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Optical Angular Momentum Transfer to Trapped Particles in Vacuum

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KEY WORDS: optomechanics, spin angular momentum, orbital angular momentum

The control over all the degrees of freedom of a macroscopic object is a precursor for exploring the transitions between the classical and quantum world. With this remit, the complete optical control over an isolated nano- or microscopic particle in vacuum is an exciting testbed for such studies, crucially minimizing decoherence. Furthermore, there is the potential for exploring the predictions of the Casimir force and quantum friction if such a particle can be set into rotation at sufficiently high angular velocities and controllably positioned next to a surface.

In this paper, we present a trapped, levitated rotating microparticle confined in an optical potential, rendering it well isolated from the thermal environment [1]. Rotation is induced by transferring spin angular momentum to a trapped vaterite particle approximately 4.4um in diameter. Interesting rotation dynamics are seen and rotation rates up to 10MHz observed. This approach opens up a powerful route to explore the fascinating predictions of quantum friction, which may ultimately have a profound impact upon nanoscale devices [2].

We support our experimental work by modelling the dynamics of a birefringent particle in an optical potential and subject to position- and orientation-dependent torque. To simplify the system while maintaining its main optical properties, we consider the induced polarization of an anisotropic dipole corrected for the anisotropic radiative process. The optical forces and torques are calculated by generalizing the cycle-averaged Lorentz force and torque to account for the anisotropy owing to birefringence. The work is extended to describe the transfer of orbital angular momentum from single ringed Laguerre-Gaussian beams to trapped silica particles.

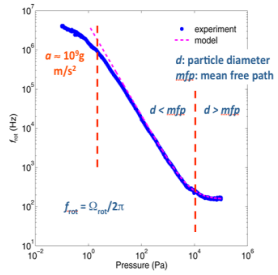

Figure 1: Rotation rate versus pressure for a 4.4.um vaterite particle.

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Nano-Optical Trapping: Recent developments and applications to quantum physics

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KEY WORDS: Nano-optical tweezers, surface plasmons, vacuum trapping, Optomechanics

Extending optical trapping to the true nanometre scale offers unprecedented opportunities in many fields of science, where nano-optical tweezers would allow the ultra-accurate manipulation of single nano-objects. To this aim we have developed parallel strategies aiming at trapping nano-objects both in solution and vacuum. We here report on our recent advances in each of those and discuss their application, in particular to quantum physics.

Our paper is organized in two main parts. The first part focuses on plasmon-assisted nano-trapping in which trapping is achieved in the optical near field of a plasmonic nanostructure. We recently extended the concept of Self-Induced Back Action (SIBA) trapping at the extremity of a near field scanning optical microscopy (NSOM) probe. The nano-optical trap is built by engineering a bowtie plasmonic aperture at the extremity of a tapered metal-coated optical fiber. Both the trapping operation and monitoring are performed through the optical fiber making these nano-tweezers totally autonomous and free of bulky optical elements. This configuration enables to trap small dielectric objects in the 10nm range and to move them in 3D with nanometer accuracy [1]. More recently, we also studied how plasmonic optical forces can be exploited to assist the immobilization of artificial atoms in a plasmonic hotspot. In particular, we demonstrate deterministic positioning of individual nanodiamonds hosting a single nitrogen vacancy (NV) in the gap of a gold nanoantenna and characterize their optical coupling through fluorescence lifetime measurements [2, 3].

The second part of our paper focuses on vacuum trapping and cooling of single nanoparticles. In our experiment, a 150nm silica nanoparticle levitates in the focus of a tightly focused laser beam. We show its motion can be cooled down along all three spatial axes by applying parametric feedback acting on the trapping laser intensity [4]. Due to the absence of clamping, we reach mechanical quality factor Q as high as 10^8 at pressure of 10^{-6}mBar. This unprecedented Q confers the levitating nanoparticle a huge sensitivity to its environment. In particular, we demonstrate that the thermal energy of remaining molecules is sufficient to drive the nanoparticle motion in a nonlinear regime [5].

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Collective Excitations of Hydrodynamically Coupled Driven Colloidal Particles

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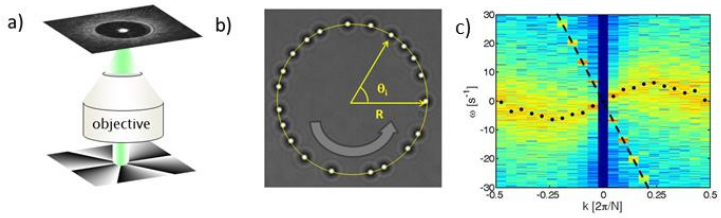
KEY WORDS: Non-equilibrium statistical mechanics, driven dissipative colloidal suspensions, optical vortex, holographic optical tweezers.

A single colloidal particle trapped in an optical vortex experiences two optical forces: a gradient force confining it to motion along a finite width ring of light, and radiation pressure driving it along the perimeter of the ring [1]. As a result, the particle rotates, at constant angular velocity with thermal fluctuations. When a second particle is introduces to the vortex trap the two particles pair due to a pseudo-potential caused by the interplay between hydrodynamic interactions and the curvature of the particles' trajectory [2]. We study the collective excitations of many colloidal particles driven in an optical vortex trap. We find that even though the system is overdamped, hydrodynamic interactions due to driving give rise to non-decaying excitations with characteristic dispersion relations. The collective excitations of the colloidal ring reflect fluctuations of particle pairs rather than those of single particles [3].



Polystyrene bead trapped in an optical vortex (a) rotate along the ring of light due to optical forces (b), and interact hydrodynamically. As a result the exhibit non-decaying oscillatory motion in the co-rotating frame with typical dispersion relations (c).

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Simultaneous three-dimensional photodiode tracking of multiple traps and biological applications

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Multiple-beam optical traps facilitate advanced trapping geometries and exciting discoveries. However, the increased manipulation capabilities come at the price of more challenging position and force detection. Due to unrivaled bandwidth and resolution, photodiode based detection is preferred over camera based detection in most single/dual-beam optical traps assays. However, it has not been trivial to implement photodiode based detection for multiple-beam optical traps. We developed a simple and efficient method based on spatial filtering for parallel photodiode detection of multiple traps [1,2,3], this technique enables fast and accurate 3D force and distance detection of multiple objects simultaneously manipulated by multiple-beam optical tweezers. The talk will also present novel bio-applications of this technique, for instance how multiple traps in connection with confocal microscopy can be used to investigate the forces exerted by rotating actin containing filopodia from cancer cells [4], and how multiple traps can be used to manipulate vesicles and gold nanoparticles and perform a plasmon mediated fusion of selected vesicles.

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Fabricating Microscopic Tools for Imaging, Force Sensing and Actuation

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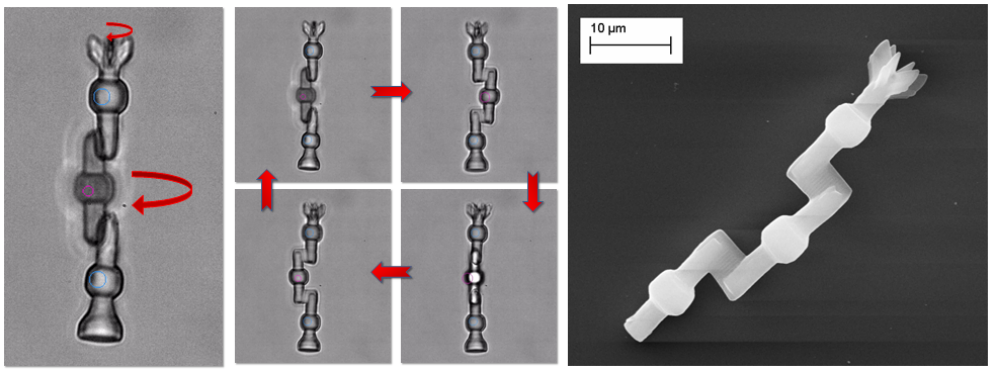
KEY WORDS: Direct laser writing, holographic optical tweezers, micro-robotics, scanning probe microscopy.

Direct laser writing is a powerful and flexible tool with which to create 3D micro-scale structures with nanoscale features. These structures can then be dispersed in aqueous media and dynamically actuated in three dimensions using optical tweezers[1,2]. The ability to build, actuate and precisely measure the motion of complex microscopic structures heralds a variety of new applications - optically actuated micro-robotics.

In this presentation I will describe how these devices are designed, fabricated and controlled. Once trapped, we are able to accurately measure[3] the translational and rotational Brownian motion of the structures in real-time (at up to 2kHz) in three dimensions using high-speed video stereomicroscopy. This enables their motion to be controlled automatically using feedback[4,5] transforming the structures into quantitative tools[6]. I will discuss a range of applications, including the imaging of surface topography inside a sealed micro-fluidic chamber[7,8], and work towards the controlled rotation of cells about an arbitrary axis.



Optical (left and middle) and Scanning Electron Microscope (right) images of a structure designed so that it can be rotated about an axis orthogonal to the optical axis of the trapping beams. It is held using three optical traps, one centered on each spherical handle.

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Holographic fabrication of 3D microstructures for bacteria-assisted delivery of colloidal cargoes

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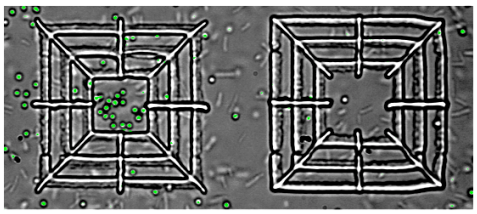
KEY WORDS: bacterial motility, colloidal transport, 3D microfabrication

The possibility of exploiting motile microorganisms as tiny propellers represents a fascinating strategy for the transport of colloidal cargoes. However, delivery on target sites usually requires external control fields to steer propellers and trigger cargo release. The need for a constant feedback mechanism prevents the design of compact devices where biopropellers could perform their tasks autonomously. Here we show that holographically designed 3D microstructures can define accumulation areas where bacteria spontaneously and efficiently store colloidal beads[1]. The process is stochastic in nature and results from the rectifying action of an asymmetric energy landscape over the fluctuating forces arising from collisions with swimming bacteria. As a result, the concentration of colloids over target areas can be strongly increased or depleted according to the topography of the surrounding structures. We show that it is possible to define a inhomogeneous effective temperature that is reduced by the local value of the external force. As a consequence, transitions rates over energy barriers with asymmetric slopes can be unbalanced[2]. Besides the significance to technological applications, our results provide new insights into the structure of stationary probability distributions in active systems.



Swimming bacteria deliver colloidal tracers (in green) inside collecting structures (left) while depleting colloidal concentration over structures of opposite simmetry (right).

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Force detection on non-spherical samples with holographic optical tweezers

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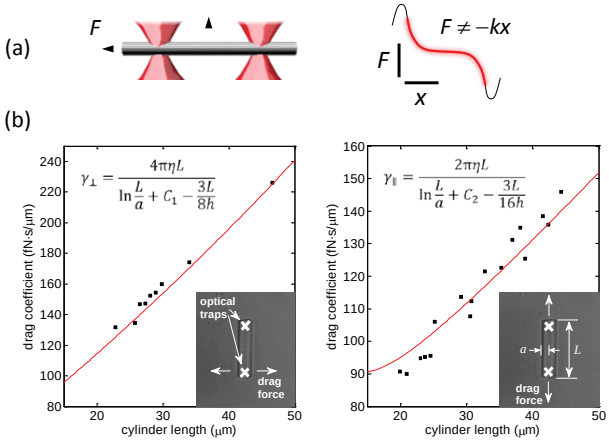
KEY WORDS: holographic optical tweezers, force measurement, irregular samples

Force measurements based on trap stiffness calibration determine the force through an indirect route, by assuming a linear relation with the sample position. Alternatively, force can be assessed directly from the changes in the light momentum of the trapping beam, with no need of in situ calibration; additionally, measurements are not restricted to certain properties of the sample or the surrounding medium: spherical particles, homogeneous medium, Gaussian beams, etc. Initially conceived for the counter-propagating beam geometry, we recently showed the combination of the direct force method with optical tweezers[1,2]. This arrangement easily allows the use of other technologies such as spatial light modulators or acousto-optic deflectors for the generation of multiple traps or exotic beams.

We show that force measurements are compatible with holographic optical tweezers by simultaneously applying a force to multiple holographically-trapped micro-beads through a constant flow. We are also able to satisfactorily measure forces exerted on non-spherical objects such as micro-cylinders, held by two permanent traps, in order to assess their transversal and longitudinal drag coefficients[3]. In conclusion, momentum measurements permit the determination of forces when trap stiffness calibration is not possible, as with irregular beams or samples.



(a) Cylindrical samples are held with two holographic optical traps and drag forces are applied to them in the transversal and longitudinal directions. In these cases, the relationship between the force and the displacement of the particles is generally non-linear and force measurement methods based on stiffness calibration cannot be used. (b) We measured the force from light momentum changes to assess the transversal (left) and longitudinal (right) drag coefficients for several cylinders of varying lengths. Red lines correspond to the theoretical hydrodynamic model described by the equations.

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Direct measurement of axial optical forces

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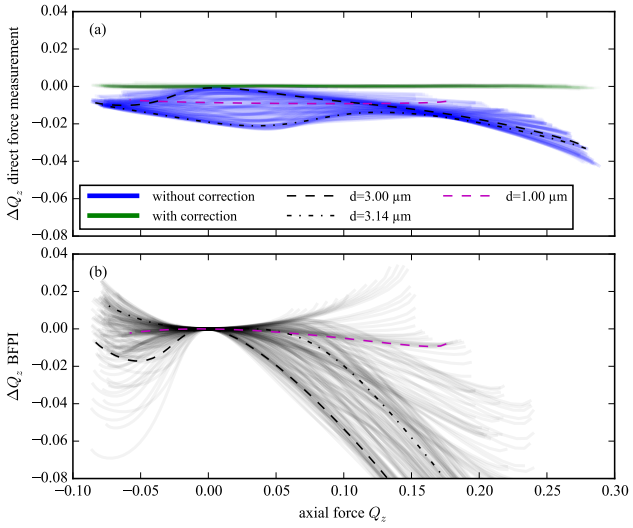
KEY WORDS: force measurement, holographic optical tweezers, photonic force microscopy

Measuring forces on the micro-scale with the aid of optically trapped particles is a valuable tool that provides insight to mechanical properties and processes taking place, e.g., in cells, micro-organisms or on the molecular level. Directly measuring the optical force based on the change of momentum of the in- and outgoing light is independent of particle size, shape, and beam shape and thus overcomes many of the limitations of force measurements based on position measurements, which require a frequent calibration.

We validated the achievable accuracy for direct force measurements in the axial direction for a single beam optical tweezers setup, based on numerical simulations and experimental investigations of situations, where the true force is known. We find that for typical experimental situations a good accuracy with an error of less than 1% of the maximum force can be achieved, independent of particle size or refractive index, provided that the total amount of light scattered in the backward direction is also taken into account, which is easy to accomplish experimentally. Due to the inherent particle shape independence of the direct force measurement method, these findings support that this method provides accurate results for 3D force measurements for particles of arbitrary shape.



Numerical simulation of the achievable accuracy for axial force measurements (polystyrene beads with various sizes from 0.5 – 4 µm). (a) Omitting the light scattered in the backward direction leads to a significant error, which can be strongly reduced by also taking into account the *total* amount of backward scattered light. (b) Error for force measurements based on position measurements by back focal plane interferometry (BFPI), assuming a linear relationship between force and BFPI signal.

Mapping Mechanical Properties of the Extra Cellular Matrix Surrounding Cells Cultured in 3D

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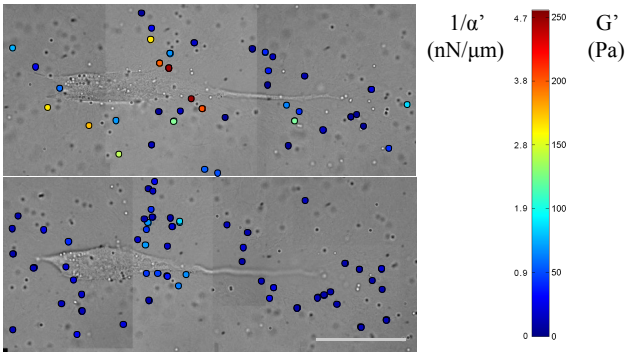
KEY WORDS: Engineered tissue, 3D live cell culture, stiffness measurement, optical tweezers, microrheology.

There is a preponderance of evidence for roles of bulk stromal stiffness in cell regulation, but little is about the pericellular mechanical microenvironment. To investigate these roles we culture cells within 3D extracellular matrix (ECM) hydrogels. We implement automated optical tweezers active microrheology (aAMR) to probe extracellular stiffness and map it in the volume surrounding cells. Our aAMR uses optical forces acting on microbeads to measure the complex response function of the ECM surrounding each bead. Many can be probed in one hour to map the mechanical landscape and to seek correlations between local stiffness and cell properties such as contractility, signaling and differentiation. For example, we will present our study of primary human aortic smooth muscle cells (AoSMCs) cultured within a type 1 collagen hydrogel. Typical stiffness (real component of the shear modulus) ranges from over 500 Pa to less than 20 Pa around each cell. We hypothesize this order-of-magnitude variability in stiffness is mediated by cell contractile forces acting on the non linear matrix material. To test this, we treated cells with inhibitors of contractility such as blebbistatin or the ROCK Inhibitor Y-27632 and observed cell relaxation coincident with a distribution of softer values ranging from 100 to 10 Pa. We previously reported force-dependent stiffness around primary mesenchymal stem cells and invasive capillaries, however in those studies approximately 10 minutes were required per bead measurement. In contrast, our aAMR only requires 10 to 30 seconds depending on the parameters of the frequency sweep. In summary, aAMR is a new research tool for studying the interplay between viscoelastic properties, forces and signaling at the ECM-cell interface.



Pericellular stiffness surrounding a human Aortic Smooth Muscle cell is measured by automated optical tweezers active microrheology. The cell is cultured within a type 1 collagen hydrogel containing 2 micron diameter polystyrene beads. Before *(top)* and after *(bottom)* the addition of an inhibitor to cell contractile forces. Force-mediated stiffening is observed. *Scale bar = 50 μm.*

Particle Trapping, Optomechanics and Chirality in Microstructured Optical Glass Fibres

P. St.J. Russell, A. Butsch, D. S. Bykov, T. G. Euser, J. R. Koehler, R. E. Noskov, G. K. L. Wong and X. M. Xi

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KEY WORDS: Optomechanics, orbital angular momentum, laser trapping & delivery of particles

Recently a special PCF with a three-bladed propeller-like core, when twisted, was shown to support a pair of helical Bloch modes whose dominant azimuthal harmonics have orbital angular momentum order $\ell = \pm 1$ [1]. Unlike in untwisted fibres, the spatially periodic factor describing the Bloch field spins at the same rate as the fibre twist. The two Bloch modes have opposite signs of azimuthal group velocity and are non-degenerate, i.e., their axial propagation constants are different. This means that the twisted "propeller PCF" preserves the chirality of the OAM, i.e., it inhibits scattering between an order +1 mode and an order –1 mode. Since twisted PCF can readily be produced in a fiber drawing tower, the OAM order can be transmitted undistorted over long distances. Also, inhibition of scattering between $\ell = \pm 1$ modes means that the twisted fibre acts like a topological insulator[2].

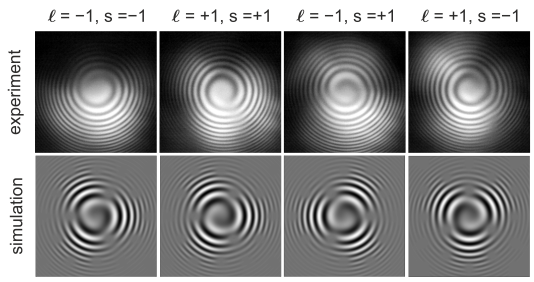

Figure 1: Interference patterns transmitted by twisted PCF, created by interference with Gaussian beam. The OAM sign is preserved [1].

In the field of optomechanics, giant optomechanical nonlinearities have been measured in a unique capillary fibre containing two parallel nano-webs (i.e., membranes) of glass. This structure, when evacuated to μbar pressures, undergoes noise-seeded Raman-like oscillations when pumped with only a few mW of CW light, resulting in generation of an optical frequency comb spaced by ~6 MHz: it behaves like an artificial Raman-active "molecule" [3].

Hollow-core photonic crystal fibre has been shown to be effective for optically trapping and propelling micron-sized particles over long distances. If charged, such a "flying particle" allows electric field mapping over long distances and is suitable for otherwise inaccessible or harsh environments[4]. Recently it was shown that a coherent superposition of forward-propagating LP_{01} and LP_{11} modes, balanced by a backward-propagating LP_{01} mode, can be used to create a series of trapping positions spaced by half the LP_{01}/LP_{11} beat-length (~1 mm). A trapped microparticle could be moved to and fro along the fibre by varying the relative LP_{01}/LP_{11} phase. This mode-based "optical conveyor belt" combines long range transport of microparticles with a positional accuracy of about 1 μm [5].

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Surface plasmon resonance spectroscopy combined with optical tweezers for nanoscale sensing applications

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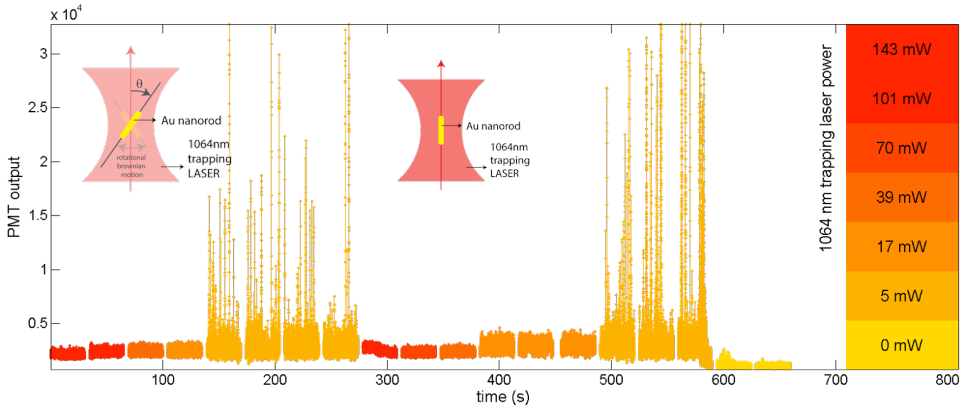
KEY WORDS: metallic nanoparticles, localized surface plasmon spectroscopy, optical tweezers, Brownian dynamics

Localised surface plasmon resonances (LSPR) are coherent oscillations of surface conduction electrons and are responsible for local optical field enhancements and strong wavelength dependent scattering in metallic nanoparticles. The peak wavelength, shape and intensity of the scattering spectra of metallic nanoparticles thus depend on several factors including the size, shape, orientation, and optical constants, as well as the surrounding dielectric environment. In this study we will explore the possibility of using plasmonic effects to improve optical trapping at the nanoscale as well provide new modes of sensing for particle dynamics through LSPR spectroscopy.



