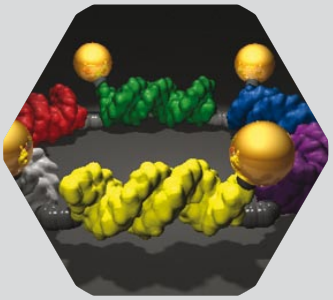
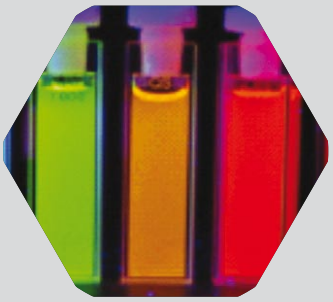
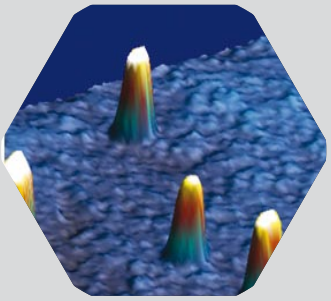






2006

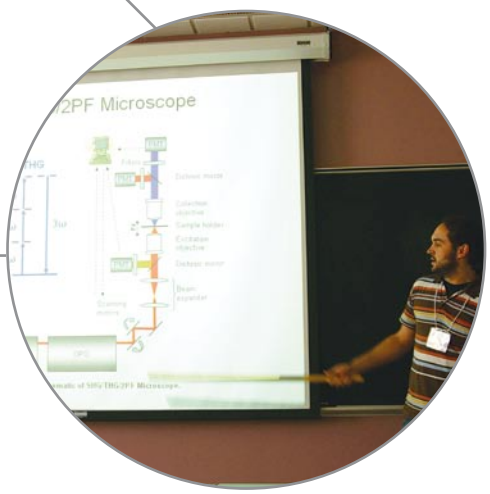
Junior Nanotech Network

A Self-Organized Graduate Student Exchange Program

CeNS Center for NanoScience

McGill Institute for Advanced Materials

Research Projects



Scientific Discussions

Stimulating Lectures

The Junior Nanotech Network – A Self-Organized Graduate Student Exchange Program

Driven by the tremendous progress, nanoscience and biotechnology are converging towards an exciting new discipline: NanoBioTechnology. Novel insights in the unique properties of living and non-living matter at the nanometer scale emerge by the combination of these two intrinsically powerful and dynamic disciplines. Potential applications are plentiful and will shape various areas such as nanoelectronics, nanomedecine and optoelectronics. For this challenging research, joint efforts of scientists trained in different disciplines and a strong international network are of utmost importance.

Top-Level Research

The Junior Nanotech Network (JNN) was initiated to meet these new requirements for top-level research. The exchange program was established between Ph.D. students from the McGill Institute for Advanced Materials (MIAM) at McGill University in Montréal, Canada, and the Center for NanoScience (CeNS) at the Ludwig-Maximilians-University in Munich, Germany. The symmetrical project consisted of a three-week visit of the young scientists from CeNS in Montréal in June 2006, followed by a return visit of the Canadian students in Munich in autumn of the same year. The scientific program combined lectures from internationally renowned speakers with an extended period of laboratory work.

Learning From Peers

The new and particularly interesting approach of the JNN is the self-organized character of the project. This goal was realized by giving the students the chance to conceive, teach, and supervise the scientific experiments in an independent manner. The laboratory work was carried out by the guest students under the supervision of the host students. In this way, students coming from the different areas of NanoBio-Technology were taken to the cutting-edge of a variety of domains via the guidance of the host students. The scientific projects allowed the transfer of broad knowledge and new technical skills and gave the hosts the opportunity to demonstrate their advanced research to a small and enthusiastic audience.

Intercultural Exchange

The scientific program was complemented and enriched by the social, cultural and outdoor activities which were organized by the host students. To guarantee social integration of the guests, the visiting researchers were housed at the homes of their local exchange partners. This close interaction fostered the intercultural exchange and stimulated scientific informal discussions. Combining joint lab work with common leisure activities enabled the creation of strong bonds between the young people from Canada and Germany.

Collaborations

The gain in scientific and intercultural competence has created the fertile soil for the establishment of future collaborations between the students, some of which are very prospective to be realized in the near future. These concrete plans for joint scientific work, but also the newly initiated friendships, demonstrate the success of the Junior Nanotech Network.

