molecule in nucleotide and nucleotide-free assays, we expect to learn more about the cycle of events necessary for kinesin to move. An understanding of how nature achieves this motion on the nanoscale will be useful in two ways. First, this understanding will help to deal with diseases for which kinesin’s malfunction is thought to be the cause and second it will allow the production of artificial nanomachines in the future based on the same principles.
In optical tweezers, thermal drift is detrimental for high-resolution measurements. In particular, absorption of the trapping laser light by the microscope objective that focuses the beam leads to heating of the objective and subsequent drift. This entails long equilibration times which may limit sensitive biophysical assays. Here, we introduce an objective temperature feedback system for minimizing thermal drift. We measured that the infrared laser heated the objective by 0.7K per watt of laser power and that the laser focus moved relative to the sample by \(\approx 1\) nm/mK due to thermal expansion of the objective. The feedback stabilized the temperature of the trapping objective with millikelvin precision. This enhanced the long-term temperature stability and significantly reduced the settling time of the instrument to about 100 s after a temperature disturbance while preserving single DNA base-pair resolution of surface-coupled assays. Minimizing systematic temperature changes of the objective and concurrent drift is of interest for other high-resolution microscopy techniques. Furthermore, temperature control is often a desirable parameter in biophysical experiments.
P8 – Quantitative Analysis of Fluctuations and Irreversibility of Optically Trapped Microspheres

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We present an experimental verification of several universal theorems of stochastic thermodynamics by means of controllable optical traps, so-called “optical tweezers”. The theoretical and experimental investigation of this novel branch of modern thermodynamics promises in various ways to be of great benefit for the quantitative understanding and future application of processes at the micro- and nanometre scale in the fields of physics, biology, chemistry and applied technology. Utilizing the formal framework established by U. Seifert and others around 2005 and following the experimental approaches of G. M. Wang et. al first published in 2002, we experimentally demonstrate the validity of several pertinent fluctuation theorems for special non-equilibrium states of optically trapped colloids. Our results in this context exceed the scope of Wang et al. 2002-2005 by a considerable margin. Fluctuation theorems which came into the focus of theoretical research about 15 years ago describe the emergence and quantitative evolution of macroscopic irreversibility from the microscopic point of view of stochastic thermodynamics. Moreover, we present a new method for the experimental determination of the radius and the temperature of a single optically trapped colloid by directly analyzing the thermal equilibrium fluctuations of the bead. This technique may be utilized for the optimisation of several other optical tweezers experiments which show a high requirement for quantitative precision.
Towards Nanonewton Forces: Optical Trapping of Anti-Reflection-Coated Titania Microspheres

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The maximum force that can be generated by optical tweezers is limited by the trap efficiency and light power used. Increasing the light power eventually leads to heating and photo damage in case of biological applications. Increasing the refractive index mismatch between the trapped microsphere and its surrounding medium increases the trap efficiency, however, there is an upper limit to this mismatch since the destabilizing scattering force increases stronger with the mismatch than the stabilizing gradient force. Here we fabricated anti-reflection-coated, high refractive index particles composed of titania that cannot be trapped by a single gradient trap unless coated. We find an almost two-fold improvement in trapping efficiency compared to polystyrene microspheres in agreement with Mie theory calculations. Using these coated microspheres, we expect to achieve forces of up to a nanonewton with a 4 W laser. Optical tweezers experiments in the nanonewton force range, for instance protein unfolding or intra- and intercellular measurements, with sub-piconewton resolution are therefore feasible.
P10 – Force Generation by Type IV Pili of *Neisseria Gonorrhoeae*