Time resolved fluctuations in the scattering intensity of an optically trapped Au nanorod for different trapping powers. Large fluctuations in the intensity at low trapping powers are associated with reorientation of the rod in the trap.

Non-conservative acoustic manipulation

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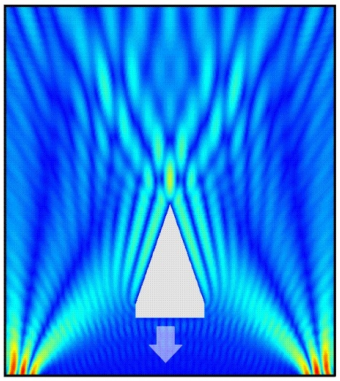
KEY WORDS: acoustic manipulation, non-conservative forces, orbital angular momentum, tractor beams

The ability of waves to carry momentum has been behind many breakthroughs in physics, including the understanding of the fundamental nature of light and the development of quantum mechanics. This momentum-carrying ability has allowed many different kinds of waves to be used to manipulate matter, with analogous experiments, and accompanying descriptions, being possible through mechanisms as diverse as optics and ultrasonics. In two recent experiments we have shown the use of non-conservative force transduction in acoustic fields to transfer angular momentum and to produce an attractive force *against* a net momentum flux [1,2].

Despite fundamentally different physical natures and wavelengths, highly analogous acoustic and optical manipulation experiments are possible due to equivalent separation of effects into gradient force, radiation pressure and angular momentum. Due to its scalar nature, acoustic waves are not able to carry spin angular momentum, but we have shown that it is possible to measurably transfer orbital angular momentum from an acoustic beam to even macroscopic objects, simultaneously making use of two non-conservative mechanisms: levitation by acoustic radiation pressure and rotation by transfer of azimuthal momentum components [1].

Most commonly in optical and acoustic trapping, gradient-induced trapping mechanisms, such as optical tweezers or ultrasonic standing waves, are what are used to manipulate matter. In such traps, it is reasonable to describe the situation in terms of potential energy landscapes, an approach that has been extended to applications such as cell sorting [3]. However, most particularly in the ultrasonic manipulation community it is conventional (since 1934 even [4]) to refer to the forces, even in a standing wave gradient trap, as radiation forces. Even if the correct form of Gorkov's equations [5] are used, this *lax* use of terminology can lead to significant confusion.



Acoustic pressure field which results in an attractive force (arrow) towards the acoustic source.

In a recent publication we have set out an experiment which helps to clarify the distinction between the role of conservative and non-conservative forces, by first arguing that the concept of a "tractor beam" is one which requires a non-conservative force, then by demonstrating the attractive force produced in such an arrangement, even against a net momentum flux.

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STED nanoscopy combined with optical tweezers reveals protein dynamics on densely covered DNA

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Dense coverage of DNA by proteins is a ubiquitous feature of cellular processes such as DNA organization, replication, and repair. We present a single-molecule approach capable of visualizing individual DNA-binding proteins on densely covered DNA and in presence of high protein concentrations. Our approach combines optical tweezers with multicolor confocal and STED fluorescence microscopy. Proteins on DNA are visualized at a resolution of 50 nm, a 6-fold resolution improvement over confocal microscopy. High temporal resolution (<50 ms) is ensured by fast one-dimensional beam scanning. Individual trajectories of proteins translocating on DNA can thus be distinguished and tracked with enhanced precision. We demonstrate our multimodal approach by visualizing the assembly of dense nucleoprotein filaments with unprecedented spatial resolution in real time. Experimental access to the force-dependent kinetics and motility of DNA-associating proteins at biologically relevant protein densities is essential for linking idealized *in vitro* experiments with the *in vivo* situation.

Optical Forces in "Slow-" and "Fast-Light" Media

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KEY WORDS: optical forces, optical trapping, phase and group velocity

Recent work has shown that the strength of optical forces exerted on a particle immersed in a dielectric background medium depends, under most circumstances, on both the refractive index and the group index of both the particle and of the background material.[1] This circumstance allows laboratory control of the strength of optical forces, with potentially important implications for optical trapping and micromanipulation.

As a first experimental study of such light-induced forces we have investigated the effect of dispersion on the mechanical dragging of light. When light enters a non-dispersive medium with refractive index n, its phase velocity decreases to c/n. If one now moves the medium with speed v in the direction of propagation, light is dragged with the medium and its speed with respect to the lab frame increases. This velocity is given by relativistic addition of the two velocities: v and c/n. In this case, one cannot increase the speed of light by more than the speed of the medium. Also, the maximum speed is limited by the speed of light in vacuum, c, no matter how large the refractive index is and how fast one moves the medium. However, by using a highly dispersive medium, one can exceed these limits. In fact, the effect of dispersion in light dragging is a manifestation of the Doppler effect.

We used rubidium gas, confined in a cylindrical cell, as the dispersive material. By heating the rubidium cell and tuning the laser at a particular point in the transmission spectrum of the D2 line, we achieved a large group index of $n_g = 160$. Through this mechanism we were able to increase or decrease speed of light by 160v, depending on the direction of motion. Here, the speed v of motion for the rubidium cell is 1 m/s. To the best of our knowledge, this is the largest dragging effect ever reported. In the future we plan to use electromagnetic induced transparency to obtain a much larger group index. Then, by dragging photons in opposite directions, we should be able to reduce speed of light by a huge amount and eventually to stop it. An important point about our experiment is that we are using a cw laser and reducing phase velocity of light rather than its group velocity, which many groups in the world are working on that.

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About fast optical traps and fast probe fluctuations in fast biological processes

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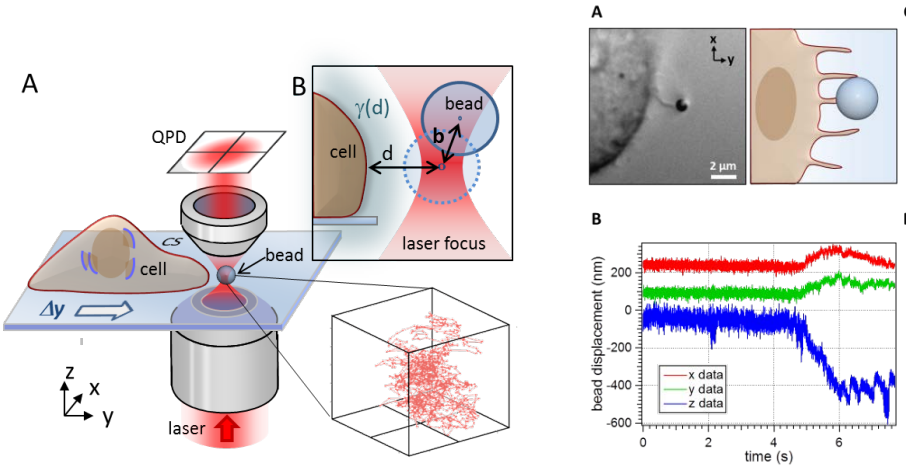
KEY WORDS: Photonic Force Microscopy, optical line traps, particle tracking, particle coupling, thermal fluctuations, helical bacteria, molecular motor transport, local viscosities.

Thermal energy fluctuations resulting in Brownian motion of molecules and particles is the basis for all kinds of cellular processes. However, fluctuations in position and orientation often are the faster, the smaller they are. This requires fast optical manipulation and tracking technology, which is in our case enabled by dynamic optical traps and 3D back focal plane interferometry.
In this talk I will present and discuss some applications of particles interacting with their local environment. This includes long-range coupling of particles to the cell membrane, hydrodynamic and plasmonic particle coupling in a single optical potential, and, a quickly deforming bacterium, which can be modeled as a chain of softly interacting particles. In all examples important information is extracted from fast correlated processes on a very small scale.



Fast 3D position tracking of trapped particles nearby cell membranes.

Optical Tweezers for Local Stimulation of Neuronal Cells *in vitro*

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KEY WORDS: optical manipulation, local drug delivery, live neurons, liposomes

Local stimulation and probing of neurons *in vitro* helps to mimic and understand the interaction between neurons with their microenvironment at single cell level. Based on Optical Tweezers Microscopy (OTM) we have developed a novel approach to release active molecules to a specific compartment of the neuron. Here we discuss the principle and the potential applications of the OTM technique to single cell studies in general.

OTM allows trapping and manipulation, with sub-micrometric precision, of a wide range of particles (silica/latex beads, liposomes, biodegradable polymer beads, quantum dots, nanoparticles) in physiological environment. These particles can be easily functionalized to carry almost any type of molecules, proteins included, thus covering a wide range of applications. Moreover, since the manipulation is operated on an optical microscope platform, high spatial-resolution phase contrast or/and fluorescence imaging (TIRF, FRET) can be run in parallel to optical manipulation. We will discuss experimental examples using OTM, their advantages and limitations. Since molecular gradients have a great importance for neuronal stimulation, a particular attention will be given to the possibility to control the number of the delivered molecules and their distribution in space and time.

Using OTM and microbeads functionalized with Brain-Derived Neurotrophic Factor (BDNF) as stimulus, or Bovine Serum Album (BSA) as control, we positioned the microbeads on dendrites at a specific distance from the cell body. We showed that a single bead was able to activate the BDNF receptor TrkB, inducing its phosphorylation. BDNF beads induced also c-Fos translocation into the nucleus while control beads did not, indicating that the whole pathway was activated. Moreover, we found that the BDNF beads stimulate an increase of the Calcium level both in the cell body and in the stimulated dendrite. By positioning the beads in the proximity of the growth cones (GC) we observed that BDNF beads significantly influenced the development of these structures. Finally, since the protein was tightly attached to the bead and this could not pass through the neuronal membrane, we concluded that BDNF not necessarily needs to be endocyted to trigger the neuronal pathway.

Liposomes represent another example of vectors carrying active molecules. In our experiments we used micron size liposomes to encapsulate guidance molecules as Sema3A and Netrin-1. Liposomes were trapped and manipulated near the GC of hippocampal neurons and the molecules released by liposome membrane photolysis and then we observed the morphological changes induced by the released molecules to the GC. To determine the speed of action and efficiency of these guidance cues we developed an experimental procedure to deliver controlled amounts of these molecules. Liposomes encapsulating 10–104 molecules of Sema3A or Netrin-1 were manipulated with high spatial and temporal resolution by optical tweezers and their photolysis triggered by laser pulses. Guidance molecules released from the liposomes diffused and reached the GC membrane in a few seconds. Following their arrival, GCs retracted or grew in 20–120 s. By determining the number of guidance molecules trapped inside vesicles and estimating the fraction of guidance molecules reaching the GC, we showed that much less Netrin-1 molecules reaching the GC were necessary to induce growth than Sema3A molecules to induce retraction.

Acoustic Force Spectroscopy

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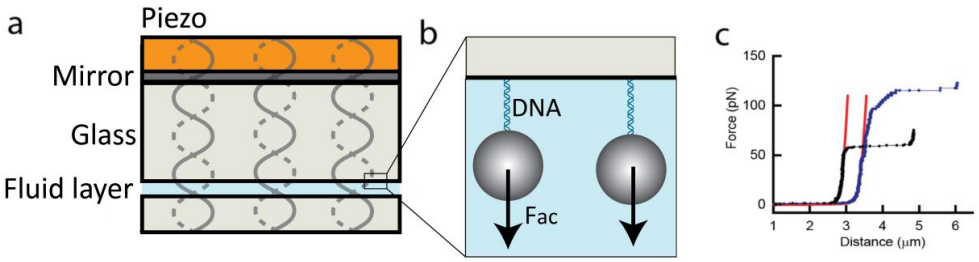
KEY WORDS: Force spectroscopy, Single molecule, DNA, Acoustic manipulation

Force-spectroscopy has become an indispensable tool to unravel the structural and mechanochemical properties of biomolecules. Here we extend the force-spectroscopy toolbox with an acoustic manipulation device, *Acoustic Force Spectroscopy* (AFS), which consists of a resonator integrated into a micro-fabricated fluidic chip. An acoustical pressure gradient is created homogeneously throughout the sample enabling to exert forces on DNA-tethered microspheres. By changing the amplitude of the driving voltage the pressure gradient can be altered, allowing sensitive control of the force applied to the DNA molecules. This approach allows exerting acoustic forces from sub-pN to hundreds of pN applied to thousands of biomolecules in parallel, with sub-millisecond response time and inherent stability. We demonstrate that AFS is an accurate single-molecule technique providing insight into protein-DNA and protein-protein interactions by force-extension, constant-force and dynamic force spectroscopy measurements AFS can be readily integrated in lab-on-a-chip devices, allowing cost-effective and massively parallel applications.



a) The AFS device consists of two glass plates with a fluid chamber in between. For illumination purposes, the upper glass slide has a sputtered mirroring aluminum layer on top. The piezo plate is glued on top of the mirror and driven by an oscilationg current creating a standing wave over the fluidic chip. b) DNA tethered microspheres experience a force directed to the closest node of the standing acoustic wave. c) A typical force distance measurement performed on torsionally unconstrained (black data) and torsionally constrained (blue data) DNA showing clearly that AFS is able to overstretch both molecules, the unconstraint molecule overstretches at approximately 65 pN and the constraint molecule overstretches at approximately 110 pN. The red lines are fits to the data using the extensible worm-like chain model.

Thermooptical Broom for Rapid Biomolecular Accumulation

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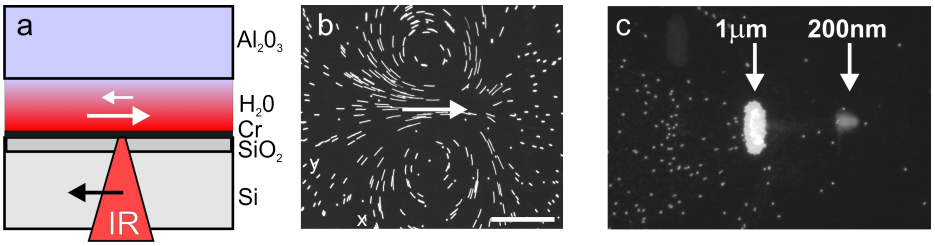
KEY WORDS: thermophoresis, optothermal pumping, thermal trap

Thermophoretic force moves molecules and particles along temperature gradients and builds typically a weak concentration gradient[1]. In order to enhance the concentration for a few orders of magnitude, i.e. to trap molecules, thermophoresis has to be combined with fluid flow[2]. We show that heating by repeated unidirectional movement of elongated laser spot gives rise to a strong thermophoretic trapping and additionally induces a global fluid flow that efficiently "feeds" the trap. Such thermal broom can deplete micro particles and molecules from large surrounding region and accumulate them within seconds into 10 µm small spot.

We demonstrate the thermal trapping by 60-fold accumulation of 5.8 kbps dsDNA and by achieving close-packed arrangement of colloidal particles from an initial dilute suspension. Because trapping efficiency strongly depends on thermophoretic properties of particles it is possible to tune the trapping regime so that only certain type of particles is trapped. As an application, we show accumulation and spatial separation of a binary colloidal mixture.



a) Chamber cross-section. b) Fluid flow visualized by 1 µm fluorescent tracers. c) In-situ spatial separation of 200 nm and 1 µm beads.

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Dielectrophoretic and photophoretic control of liquids

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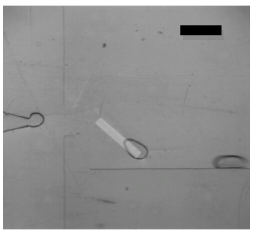
KEY WORDS: optofluidics, dielectrophoresis, droplets, photophoresis

The control of fluids by light has from the beginning been a vital part of the novel field of optofluidics and can be regarded as a key technology to the realization of integrated Lab-on-a-Chip devices. While optical tweezers have been shown to be able to handle transparent aerosols, different techniques must be developed for absorbing or partly absorbing liquids [1].

In this contribution, we present two novel techniques to control liquids based on light induced phoresis. In the first example, photophoresis is employed, describing the movement of particles in a thermal gradient. Therefore, this technique is especially suited for absorbing media, where the thermal gradient is created all-optically [2]. Using the repelling forces of a high intensity light sheet, interaction of absorbing airborne droplets with light was observed for the first time, paving the way towards a new microscopic toolset for the control of absorbing droplets.

Light-induced dielectrophoresis on the other hand allows for the combination of high throughput with optical control [3]. An incident light pattern is converted into a highly-modulated electric field by a photovoltaic lithium niobate crystal. We present the integration of this functional surface into a PDMS-made droplet generator device. Depending on the electrical properties of the droplets created, the electric field gradients act either repelling or attracting, thereby enabling the construction of *virtual rails* on which droplets are traveling as well as *virtual barriers*, by which they are deflected. This effect can be utilized for droplet sorting, splitting or merging, where the geometry of the underlying network is solely created by light.



Droplet routing on an attracting virtual rail (indicated by white bar); droplets are created by Novec 7300 flowing in tetradecane. Flow direction is from left to right, black scale bar represents 300 µm.

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Soft matter based chiral optomechanics

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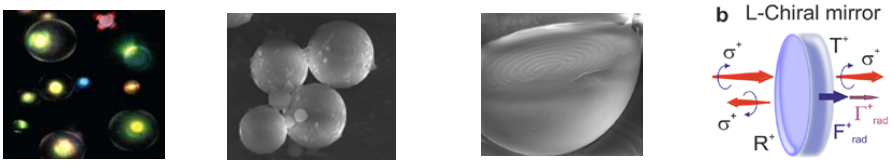
KEY WORDS: Chiral microparticles, optomechanics, optical force and torque

Soft matter based chiral optomechanics has been investigated exploiting solid chiral microparticles. These particles have been developed exploiting liquid crystals water emulsions. Micrometer-sized droplets of a photo-polymerizable cholesteric liquid crystal (CLC), based on a nematic reactive mesogen doped with a chiral agent, are first produced in water. Irradiation with UV light results in chiral solid micro-particles preserving both the shape of the precursor droplets and their internal self organised supramolecular arrangement[1]. Because of the chiral internal structures of the micro-particles, unique capabilities have been observed in optical manipulations experiments addressing novel issues for chiral optomechanics and microphotonics[2].

Depending on the internal geometry, chiral microparticles work as chiral microresonators. The inner Bragg structure of the particle and the corresponding selective reflection for light wavelength in the stop band, allows spin angular momentum transfer only from the light component having spin parallel to the particle handedness. Optomechanical effects originating from the coupling of transferred linear and angular momentum mediated by the particle chirality have been investigated. Their control by means of the particle reflectance and light spin enables to observe unique behavior.

Acknowledgements: MPNS COST Action 1205 "Advances in Optofluidics: Integration of Optical Control and Photonics with Microfluidics".



a) Optical microscope and SEM images of chiral microparticles. b) Chiral optomechanics

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Conditions of Rotaion Driven by Light with no Angular Momentum

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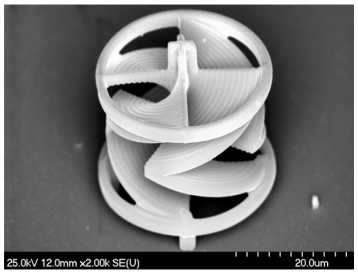
KEY WORDS: light driven rotation, photopolymerization

Light-induced rotation is an interesting phenomenon with important applications. In this work we determine the conditions for rotation induced by collimated light carrying no angular momentum. As opposed to the well discussed arrangement where the rotation axis is parallel to the illumination axis (in analogy with a propeller or windmill), here we explore the geometry in which the rotation axis is perpendicular to the direction of the propagation of light. We studied the rotation of objects of different shapes and optical properties under uniform illumination. This arrangement may be important for practical purposes: rotors could be arranged to roll in arbitrary directions as long as their rotation axes are in a plane perpendicular to the direction of a single shared light beam (e.g. robots moving in independent directions on a flat surface could be supplied with energy by the same light source illuminating the area of motion perpendicularly from above – similar to the non-rotating wedge shaped particles introduced recently[1]).

We will discuss the conditions of rotation concerning both the geometry and the material of the rotor. A general rule is formulated about the properties of the light scattering process to maintain sustained rotation.



Rotor built by photopolymerization capable of light driven rotation.

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Optical manipulation of neuronal function

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KEY WORDS: Live cells, holographic projection, two-photon microscopy, dendrotomy

Light is now an indispensable tool among neuroscientists, particularly for visualisation and characterisation of cells/proteins as well as temporal functional imaging. Within fundamental optical limits, imaging using the visible and near-infrared electromagnetic spectrum provides a good spatial range for studying single neuronal cells up to interconnected neurons in a circuit. Here, we describe two light-based techniques to elucidate how neurons process inputs and yield an output. First, we describe our technique that uses patterned light to photo-induce synaptic inputs and analyze how the spatio-temporal organization of these inputs cause the neuron to fire an output [1]. We use a dynamically programmable hologram to produce 3D light patterns that can induce targeted and highly localized synaptic inputs along the dendritic tree of a neuron. **Fig. 1.(a-d)** show the response of a single neuron with five (5) inputs that are randomly activated in time. The output of the neuron is described as a voltage spike (or action potential) where our data shows a minimum of three (3) simultaneous inputs for the neuron to fire an output. Second, we show how we use light as an ultra-sharp scalpel to cut dendrites. Our technique uses non-linear light-tissue interaction to dynamically prune the neuron's dendritic tree [2]. **Fig. 1.(e-g)** show successful laser dendrotomy of a neuron's main apical dendrite. The neuron's output and overall function in a neuronal circuit are said to be dependent on the spatial extent of its dendritic tree. Our pre- and post-cut characterisations of the neuron's output firing characteristics confirm numerically predicted changes in the neuron's output firing pattern. As such, highly targeted laser dendrotomy allows us to study morphology-dependent neuronal function. These experiments demonstrate how we can optically manipulate neuronal function to aid our understanding of how the brain works.