McGill

Montreal, Canada

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Munich, Germany

Research Projects

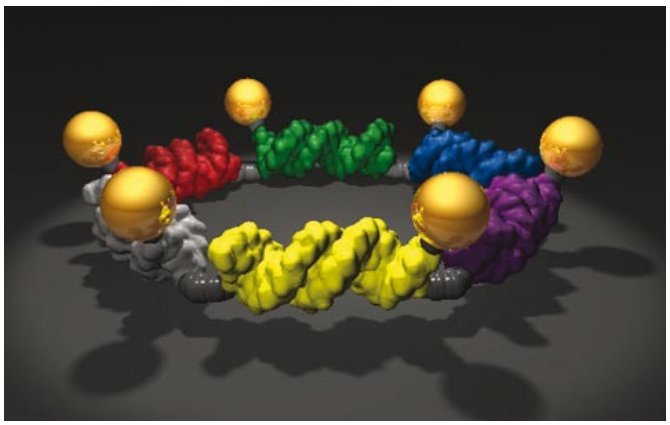
Content

Gold-DNA Assemblies

[Faisal A. Aldaye, Emily Cranston, Hua Yang]

An ongoing project in our laboratories deals with the use of DNA as an architectural component for the organization of nanoparticles into predefined assemblies. DNA holds great promise as a building block towards the construction of such constructs because of its sequence programmability, selective molecular recognition ability, and the relative rigidity of its double-helical form. Our approach involves labeling each nanoparticle with a DNA-containing molecule that provides it with an exact address that dictates its final position within the assembly.

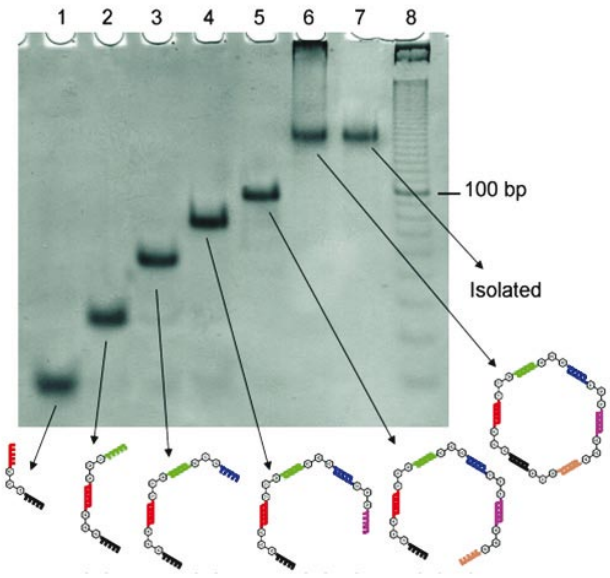
In an attempt to provide the students with the expertise required to construct such a DNA scaffold, and its subsequent use for the organization of nanoparticles, the training sessions in our laboratories were divided into three main modules. In the first part, work was undertaken to construct a hexagonally shaped, two-dimensional DNA containing template. This specific template was shown to organize six gold nanoparticles into a well-defined hexagonal arrangement (Angew. Chem. Int. Ed. 2006, 45 (14), 2204-2209). The self-assembly was studied sequentially and then characterized using native polyacrylamide gel electrophoresis (PAGE), while the theory behind the purification of the DNA scaffold was demonstrated using buffer-based electro-elution (as shown in the figure below).



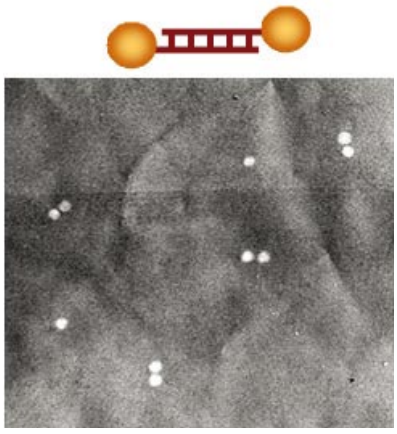
Visualization of gold-DNA assemblies.

Functionalizing DNA

In the second part, the students learnt how gold is functionalized with DNA strands. All the necessary tools were provided using a proof of concept system in which dimers of gold nanoparticles are assembled using a single double helix of DNA. Two separate batches of 5nm gold nanoparticles were mono-functionalized with complementary strands of DNA, respectively, and the purification of the mono-functionalized adducts was demonstrated practically using agarose gel electrophoresis. Such a purification of short DNA stranded gold adducts is made possible using methodology developed in our laboratories that involves helper extension strands. In the third and final part, the complementary mono-functionalized gold constructs were hybridized to each other, forming dimers of gold nanoparticles as templated by the single double helix of DNA. This assembly was characterized using the two imaging methods of atomic force microscopy and transmission electron microscopy (TEM).



PAGE of different assembly steps.



TEM image of gold-DNA assemblies.