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Type IV pili are major bacterial virulence factors supporting adhesion, surface motility, and gene transfer. During infection they mediate attachment to mammalian host cells and elicit downstream signals. The polymeric pilus fiber is a highly dynamic molecular machine that switches between elongation and retraction. We used laser tweezers to investigate the dynamics of individual pili of the human pathogen *Neisseria gonorrhoeae*. We found that the retraction velocity of bacteria adhered to an abiotic surface is bimodal and that the bimodality depends on force and on the concentration of the putative motor protein PilT [1]. When adhered to host cells the bimodality persisted at higher forces compared to an abiotic environment. This increase in average velocity is consistent with an up-regulation of PilT due to interaction with host cells. Bacteria generated considerable force during infection but the maximum force was reduced from (120±40)pN on abiotic surfaces to (70±20)pN on host cells, most likely due to elastic effects. Velocity and maximum force of pilus retraction were independent of the infection period within 1h and 24h post infection [2]. Thus the force generated by type IV pili during infection is high enough to induce cytoskeletal rearrangements in the host cell.

P11 – Quantifying and Pinpointing Sources of Noise in Optical Tweezers Experiments

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Optical trapping techniques have evolved to the point where quantitative force measurements on biological systems can be performed down into the femtonewton range. As resolution is constantly improving, the pinpointing and elimination of noise sources become increasingly important. Allan-variance analysis is ideally suited for this task; adjacent time series are recorded and the variations between observation intervals are calculated [1].

Here, we provide a comprehensive toolbox consisting of: a reliable data-streaming acquisition software that allows for streaming long adjacent time series (~30min), even with high sampling frequencies (>80kHz); an analysis software (Matlab-based) with calculation times of only a few seconds; and fitting scripts to extract parameters of noise and drift sources. Furthermore, the validity and robustness of Allan-variance analysis is demonstrated in data obtained from our optical-tweezers setup wherein laboratory-specific noise sources are detected. In addition, a detailed Allan-variance analysis clarifies the difference between two common detection systems, a quadrant photodiode and a position-sensitive photodiode [2, 3]. Lastly, we demonstrate how our toolbox can be applied to position-time traces of optically trapped spheres to determine the optimal calibration interval for any setup, suitable settings for variance and update rates in force-feedback loops, and variations due to the geometrical constraints of the sample chamber [4]. Allan-variance analysis can be used as a standard tool enabling reliable comparison of different optical-tweezers setups and the precise quantification of noise and drift sources, which are particularly relevant in high precision experiments with individual biomolecules.

The Forces of Interaction Between Poly(2-vinylpyridine) Brushes as Measured by Optical Tweezers

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Forces of interaction within single pairs of poly(2-vinylpyridine) (P2VP) grafted colloids have been measured by optical tweezers (OT) with an extraordinary resolution of ±0.5 pN [1]. Parameters to be varied are the concentration and type of salt (KCl, CaCl₂, and LaCl₃) of the surrounding medium as well as its pH. The observed force-distance relation is quantitatively described by the Jusufi model [Colloid Polym. Sci. 2004, 282, 910-917] for spherical polyelectrolyte brushes which takes into account the entropic effect of the counterions and enables one to estimate the ionic concentration inside the brush [2]. The transition from an osmotic to the salted brush regime is analysed in detail. For the scaling of the brush height a power law is found having an exponent of 0.24±0.01 which ranges between the values expected for spherical and planar brushes [3,4]. At pH 4 a strong transition from a brush to a pancake conformation takes place.

References
P13 – Optical Tweezers as Means to Stimulate Hippocampal Neurons With BDNF Coated Beads

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Brain derived neurotrophic factor (BDNF) is a small secreted protein that acts as neurotrophin and plays important roles in the development of nervous system in vertebrates. Secretion of BDNF occurs from both dendrites and axons and is regulated in an activity-dependent manner. Its effects, mediated mainly by TrkB receptor, are partially contrasting since BDNF can induce both survival, growth, differentiation and cell death [1]. This dichotomy can be ascribed to the differences between neuron types, to the activation of different pathways, or to the fine regulation of its local availability (stimulation of soma or dendrites can produce opposite effects)[2]. To better understand this process and its molecular mechanisms, we used 1.5 µm diameter silica beads functionalized with BDNF to stimulate precise domains of hippocampal rat neurons and transported them to the site of stimulation by means of optical tweezers [3]. We demonstrate that BDNF, even if it is covalently bound to the beads, preserves its biological activity and is able to activate TrkB receptor. BDNF-coated beads, in fact, induce the translocation into the nucleus of c-Fos (a transcription factor) and lead to increase in calcium levels both in the soma and in the dendrites [4]. In this work we show a novel application of optical tweezers for localized delivery and stimulation of neurons.