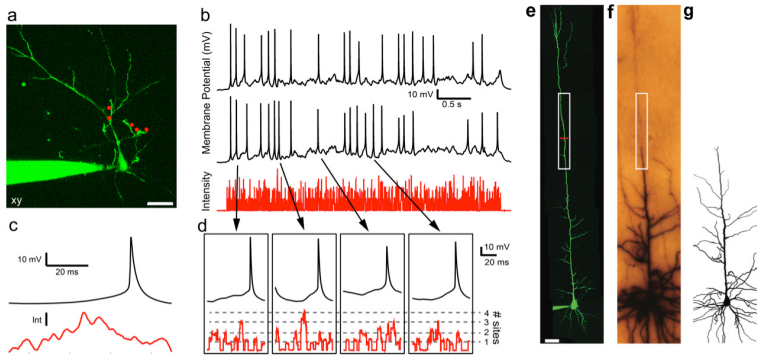

Figure 1. (**a**) Four-dimensional photostimulation of neurons with five (5) photo-induced spatially distinct synaptic inputs. (**b**) The spatio-temporal synaptic input pattern induces neuronal firing of action potentials plotted as a voltage response of the neuron as a function of time (**c**) Average input/output response taken from traces in (**b**). (**d**)Magnified trace of selected action potentials in **b**. (**e**) Dendrotomy of a dendrite of a neuron. Scale bar 50 µm. (**f**) Bright-field image of biocytin stain after histology with the same boxed region in a indicated. (**g**) Neuronal reconstruction based on **e** and **f**.

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Laser-guided AFM: Improved Single Molecule Force Spectroscopy

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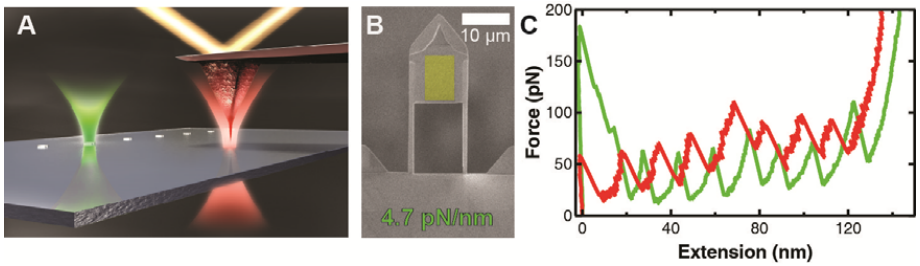
KEY WORDS: Single molecule force spectroscopy, atomic force microscopy, biophysics

A more precise and stable AFM is critical to many applications including single molecule force spectroscopy (SMFS). For instance, the equilibrium between folded and unfolded states is sensitive to sub-pN changes in F, so force stability is critical. Yet, unwanted instrumental drift leads to motion of the sample relative to the cantilever (position drift) or bending of the cantilever itself (force drift). Moreover, to resolve briefly populated folding intermediates, we sought to improve precision and stability without sacrificing temporal resolution.

By adapting ideas from optical trapping, we developed an ultrastable AFM in which the tip and the sample positions are independently measured by, and stabilized with respect to, a pair of laser foci in three dimensions (3D). These lasers establish a local reference frame that is insensitive to long-term mechanical drift of the AFM. The tip and sample's 3D positions were stabilized to ~0.3 Å (Δf = 0.01–10 Hz) at ambient conditions. Tip-sample stability, as demonstrated by imaging, showed ~4 Å lateral drift over 80 min, a 100-fold improvement.[1]

However, positional drift was only half the problem; force drift also occurs via uncontrolled deflection in the zero-force position of the cantilever. The primary source of this force drift in liquid for a popular class of soft cantilevers was their gold coating. Removing the gold led to ~10-fold reduction in reflected light. Yet there was no loss in positional precision on short-time scales (1 ms) when using soft cantilevers (6 pN/nm). A majority of these cantilevers achieved sub-pN force precision over a broad bandwidth (0.01–10 Hz).[2]

We achieved further improved short-term precision while maintaining excellent long-term stability by modifying commercially available cantilevers with a focused ion beam (FIB). Our process led to a ~10-fold reduction in both a cantilever's stiffness and its hydrodynamic drag. We also preserved the benefits of a highly reflective cantilever while mitigating gold-coating induced long-term drift by preserving a small gold patch at the end of the cantilever. As a result, we extended AFM's sub-pN bandwidth by a factor of 50 to span five decades of bandwidth ($\Delta f \approx$ 0.01–1,000 Hz). Measurements of mechanically stretching individual proteins showed improved force precision coupled with state-of-the-art force stability.[3]



(**A**) Laser-guided AFM. (**B**) Micro-machined cantilever. (**C**) Improved SMFS compared to prior advanced data.

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Fabrication and Surface Functionalization of Highly Birefringent Rutile Particles for Use in an Optical Torque Wrench

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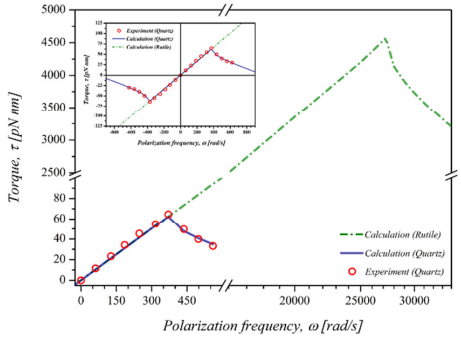
KEY WORDS: Force and torque spectroscopy, optical tweezers, biomolecules, micro/nano fabrication, surface functionalization

The optical torque wrench (OTW) allows the direct application and measurement of torque on biomolecules, such as DNA or DNA-protein complexes, or rotary motors like the F_0F_1-ATP-synthase or the bacterial flagellar motor[1]. The applicable torque of the OTW is a function of the size and birefringence of the particle[2]. Quartz has proven a convenient material, but its quite low birefringence limits full investigation of torque-speed relationships of diverse biological systems. In contrast, rutile exhibits a much higher birefringence - exceeding that of quartz by a factor of 30 - but its utilization has been infrequent because of the difficulties in optical trapping and fabrication.

To enhance the applicability of the OTW, we have improved both the design and fabrication of cylindrical rutile particles. We have employed finite element method calculations to determine the optimal dimension of stably trappable rutile cylinders. To obtain rutile cylinders with the optimal dimensions, we developed a protocol for full control of size and sidewall angle. In our fabrication protocol, a chromium etch mask provides increased resistance to dry etching and allows the fabrication of structures with both high aspect ratio and anisotropy. Also, the sidewall angle of cylinders can be readily tuned by adjusting a single process parameter, namely the oxygen flow rate during dry etching. The fabricated cylinders were characterized in the OTW setup to reveal their linear and angular trapping properties. The fabrication process is compatible with common chemical functionalization procedures and permits covalent biomolecule attachment. To enhance biomolecule coverage, we used ethanolamine and poly(ethylene glycol) as biomolecular crosslinkers to obtain homogenous and dense coatings. Our recent results, in which we use functionalized, trapped rutile cylinders to study single biomolecules and motor proteins, will be presented.



Values of torque versus input laser polarization modulation frequency, for quartz and rutile cylinders with the same size (diameter: 250 nm, height: 650 nm)

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[2] Z. Huang, et. al., *ACS Nano*, **5** (2011) 1418-1427.

Optically-driven actuators using flexible photonic crystals

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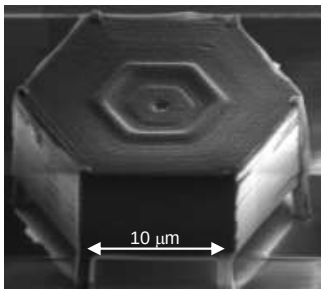
KEY WORDS: Dipole force, radiation pressure, photonic crystal, microfabrication

The optical dipole force is particularly significant for particles a fraction of a wavelength in size, at the boundary between Rayleigh and Mie scattering, where each extra atom contributes to the amplitude of the scattered field rather than its intensity. The steepest optical gradients occur with the counter-propagating beams of standing waves; and their intensities may be resonantly enhanced by the use of a photonic cavity. Optical forces should thus be at their most significant when they act upon the sub-wavelength components of a photonic crystal. Such structures resemble the free-space patterns formed by self-organization of cold atom clouds through collective atomic recoil within optical cavities[1-4].

In order to respond to the optical forces, the structure within which they occur must be free to move. We are therefore exploring the microfabrication of flexible photonic crystals by 3-D scanning multi-photon photopolymerization, using a commercial *Nanoscribe* apparatus[5] and critical-point drying to form 3-D photonic structures from materials that have optical and mechanical properties similar to *Plexiglass*.

For resonant structures, the refracting elements that shape and respond to the optical field lie at the nodes or antinodes of the optical field. For a force to result, the structures must therefore be aperiodic or illuminated off-resonance, so that the field maxima and minima are shifted relative to the structure and the field strength varies from layer to layer. In principle, with a structure whose shape or periodicity varies from place to place, different illumination wavelengths could address different spatial regions.



Early attempt to write a flexible 1-D photonic crystal by scanning multi-photon photopolymerization.

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Thermorheology of Living Cells — Impact of Temperature Variations on Cell Mechanics

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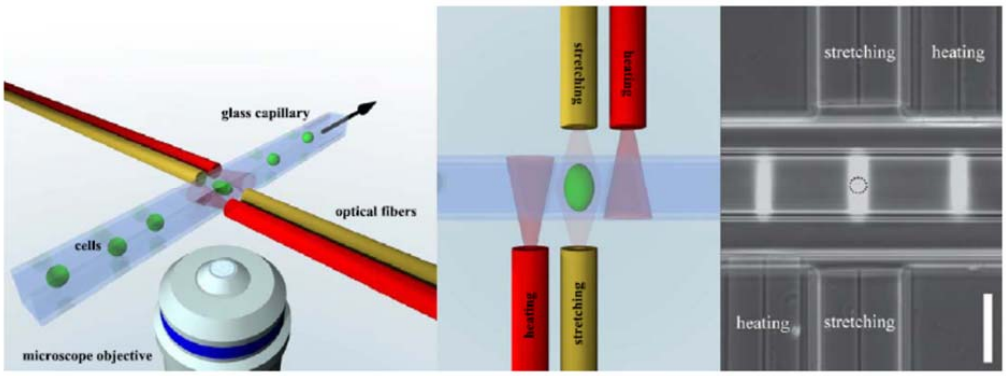
KEY WORDS: Optical traps, cell mechanics, time-temperature superposition,

Upon temperature changes, we observe a systematic shift of creep compliance curves J(t) for single living breast epithelial cells. We use a dual-beam laser trap (optical stretcher) to induce temperature jumps within milliseconds, while simultaneously measuring the mechanical response of whole cells to optical force. The cellular mechanical response was found to differ between sudden temperature changes compared to slow, long-term changes implying adaptation of cytoskeletal structure. Interpreting optically induced cell deformation as a thermorheological experiment allows us to consistently explain data on the basis of time–temperature superposition, well known from classical polymer physics. Measured time shift factors give access to the activation energy of the viscous flow of MCF-10A breast cells, which was determined to be ~80 kJ/mol. The presented measurements highlight the fundamental role that temperature plays for the deformability of cellular matter. We propose thermorheology as a powerful concept to assess the inherent material properties of living cells and to investigate cell regulatory responses upon environmental changes[1].



Modified Optical Stretcher: Two heating fibers (red) have been added to the setup.

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[1] Tobias R. Kießling, Roland Stange, Josef A. Käs, Anatol W. Fritsch, *Thermorheology of living cells—impact of temperature variations on cell mechanics*, New Journal of Physics, Volume 15, Issue 4 (2013)

Characterising Conical Refraction Tweezers

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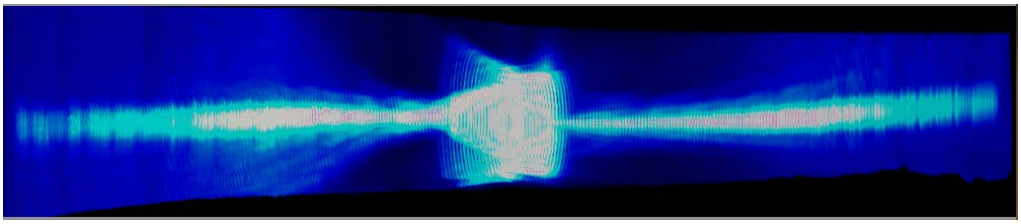
KEY WORDS: Optical Tweezers, Conical Refraction, Aerosols, Aerosol Lasers, Biofuels

Beam shaping techniques for optical manipulation are now very sophisticated, but there are still opportunities for 'natural' beam shaping to offer simple but flexible solutions that are not easily available using spatial modulation techniques. One such example is the use of biaxial crystals to form 'conically refracted' beams, which form a natural optical bottle with a controllable opening [1]. They also exhibit interesting polarization properties, and as such offer an interesting extension to the optical toolbox.

Here we examine the trapping abilities of such tweezers [2]. The beams can be characterized as having three different planes of interest: an upper and lower 'Raman' spot and an intermediate 'ring' plane called the Poggendorf plane. We find that in general the lower Raman spot and ring plane can be thought of as 3D optical tweezers, while optical levitation dominates in the upper spot, and far lower trap stiffnesses are measured. Due to the polarization properties of the beam the ring plane offer rotational control of particles.

We present data on trapped silica microspheres and some preliminary data looking at the interaction of airborne particles with such beams.

Additionally we present some other recent results on airborne tweezers from our group, in which we have demonstrated the ability to form lasers from optically trapped liquid droplets [3] and work exploring the evaporation of volatile biofuel-gasoline mixtures, and compare optical tweezers with electrodynamic balance measurements.



Optical cross section of a conically refracted beam

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Optical trapping: from the study of complex dynamical systems to spatial optical solitons

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KEY WORDS: Optical forces, ratchets, nano-suspensions, nonlinear optics, spatial solitons

Optical trapping and manipulation of microscopic objects with laser light is nowadays a well-established technique. Moreover, the possibility of tailoring light distributions with a dynamic control like interferometric traps, holographic optical tweezers, generalized phase contrast and time-sharing traps with acousto-optic modulators, for instance, have widely extended the capabilities and applications of these techniques to many scientific areas. One of them is the study of complex dynamical systems, which constitute the basis to explain the functioning of some mechanisms in nature, and can also be applied to develop practical tasks or even new devices. Such is the case of the ratchet effect, which explains the operation of some molecular motors in eukaryotic cells[1] and is also the working principle of some transport devices that allow, for example, the removal of vortices in low temperature superconductors[2]. A brief overview of our work on optical ratchets[3,4], which allow the directed transport of mesoscopic particles resulting from an unbiased external oscillating force due to a spatial symmetry breaking in the optical potential, will be presented. As the optical force and potential depend on the size of the particle with respect to the characteristic size of the light pattern, we can establish the conditions for observing simultaneous currents in opposite directions for particles of different sizes in a given light pattern and current reversals for a given particle by varying the magnitude and period of the external force.

On the other hand, when the size of the particles is well below the wavelength of the trapping beam, as in a suspension of nanoparticles, the gradient optical force causes a collective response and the medium behaves as an effective medium with a self-focusing non-linearity[5]. Although this behavior was first discovered in the early 1980's, we have revisited the subject and demonstrated the generation of (2+1)D spatial optical solitons over distances that exceed by two orders of magnitude all the previous reports. We also show their use as waveguides for a weaker probe beam[6] and study soliton interactions in different regimes leading to new effects.

Acknowledgments

This work was partially supported by DGAPA-UNAM Projects PAPIIT IN115614 and IA103615, and CONACYT Mexico, grant CB-2009-132527.

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Manipulating and displaying the matter in 3D

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A challenging step forward would be the availability of a fast, high-throughput and label-free system for the measurement of physical parameters and visualization of the 3D shape of biological specimens. We report in this talk on a quantitative imaging approach to estimate simply and quickly the biovolume of various cells, combining the optical tweezers technique with digital holography, in a single and integrated set-up for a biotechnology assay process on the lab-on-a-chip scale. This approach can open the way for fast and high-throughput analysis in microfluidic label-free "cytofluorimeters" and prognostic examination of motile cells or for detecting various blood diseases by morphology identification for RBCs, thus allowing relevant advancements in biomedical sciences.

Behaviour of Nonspherical Particles in Laser Beams

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KEY WORDS: optical force & torque, nonspherical particles, hydrodynamic synchronization

Optical trapping of a particle is based on exchange of linear momentum between incident photons and the particle which leads to an optical force acting upon the particle. In the case of a nonspherical particle, this optical interaction is inevitably enriched by the exchange of angular momentum between photons and the particle. Consequently, the optical torque orients the particle in the laser beam. However, particle orientation strongly influences the trapping force and equilibrium position of the particle in the beam and thus makes the particle behaviour more complex.

In this contribution we demonstrate how a natural nonspherical shape of gold nanoparticles enables their three-dimensional trapping even in a single focused beam with numerical aperture as low as 0.2-0.37 (see Fig. 1). In the case of larger particles of spheroidal shape, we demonstrate their rotation due to transfer of spin and orbital angular momentum. In the studied geometry the unit change of the beam topological charge causes an order of magnitude faster particle rotation in comparison to beam polarization switch from linear to circular [2]. In the case of several rotating particles (see Fig. 2) we investigate their hydrodynamic interaction leading to synchronization of their rotations.

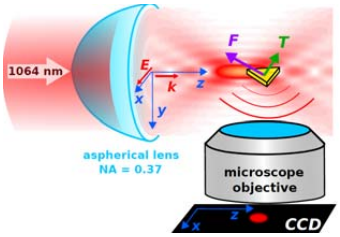

Fig. 1. A naturally shaped gold nanoparticle (yellow triangular prism) is trapped laterally on the optical axis and longitudinally slightly behind the beam focus (bright yellow spot). The longitudinal position of the NP strongly depends on its orientation with respect to the direction of beam polarization and propagation. The objective and the CCD camera were placed perpendicularly to the direction of beam propagation and image the nanoparticle as a bright spot on the dark background [1].

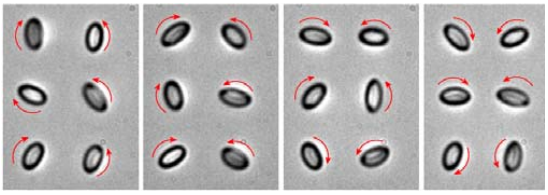

Fig. 2. Sequence of images demonstrating rotation of six oblate spheroidal polystyrene microparticles 3D trapped in six focused vortex beams. The particles in the left column rotate in the opposite direction to the particles in the right column. Each spheroid is trapped in a focused vortex beam with topological charge -2 (left column) and 2 (right column). The mean power per trap is approximately 2.5 mW. Particles volume corresponds to a sphere of radius 0.85 μm and spheroids aspect ratio is between 1.7-2.2.

The authors acknowledge support from CSF (GA14-16195S, GA14-36681G), MEYS (LD14069, LO1212) and EC (CZ.1.05/2.1.00/01.0017).

[1] O. Brzobohatý et al., *Three-dimensional optical trapping of a plasmonic nanoparticle using low numerical aperture optical tweezers*, submitted to Scientific Reports

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Force measurements with optical tweezers inside living cells

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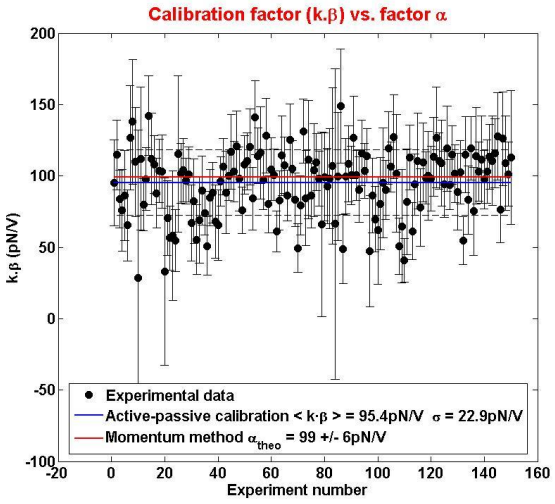
KEY WORDS: Optical trapping, optical tweezers, optical manipulation, back-focal-plane interferometry, force calibration

Forces exerted by optical tweezers can be measured by tracking the rate of momentum transfer between the trapping beam and the sample[1-3]. Although it has not been brought to its full potential yet, probably due to practical difficulties when combined with high-NA optical traps, this route shows promising to overcome some important limitations of the typical approach based on trap stiffness calibration. Using this approach, we show here the feasibility of measuring forces on arbitrary biological objects inside cells without an *in situ* calibration. The instrument can be calibrated by measuring three scaling parameters that are exclusively determined by the design of the system. The conversion factor from volts to piconewtons is thus independent of the physical properties of the sample and the surrounding medium. We prove that this factor keeps valid inside living cells through the comparison with an alternative calibration method developed in recent years for viscoelastic media. Finally, we apply momentum-based measurements to determine the stall forces of both kinesin and dynein in living A549 cells.



Experimental results show that the sensor's absolute calibration equals the conversion factor from volts to piconewtons obtained through an alternative method for viscoelastic materials.

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Optical sculpting of ultra-low interfacial tension oil-in-water emulsion droplets

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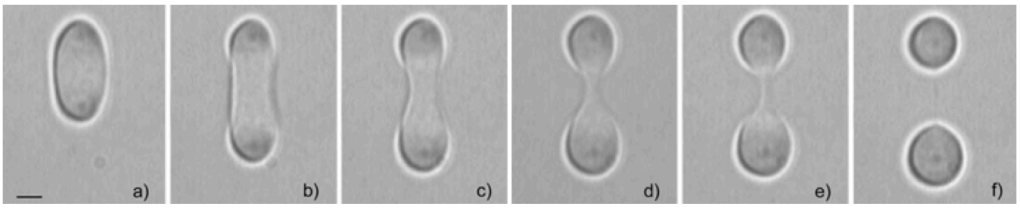
KEY WORDS: Holographic optical tweezers, optical sculpting, ultralow interfacial tension, emulsion

We have developed a novel platform for the production, optical sculpting, volumetric reconstruction and compositional anaylsis of ultra-low interfacial tension oil-in-water droplets in the 1-10 micron regime.

We discuss the optical sculpting methods used for the trapping and shaping of individual droplets and for the formation of networks of interconnected droplets. We present the results of both experimental and corresponding modeling work[1] and discuss the applications and limitations of the techniques used.



Formation of a simple network from a single emulsion droplet using two HOTs with increasing separation.