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Mesoscopic Properties in 2-dimensional Electron Systems

[Alistair Armstrong-Brown, Sophie Avesque]

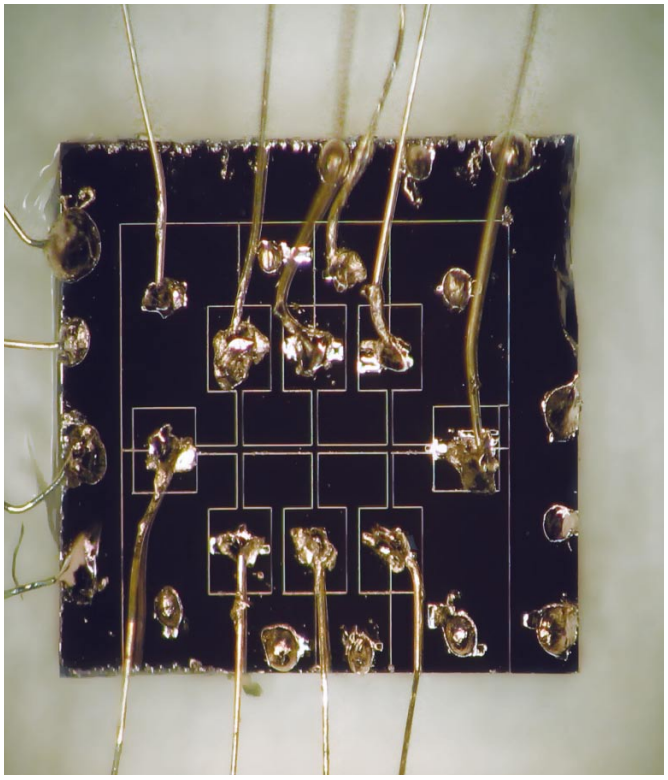
We proposed an experiment to measure mesoscopic properties in a 2-dimensional electron gas (2DEG) in the quantum Hall regime. We started with a blank wafer containing the 2DEG and went through the whole processing during the first day and performed some low temperature measurements during the second day.

Sample Preparation

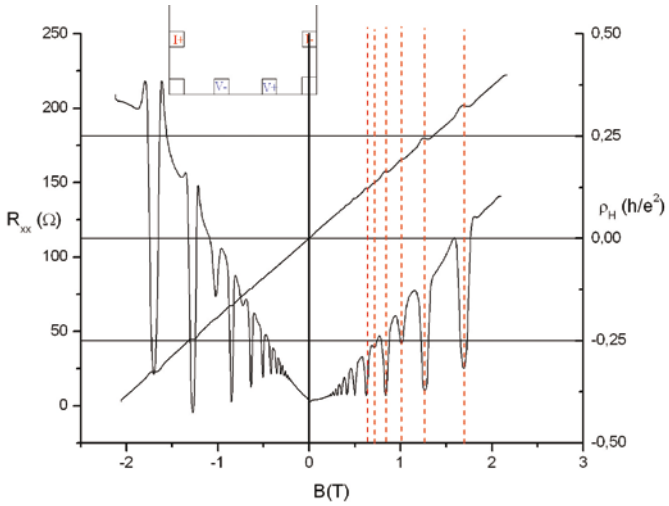
The generic shape used for the quantum Hall effect measurements is called a "Hall Bar" pattern. Using a novel patterning technique, a diamond scribe is driven by motors in the X and Y directions to scratch a pattern onto the surface. The scratching process depletes the 2-DEG beneath the surface in the desired shape. Conversely from clean-room lithographic processes this can be performed in a few minutes. The second step was to contact the 2DEG by diffusing Indium into the sample using a thermal annealing process (400°C for 25 minutes). The final step was to wire the sample to the sample holder.

Measurement

The measurements were performed in a Helium 3 system at 300mK in magnetic fields up to 9T. These quantum Hall effect measurements allowed us to extract information about the 2-dimensional electron gas: density, transport mobility and the quantum lifetime. In addition, we deposited an Indium gate on top of the Hall bar that allowed us to change the electron density by applying a negative gate voltage.



Sample with scratched-in Hall bar pattern and voltage contacts.



Sample resistance versus magnetic field. Measurements are performed at ultralow temperatures (300mK).

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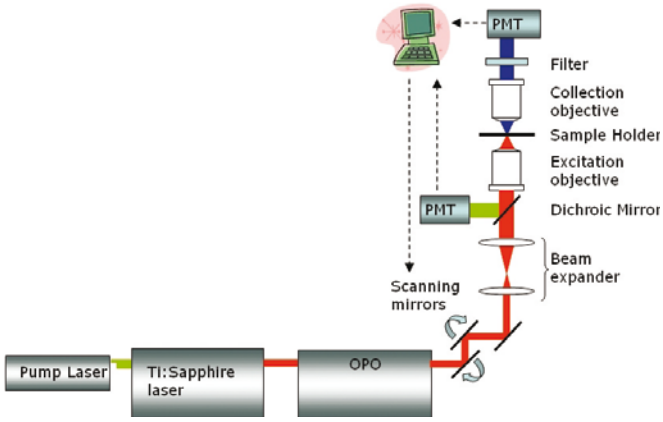
Third Harmonic Generation Imaging of Gold Nanoparticles [Jonathan Belisle]