Bibliography
P14 – Dual-Trap Optical Tweezer for Single Molecule Studies of Transcription

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Observing fundamental cellular processes one molecule at the time requires the ability to accurately monitor and manipulate individual enzymes under physiological conditions. Here, we report the construction of a high-resolution dual-trap optical tweezer setup with an integrated flow system to investigate the dynamics of processive molecular motors. Splitting a 1064 nm solid-state laser beam by polarization generates two optical traps, each independently maneuverable by either a piezo-driven mirror or an acousto-optical deflector. We present a detailed analysis of the performance of the instrument, including a careful analysis and subsequent reduction of the cross-talk between the two polarization states caused by various optical elements.

Transcription, the generation of RNA from DNA by RNA Polymerase, is one of these key biological processes that can now be studied with very high precision. With a step size of 0.34 nm (1 bp), the movement of RNA Polymerase requires extraordinary force resolution and instrument stability for observation. We will use the aforementioned instrument to study the dynamics of different RNA Polymerases under varying conditions. We hope to ultimately demonstrate that the comparison of the dynamics of RNAPs from all domains of life deepens our understanding of the transcription process and allows us to assess the evolutionary advantages for combining or separating special domains of an enzyme for regulatory purposes.
P15 – Interaction of XMAP215 With Dynamic Microtubules Studied With Optical Tweezers

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The protein XMAP215 binds to the end of growing microtubules and changes the polymerization rate, promoting faster elongation. To investigate the addition of tubulin dimers to the plus end of the microtubule by XMAP215 and the dependence of the addition on the applied force, XMAP215 is tethered to a microsphere held by an optical trap. Because XMAP215 remains at the microtubule end for several rounds of tubulin addition, one can use it as a handle to hold the microtubule tip. In this way one can assess the force exerted by polymerizing microtubule and explore changing its growth dynamics under applied load with high temporal and spatial resolution.
P16 – Using Optical Tweezers to Study the Elementary Events Underlying Force Generation in Neuronal Lamellipodia

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The propulsion of the leading edge of neuronal lamellipodia is a complex process in which the polymerization of actin filaments towards the cell membrane is a major component [1,2]. This process is at the origin of force generation in neurons. By using optical tweezers, we have characterized the dynamics by which lamellipodia of Dorsal Root Ganglia neurons exerted force on encountered obstacles such as silica beads. To determine the displacements produced by elementary events behind force generation, the stiffness of the optical trap was kept as low as 0.015 pN·nm⁻¹, so that the underlying motion could occur in an unhindered fashion [3]. At such a low stiffness the bead held in the optical tweezers necessarily fluctuate with large amplitude possibly masking the underlying biological events. Because of the presence of adhesion forces, beads in close contact with a lamellipodium could seal on its membrane so that the standard deviation of Brownian fluctuations could be reduced by 10 times. In several experiments, the bead remained within 300 nm from the center of the optical trap where the voltage sensitivity of the detector and the trap stiffness is constant. Under these conditions, if the lamellipodium pushed the bead, discrete jumps could be detected. Jumps were detected using an algorithm based on nonlinear diffusion filtering [4]. Briefly, the original signal was smoothed in order to obtain a smooth piece-wise (regularized) trace where the discrete jumps were enhanced and then detected. These jumps occurred within 1 ms and had an amplitude varying from 5 to 20 nm. When the lamellipodium retracted, pulling the beads with it, no discrete events were observed. These discrete events were not observed in the presence of Latrunculin A, a blocker of actin polymerization or when neurons were fixed with paraformaldehyde. These jumps show that force generation in lamellipodia is a discontinuous process in which bursts of actin polymerization and depolymerization alternate continuously. In future we will explore changes in the characteristics of these jumps by pharmacologically altering the membrane rigidity to understand the role of the membrane in this process.