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Multimode Fibres: Seeing through chaos

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Small, fibre-based endoscopes have already improved our ability to image deep within the human body. Current fibre-based devices consist of fibre-- bundles in which individual fibres represent single pixels of the transmitted image. A novel approach introduced recently[1] utilized disordered light within a standard multimode optical fibre for lensless imaging. Importantly, this approach brought very significant reduction of the instrument's footprint to dimensions below 100μm. Such device may be used for imaging of structures deep inside living organisms directly through centimeters of living tissues without bringing about their extended collateral damage. In Neuroscience, this technology may assist to address important unanswered questions related to formation and recall of memories as well as onset and progression of severe neuronal disorders such as Alzheimer's disease.

The two most important limitations of this exciting technology are (i) the lack of bending flexibility (imaging is only possible as long as the fibre remains stationary) and (ii) high demands on computational power, making the performance of such systems slow.

We discuss routes to allow flexibility of such endoscopes by broader understanding of light transport processes within. We show that typical fibers retain highly ordered propagation of light over remarkably large distances, which allows correction operators to be introduced in imaging geometries in order to maintain high-quality performance even in such flexible micro-endoscopes.

Separately, we introduce a GPU toolbox[2] to make these technique faster and accessible to researchers. The toolbox optimizes acquisition time of the transformation matrix of the fibre by synchronous operation of CCD and SLM. Further, it uses the acquired transformation matrix retained within the GPU memory to generate any desired holographic mask for on-the-fly modulation of the output light fields. We demonstrate the functionality of the toolbox by displaying an on-demand oriented cube, at the distal end of the fibre with refresh-rate of 20ms.

References:

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Real-time Infrared Video from a Pixel

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KEY WORDS: Infrared imaging, single-pixel camera, computational imaging,

Conventionally, images are recorded on cameras utilising a pixelated optical sensor. Imaging in the visible can be performed at low-cost due to widespread demand for silicon-based sensors; meanwhile to perform imaging at non-visible wavelengths requires the use of different substrate material sensors, which come with a higher price tag. An alternative imaging approach replaces the sensor with a device capable of applying a series of spatial filters and a single-pixel detector to measure the reflected intensities. Combining knowledge of the spatial masks applied and the associated intensities allows the image to be deduced utilising a computer algorithm. In this work we demonstrate a so-called 'single-pixel camera' capable of producing continuous real-time video at visible and shortwave infrared wavelengths simultaneously. We anticipate these low-cost, non-visible imaging systems to have important applications in microscopy, night vision, gas sensing and medical imaging.

A number of closely related approaches use spatially structured illumination[123] or structured detection[45] and a single-pixel detector to deduce an image. Naturally, a trade-off exists between acquisition time and image resolution, which results from the finite display rate of the spatial light modulator. One approach relies on utilising compressed sensing[6], however the associated reconstruction times greatly exceed the acquisition time thereby prohibiting use in real-time video systems. Utilising our prototype (Fig.1a) we demonstrate an approach for increasing the spatial resolution whilst maintaining high-frame rate video by instead modifying the mirror binning (Fig.1b). Moreover we can employ an evolutionary algorithm to better select the most significant patterns for further increasing the frame-rate of real-time video of colour (Fig.1c) and shortwave infrared (SWIR) video.

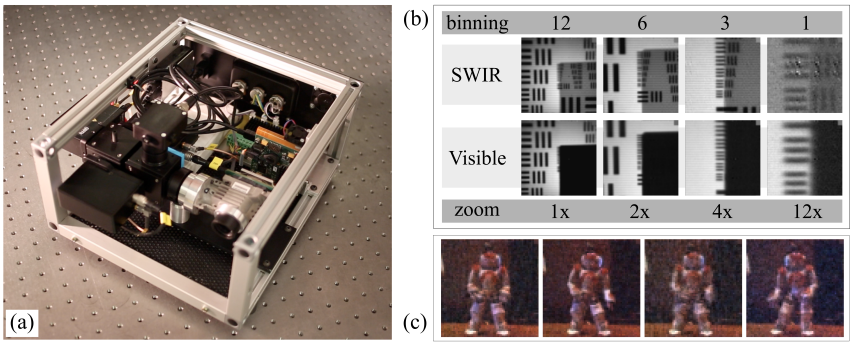

Fig.1: (a) Photograph of the single-pixel camera prototype containing a high-speed DMD, 3 spectrally filtered photomultipliers and an InGaAs photodiode. (b) Experimental demonstration of 'zoom' functionality described in text. (c) Sample of four consecutive colour video frames.

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Holographic Electron Beam Shaping

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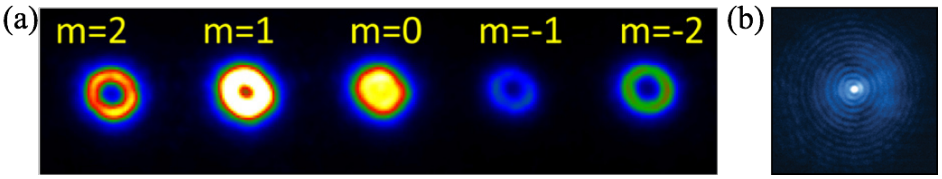
KEY WORDS: Electron beams, vortices, Bessel beams,

The wavefunction corresponding to free electrons, is a solution to the Schrödinger equation, may possess specific intensity and phase distributions. Electrons with a wavefuntion having a helical phase $exp(il\phi)$ is an interesting example. These electrons possess a magnetic moment $l\mu_B$ and an intrinsic orbital angular momentum (OAM) value of $l\hbar$ along the propagation axis, where μ_B is the electron Bohr magneton[1]. This is in addition to the spin magnetic moment, which can only take two values, $\pm\mu_B$. Therefore, such electron beams can be used to explore magnetic dichroism, a property that has not yet been fully investigated in materials science. Several techniques analogous to the optics counterparts, such as spiral-phase plate[2], amplitude mask[3], spin-to-OAM converter[4] and astigmatic lenses, have been proposed or implemented to shape the conventional electron beam into an electron beam carrying OAM. All of these techniques, however, have a low conversion efficiency. We have recently developed a novel method, which leads to a precise control of electron wavefunction' phasefront when it passes through a silicon nitride substrate [5]. Indeed, the electron phasefront is tailored by elastic electron-electron scattering in the membrane, thus it results in bright electron beam shaping. We have successfully generated a pure-phase kinoform for electron beams in the middle energy range of 200 keV with a conversion efficiency about 40%.

Our recent achievement on generating electron beams having Bessel[6], Laguerre-Gauss distributions, and electron beam with the highest OAM value[7] will be the subject of my talk.



(a) Diffracted electron beams from a purely blazed phase hologram. The achieved conversion efficiency is higher than 35%. (b) Intensity distribution of the generated electron Bessel beam of the first kind. This beam is shown to be diffraction-free for a certain region.

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Optical Angular Momentum of Light in Laser Micromanipulation and Applications

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KEY WORDS: Optical angular momentum, optical tweezers, live cells

The way light can apply forces to a microscopic object is easily understood as an exchange of momentum between the light beam and the object. This applies both to linear momentum and to angular momentum exchange. Methods based on these phenomena promise high flexibility and an opportunity for driving these objects in microfluidic devices or even inside a biological cell. Optical drive of micron scale devices promises the ability to carry out measurements and operations on microscopic systems in a flexible way. The energy that is needed can be transmitted without harm through many materials including a membrane of a cell.

The use of the angular momentum of light enables introduction of rotation of microscopic objects. Quantitative measurements of this rotation are possible through a measurement of the change of polarisation state of light after passing through the object. The transfer of the angular momentum can then be used for several applications in biology and medicine. One of such application is microrheology of complex fluids that exhibit both viscous and elastic behaviours and it has been and continues to be one of the most important subjects that can be applied to characterize the behavior of biological fluids such as cytoplasm in cells enabling quantitative studies of biophysical properties of cells. Optical tweezers and fluorescence correlation spectroscopy have been used for such studies. However, it is extremely difficult to make rapid measurement of viscoelastic properties inside living cells. Due to the complicated components in the cell, by using giant unilamellar vesicle (GUV) as a model we propose to develop a microrhometer based on optical tweezers where angular momentum of light is used resulting in rotation of the probe. Using this method we investigate the viscoelastic properties of the fluid inside liposome.

Determination of Colloidal Osmotic Equilibrium of State by Optical Trapping

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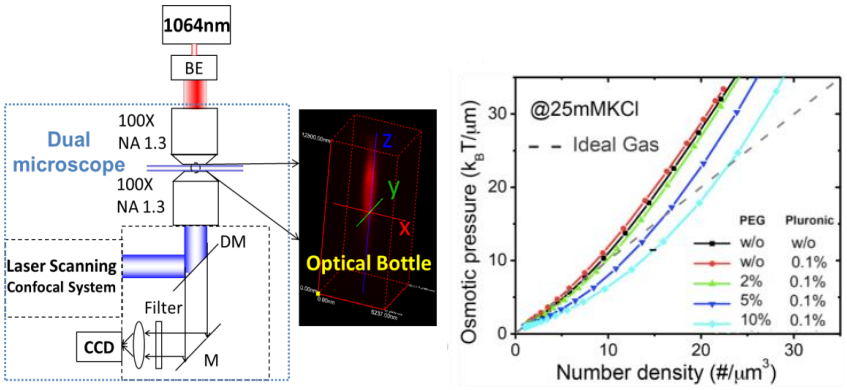
KEY WORDS: Optical bottle, colloidal osmotic equation of state.

Colloidal particles in suspension reach an equilibrium density distribution under a potential force. The shape of the particle number density profile depends on the external force and the colloidal interactions between the particles.

This paper reports a study that uses optical gradient force to create number density distributions of colloidal nanoparticles transiently confined in an optical trap, i.e., the optical bottle. Laser scanning confocal fluorescence microscopy is used to image the number density distribution of 100 nm fluorescent polystyrene particles in an IR optical trap, shown in the figure below. From the Boltzmann distribution of the particle number density of a dilute suspension of nanoparticles in the optical trap, the potential energy landscape of the nanoparticles in the optical trap can be determined [1]. From the known potential force and the particle number density distribution of interacting colloids, the osmotic pressure as a function of particle number density, i.e., the osmotic equation of state of the colloids can be calculated using the equation of osmotic equilibrium derived by Einstein in his 1905 paper on Brownian motion [2]. Osmotic equation of state and chemical potentials of charge stabilized colloidal particles interact by the screened Coulomb repulsion and by the polymer-induced depletion attraction, shown in the figure below, will be discussed in this paper.



Experimental setup (left); 3D image of optically trapped nanoparticles (center); equation of state (right).

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Glycolytic oscillations in single yeast cells

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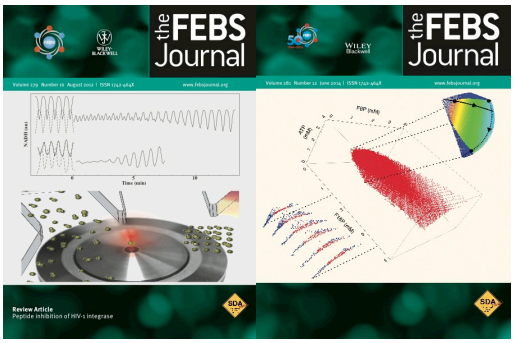
KEY WORDS: Glycolytic oscillations, live cells, optical tweezers, microfluidics, single cell analysis

Yeast glycolytic oscillations have been studied since the 1950s in cell-free extracts and intact cells. For intact cells, sustained oscillations have previously only been observed at the population level, i.e. for synchronized cultures at high biomass concentrations. Using optical tweezers to position yeast cells in a microfluidic chamber, we were able to observe sustained oscillations in individual isolated cells. Using a detailed kinetic model for the cellular reactions, we simulated the heterogeneity in the response of the individual cells, assuming small differences in a single internal parameter [1]. This is the first time that sustained limit-cycle oscillations have been demonstrated in isolated yeast cells. The precise conditions for autonomous glycolytic oscillations were also investigated. Hopf bifurcation points were determined experimentally in individual cells as a function of glucose and cyanide concentrations. The experiments were analyzed in a detailed mathematical model and could be interpreted in terms of an oscillatory manifold in a three-dimensional state-space; crossing the boundaries of the manifold coincides with the onset of oscillations and positioning along the longitudinal axis of the volume sets the period. The oscillatory manifold could be approximated by allosteric control values of phosphofructokinase for ATP and AMP [2].



(Left) Schematic of experimental procedure and different types of single cell oscillation.
(Right) Oscillatory manifold in three-dimensional state-space (phosphofructokinase, ATP and AMP).

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Measuring Inter-Bacterial Forces with Holographic Tweezers

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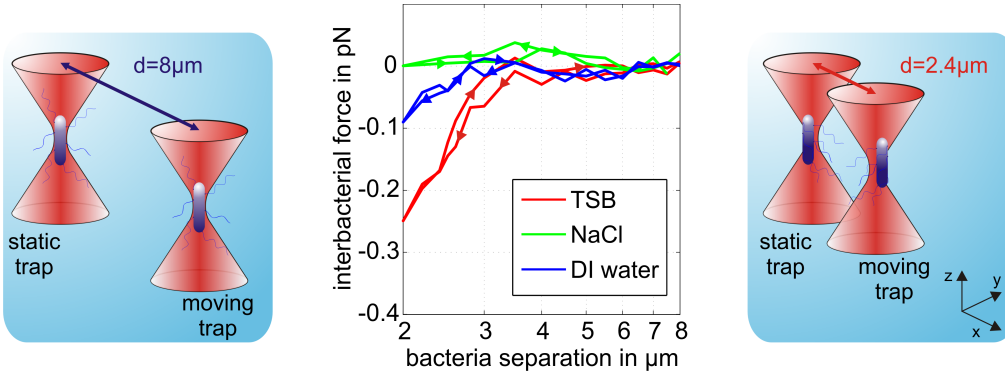
KEY WORDS: Force measurement, holographic optical tweezers, Bacillus subtilis,

Aggregation of bacteria plays a key role in the formation of many biofilms[1]. The critical first step is cell-cell approach, either via swimming motility or through Brownian motion[2]. And yet, the ability of bacteria to control the likelihood of aggregation during this primary phase is unknown. We use optical tweezers to measure the force between isolated Bacillus subtilis cells during approach. As we move the bacteria towards each other, bacterial swimming initiates the generation of repulsive forces at bacterial separations of ~3µm. Moreover, the motile response displays spatial sensitivity with greater cell–cell repulsion evident as inter-bacterial distances decrease[3].

Our experiments demonstrate that repulsive forces are strongest in systems that inhibit biofilm formation (Tryptic Soy Broth), while attractive forces are weak and rare, even in systems where biofilms develop (NaCl solution). These results reveal that bacteria are able to control the likelihood of aggregation during the approach phase through a discretely modulated motile response.



Two individual bacteria are held in separate traps and approach each other in a step-wise fashion. At each set distance we measure the force between the bacteria. The force lines represents the combined average of six separate bacteria pairs for varying media – Tryptic Soy Broth (TSB), NaCl solution and deionised water. The repulsive force on approach is strongest for bacteria suspended in TSB, decreases for nutrient-deprived deionized water and disappears for bacteria in NaCl solution[3].

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Engineered optical trapping forces with Mie scattering interferometry

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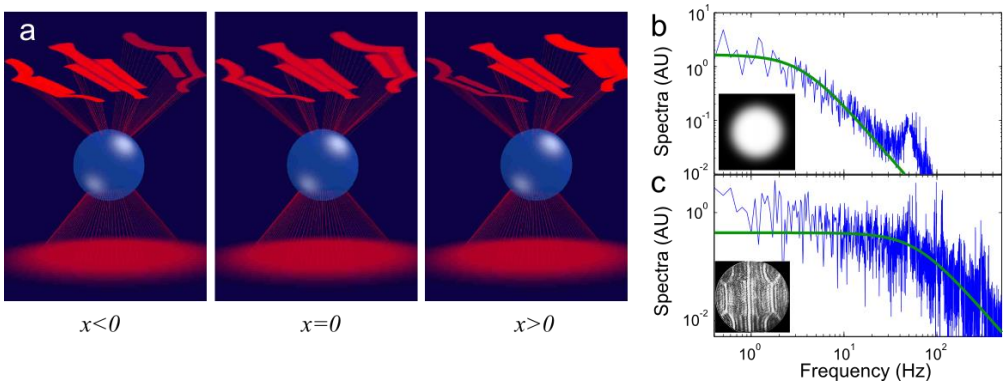
KEY WORDS: Optical force, Mie scattering, trap stiffness,

While most optical trapping experiments confine dielectric particles near the focus of a Gaussian laser profile, the use of different trapping profiles allows new capabilities and has allowed novel optical trapping forces to be demonstrated[1, 2]. In principle, it also allows complete optimization of the trapping forces, which could drastically improve trapping performance and enable important new applications in biophysical research[3].

Here we introduce and demonstrate a new approach, referred to as Mie interference for stiffness-enhanced trapping (MIST), which allows order of magnitude enhancements in trap stiffness over use of a Gaussian profile. In this approach, the trapping light is tailored such that the Mie scattering of a large dielectric sphere transforms the field into well separated output beams. Small particle displacements then modulate the power in these outputs via constructive or destructive interference. This induces an optical force with qualitatively different characteristics than conventional optical traps that rely on deflection of the transmitted rays. We experimentally demonstrate MIST with phase-only control of the trapping light, and trap 10μm silica particles with stiffness 17 times higher than achieved with a Gaussian trap of similar power. This is in reasonable agreement with theory, which predicts an enhancement factor of 25 in the absence of aberrations. Further refinements are predicted to allow an absolute trap stiffness that surpasses the existing state-of-the-art[4]. MIST can be incorporated with relative ease into any existing holographic optical tweezers setup, and consequently could see widespread applications.



Preliminary experimental demonstration of MIST. (a) The scattering particle reshapes the phase-modulated trapping profile into separated beams. The power of these outputs is modulated by small displacements, resulting in a trapping force based on interference rather than deflection of light rays. This allows. Trapped thermal spectra measured with Gaussian trap (b) and MIST (c) show that MIST increases the corner frequency, and therefore the trap stiffness, by a factor of 17. This result is also consistent with measurements of the force-displacement curve. The insets show the respective intensity distributions after interaction with the particle.

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Controlling dispersion forces between small particles with artificially created random light fields

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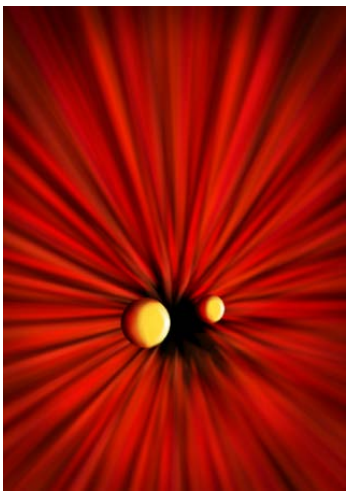
Appropriate combinations of laser beams can be used to trap and manipulate small particles with "optical tweezers" [1,2] as well as to induce significant "optical binding" forces between particles [3-4]. These interaction forces are usually strongly anisotropic depending on the interference landscape of the external fields [5]. This is in contrast with the familiar isotropic van der Waals and, in general, Casimir-Lifshitz interactions between neutral bodies arising from random electromagnetic waves generated by equilibrium quantum and thermal fluctuations [6-8]. The external control of isotropic interaction forces between particles can influence dramatically the macroscopic properties of colloidal suspensions and is of key importance not only for Biological or Materials Science applications but also to address fundamental questions about the nature of liquids, crystals, and glasses [9]. In the present work we show, both theoretically and experimentally, that artificially created fluctuating light fields can be used to induce and control dispersion forces between small colloidal particles. Using optical tweezers as gauge, we present experimental evidence for the predicted isotropic attractive interactions between dielectric microspheres induced by laser-generated, random light fields. These light induced interactions open a path towards the control of translational invariant interactions with tuneable strength and range in colloidal systems.



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Scanning Traction Microscopy

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KEY WORDS: Force measurement, extracellular matrix, live cells, confocal microscopy

Traction force microscopy is an established method for imaging stresses built up over time in two- and three-dimensional cell cultures[1]. It is based on microscopic measurement of the displacement of fluorescent beads embedded in a medium of known stiffness in response to stresses exerted by cells on the culture matrix. In the research presented here, we report an optical tweezers based approach to the inverse problem: given application of a known force to a given location in the culture matrix, can we determine the stiffness at that location? This research is motivated by efforts to understand the effects of matrix stiffness on normal and abnormal tissue development, especially with regard to cancer research. For example, in mammary gland tissue cultures the differentiation of epithelial cells into ducts or acini (milk producing structures) is correlated at least in part with the local stiffness of the extracellular matrix[2]. By combining optical tweezers and nonlinear scanning microscopy in a single instrument it is possible to simultaneously measure the stiffness and structural organization of cells and their 3D extracellular matrix (ECM). The matrix is seeded with the appropriate cells as well as fluorescent microspheres. The cells are imaged using endogenous sources of two-photon fluorescence (TPEF) and reflectance confocal. The surrounding collagen in the matrix is mapped with second harmonic imaging. The location of the fluorescent probe beads is determined using TPEF. These beads are subsequently imaged via TPEF at low scan rates and high power so that during imaging they are moved in the matrix gel by optical tweezer forcing. The size and shape of the resulting forced bead images can yield the local matrix stiffness and degree of inhomogeneity.

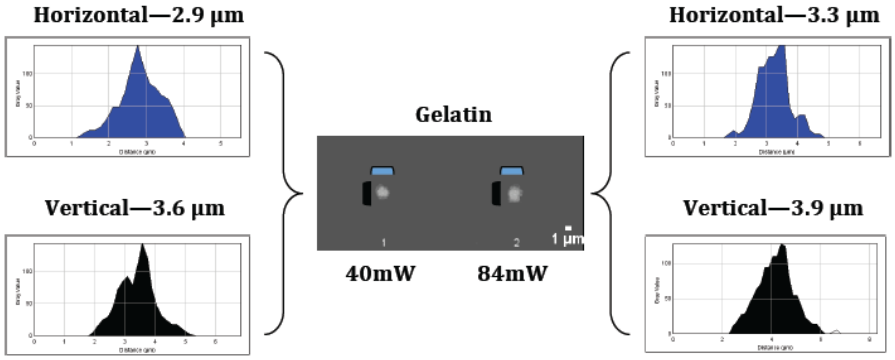

Illustration of 2-photon bead apparent image size of 2µm bead. Scans were performed on bead embedded in 0.07g/ml gelatin gel at 40 and 84 mW laser power. Measurements based on vertical and horizontal and vertical lengths are shown based on the 2-photon bead image intensity. Images were all normalized between 0 and 255 color/intensity value in grayscale

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Non-conservative optical action

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KEY WORDS: Electromagnetic forces, vortices, light-matter interaction.