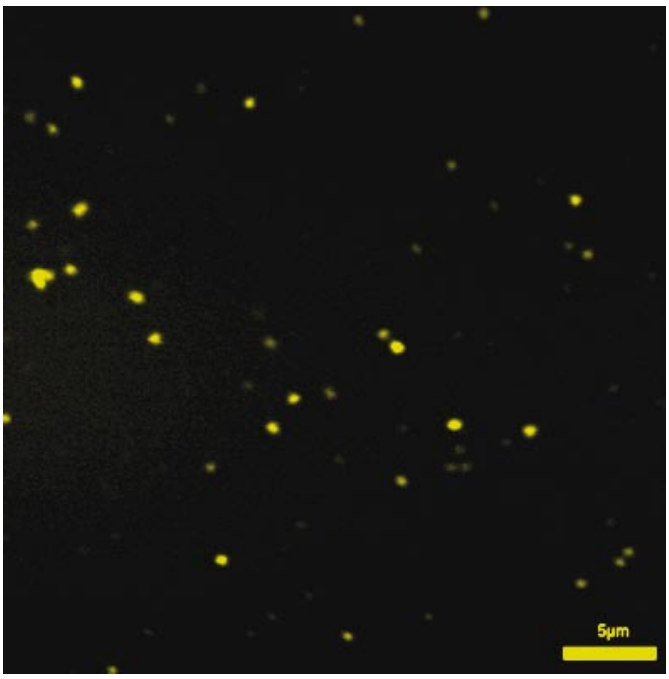
We imaged gold nanoparticles deposited on a cover glass by Third Harmonic Generation (THG) using our multiphoton laser scanning microscope as verification based on a recent report in the literature of THG imaging of such samples. Working with THG makes it easy to adjust the sample height as the THG signal produced at the air-glass interface is readily detected. The figure bottom left shows a THG image obtained from 40nm gold nanoparticles using an excitation wavelength of 1200nm.

Calculating Spatial Resolution

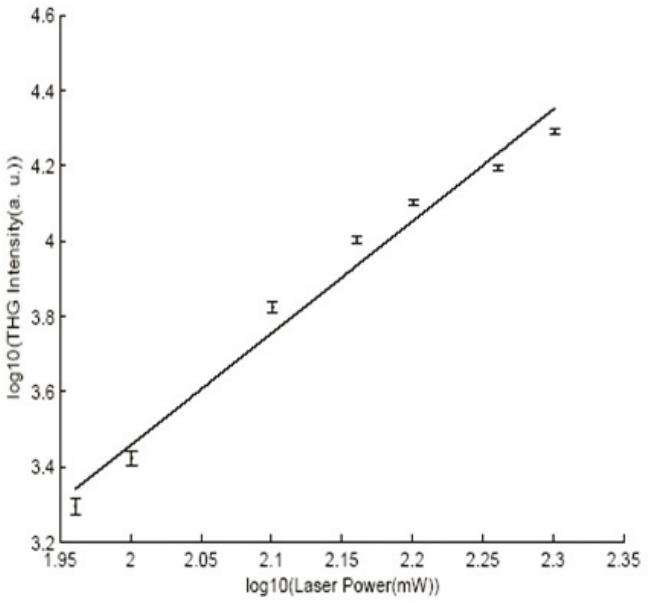
The 400nm THG was then collected using a 400/20 filter. By applying a Matlab algorithm that locates peaks within the image and fits Gaussian functions to them, we were able to measure the signal-to-noise ratio (S/N) and the full-width-at-half-maximum (FWHM). We obtained a S/N of 154 and a FWHM of 0.58μm. We therefore conclude that the practical radial resolution of the microscope at this wavelength is 0.58μm. Study of the peak THG intensity as a function of laser power for many particles was fitted in order to verify the cubic intensity dependence of the THG emission. We obtained a value of 3.0 ± 0.4 for the slope of the THG intensity (logarithmic scale) versus laser power (logarithmic scale). The corresponding plot is shown in the lower right figure.



Schematic of THG/MPF microscope.



THG image of 40nm gold nanoparticles. Excitation wavelength λ=1200nm and emission filter 400/20.



THG signal (log. scale) versus laser power (log. scale). A linear regression best fit yields a slope of 3.0 ± 0.4.