References
Light beams with screw phase dislocations are known to carry optical orbital angular momentum. A novel field of applications for these beams are optical tweezers where angular momentum is transferred to microscopic samples e.g. to drive micro machines.

A screw phase dislocation (optical vortex) possesses a topological charge, equal to the integer \( m \), where \( m \) is defined by the \( 2m \) phase change on any closed circuit around the dislocation center. The topological charge also indicates the optical orbital angular momentum, which is given as \( m \hbar \) per photon. The sign of \( m \) is defined by the handiness of the screw-like surface of fixed phase in space. It is a well known and often used fact, that the sign of the topological charge of a vortex beam is reversed when it is reflected by a mirror. Since the direction of propagation also reverses in normal reflection, orbital momentum is conserved. The situation is different for a phase-conjugating mirror. Due to the time reversal property of the phase-conjugating mirror, the incident and reflected wavefront surfaces match perfectly. As a result, the topological charge does not change sign and the optical orbital angular momentum is reversed. Hence, the difference in angular momentum of \( 2m \hbar \) per photon needs to be transferred to the phase-conjugating mirror.

In this contribution we demonstrate a self-pumped photorefractive phase-conjugating mirror that is used to investigate these fundamental characteristics. It is shown that this implementation of a phase-conjugating mirror is suitable to produce very stable, high-fidelity phase conjugation of vortex beams. We directly compare the reflection properties of a conventional mirror to that of a phase-conjugating mirror. In front of the phase-conjugating mirror a stationary three dimensional interference pattern is created by two counter propagating vortices of equal charge. This three dimensional interference pattern is studied and applications in optical traps are suggested.
P18 – Multiple Traps in a Counter-Propagating Optical Trap Configuration Based on Optical Phase Conjugation

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Counter-propagating laser beams that compensate for axial scattering forces resulting from radiation pressure of light are widely used for acceleration and trapping of micro- to nanometer-sized particles [1]. One elegant method to realize such counter-propagating optical traps is based on a photorefractive phase-conjugating mirror which inverts wave vector and phase of incoming light [2, 3]. For counter-propagating optical traps, the laser beam is phase-conjugated after transmission through the intended trapping plane. The resulting phase conjugate beam travels back the optical path and exactly overlaps with the first beam. Hence, a counter-propagating beam trap builds up, which is intrinsically self-aligning and can be generated with low numerical aperture microscope objectives. This is important for application which require long working distances such as generalized phase contrast [4] or when low intensities are needed (trapping of living cells [5]).

We successfully implemented a single beam optical trap configuration based on optical phase conjugation and currently investigate a dual trap geometry which provides trapping of two particles simultaneously. These optical traps are characterized and compared with competing methods by measurement of trap stiffness and trapping potential wells.

The current experimental set-up should be extendable to complex light fields such as beams carrying angular momentum or non-diffracting beams. Coherent superposition of the incoming beam and its counter-propagating phase conjugate in the trapping plane can lead to expanded novel 3D-trapping structures.

Fluid flows on microscopic scales are important for cell-cell interactions and could also be of future interest for mixing processes on small ranges. For a general study on these flows we chose *Bacillus subtilis* because of its well understood flagella motor as a model system to get a better understanding of hydrodynamic interactions between several bacteria.

Flow fields created on the one hand by a single bacterium [1] and on the other hand by large colonies of bacteria [2] which show cooperative behaviour have been investigated recently. Our system enables us to study the interesting region in between to get a better understanding when and how this behaviour appears and most importantly to study flows created by well defined arrangements of bacteria. For this purpose we implemented a Holographic Optical Tweezers system [3] that traps up to about 100 bacteria simultaneously in all 3 dimensions at a wavelength of $\lambda = 1064$ nm. At this wavelength photodamage to *Bacillus subtilis* [4] is minimal and fluid flows can be studied as unaffected and natural as possible.