Electromagnetic waves carry momenta. The associated conservation laws, in both propagation and scattering, provide insights into phenomena such as spin transfer and power flow which, in turn, are essential for developing novel sensing approaches at nanoscales. For instance, for a wave in a pure state of polarization, the spin-orbit interaction results in a spiraling power flow, which is determined by the extent of interaction [1,2]. We will review this spin Hall effect of light and discuss some of its mechanical consequences. We will show how the phase dislocations at a vortex state can lead to nondissipative mechanical forces. The non-trivial dynamics that can be optically controlled creates an Aharonov-Bohm setting where nonconservative reaction forces can be detected experimentally [3]. Observing this dynamics clarifies the role the finite wave-packets on the transfer of wave's canonical momentum.

In complex interacting systems, the reconfiguration of the electromagnetic field in space and time leads to unique non-equilibrium dynamics [4]. Remarkably, torques can be induced on spherically symmetric, optically isotropic, and lossless objects. In this case, the interplay between conservative and nonconservative contributions can be controlled by the polarization of the incident field [5,6].

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OAM measurement of elliptical Ince-Gaussian beams

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KEY WORDS: Orbital angular momentum, vortices, Ince-Gaussian beams

The manipulation of fundamental properties of light allows the creation of a diversity of complex photonic structures. These tailored light fields enable many applications in biological, medical, and technical fields and have been exploited for optical micromanipulation in numerous configurations [1-2]. Besides well-known Laguerre- and Hermite-Gaussian beams, especially Ince-Gaussian (IG) beams as solutions of the paraxial Helmholtz equation in elliptical coordinates are of fundamental interest, as they already include the former classes and are therefore more general solutions with special transversal and longitudinal light distributions. Helical Ince-Gaussian beams exhibit spatially separated phase singularities within their transverse elliptical light distribution and therefore may carry orbital angular momentum (OAM). But as the spatial distribution of singularities in these beams is distributed and varies with increasing ellipticity, non-integer values of OAM have been predicted for these modes [3].

We show in this contribution the analysis of the spatially distributed OAM of Ince-Gaussian beams by implementing a single slit diffraction method, which has been already employed for Laguerre-Gaussian and Bessel beams [4]. For the case of distributed OAM for helical IG modes of different ellipticities we demonstrate excellent agreement of our measurements with theoretical predictions. Fig. 1 shows an overlay of the intensity and phase distribution of a higher order IG beam with ellipticity $\varepsilon = 20$. Several phase singularities (indicated by red circles) are distributed along the horizontal symmetry axis of the beam. This leads to a spatial variation of the inclination of the phase front along the azimuthal coordinate η which is indicated in the figure by comparison of the phase gradient at the positions 1 and 2.

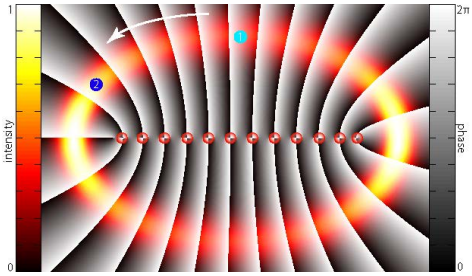

Figure 1: Overlay of intensity and phase distribution of higher order helical Ince-Gaussian beam.

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Two Dimensional Optical Rocking Ratchet

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KEY WORDS: holographic optical tweezers, non-linear dynamics, ratchets, optical sorting

Optical micromanipulation represents a versatile tool to study the complex dynamics of microscopic particles constrained to move in energy potential landscapes[1–3]. Based on these techniques, in this work we present an experimental realization of a fully reconfigurable two dimensional optical ratchet[4-7]. This consists in microscopic dielectric particles which are forced to move periodically on a two-dimensional periodic optical potential with a mirrored asymmetry. The characteristic behavior of this system is the non-trivial transport given by the dynamic coupling of the spatial asymmetric and the time-dependent non-biased forces. The time-dependent force is given by a relative displacement of the sample cell respect to the pattern of light which remains static, while the potential is created by means of the overlapping of two identical lattices of optical tweezers with orthogonal polarizations. The asymmetry of the resulting energy potential is tailored in situ by shifting one of the patterns respect to the other and changing their relative power. We obtain a current of particles that depends strongly on the shape of the energy potential and on the magnitude, periodicity and direction of the drag force. With this setup we are able to observe the absolute transversal mobility (see Fig. 1) previously predicted in theoretical models[8]. Moreover, we controlled the motion of a dense sample of particles in a two dimensional space by only changing the asymmetry of the pattern of light.

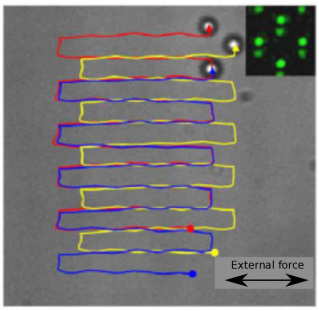

Fig. 1. Absolute transversal mobility in a 2D optical rocking ratchet. While the time-periodic drag force acts along the horizontal direction, the particles move upwards, perpendicularly to this force. The inset shows the asymmetric unit cell of the 2D optical lattice.

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Vesicle fusion mediated by optical trapping of metallic nanoparticles

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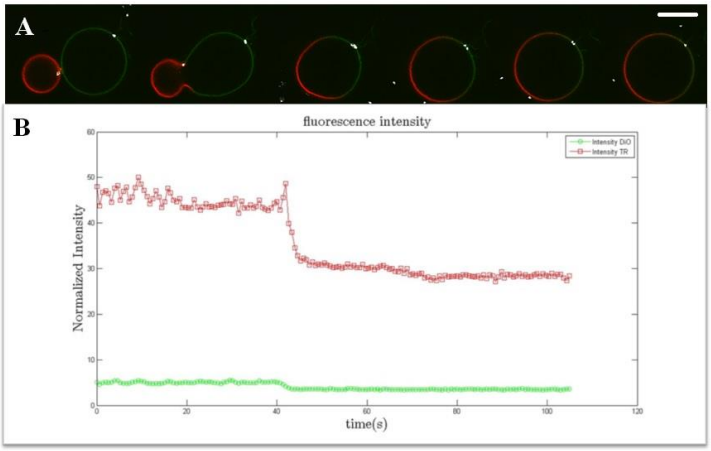
KEY WORDS: GUVs, optical tweezers, membrane fusion, nanoparticles, plasmonic heating

We developed a novel approach for membrane fusion of Giant Unilamellar lipid Vesicles (GUVs) by exploiting the localized heating generated by an optically trapped gold nanoparticle (AuNP). We previously showed that GUVs can be stably trapped by optical tweezers when their cargo has a sufficiently high refractive index compare to their surrounding buffer[1] and that optically trapped AuNPs can generate enough localized plasmonic heat to disrupt membranes[2]. We used optical tweezers to position two selected GUVs into close proximity. Then a AuNP, seen as a bright spot between the two GUVs in Fig. 1A, was trapped and held in the contact area between the two GUVs. The laser heated the AuNP enough to eventually lead to fusion of the adjacent membranes, thereby fusing also the lumen of the two GUVs. As the optical trap was implemented in a confocal microscope, we were able to simultaneously image the whole fusion process of fluorescently stained vesicle membranes or vesicle lumens with high resolution. We quantified the time scales for mixing of lipid fluorophores incorporated within the GUVs (see Fig. 1B) and found the time scales consistent with diffusional mixing in lipid bilayers. As expected, we found a linear relation between the mixing time and the relative increase in area for the individual GUV fusion pairs, hence, the fusion time scale for mixing depends on the relative size of the GUVs. Using this novel approach, it is possible to fuse any two individual vesicles in a controlled manner which has great implications for small scale and controlled mixing of reactants contained in separate GUVs.



A) Frame by frame illustration of vesicle fusion, the trapped AuNP is seen as a white spot between the vesicles. Scale bar is 10µm. B) Fluorophore intensity versus time for the same experiment. The fusion is visible as a dramatic decrease of fluorescence.

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Efficient illumination of spatial light modulators for optical trapping and manipulation

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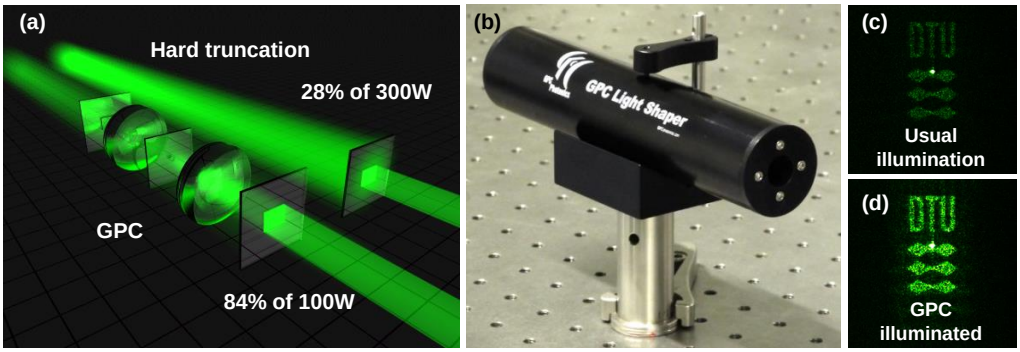
KEY WORDS: Beam shaping, illumination, spatial light modulators, GPC

Energy efficiency is always desirable. This is particularly true with lasers that find many applications in research and industry. Combined with spatial light modulators (SLMs) lasers are used for optical trapping and manipulation, sorting, microscopy or biological stimulation[1]. Besides efficiency, one wants to uniformly illuminate a specific shape such as the addressable area of an SLM. The common practice of truncating an expanded Gaussian source, however, is inefficient[2].

The Generalized Phase Contrast (GPC) method enables illumination that inherits the efficiency advantages of phase-only light shaping while maintaining the speckle-free, high-contrast qualities of amplitude masking. Compared to a hard truncated Gaussian, a GPC Light Shaper (LS) saves up to 93% of typical losses[3]. We experimentally demonstrated shaped illumination with ~80% efficiency, ~3x intensity gain, and ~90% energy savings[4]. We have also shown dynamic SLM-generated patterns for materials processing and biological research.

To efficiently illuminate an SLM, we used a compact pen-sized GPC-LS in place of an iris. For the same input power, hologram reconstructions are ~3x brighter or alternatively ~3x more focal spots can be addressed. This allows better response or increased parallel addressing for e.g. optical manipulation and sorting. Simple yet effective, a GPC-LS could save substantial power in applications that otherwise truncate lasers to a specific shape.



To obtain a uniformly illuminated rectangle with 84W, up to 216W is normally blocked. GPC, on the other hand can use 84W out of 100W, saving 200W (93%) (a). The compact GPC-LS is shown with an enclosure that prevents dust (b). Alternatively, a GPC-LS could increase the brightness by ~3x for the same input power as seen in the holographic reconstructions (c-d).

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POSTERS

Direct visualization and quantification of Doc2b-mediated membrane hemifusion

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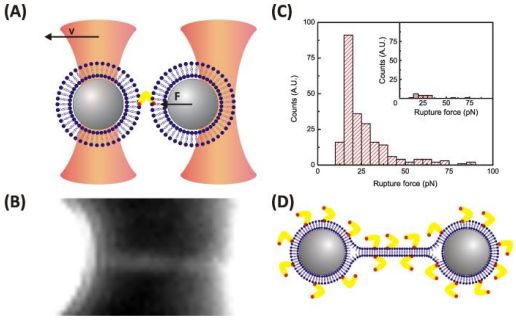
KEY WORDS: Optical tweezers, fluorescence, force spectroscopy, membrane fusion, Ca^{2+} sensors

The Doc2b protein plays a crucial role in regulating exocytosis by coupling calcium signals to secretory events. The exact mechanism remains debated: Doc2b either inhibits SNARE-mediated membrane fusion at low Ca^{2+} concentrations or it directly enhances membrane remodeling at elevated Ca^{2+} concentrations. Using a combination of optical trapping and fluorescence microscopy, we study membrane-membrane interactions between two optically trapped micrometer-sized lipid-coated polystyrene beads. The beads are brought in contact allowing protein-lipid and lipid-lipid interactions to occur. One bead is then retracted while monitoring the associated force. Fluorescent labelling of both the lipids and Doc2b enabled imaging of protein binding and lipid remodeling. We show that in the presence of Doc2b, the anionic lipid phosphatidylserine and Ca^{2+} a micrometers-long membrane stalk forms between the membranes with a stability of multiple minutes. Doc2b efficiently coats the entire lipid complex. Lipid mixing but no content mixing occurs between the two membranes, indicating that Doc2b enhances the formation of a hemifusion intermediate. Formation of this complex is associated with a force up to 250 pN. We conclude that Ca^{2+}-activated Doc2b reduces the energy barrier for membrane fusion by promoting and stabilizing a hemifused state. In living cells, this mechanism may occur in conjunction with progressive zippering of the SNARE complex, together driving efficient Ca^{2+}-secretion coupling.



Doc2b binds to lipid bilayers and induces membrane hemifusion. (A) Schematic representation of our assay, where two optically-trapped lipid-coated polystyrene beads are brought into contact in the presence of Doc2b, PS and calcium to allow protein-lipid interactions to occur. The optical traps give us a direct measure of the forces involved in the process. Additionally, we use fluorescent lipids and Doc2b-eGFP to directly visualize the interactions. (B) Fluorescence image obtained using Doc2b-eGFP and dark lipids. (C) Distribution of rupture forces of membrane stalks measured at a bead retraction speed of 7 µm/s. The large peak in the distribution around a force of 15-20 pN is the background signal (also presence in the absence of protein), while the long tail corresponds to protein-specific fusion events, showing that it requires forces up to 80 pN to break these membrane stalks. (D) Schematic representation of the hemifused state, where the outer layers of the original lipid bilayers have been fused together but the inner layers are still intact and thus lipid mixing can occur but not content mixing.

Flying particle sensors in hollow-core photonic crystal fibre

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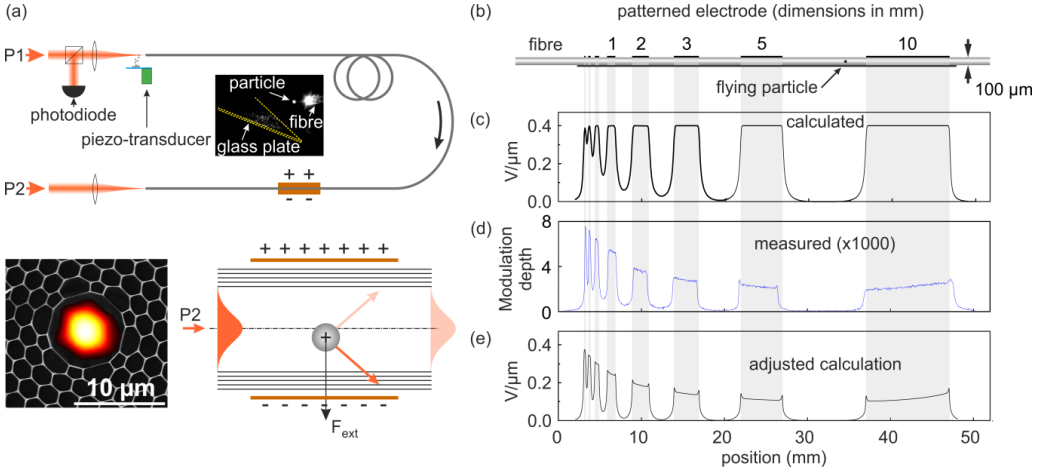
KEY WORDS: Optical trapping, fiber sensors, electric field sensing, temperature sensing.

We introduce a 'flying-particle' fibre optic sensor[1], based on a microparticle that is optically trapped and moved to and fro inside a hollow-core photonic crystal fibre (HC-PCF)[2]. In a proof-of-principle experiment, the transverse displacement of a charged microparticle, detected by changes in the transmitted light signal, is used to map with a spatial resolution of 50 µm the electric field pattern near the surface of a multi-element electrode. The frequency response of the system to a modulated electric field is in excellent agreement with theory. In a further demonstration, Doppler-based velocity measurements[3,4] on an optically propelled microparticle are used to map the gas viscosity (and thus resolve the temperature profile) along a length of HC-PCF. Flying-particle sensors represent a new paradigm in reconfigurable fibre sensors, potentially allowing multiple physical quantities to be mapped with very high positional accuracy over km distances. The technique is also ideal for use in highly radioactive environments where conventional solid-glass cores rapidly darken due to radiation damage.



(a) Top: Schematic of the set-up. In the dual-beam optical trap the power of the forward and backward propagating beams (P1, P2) can be adjusted independently. The transmitted power P2 is monitored by a photodiode. Lower left: SEM image of a HC-PCF with superimposed mode intensity profile. Lower right: Schematic of the particle in the fibre. (b) Cross-section of a patterned electrode with the fiber clamped between its plates. (c) Calculated electric field pattern for a straight electrode array. (d) Electric field pattern measured using the flying particle sensor. (e) Calculated field, adjusted for a slight bend in the electrode plates.

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A Microfluidic/Optical Tweezers-based System for FRET-Sensor Development

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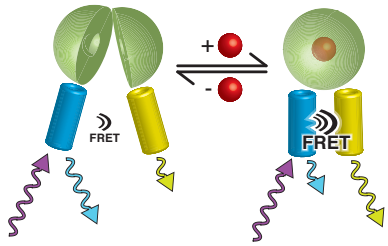
KEY WORDS: optical tweezers, microfluidics, sorting, FRET, biosensor

Genetically encoded fluorescent biosensors are sensors that allow for non-invasive, real-time monitoring of small molecules, at a subcellular level in living organisms. The high spatial and temporal resolution provided by these sensors make them ideal tools for studying highly transient and dynamic biological processes at the same time as the genetically encoded and non-invasive nature allows for stable long-term measurements[1,2]. Although it would be possible to make such sensors that specifically report concentrations of virtually any physiologically relevant molecule or even physical property, they have since their introduction in the late 1990s only seen a relatively modest use The main reason for these sensors not being more widespread and used today is the extremely labor intensive and semi-rational design process for development and optimization, often involving making and screening through thousands of constructs in order to find a working sensor[3].

Currently we are developing a platform to facilitate the process of making and optimizing fluorescent biosensors. Even though the platform will be very generic, we will initially focus on a subset of fluorescent biosensors based on Förster Resonance Energy Transfer (FRET). FRET-based biosensors (or FRET-sensors) have the additional advantage of being ratiometric, since sensor activation results in changed energy transfer between the two fluorophores used, resulting in opposite changes in their relative fluorescence intensities. The platform we design is based on a combinatorial assembly-based cloning-method to generate a library of putative sensor constructs expressed in yeast, combined with a microfluidics/optical tweezers-based system for trapping, screening and sorting the yeast library at single-cell level.



Schematic drawing of a FRET-sensor illustrating how ligand binding results in a change in FRET between the two fluorophores (yellow and blue) resulting in opposite changes in fluorescence intensities of the two fluorophores.

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Viability of bacterial cells in optical tweezers

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KEY WORDS: viability, live cells, holographic optical tweezers,

Optical trapping is an excellent tool for manipulation of microscopic objects. On one hand this manipulation capability opens the window towards novel ways of cellular studies. However the photodamage induced by high power trapping lasers in live cells limits the usability of optical tweezers in biological experiments.

The progress in the field of optical trapping brought in new and improved techniques, such as holographic tweezers, that extend the manipulation capabilities. These techniques may be used to minimize the harmful effects on live cells.

We have studied *E. coli* bacteria in optical tweezers in order to compare various trapping configurations in terms cellular viability. We used the waggling motion of swimming bacterial cells to determine how long the cell stays alive in the trap. We used two trapping configurations: a) a single trap arrangement where the cell orients along the optical axis of the trap, and b) a double trap arrangement where a cell is grabbed by two traps at the cell poles (the extremes of the cell body). In this latter configuration the cell body was less exposed to the laser light.

Our results may help in designing experiments with live cells where extended timescales and minimal photodamage is necessary.

Investigation of the Synchronization Mechanism of Glycolytic Oscillations in Individual Yeast Cells

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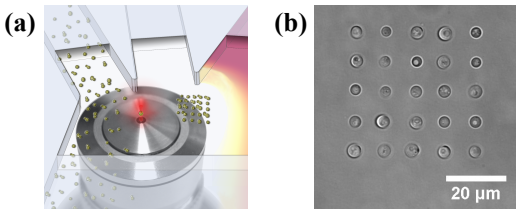
KEY WORDS: Optical tweezers, microfluidics, fluorescence microscopy, single-cell analysis, synchronization

Rhythms occur in a wide range of systems in biology and one of the most studied is that of glycolytic oscillations in yeast. These oscillations have been intensively studied for more than 50 years in dense populations of cells[1]. However, studying oscillations from millions of cells in a population, some kind of synchronization of the oscillations is required. The disadvantage of studying oscillations from synchronized populations is that only the average behavior of the population can be investigated, and possible heterogeneous behavior of the individual cells will be lost. For this reason, despite intense efforts, it was for long not known whether the onset of oscillations was a collective property that required a high cell density, or if individual cells could oscillate also in isolation[2-3]. The mechanism of synchronization has also been a matter of debate. In recent work we showed that cells in isolation indeed can oscillate[4-6] and that the requirements for oscillations to arise in individual cells is less restrictive than the requirements for synchronized oscillations to be detected in populations[7]. This suggests that coherence of oscillations in populations is the result of synchronization of individual oscillators via a so-called Kuramoto transition[8].

To elucidate the synchronization mechanism, we used optical tweezers to position yeast cells in arrays inside a microfluidic flow chamber. The responses of individual cells to periodic perturbations of the extracellular environment were then measured and the mechanism behind synchronization of glycolytic oscillations determined. The robustness of the mechanism with regards to cell heterogeneity was also evaluated.



(a) Schematic of the experimental procedure, where optical tweezers are used to position individual yeast cells at the bottom of a microfluidic flow chamber. (b) Image showing a typical cell array used in the experiments. The cell-cell distance is set to10 µm to avoid cell-cell interactions and synchronization.