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Quantum Dots - Synthesis and Biofunctionalization

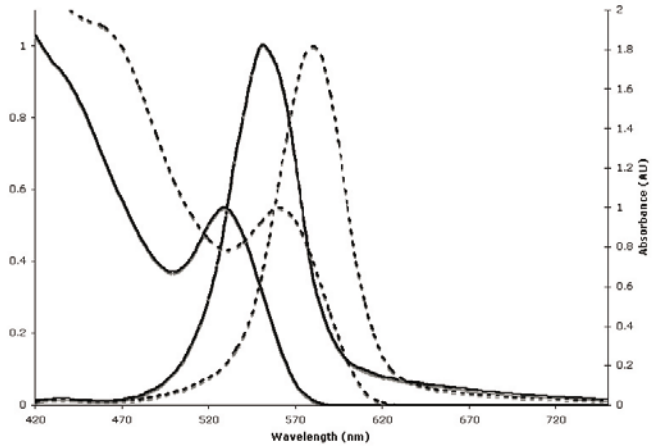
[Samuel Clarke]

We were able to build and modify quantum dot (QD) probes in order to target them to living systems. This is one of the major research areas of the Nadeau laboratory. Currently, one of the major hurdles is the instability of the QD probes. For this reason, we are actively exploring the use of novel materials to engineer ultrastable QD constructs.

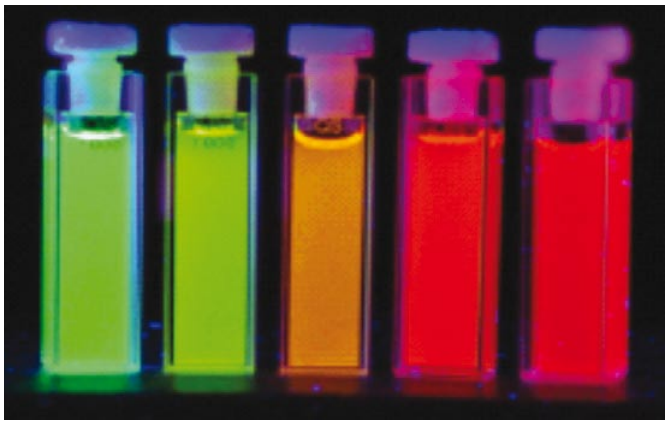
Synthesis and Solubilization

Quantum dots are nanoscale semiconductor particles. These particles have unique optical properties that make them highly fluorescent. The figure below shows the representative optical characteristics for two distinct batches of QDs. Of particular interest is the wide absorption spectrum and narrow emission. The size and color of the particles can be tuned during the synthesis to produce QDs that emit light over the entire visual spectrum. Our lab has recently adopted a new procedure for the synthesis of cadmium selenide (CdSe) QDs. During our synthesis experiments the noncoordinating solvent octadecene and a two-step, single flask method were used to synthesize QDs of various sizes and compositions.

This QD synthesis procedure yields QDs that are not soluble in water. We performed ligand-exchange reactions with a variety of hydrophilic molecules to render them soluble in aqueous solvents. The ligand exchange reactions often use thiol compounds that preferentially bind to the nanoparticle surface. A drawback of this technique is that the thiol bonds can quench the fluorescence of the QDs. For this reason, we investigated the performance of alternative ligands that better preserve the intrinsic QD fluorescence.



Emission and absorption characteristics for two batches of QDs.



When excited by ultraviolet light, QDs emit light. The color is dependent on the size of the particles that can be precisely tuned during synthesis.

Coupling of QDs to Biomolecules and Cellular Uptake

We performed a number of experiments designed to target the QDs to living cells. We attached the molecules dopamine and folate to QDs in order to target them to living cells with these receptors. There is a variety of coupling strategies for QDs and we used carbidiimide chemistry to attach the molecules via amide bond formation. Our laboratory has several cell lines with dopamine receptors and folate receptors, and these cells will be used to examine the cellular trafficking patterns of the QD conjugates. Cellular uptake is assessed with fluorescence and confocal microscopy. QDs targeted to cells in this fashion are useful for a number of reasons including stable fluorescent imaging. Furthermore they act as intracellular sensors of certain cell processes.

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Detecting Movements of Single Electrons Using an Atomic Force Microscope [Lynda Cockins]

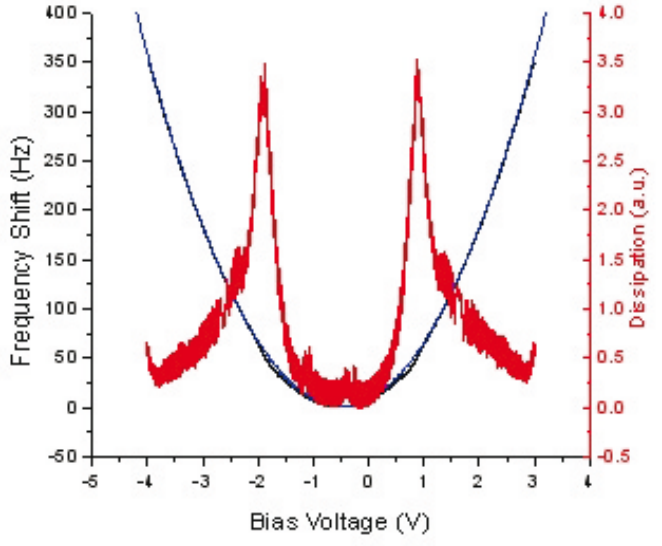
Peter Grütter's group at McGill University provided the German students with a technique to measure single electron tunneling events by using an atomic force microscope. The atomic force microscope consists of a small (micron scale) cantilever with a tip on the end which is brought into close proximity (nanometer scale) to a sample. The forces between cantilever tip and sample can then be measured by optically detecting changes in the resonance frequency of the cantilever.