We use our tweezers system as a tool to adhere multiple bacteria at the same time to polystyrene coated glass surfaces. This robust technique provides us a good basis for further studies on hydrodynamic interactions between bacteria and their influences on fluid mixing.

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P20 — Optical Force Based Investigations of Cell Mechanical Concepts During Phagocytosis

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A central mechanism of the mammalian immune system is the internalization of bacteria by macrophages during phagocytosis. However, the mechanical properties of phagocytosis are largely unknown, in particular when mediated by cellular tentacles like filopodia. We used optical tweezers-based microscopy to investigate different mechanical concepts of the cell to take up 1 µm beads, which serve as synthetic bacteria. The motion of an optically trapped bead was tracked interferometrically in 3D with nanometer precision at a microsecond timescale. On the one hand, the measurement of the thermal bead fluctuations during the binding to the cell membrane enabled the observation of individual receptor-ligand bond formation. On the other hand, the measurement of the mean bead displacements allowed determining retraction forces of filopodia at various retraction speeds. We measured F-actin dependent 36-nanometer steps inside living cells during filopodia retraction likely belonging to actin-based molecular motors [1]. Steps remained clearly visible even at force regimes clearly beyond the stall force of a single myosin motor. This seems to indicate a kind of inter-motor coupling, a phenomenon which we try to explain by a stochastic multi-state model. We want to combine the position detection of the bead with fluorescence microscopy techniques to investigate the reorganization of the cell during filopodial retraction and the underlying concepts of phagocytosis.

P21 – A Force Detection Technique for Molecular Experiments in Living Cells Using Gradient Optical Traps

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The powerful results in the molecular and cellular domain that are currently obtained using optical tweezers are leading to an increasing interest in this biophysical tool. Once calibrated, laser traps can be used to accurately measure the forces and displacements involved in many different molecular processes. Unfortunately, standard force detection techniques are not suitable for experiments inside living cells. For instance, in the study of the forces involved in the intracellular transport, vesicles or subcellular structures that need to be used as handles to interact with motor proteins propelling them along the cytoskeletal filaments may not be spherical, and their sizes are usually unknown. Furthermore, the cytoplasm is an optically and mechanically nonhomogeneous medium. As a consequence, in vivo experiments generally do not meet the requirements for current force calibration methods, so forces cannot be accurately measured.

Here, we show a force detection technique for gradient optical traps based on the measurement of the change in momentum of the photons of the trapping beam. A duolateral position sensing detector records a signal which is proportional to the force exerted on the sample, in spite of the properties of both the trapped particle and the beam. Thus, the system does not demand a specific calibration in every experiment; it provides a correct force reading under any circumstance.
**P22 – Manipulating Microscopic Objects Using Combined Elliptical and Point Optical Tweezers**

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Single beam point optical tweezers were modified to manipulate microscopic objects. Idea to this kind of object manipulation came from article written by Mohanty and colleagues [1]. Second line was designed with Zemax optical designing software and built to produce elliptical tweezers with two cylindrical lenses. We used Nd:YAG laser with 1064 nm wavelength. Laser light was split to two lines and combined before objective with polarizing cube beam splitters. Microscope objective was water immersion objective (Olympus LUMPLFL 100X W/1.00). With this new setup single red blood cell could be rotated with combined tweezers by rotating cylindrical lenses. Maximum of seven red blood cells were trapped to the elliptical tweezers simultaneously in PBS solution when optical power in focal plane was ~ 13 mW (figure 1). When optical power was reduced to ~ 5 mW, the last four remaining cells escaped from elliptical tweezers.

![Figure 1: Seven red blood cells trapped in elliptical tweezers.](image)