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Measuring Dynamic Forces using Optical Tweezers

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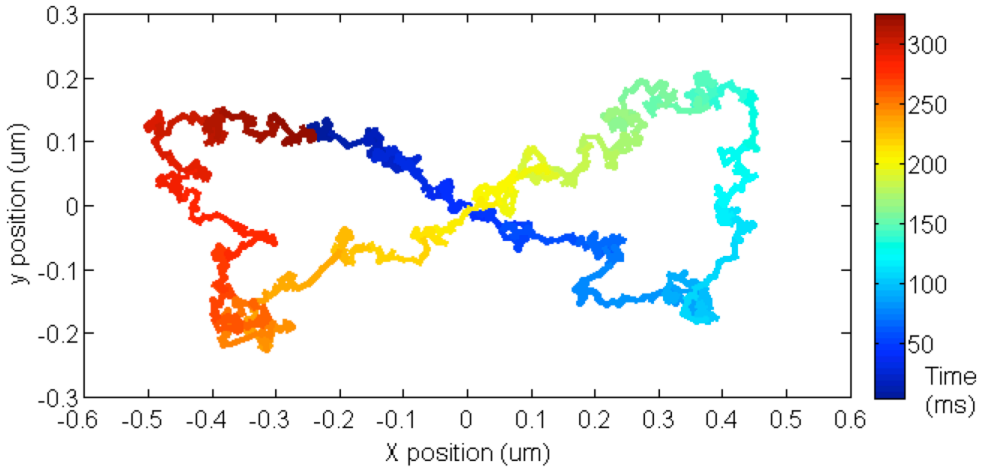
KEY WORDS: Low Reynold's number hydrodynamics, holographic optical tweezers.

Optically trapped microspheres are widely used as extremely sensitive femto-Newton scale force transducers[1,2]. When subjected to an external force, the microsphere translates until the Hookean optical restoring force of the trap balances the external force. At this point, the magnitude and direction of the external force vector is encoded by the particle's position (once thermal motion is averaged away). However, this situation becomes more complicated when such a bead is subjected to a time varying external force and never reaches a static equilibrium. In this case, the changing balance between optical, external, and hydrodynamic friction forces govern the trajectory of the particle, and hysteresis means that recovery of the external force now requires knowledge of the particle's position and its velocity.[3,4]

In this work we generate a time-dependent external force on an optically trapped 'probe' bead by driving an 'actuator' bead in a sinusoidal fashion in close proximity. We ensure that the both traps are uncoupled by using independent lasers. The motion of the actuator then exerts a time varying hydrodynamic flow on the probe bead, and we explore the resulting trajectories that are executed by the probe under a range of time varying configurations of the actuator.



The figure shows the trajectory of an optically trapped microsphere within a static optical trap as another microsphere is translated in a sinusoidal fashion nearby. The changing hydrodynamic force on the trapped bead causes it to map out a 'figure of eight' path in the optical trap. The colour of the each point represents the time of each measurement.

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Building and calibrating a low cost optical trap.

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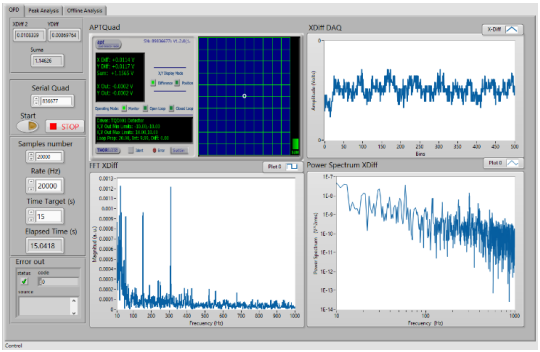
KEY WORDS: Optical Tweezers construction, Force Measurement, low cost optical traps.

We have implemented an optical tweezers setup able to confidently trap spheres in sizes ranging from 4 to 7 µm using the second harmonic of a Nd:YAG laser. A LabVIEW program allows us to acquire the signal from the light scattered by the trapped particle via a four quadrant photodiode and, processing such signal, is it possible to obtain the vibrational frequencies of the trap. Our main constriction in building this setup was a limited budget, so using in many cases second hand equipment (such as microscope objectives, optical mounts and data acquisition system), we have reduced the cost of the setup having a fully functional system (including laser) with a cost of around $9 000.00 US dollars[1]. The only cost not included here is the LabVIEW software.

The calibration of the system was performed using different methods (and software routines) that can already be found in the literature[2,3], such as power spectrum analysis, variance calculation, equipartition theorem and Boltzmann Statistics.



Virtual Instrument Main Panel for data acquisition of the scattered light and the calculation of vibrational frequencies of a trapped single glass sphere.

We also have measured force and spring constants using pure hydrodynamic measurements and compared such values with the ones obtained via analysis of scattered light such that we can confront the values obtained by these different approaches. From the obtained measurements, we can also reconstruct the potential well in which the spherical particles are trapped and we have been able to reconstruct by these methods, an asymmetry of the mode presented in the laser we use, which is mapped onto the potential well created by the trap setup.

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POSTERS

Optical trapping and rotation of large asymmetric absorbing media in Laguerre-Gauss modes

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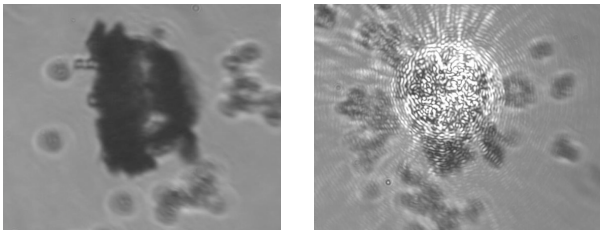
KEY WORDS: Absorbing media, orbital angular momentum, holographic optical tweezers.

We show the trapping and rotation of opaque graphite through adhesion with optically trapped polystyrene spheres. There are clear and distinct advantages to manipulating either refractive or absorbing materials, and incorporating both types of materials leads to more effective overall manipulation. Our graphite-polystyrene technique results in increased rotation rates of the conglomerate system over that of polystyrene alone, and in addition enables rotation of absorbing objects that are significantly larger than the beam mode size. The size of trapped absorbing media has previously been limited to less than the mode size.[1] In this conglomerate system, the trapping occurs because the refracting spheres are drawn towards the location of the laser mode, and rotation happens through orbital angular momentum transfer from a Laguerre-Gauss (LG) mode to the absorbing graphite.

An example of the graphite-polystyrene system in an LG mode with azimuthal index l equal to 10 is shown in the figure below. On the left is a still image of a rotating chunk of graphite with some polystyrene spheres visibly attached; the other significantly out-of-focus spheres are free in the fluid. The entire system is rotating at 0.2 Hz. On the right, reflection of the LG mode off of the adjacent glass surface is shown to provide a comparison between approximate laser mode size and the size of the graphite chunk. In the same laser mode, polystyrene spheres alone rotate about three times more slowly than the combined graphite-polystyrene system. We are able to trap and rotate absorbing objects up to 3 times the laser mode size in the sample at 2-3 times the rotation rates of refracting media alone.



Left: large 75 μm piece of graphite with polystyrene spheres adhered to it, rotating at approximately 0.2 Hz. Right: reflected light from LG mode visible on the same rotating cluster.

POSTERS

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An optofluidic gate as a light induced membrane: Enhancements in optical force chromatography for microfluidic systems

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KEY WORDS: optical chromatography, optofluidics, momentum transfer, holographics

Nanoparticles, especially in the biotechnological and pharmaceutical fields possess significant potential for future applications. However, undefined and heterogenic particle populations demand for analytical/characterization tools and advanced manipulation equipment for a focused and controlled application.

Since the discovery was made that photons can be used to manipulate particles in the nano- to microscopic size regime through momentum transfer[1], research efforts have focused on different application and innovations using this approach[2,3]. Our studies focus on advancements in optical force chromatography (OFC) achieving separation of heterogeneous mixtures in a liquid medium by application of optical forces counteracting well-defined fluidic drag forces[4]. Combining the principles of eluting particle separation[5] with computer-addressed spatial light modulators (SLM), new and fine-tuned membrane-like modes for separating, sorting, concentrating and filtering nano-structured particles are expected[6].

Performance and limitations of the supposed experimental setup (Fig.1) will be evaluated referring to results on standard latex beads (50-1000nm) using Dynamic Light Scattering (*Zetasizer*® from Malvern Instruments) and Particle Tracking Analysis (*NanoSight*® a Malvern Instruments Company) as reference techniques.

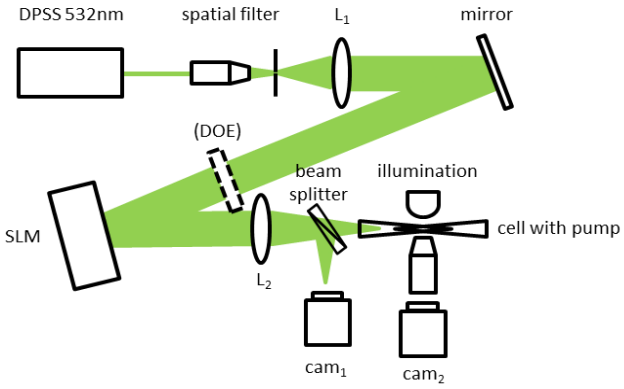

Figure 1 Block diagram of the experimental apparatus

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Stretching of Single DNA Molecules under Pressure-Driven Flow in Straight and Curved Microfluidic Channels

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KEY WORDS: Microfluidics, DNA mapping, fluid dynamics, Brownian motion.

Microfluidic channels are investigated here with a view to unravel and deliver long genomic DNA strands (>20μm) into channels for subsequent optical interrogation. Previous studies have focused on devices where strands are stretched under sudden shear and elongational microflows, but unfortunately there is a potential for strand fragmentation[1]. We investigate differently shaped microchannels to deliver intact strands for subsequent genome mapping.

This unravelling and stretching of DNA is based on the shear forces present in a pressure-driven laminar flow inside a microchannel of dimensions comparable to the length of the DNA. However, diffusion induced by Brownian motion tends to accumulate DNA in the region of maximum flow velocity at the centre of the channel where shear forces vanish and DNA strands start to coil up again. We have found experimentally that this can be mitigated by employing curved microfluidic channels[2]. In particular there is evidence that serpentine-shaped channels deliver more fully extended DNA strands than simple straight channels.

To understand the mechanism behind this improvement we perform numerical simulations combining a computational fluid dynamics (CFD) model of the microchannel with Brownian dynamics of a coarse-grain model of λ-DNA molecules[3], see Fig. 1(a). Comparing the simulations of a serpentine channel with those of a straight channel supports the experimentally found improvement of DNA stretching in the former, Fig. 1(b), with 17% of DNA molecules stretched to >15 μm length (>75% of maximum extension) compared to 5% in the case of a straight channel. A detailed analysis of the DNA dynamics reveals that the elastic molecular forces opposing the stretching of the molecule are pulling the DNA out of the central flow line towards the inside of a microchannel bend and thus into regions of larger shear forces. This gives rise to larger average DNA extension but it can also be seen in a modified spatial distribution of the molecules over the channel cross section at the output.

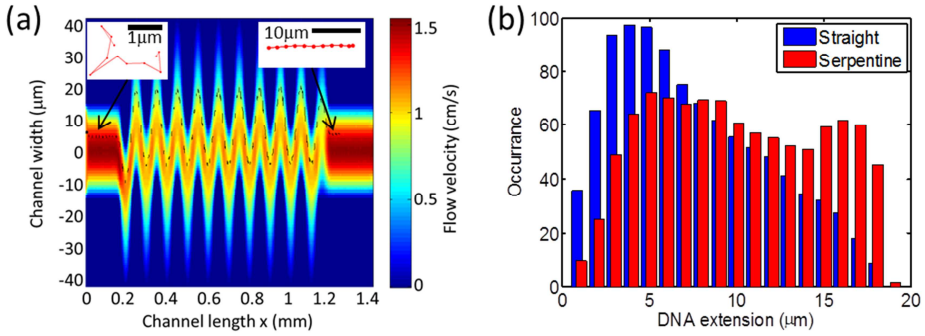

Fig.1. (a) CFD model of a serpentine microfluidic channel with a sample simulation of DNA dynamics. Insets show the shape of DNA before and after the channel. (b) Distribution of single λ-DNA molecule extension after 1.4 mm straight and serpentine channels (1000 simulations each).

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POSTERS

Kicking optically trapped particles

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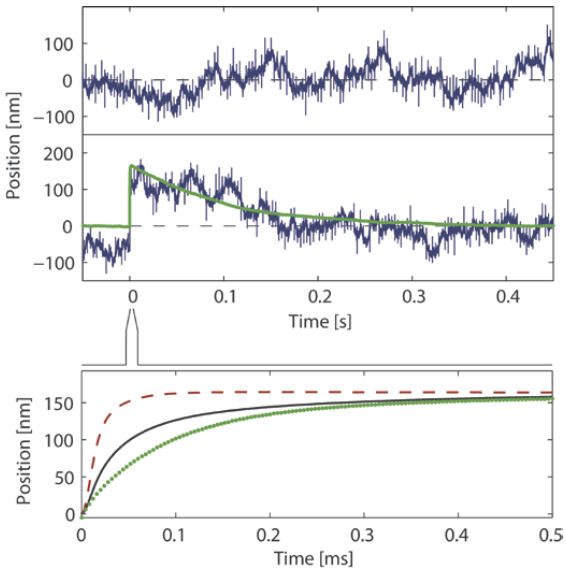
KEY WORDS: sub-nanometer resolution, short time scale diffusion, hydrodynamics, Stokes-Boussinesq.

Recent advances in experimental technologies have spurred increasing interest in the short time dynamics of diffusive motion of small microscopic object[1]. However, as the time scale get shorter and shorter the length of each diffusive step becomes smaller. For a 10µm sphere in water, nanometer and µs resolution is required to observe the minute deviations from classical Stokes-Einstein diffusion.

In this contribution, we present a simple technique, where a trapped particle is repetitively kicked by a short laser pulse orthogonal to the trapping laser. Using fast (250 kHz) detection of the position of the particle, accurately (nanosecond) synchronized to the kicking laser pulses, we construct a coherent superposition of the displacement. This result in a complete suppression of the Brownian motion of the particle and an increase in the resolution by $\sqrt{n}$, where n is the number of repetitive kick pulses.



In results present here, we obtain a temporal resolution of 4µs and we can determine the position of the 10µm sphere with a resolution of 0.7 nm. The data depicts the diffusive motion of the particle, the motion following 1 kick pulse, and the averaged motion corresponding to 1200 kick pulses (green curve). The decay of the averaged curve closely follows the ~80ms time constant given by force constant of the trap and the Stokes friction. However, as depicted in the bottom frame, the rise time of the motion (green line) neither follows the Stokes-Einstein friction (red dash) nor the Stokes-Boussinesq[1] (blue) expression for the friction. We will discuss the implications of the measurements and the limitations of the theoretical models.

The upper panel shows the diffusive motion of a 10µm polystyrene sphere held in a counter propagating optical trap with a force constant κ=0.88 pN/µm. Below is the trace after the sphere was kicked by a ns laser pulse at 532 nm. The green curve corresponds to an average of 1200 traces coherently added using the laser to synchronize the traces. Bottom panel shows the rise time of the averaged trace the first 0.5 ms compared to the theoretical predictions obtained using either the simple Stokes expression for the friction or the more refined Stokes-Boussinesq expression[1].

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POSTERS

Gold-coated microtools for localized fluorescence enhancement

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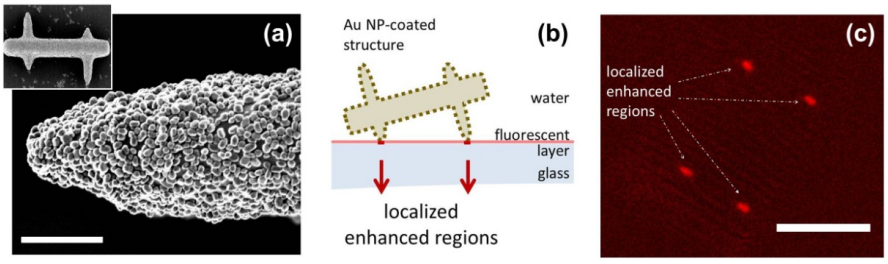
KEY WORDS: Two-photon polymerization, microstructure fabrication, gold nanoparticle, surface functionalization, fluorescence enhancement

There is an increasing interest in functionalized complex microstructures for micro- and nanotechnology applications in biology. Particularly in imaging, metal-enhanced fluorescence (MEF), achieved by microscopic surfaces coated with metal nanoparticles (NPs) or films has been applied recently on cells observing otherwise weakly detectable signals [1-2]. We introduce the combination of microstructures made of SU-8 photoresist by two-photon polymerization and gold nanoparticles or thin gold layers as fluorescence signal enhancers. The polymer microstructures serve as platforms and can be tailor made into any shape with sub-diffraction resolution required by the actual application. We coated these platforms either with 80 nm gold nanoparticles (NP) at various surface density [3] or sputtered gold layers of high reflectivity. We demonstrated localized MEF by NP-coated microstructures equipped with tips of 250 nm radius of curvature that provided enhancement factor of more than 3. The NP-mediated enhancement factor was as high as 6 over areas of several square-micrometers when flat microstructures without tips were used. We attributed the enhancement effect to reflection of the excitation and emitted light, what is demonstrated with the use of tilted polymer platforms. According to this observation, highly reflecting gold thin films were also used for coating and provided enhancement up to 8. The coated microtools can be further developed for optical tweezers actuation and in addition to localization of MEF, targeted enhancement at desired locations will also be possible.



Fluorescence enhancement with a tipped SU-8 microstructure coated with Au nanoparticles. (a) The Au-coated tip region of the microstructure (insert: the entire structure). Scale bar: 1 μm. (b) Scheme of the microstructure arrangement over a fluorescent layer showing the enhanced regions. (c) Confocal fluorescence image of the achieved enhancement. Enhancement factor in this case is ~3.2.

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Micro-rheology of Single Microtubule Filaments and Synthesized Cytoskeletal Networks

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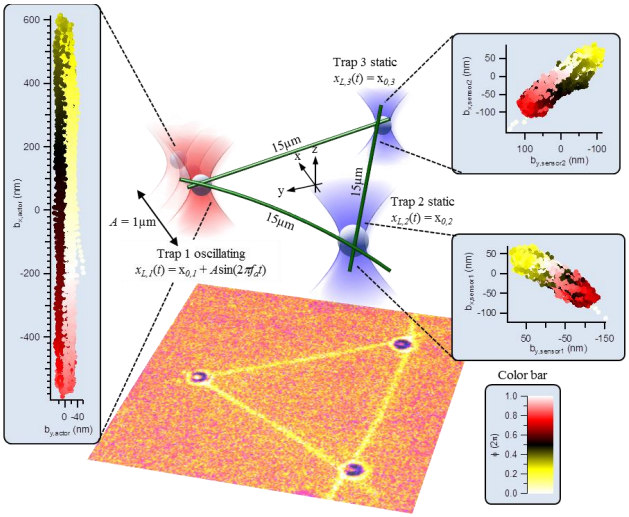
KEY WORDS: Cytoskeleton, microtubules, micro-rheology, time-multiplexed optical tweezers

The ability to sense and respond to external mechanical forces is crucial for cells in many processes such as cell growth and division. Malfunctioning of cellular mechanotransduction is often related to severe diseases like deafness or cancer[1].

Common models on mechanotransduction rely on the conversion of mechanical stimuli to chemical signals in the cell periphery and their translocation by diffusion (passive) or molecular motors (active). These processes are rather slow (~seconds) and it has been argued that the cytoskeleton itself might be able to transport a mechanical signal within microseconds via stress waves[2]. Microtubules are the stiffest component of the cytoskeleton and thus ideal candidates for this purpose.

We study the frequency dependent response of single microtubule filaments and small networks thereof in a bottom-up approach using several (N=2-10) time-multiplexed optical tweezers together with back focal plane interferometry. Small synthesized networks with a defined geometry are constructed using trapped Neutravidin beads as anchor points for biotinylated filaments. The network is then probed by a defined oscillation of one anchor (actor). The frequency dependent response of the remaining beads (sensors) is analyzed experimentally and modeled theoretically over a wide frequency range.



Equilateral network cell of three 15µm long microtubules created by time-multiplexed optical traps. Trap 1 is oscillated at f = 100Hz. Position histograms of bead trajectories as response to the physical stimulus are shown color coded over the current phase ϕ of the oscillating trap.

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POSTERS

Shaping UV Light for 3D Printing of Microfluidics

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KEY WORDS: Stereolithography, digital micromirror devices, microfluidics.

Stereolithography requires precise control of a laser focus to polymerize a photochemical. Areas exposed to the laser form a solid structure, enabling 3D printing of objects with a resolution comparable to the beam waist. Usually, the laser focus is scanned across a predetermined path to form the structure. In this work, we build a 3D printer that replaces the scanning process with a series of 2D images projected by a digital micromirror device which allows a more rapid fabrication. In addition, 3D structures can be printed in the bulk fluid without the need for any intermediate processing steps. 3D printers are becoming common laboratory tools for a variety of applications[1].We demonstrate the resolution limits of our printer, as well as a variety of structures and applications which should prove useful in microfluidics, micro-structured materials, cell scaffolds, and bespoke precision labware.

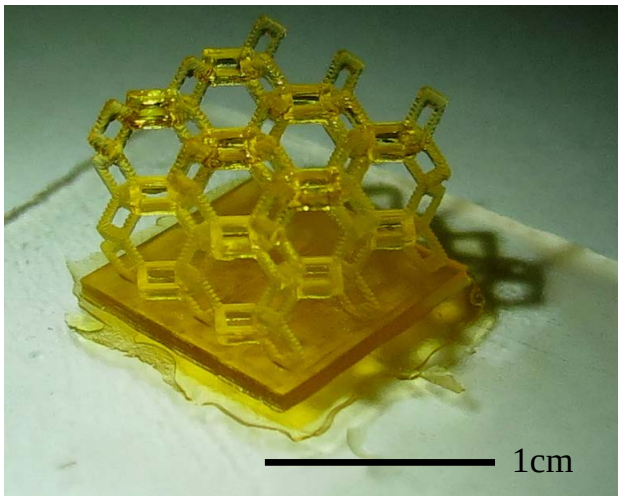

Figure: high resolution 3D printed lattice structure created by our printer.