Two types of measurements can be performed depending on whether the sample is being raster scanned beneath the cantilever or held in the same x-y position with respect to the cantilever, the former providing spatial resolution as in imaging, while the latter provides spectroscopic data as a function of voltage or tip-sample separation.

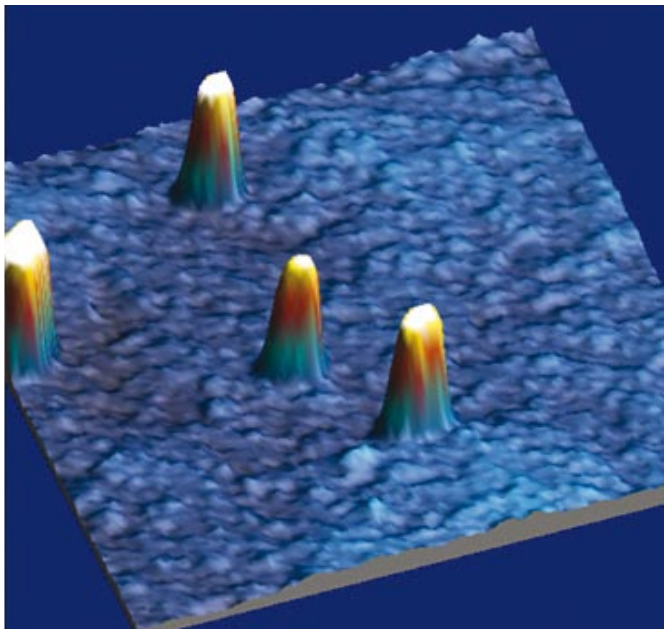
Self-Assembled Quantum Dots

The German students studied a sample consisting of self-assembled InAs quantum dots situated above a 20nm barrier that separated the quantum dots from a planar electron gas (a resource of electrons). In the quantum regime it is possible for small entities, in our case electrons, to tunnel through barriers if the barrier is small enough. Here, this means that the barrier must be neither too thick nor require too much energy to tunnel into.

After pre-cooling our system down to 77K, we proceeded to cool our InAs quantum dot sample to 4.5K. By applying a voltage between tip and sample at this temperature we can provide the electrons with the right energy that they need to tunnel from the electron gas layer into the top layer where the quantum dots exist. This occurrence is marked by the presence of peaks in the dissipation signal as the voltage is swept while the cantilever tip is held at a constant distance above a quantum dot. The figure on the lower right shows an image taken with the German students at liquid nitrogen temperature, while the upper figure shows the spectroscopic curves obtained during a voltage sweep. In the upper figure, note the correlation between peaks in the dissipation with jumps in the frequency shift of the cantilever. This is the signature of a change in the detected force due to the tunneling electron.



Response of the cantilever to a voltage sweep while positioned over a quantum dot. The electrostatic force is quadratically dependent on the voltage, which is apparent in the frequency shift of the cantilever (black). The blue curve is a parabolic fit. The dissipation (red) refers to the energy required by the cantilever to maintain its oscillation amplitude.



Atomic force microscope image taken of an InAs quantum dot sample. The dots are approximately 10nm heigh.

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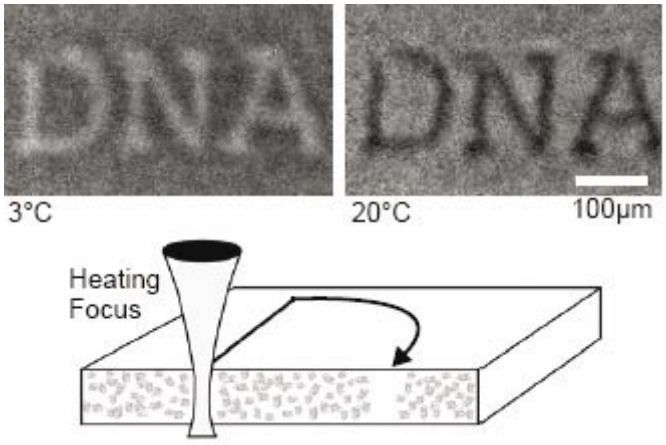
All-Optical Molecule Manipulation by Thermal Gradients on the Micron Scale [Stefan Duhr]

Molecules move along temperature gradients, mostly from hot to cold, but also the opposite is observed. The effect is called thermophoresis, Soret-effect or thermal diffusion. By infrared laser heating we create strong μm-sized temperature gradients of 10^6K/m. This all-optical method is a very versatile tool to manipulate concentration patterns in solution. We use single particle tracking or analyze the buildup of the concentration gradient to obtain information about the movement of molecules in these gradients. With a recent theoretical description of this effect [Duhr and Braun, PNAS in press] it is possible to measure the entropy of solvation of a molecule and thereby determine the surface properties as well as effective charge.