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Implementation of Force Measurements based on the Detection of Light Momentum Changes in Optical Tweezers

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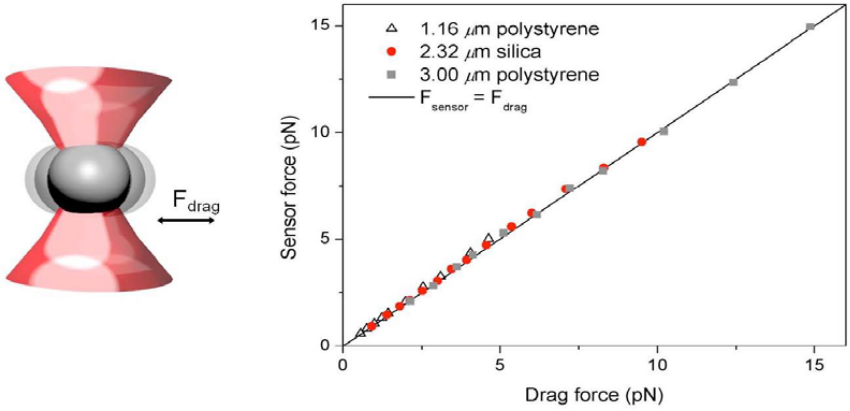
KEY WORDS: Optical trapping, optical tweezers, optical manipulation, back-focal-plane interferometry, force calibration

In this work, we present and discuss several issues that one should consider when implementing the light momentum change method for measuring forces in an optical tweezers setup. A system based on this principle provides a direct determination of this magnitude regardless of the positional response of the sample under the effect of an external force, and it is therefore to be preferred when in situ calibrations of the trap stiffness are not attainable or are difficult to achieve. The possibility to obtain this information without relying upon a harmonic model of the force is more general and can be used in a wider range of situations. Forces can be measured on non-spherical samples or non-Gaussian beams, on complex and changing environments, such as the interior of cells, or on samples with unknown properties (size, viscosity, etc.). However, the practical implementation of the method entails some difficulties due to the strict conditions in the design and operation of an instrument based on this method. We have focused on some particularly conflicting points, such as determining a process to systematically set the correct axial position of the system. We further analyzed and corrected the non-uniform transmittance of the optics and we finally compensated for the variations in the sensor responsivity with temperature. With all these improvements, we obtained an accuracy of ~5% in force measurements for samples of different kinds.



Experimental results show that the sensor output is equal to the drag force applied on samples of different sizes and materials.

POSTERS

Anti-reflection coated particles for enhanced optical manipulation of cells

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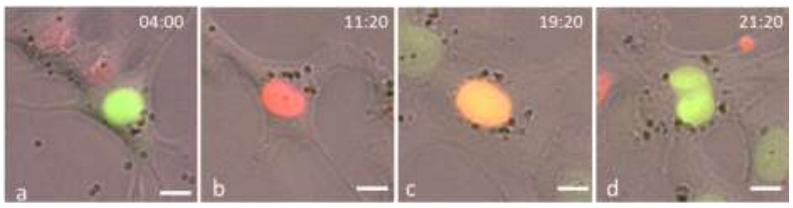
KEY WORDS: Force measurement, intracellular dielectric tagging, live cells

Optical Trapping has become a powerful technique throughout the natural sciences. To date trapping forces have been limited to the pN range, not yet reaching the nN range achieved by atomic force microscopy (AFM). To overcome this barrier many alterations to the optical trapping set-up have been proposed such as, increasing the laser power, beam shaping or correcting deleterious effects such as spherical aberration[1]. However, these approaches have the caveat of either increased heating of the sample or increased cost and complexity of the optical system. It has been shown recently by Shäffer and Jannasch that by optimising the properties of the trapped particle this limit can be overcome[2]. They synthesised high refractive index anatase titania particles that were coated with amorphous titania to reduce the scattering force and therefore permitting forces in the nN range.

We have reproducibly synthesised anti-reflection (AR) coated particles and will present the first study using these particles for enhanced optical manipulation of live cells. We dielectrically tagged Chinese hamster ovary (CHO) cells with AR particles or with Silica particles of the same size. The particles are incubated with the cells for a period of 24 hours before experiments where the particles naturally undergo endocytosis by the cell. After incubation drag force studies were carried out. We found the Q values for cells incubated with AR particles to exceed those for native cells by over 300% and only by 50% for cells incubated with silica particles.

To ensure that this method is not detrimental to the cell health we have performed extensive cell viability studies using fluorescence staining protocols. We also conducted time lapse imaging of cells expressing fluorescent ubiquitination cell cycle indicator (FUCCI) to determine if the addition of AR particles disrupted the cell cycle as shown in the figure. We found that the cells were uncompromised during this process. This work shows there is great promise for the use of such particles for nN force level studies in biological applications.



Fluorescence and brightfield images of FUCCI expressing cells incubated with AR particles. The cell cycle was shown to be unaffected by the presence of AR particles. a) 4 hours after incubation cell at the G2 phase, b) G1 phase, c) G1/S phase and d) S phase. Scale bar 10μm.

POSTERS

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Characterising Conical Refraction Optical Tweezers

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KEY WORDS: Conical refraction, optical trapping and levitation, trap stiffness, power spectrum.

Conical refraction occurs when a beam of light travels through an appropriately cut biaxial crystal. By focussing the conically refracted beam through a high numerical aperture microscope objective, conical refraction optical tweezers can be created, allowing for particle manipulation in both Raman spots and in the Lloyd/Poggendorff rings. We present a thorough quantification of the trapping properties of such a beam, focussing on the trap stiffness and how this varies with trap power and trapped particle location.

Through the use of back focal plane interferometry and power spectral analysis, we show that the lower Raman spot can be though of as a single-beam optical gradient force trap. Radiation pressure is shown to dominate in the upper Raman spot, giving rise to optical levitation rather than gradient trapping. Particles in the Lloyd/Poggendorff rings show gradient trapping effects but with a lower trap stiffness than particles in the lower Raman spot. However, these particles benefit from rotational control.

Applications where trapping, rotation and guiding are required could, therefore, be achieved through the use of a conically refracted beam, negating the need for complex beam-shaping techniques using, for example, spatial light modulators. Additionally, 3D STED microscopy could benefit from the complex beam profile generated by the conically refracted beam due to the "hollow cone" generated at the centre. The study of photophoretic manipulation of light-absorbing particles could benefit from such conical beams, with confinement possible in the dark regions of the beam enclosed by the Lloyd/Poggendorff rings.

Circularly polarised, conically refracted beam, ~75.5µm long, propagating from left to right, focussed with a 0.9NA objective. The three trapping locations are highlighted.

Accurately Measuring the Unbinding Force of Integrin-Ligand Interactions in Effector T Cells Using Optical Tweezers

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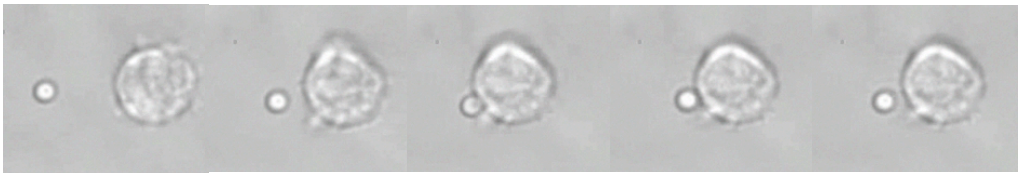
KEY WORDS: Force Measurement, Cellular Adhesion, T Cells, Live Cells, Mechanotransduction.

It has long been known that the Leukocyte Function-associated Antigen-1 (LFA-1) integrin, a beta2-integrin, is strongly expressed in effector T cells and plays an important role integrin mediated adhesion. The importance of beta2-integrin binding is demonstrated by the genetic disorder leukocyte adhesion deficiency (LAD). Previous studies have shown that LFA-1 can engage in rolling adhesions with intercellular adhesion molecule-1 (ICAM-1) under physiological shear flow[1]. The force required for the unbinding of this integrin-ligand interaction has been studied using atomic force microscopy measurements under static conditions[2]. However, the unbinding force has not previously been quantified under shear flow conditions.

Here, we present an approach to cell adhesion measurements using optical tweezers. Using micron-sized beads, coated with ICAM-1 and held in an optical trap, primary murine effector T cells were carefully probed. We developed a LabVIEW program that allows for automation of the adhesion measurement after setting parameters such as the contact time, pushing force and retraction speed. The force required to rupture the integrin-ligand bond was measured by monitoring the trapped bead displacement with a quadrant photodiode in back focal plane interferometry mode. An average Integrin-Ligand unbinding force of 18.3 ± 6.8pN was measured under static conditions, for wild type cells. We are currently measuring the adhesion properties of TTT/AAA-beta2-integrin knock in leukocytes, which display a similar reduction in integrin expression to neutrophils from LAD patients.

Our optical trapping approach to cell adhesion measurements has the advantage of allowing for the integration of shear flow in to the system to allow for more physiological relevant adhesion measurements to be made.



Frames showing (left to right) acquisition of trap stiffness, approach to cell, bead-cell contact, applying a pulling force to the bead, and integrin-ligand bond breaking, resulting in the bead jumping back in to the trap.

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Transverse optical manipulation of acoustically confined particles

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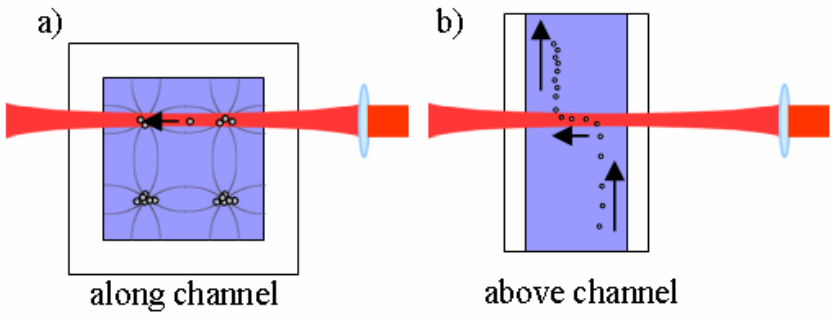
KEY WORDS: Acoustic confinement, Optical Manipulation, Force balance, Transparent acoustic element

We present a hybrid acousto-optical manipulation methodology based upon acoustic confinement and transverse optical forcing.

Existing work in combined acoustic and optical manipulation uses acoustic levitation to restrict particles to distinct planes. Optical manipulation of particles within and between these planes is possible throughout the sample volume at reduced optical powers compared to normal optical tweezers[1].

Here we present an initial study into the force balance between acoustic and optical forces for manipulating particles confined to streams. We generate an acoustic node pattern by combining a transparent acoustic transducer[2] with a symmetric acoustic cavity. Particles are confined to streams at the node locations and unrestricted optical access across the channel permits simultaneous imaging and optical manipulation of the particles. Using a loosely focused laser beam it is possible to push particles between streams in the channel. We explore selective direction of particles between streams and the sample volume.

In the future selective direction of particles between streams may provide volume manipulation by transverse optical forcing. Particle response is determined by intrinsic physical properties of the particles so it may be possible to adjust parameters for passive sorting of samples.



Particles acoustically confined to streams looking along a) and above b) the channel. Optical radiation force can direct particles between streams or reject particles to the bulk solution.

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2 G. Brodie, *{IEEE} Trans. Ultrason., Ferroelect., Freq. Con*, **61** (2014) 389-391

Light-assisted control of micro-probes in living cells: contactless, sterile probing of cell mechanics

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KEY WORDS: digital holographic microscopy, holographic optical tweezers, living cells

Optical tweezers are well known to enable the manipulation and control of objects in the range of 10nm to 10µm. Living biological cells, artificial microparticles and micro- or nanocontainers can be found in this range and are typically visualized by fluorescence microscopy. This has, however, rather high requirements with respect to preparation, handling and towards the microscopy environment. In contrast, digital holographic microscopy (DHM) allows 3D visualization and tracking of particles inside livings cells at sub-micrometer resolution and high speed without any markers[1]. Differences in refractive index between cells and particles lead to a strong contrast in DHM phase image (Fig. 1, middle column). Investigation of motility of particles inside living cells was done and compared with respect to different salt concentrations in the solution[1].

On the other hand holographic optical tweezers (HOT) enable the manipulation of multiple objects simultaneously in real-time[2]. By employing microparticles as sensor probes, one can investigate cellular properties like viscoelasticity or viscosity.

For this purpose, microparticles can be injected into cells through phagocytosis or with a glass needle[3]. Cell deformations are an important step towards information about spatio-temporally resolved biomechanical properties of cells (Fig. 2). Thus, with HOT several areas of the cell can be investigated at the same time with high spatial and temporal resolution. The ability of HOT to be combined with other microscopy technologies as e.g. DHM, fluorescence microscopy will be shown in our presentation, demonstrating its versatility for biological applications.

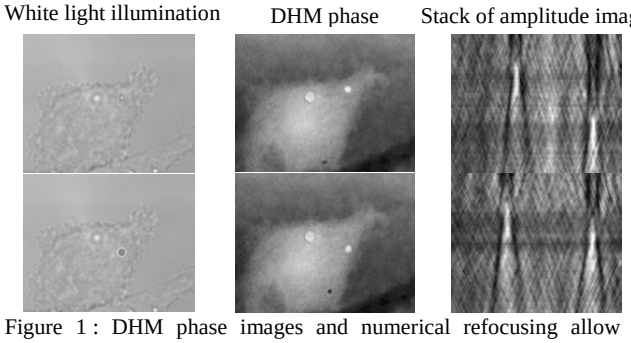

Figure 1 : DHM phase images and numerical refocusing allow quantitative phase imaging of the cell morphology as well as 3D tracking of optically manipulated particles

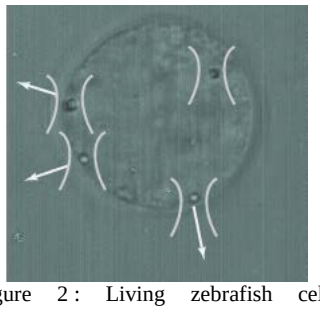

Figure 2 : Living zebrafish cell stretched by incorporated silica beads moved with HOT traps (white). Arrows show direction of deformation

[1] Á. Barroso, M. Woerdemann, A. Vollmer, G. von Bally, B. Kemper and C. Denz, *Three-Dimensional Exploration and Mechano-Biophysical Analysis of the Inner Structure of Living Cells*, Small **6**, 885–893 (2013)
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POSTERS

Using optical tweezer-based microrheology to investigate noise, fluctuations and nonlinear mechanical properties of living cells

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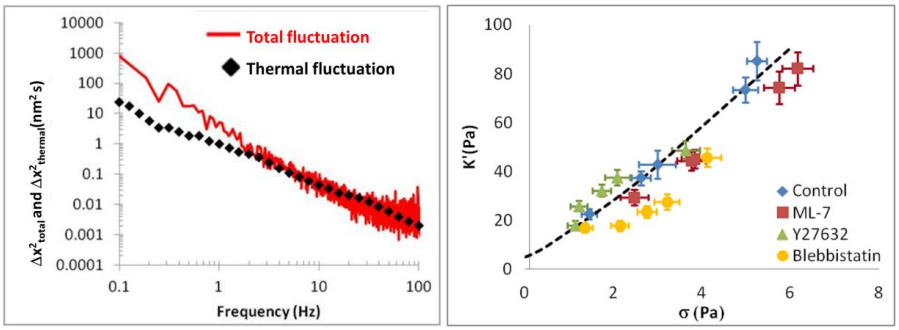
KEY WORDS: Optical tweezers, microrheology, noise, fluctuations, non-thermal, non-equilibrium, non-linear mechanical system, living cells

Living cells are a non-equilibrium mechanical system, largely because intracellular molecular motors consume chemical energy to generate forces that reorganize and maintain cytoskeletal functions. Persistently under tension, the network of cytoskeletal proteins exhibits a nonlinear mechanical behavior where the network stiffness increases with intracellular tension. We examined the nonlinear mechanical properties of living cells by characterizing the differential stiffness of the cytoskeletal network for HeLa cells under different intracellular tensions. Combining optical tweezer-based active and passive microrheology methods, we measured non-thermal fluctuating forces and found them to be much larger than the thermal fluctuating force. From the variations of differential stiffness caused by the fluctuating non-thermal force for cells under different tension, we obtained a master curve describing the differential stiffness as a function of the intracellular tension. Varying the intracellular tension by treating cells with drugs that alter motor protein activities we found the differential stiffness follows the same master curve that describes intracellular stiffness as a function of intracellular tension. This observation suggests that cells can regulate their mechanical properties by adjusting intracellular tension.



Left: power spectral densities of thermal and total fluctuations in living cells. Right: intracellular differential stiffness vs. intracellular stress for control cells and cells treated by drugs that interrupt myosin-II motor proteins [1].

[1] M-T Wei, D. Vavylonis , S. Jedlicka[1,3] H. D. Ou-Yang,[1,2] *Noise, fluctuations and nonlinear mechanical properties of living cells*, manuscript in preparation.

GPU accelerated beam shaping in multimode fibers

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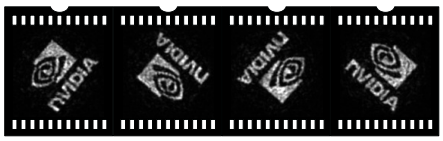
KEY WORDS: digital holography, multimode fiber, GPU

Research in life sciences increasingly relies on obtaining high spatial resolution information from systems and processes that are deep inside biological tissues. This represents a significant problem due to the intrinsic trade-off between resolution and penetration depth, a problem that requires mechanical, undisturbed delivery of light to a place of interest. Multimode fibers, as a class of miniaturized endoscopes, seems to provide an ideal solution for this problem but the complex propagation characteristics of light within multimode fibers lead to extreme computational requirements of such systems.

We discuss strategies utilizing the GPU accelerated toolbox for shaping the light propagation through multimode fiber using a spatial light modulator (SLM). The light is modulated before being coupled to the proximal end of the fiber in order to achieve arbitrary light patterns at the distal end of the fiber. The toolbox optimizes the acquisition time of the transformation matrix of the fiber by synchronous operation of CCD and SLM. Once the transformation matrix is obtained, the toolbox uses the acquired transformation matrix, retained within the GPU memory, to design, in real-time, the desired holographic mask for on-the-fly modulation of the output light field.

We present various approaches realizing beam shaping at the end of the fiber. Our initial incarnation of the system[1] includes Acousto-Optic Deflector (AOD) working in tandem with SLM. This allows complete removal of deleterious interference effects between the neighboring points by displaying the pattern points in time-discrete intervals. The disadvantage of the system is the relative complexity due to AOD presence and limited number of points that can be effectively displayed. The second approach, not requiring AOD, removes the undesired interference effects computationally using the GPU implemented Gerchberg-Saxton and Yang-Gu algorithms[2]. As a result of the computational power of modern GPUs, video-rate image control at the distal end of the fiber, virtually free of interference effects, is possible even for output patterns engineered from thousands of points.

We also discuss possible impact of the technology for structured illumination imaging applications using multimode fiber.



Pattern, consisting of 4000 points and generated in real-time, displayed at the output facet of the multimode fiber

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POSTERS

A Force Generation Toolkit for Cellular Biophotonics

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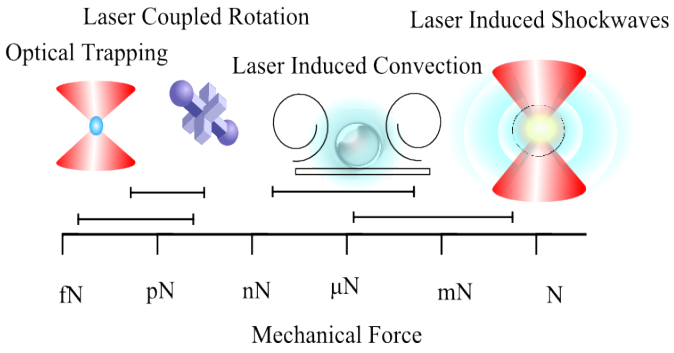
KEY WORDS: Laser induced force generation, Laser induced Shockwaves, live cells imaging, holographic optical tweezers,

Optical trapping is an excellent way to exert forces with a scale and precision useful in cell biology. Many studies have used these forces to both measure and understand cellular based processes and phenomena[1].

Practical difficulties, however, limit to pico-newtons the force range which can be generated by optical traps. Other limitations include laser power, heat generation, low refractive index contrast, and undesired secondary effects resulting in irreversible damage and even cell death.

In order to exert greater forces while at the same time maintaining flexibility, new techniques must be developed [2,3,4]. Here we demonstrate several force generation techniques which can augment traditional optical trapping and generate forces greater than pN's, while at the same time maintaining a similar optical configuration as used in traditional optical trapping experiments.



An illustration of a variety of techniques suitable for creating different forces in cell cultures all created using similar optical setups

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[4] T.M. Burns, et al. *Optical tweezers for precise control of micro-bubble arrays: in situ temperature measurement. SPIE NanoScience + Engineering* (2013)

Optical manipulation of single living cells on a stroke-on-a-chip

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KEY WORDS: lab-on-a-chip, optical tweezers, hemoproteins in live cells, absorption spectroscopy, oxygen sensor

Hemoglobins (Hbs) are vital for our survival and are interesting from many points of view, therefore, our research has been dedicated to develop tools to study the real time response of single Hbs containing functional cells to environmental changes. This has been done by combining resonance Raman spectroscopy, absorption spectroscopy, microfluidic systems as well as optical tweezers[1]. The further development of the technique was strongly encouraged by the discovery of a mammalian globin expressed in the brain and thus termed neuroglobin (Ngb). The function of Ngb is still unclear and there is a demand to investigate the protein in functional cells under in vivo like conditions[2], especially since the protein has shown to be able to hinder neuronal damage after a stroke. With this in mind we have developed an experimental platform, a so called stroke-on-a-chip (SOC) incorporating the patch clamp technique, optical tweezers, absorption spectroscopy, and oxygen sensing. As a proof of principle studies on single red blood cells (RBCs) from chicken have been performed. The oxygen content within the channels of the SOC was monitored by an oxygen sensor and the oxygen level within the channels could be changed from a normoxic value of 18% O_2 to an anoxic value of 0.0-0.5% O_2. The spectral transfer from the oxygenated to the deoxygenated state was measured by absorption spectroscopy and occurred after about 298 s. The gastight functionality of the chamber was verified by recordings of unchanged deoxygenated spectrum for 90 min without perfusion of anoxic solution into the channels. Thereafter a transfer of the oxygenated absorption spectra was achieved after 426s by exposing the cell to normoxic buffer. Successful patching and sealing were established on a trapped RBC and the whole-cell access (Ra) and membrane (Rm) resistances were measured to be 5.1 MΩ and 889.7 MΩ respectively. This shows that the developed SOC device is suitable for electrophysiological studies on viable biological cells under control of the oxygen level.

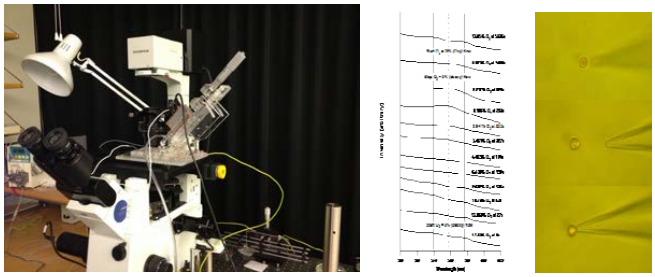

Figure 1) Left side, photograph of the microscope (Olympus XI71) with the SOC including the holder for the patch-clamp pipette. Right side microscope port is connected to the absorption spectrometer (Ocean optics HR400), behind that, the filter holder of the beam expander for the optical tweezers entering the microscope from the back can be seen. Middle, absorption spectra of a single chicken RBC, time table and value of the oxygen content of the cell. Right side, optical 3D steering of a single RBC towards the patch-clamp pipette.

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POSTERS

Optical Manipulation Proves Viscoelastic Changes during Stem Cell Differentiation

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KEY WORDS: Microrheology, optical tweezers, embryonic stem cells, differentiation

Using optical tweezers, we probed the viscoelasticity of stem cell cytoplasms. Stem cells from an embryonic stem cell culture were investigated, in this culture there existed a dynamic equilibrium between epiblast primed cells and primitive endodermal primed cells. These two sub-populations have different functional properties; however, the transcriptional differences between them are quite small. Therefore, we correlated the differentiation potential of these cells with their mechanical properties. To investigate viscoelastic properties of the stem cells we used endogenously occurring lipid granules inside the cells as handles for the optical trap[1],[2,3]. A confocal microscope with an implemented optical trap allowed us to determine whether a given cell was an epiblast cell or a primitive endoderm primed cell, which expressed a fluorescent reporter gene, Hhex.

To quantify the viscoelasticity of the cytoplasm we use the positional information of the granules to calculate the mean squared displacement (MSD) which scales with time: MSD $\propto t^{\alpha}$. The scaling exponen α contains information on the viscoelastic properties of the cells' cytoplasm: if α>1 the granule performs super-diffusion, α=1 indicates normal Brownian motion, while α< 1 indicates sub-diffusion. The cytoplasm is more viscous the closer α is to 1. After investigating ~300 granules we found that the viscoelasticity of the cytoplasm was significantly different between primitive endoderm ($Hhex^{+}$) and epiblast ($Hhex^{-}$) primed cells (α_{Hhex}^{+}=0.42± 0.19 and α_{Hhex}^{-}=0.57± 0.27, a student's T-test gave $p=9.3\times10^{-9}$). Hence, epiblast cells are significantly more viscous, less elastic, than primitive endoderm primed cells. Furthermore, we treated the cells with Latrunculin B, an actin disrupting agent. Upon this treatment, the viscoelasticity of the primitive endoderm primed cells significantly changed and became as viscous as the epiblast cells ($\alpha_{LatB,Hhex}^{+}$=0.51± 0. 4 and $\alpha_{LatB,Hhex}^{-}$=0.51± 0.3). Immuno-staining revealed that the Latrunculin treated primitive endoderm primed cells had increased levels of Nanog and decreased levels of Hhex, thereby expressing similar genes as the epiblast cells. Hence, not only viscoelasticity but also gene expression appears to be affected by Latrunculin, suggesting that the treatment reverses primitive endoderm priming.

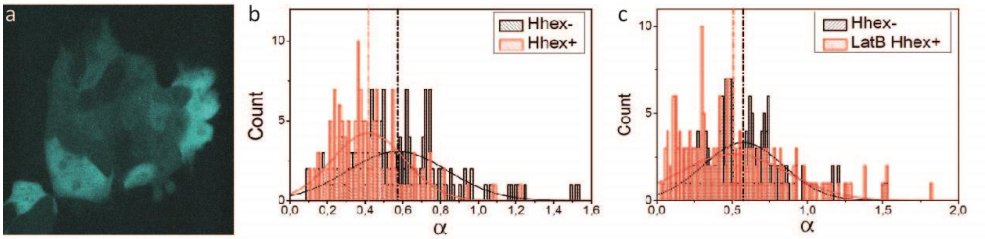

Figure 1: a) Colony of untreated Hhex positive and negative cells. Histograms of the scaling exponent α, characterizing the viscoelasticity of the cells' cytoplasm, of b) untreated and c) Latrunculin B treated primed ($Hhex^{+}$) and unprimed ($Hhex^{-}$) cells.

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[3] JH Jeon, et al., New J. Phys. **15** (2013) 045011-(1-16).

Influence of thermal noise to magnetic microparticle rotation in external magnetic field

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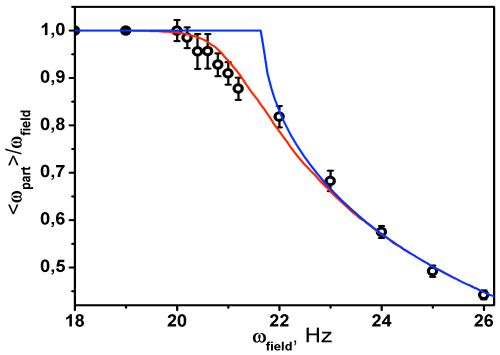
KEY WORDS: Thermal noise, vortices, magnetic microparticles, rotation of microparticles

Thermal fluctuations significantly affect the behavior of micro-scale rotating systems, as microvortexes, microbubbles in shear flow, rotating micromotors and other elements of lab-on-a-chip devices[1]. The influence of Brownian fluctuations on the motion of magnetic microparticles in rotating magnetic field is experimentally determined using optical tweezers technique. Numerical simulation of microparticle rotation in the presence of thermal noise are performed using Monte Carlo method. Experimental results are compared with the results of numerical simulations and the prediction of a general "non-thermal" theory. The results show that the presence of thermal noise changes the shape of transition between uniform and nonuniform modes of microparticles rotation.



Average rotation frequency of microparticles as a function of the external magnetic field rotation rate. Experimental results are shown by dots, blue line is theory, red line is the result of numerical simulation. Critical frequency is equal to 21,6 Hz.

In addition the coupling of translation and rotation motion of single particles was founded. This coupling often come out in form of Magnus effect, but this effect was not systematically studied at microscale. In most papers this coupling is assumed to be neglected[2], but our experimental results show that this translation-rotation coupling exist and must be considered in theoretical description of microparticle motions.

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POSTERS

Mechanical Dragging of Light by Highly Dispersive Rb Atoms (title is bold, 14-point type, centered, with capitals)

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KEY WORDS: Optical forces, slow and fast light, phase and group velocity, group index

Recently, it has been shown that dielectric particles immersed in a dispersive host medium, experience an optical force proportional to the group index of the medium[1]. In highly dispersive media this force can dominate the other forces. This implies that the mechanical action of light in highly dispersive media is completely different from that of normal media with low dispersion. As the first step towards understanding the mechanical action of light in highly dispersive media, we investigate the effect of mechanical dragging of highly dispersive media on the speed of light.

The phenomenon of light dragging by moving the host medium has been known for many years. When light enters a medium with refractive index n, its phase speed decreases to c/n. If this medium moves with speed v, light is dragged with the medium and its phase speed with respect to the lab frame changes by the relativistic addition of the two velocities: v and c/n. In general, regardless of how large the medium refractive index is, the speed of light cannot be changed by more than the speed of the medium. Also the maximum speed of light is limited by the speed of light in vacuum, c. However, by using a highly dispersive medium, where n depends strongly on the wavelength of light, one can exceed these limits. The effect of dispersion in light dragging is a manifestation of the Doppler effect. It has been shown that by using a highly dispersive medium to drag light, one can reduce group velocity of light pulses significantly[2]. This effect has been observed in a warm Rubidium vapor cell[3].

In this experiment we used Rubidium gas, confined in a cell, as the dispersive material to drag the phase velocity of light. The Rubidium cell can be translated at speed v=1 m/s. By heating the Rubidium cell, we achieved a group index of n_g=160 and thus were able to increase or decrease speed of light by n_g*v=160m/s, depending on the direction of motion. This change in speed is merely due to the dispersion effect of Rubidium atoms. We observed that dispersion has enhanced the dragging effect by two orders of magnitude. With Electromagnetic Induced Transparency technique, group indices as high as 10^7 are achievable. Then one should be able to drag light along (opposite to) the direction of propagation by a huge amount and achieve phase speeds much greater (lower) than c. An important point about this experiment is that we are using a CW laser and changing phase velocity of light rather than its group velocity, which many groups in the world are working on that.

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Thermally activated microgears spinning at the liquid-air interface

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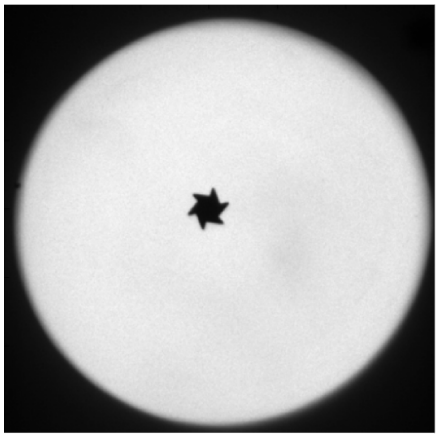
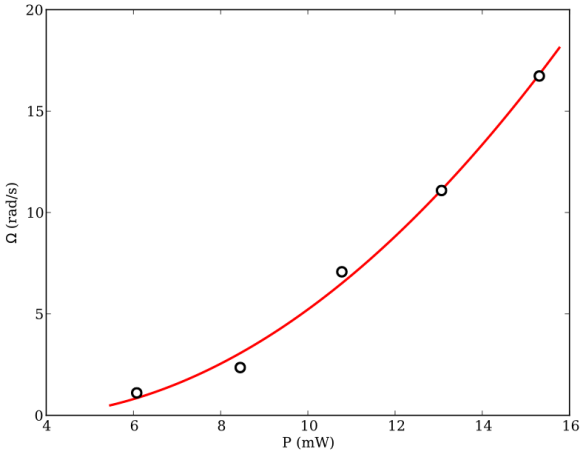
Surface tension is one of the strongest forces at the micron scale. When a colloidal particle sits at an interface, it is pulled by the liquid along the contact line, with forces tangential to the interface and orthogonal to each element of the contact line. These capillary forces are typically of the order of tens of nanonewton per micron. However, capillary forces are usually perfectly balanced leading to a zero force and torque on the suspended object. We demonstrate that small temperature gradients obtained by heating with light a micron-sized object can break this perfect balance and lead to a new and efficient mechanism for self propulsion over liquid interfaces. In particular we show that a micro-fabricated asymmetric gear, illuminated by incoherent LED light, can spin at tens of revolutions per second. This phenomenon provides a conceptually new strategy to use light as the driving force in micro-devices.

Keywords: Capillary Forces, Microfabrication, Light-Driven Microdevices, Thermocapillarity





POSTERS

Probing *Saccharomyces cerevisiae* in Different Osmotic Conditions using Holographic Optical Tweezers

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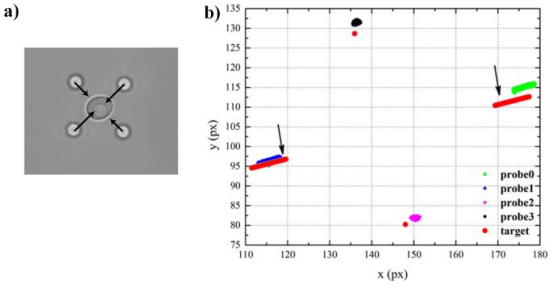
KEY WORDS: Force measurement, holographic optical tweezers, yeast cell, osmotic conditions, elasticity

Combining optical force measurements with holographic optical tweezers can be a great advantage due the fact that multiple probes or handles can be used to evaluate the forces exerted[1,2]. This technique provides a powerful and versatile platform for research in life sciences. Optical force measurements experiments rely on precise determination of the trap stiffness. This has limited the use of holographic optical tweezers systems for experiments requiring high precision measurements. The holographic optical tweezers system used has force measurement precision close to that of a single trap system. This has been achieved by using several methods for correcting different errors and limitations in the system. In particular, the used spatial light modulator has been corrected for spatial variations of the phase response, temporal variations during update and crosstalk between adjacent pixels of the spatial light modulator[3,4].

The holographic optical tweezers system was used to trap and manipulate micrometer-sized silica beads to probe yeast cells (*Saccharomyces cerevisiae*) in a non-invasive way and label free manner. Probing the cell in two opposite directions simultaneously, the obtained position data can be directly correlated to the stiffness of the cell wall/membrane. This way is used to measure the elastic modulus of the yeast cell wall/membrane in different osmotic conditions. The method can also be extended to involve different cell strains (e.g. cells lacking structural cell wall proteins).



a) Image of a yeast cell with four optically trapped silica microspheres, two are used to squeeze the cell and the remaining are only used to stabilize the cell during the measurement; b) Shows position measurements from the four optical traps recorded with the video tracking method. The arrows indicate that two probe particles are squeezing the cell, since a difference between the target and measured positions can be observable.

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Radiation Forces in Time-Dependent Optical Fields

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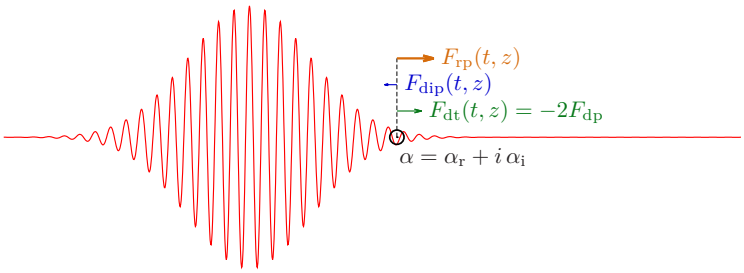
KEY WORDS: Fundamental aspects of radiation forces, time-dependent laser fields

The mechanical interaction between light and atoms, molecules or nanoparticles is usually described in two terms: the dipole force, dragging particles along the gradient of the light intensity; and the radiation pressure, pushing particles along the direction marked by the Poynting vector.

However, if the radiation field changes in time, like in a light pulse or an amplitude-modulated standing wave, additional force terms appear which are not covered by a time-dependent dipole force or radiation pressure. In fact, they sometimes even act against the intuitively expected forces.

This work presents the general idea and some simple results on this surprising and fascinating extension to optical forces, which we believed to understand so well.



Forces from a travelling wave pulse on a point-particle of polarizability $\alpha = \alpha_{\mathrm{r}} + i\alpha_{\mathrm{i}}$. The discussed time-dependent force F_{dt} reverses the effects of the dipole Force F_{dip}. For this setup, the radiation pressure F_{rp} is unchanged.

POSTERS

Plasmon-Enhanced Optical Trapping of Gold Core-Shell Nanoparticles

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KEY WORDS: Force measurement, core-shell systems, Plasmon-enhanced optical trapping

The possibility to optically trap, manipulate, characterize nanostructures is crucial for the achievement of miniaturized systems with highly advanced functionality[1]. Optical tweezers have been employed to study micro and nanoscopic particles for many technological applications such as optical microdevices, biological labeling and sensing. Metal nanoparticles are of key interest for optical trapping due to their ability to generate highly enhanced electric fields with subdiffraction spatial confinement[2,3,4,5,6,7] .

Optical trapping and manipulation of core-shell systems hold interest for many biomedical applications to control the release of drugs[8]These systems require a refractive index mismatch between particles, coating agent and solvent. So, it is crucial to evaluate the behavior of polymer capped metal nanoparticles in relation to properties such as electrolyte ionic strength, polymer-layer thickness, surface curvature[9], shape and size[5,6,7]. To this aim, we study how optical trapping changes in relation to the thickness of a polyethylene glycol shell around a gold core nanoparticle. The core nanoparticles are obtained by pulser laser ablation in colloidal dispersion with a narrow distribution of diameters (fig1a,b,c) and then coated by polymer. The trend of the k_x, k_y and k_z force costants normalized to the power at the sample, is shown in fig1d. Finally the use of Raman tweezers permits to investigate individual trapped nanostructures dispersed in solution and to characterize their chemical-physical properties.

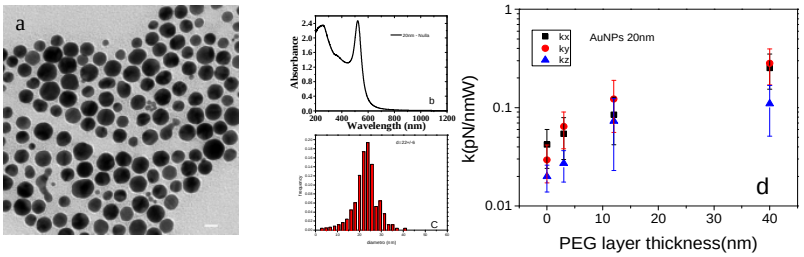

Fig.1: a) TEM image of gold nanoparticles. Scale bar:20nm; b) UV-Vis spectrum, c) histogram of size distribution and d) trend of force costants versus PEG layer thickness related to gold nanoparticles of 20 nm.

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POSTERS

High–order Gaussian beam modes and optical forces

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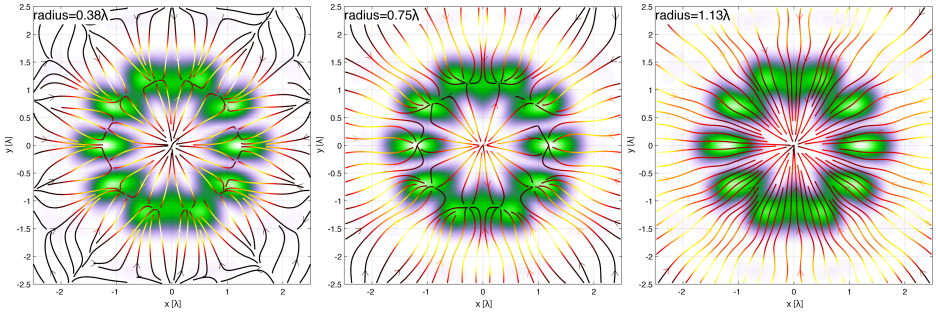
KEY WORDS: Structured light, Optical tweezers, Optical trapping theory

Three dimensional trapping in optical tweezers occurs for small particles due to large intensity gradients[1]. For larger particles, the large angle component of the impinging light dominates[2]. For these two reasons, optical traps in the mesoscopic regime generally occur when optical tweezers are produced using high numerical aperture (large angle) optics. For complex light fields it is not so clear how the intensity gradients of different modes and the high-angle requirement yield optical trapping. Here we investigate optical trapping for both the standard and elegant Ince–Gaussian modes. Standard Ince–Gaussian beams[3,4] are eigensolutions of the paraxial wave equation and so maintain their mode shape (but not size) as they propagate from the far- to near-fields. A small dielectric particle will therefore experience a broad angular spectrum of light impinging on it. Elegant Ince-Gaussian modes are not eigensolutions and evolve as they propagate from 'high-angle' spots in the far-field to tightly focused near-fields containing intricate phase structure.

The figure below shows the dependence of the force on particle size for a slice of the optical force field for the symmetric wave function component of an idealised standard Ince–Gaussian mode.



2D slice of the transverse optical forces in the focal plane for different particle sizes. As the particle size is increased the stable trapping regions in an $IG_{5,5}$ beam change. The smallest particle in this series is trapped within the bright spots of the beam. For larger particles trapping occurs at the centre of the beam.

Our work investigates the features which facilitate trapping (in both position and/or angle) in high–order Gaussian beams for different particle sizes and refractive indices. The dominant contribution of force and torque depends on a particles size/refractive index relative to the suspending medium and the feature size of the trapping beam. The large intensity gradient, high-angle scattering or a combination of both determine the properties (such as strength and overall stability) of optical traps near stationary points in complicated optical force fields.

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POSTERS

Optical Micro- and Nanofibres: Particle Trapping and Self-Assembly, and Higher Order Mode Generation for Particle Manipulation

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KEY WORDS: Optical nanofibre, optical tweezers, optical binding, higher order modes.

Optical micro- and nanofibres (MNF) have been proposed as an efficient method for multi-microparticle trapping and prolusion[1]. However, research on single-particle manipulation, the self-assembly effect of trapped particles, and the dynamical interactions between single- and multi-particles with the evanescent field of an MNF to date is not sufficient. In this work, we demonstrated a compact system of a nanofibre integrated into an optical tweezers to facilitate the study of individual and collective introduction of selected particles in the MNF evanescent field. Optical trapping and propulsion of single-particles and self-arrangement of particle chains were investigated for both fundamental and higher order modes at the fibre waist.

Speed enhancement of dielectric particle propulsion was observed in the evanescent field of the higher order modes compared to the fundamental mode[2]. Figure 1 shows the optical propulsion of a 3 µm polystyrene particle for which the speed was found to be 8 times faster for higher order mode propagation in the MNF than for the fundamental mode. The power at the waist was estimated to be 25 mW in both cases. Self-assembly of particle chains was also observed[3]. This effect was associated with optical binding. Simulations support the experimental data and provide insight into the inter-dynamics of particle assembly within the chains.

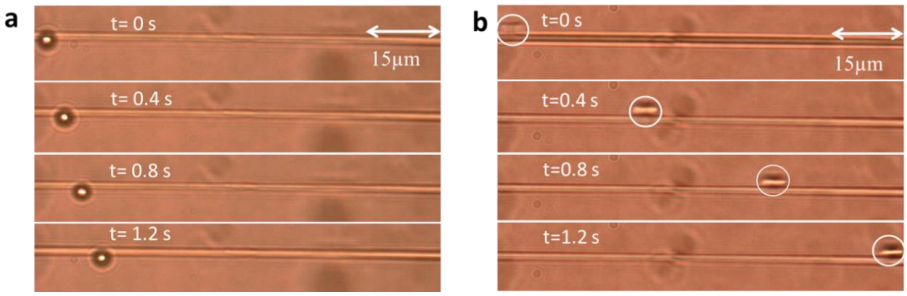

Figure 1. Microgram of 3 µm polystyrene particle propulsion under (a) fundamental mode and (b) higher order mode propagation. In both cases the waist power was 25 mW. It is clear that the speed of the particle in (b) is far higher than in (a).

This work is essential for future studies on three dimensional trapping and precise control of long-range and large scale of complex biological molecules in aqueous solutions in the vicinity of the evanescent field of an optical micro- and nanofibres.

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