An accurate determination of effective charge in solution is possible for DNA with lengths up to 50.000bp, which is a great advantage compared to electrophoresis, were the free solution mobility saturates for long DNA. In our lab we use fluorescence microscopy and microfluidic setups. The research project was composed of two parts. First, the theory of thermodiffusion was presented since thermophoresis is not well known in the scientific community as a tool for biomolecule analysis. The experimental part of the exchange program should give a general overview of the procedures used in our lab. Especially the irradiation of solutions of colloidal particle/biomolecules with coherent infrared laser was demonstrated.



Thermophoresis manipulates DNA concentration by small temperature differences in solution. A thin water film is heated by ΔT= 2K along the letters "DNA" with an infrared laser scanning microscope. For a cooled chamber at 3°C, DNA accumulates to the warm letters showing thermophilic behavior. At room temperature, DNA is thermophobic, seen by dark letters in DNA fluorescence. Thermophilicity at low temperature is attributed to positive entropy of hydration, whereas thermophobicity is explained by the negative entropy contribution of ionic shielding. Measurements were performed in a 60μm thin chamber with 50nM of 1000bp DNA in 1mM TRIS, where every 50th base pair labeled with TOTO-1.

Experimental Setup

In general, we use a fluorescent microscopy setup consisting of a high power light emitting diode (LED) for excitation, a 40x oil objective and a charge-coupled device (CCD) camera for imaging. We observed the fluorescence of biomolecules or colloidal polystyrene particles in microfluidic chambers. The setup was equipped with two laser scanning mirrors, in order to couple in and move an infrared laser. The laser beam is focused into the microfluidic chamber by an infrared corrected lens. The combination of a fluorescence microscope with an infrared laser allows the creation and measurement of temperature distributions on the micron scale.

The experiments we performed checked for concentration dependencies of thermophoresis where we could show a distinct behavior of molecules at high concentrations. We also analyzed the dependency of molecule velocity on the temperature gradient and searched for nonlinear dependency at strong thermal gradients. Furthermore, we analyzed if the thermophoresis of DNA molecules of different length is different enough to separate these molecules from each other. To resume, the project gave the opportunity to share the knowledge about thermophoresis and to establish this technique as a tool for molecule analysis in other research groups.

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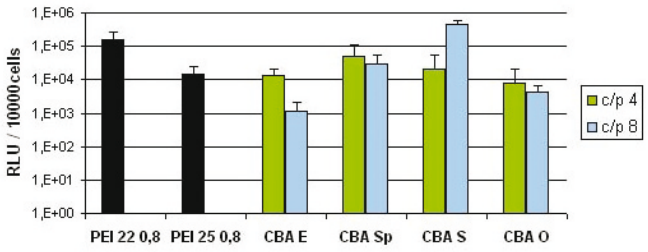
Development of Non-Viral Vectors [Carolin Fella, Verena Ruß]

At present a deciding factor for progress in somatic gene therapy is the development of efficient and safe delivery systems. While several efficient vector systems are available for ex vivo gene therapy, in vivo gene therapy is strongly hampered by the lack of efficient and target-specific vectors. However, a series of therapeutic applications can only be approached by in situ modification of patient cells, and also from the practical and economic point, in vivo gene transfer seems much more attractive.

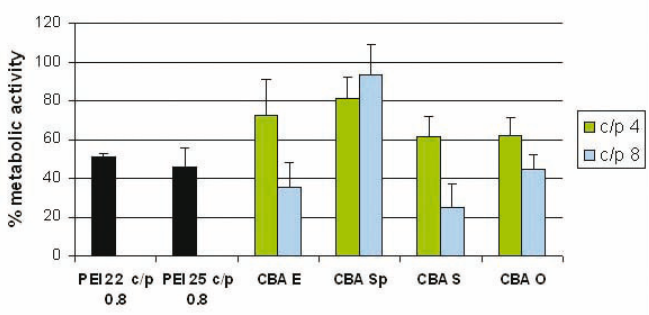
Improving Non-Viral Gene Transfer Systems

Our efforts are focused on the improvement of nonviral gene transfer systems for in vivo gene therapy. DNA complexes are characterized with regard to physical parameters (high solubility, small size, low charge) as well as minimum unspecific interactions with body fluids, tissues or cells (e.g. minimum inhibition by blood components). Despite their ability to transfer plasmid DNA into a variety of cell lines, surprisingly little is known about the mode of entry and intracellular trafficking of non-viral gene transfer particles. Uptake into cells seems to be no limiting step, but cytoplasmic release and entry of DNA into the nucleus are bottlenecks in gene transfer.

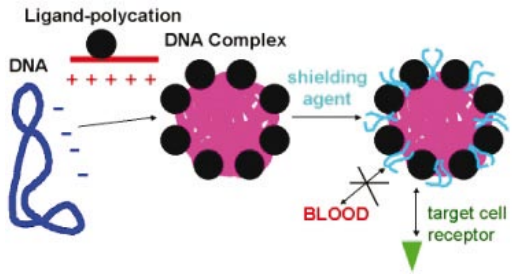
Our two-day research project included particle formation of different non-viral vectors and transfection efficiency and toxicity studies. Readily formed complexes are added to human hepatoma cancer cells. Transfection efficiency could be evaluated 24h after transfection by measuring luciferase reporter gene expression using a Luminometer. In parallel, toxicity of complexes on these cells is determined by an MTT-assay. After transfection, MTT (thiazolylblue) is added. Depending on metabolic activity, the substrate MTT is transformed to a blue dye. Its emission intensity is determined by visible spectroscopy and correlates with the viability of transfected cells. In addition, we carried out size and zeta potential measurements of particles by DLS (biophysical characterization).



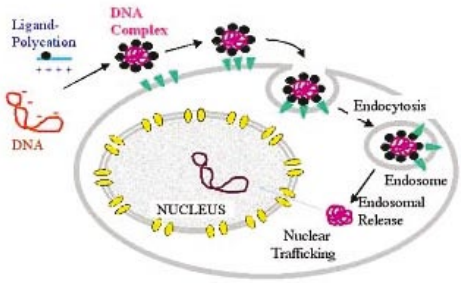
Transfection efficiency of CBA conjugates on murine neuroblastoma cells. Various conjugate/plasmid - ratios (c/p-ratio) of different polyplexes (ligand containing, PEG-shielded or non-shielded particles).



Cytoxity after transfection on murine neuroblastoma cells. Various conjugate/plasmid - ratios (c/p-ratio) of different polyplexes (ligand containing, PEG-shielded or non-shielded particles).



Formation of a non-viral gene-transfer particle.



Stepwise uptake of non-viral gene-transfer particles into a cell.

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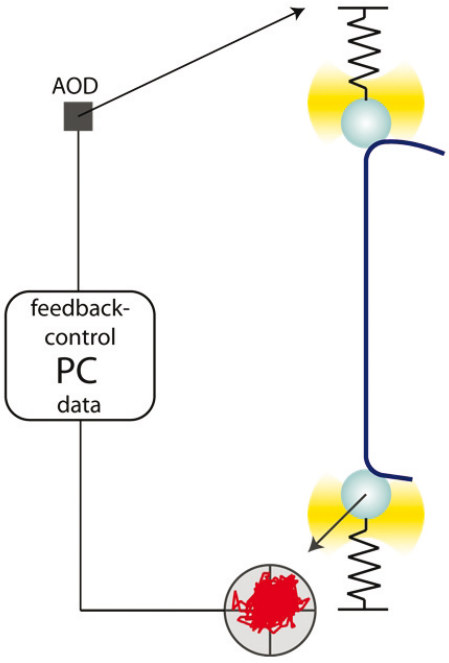
Contact

Stretching Biological Polymers in Optical Tweezers

[Melanie Reisinger, Philipp Feldpausch]

Optical tweezers provide a powerful tool to manipulate single molecules. By focusing a laser beam with Gaussian intensity-profile through an objective with high numerical aperture, a three-dimensional intensity gradient is built which can be used to hold and manipulate small particles, e.g. polystyrene beads (Ø≈1μm). For small displacements of the particles out of the centre of this trap, the trap acts as a Hookian spring. A restoring force which is proportional to the displacement is acting on the particle. Combined with a detection system providing a nanometer-resolution, optical traps are perfectly suited to measure forces in the piconewton-regime (10^{-12}N).

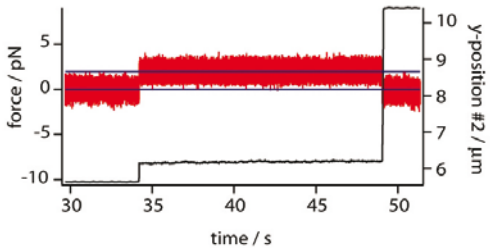
The goal of this research project was to give insight in the operating mode of different types of optical tweezers and to become familiar with the handling of this beautiful and complex experimental technique. We chose two prominent biopolymers – DNA and actin filaments – to be stretched using two different optical tweezers setups.
The stretching of actin filaments was performed with an experimental setup which generated two independent optical traps by switching the laser beam very fast (100kHz) between two different positions. However, the overstretching of DNA was performed on an optical tweezers setup comprising one laser trap and a micro glass pipette.



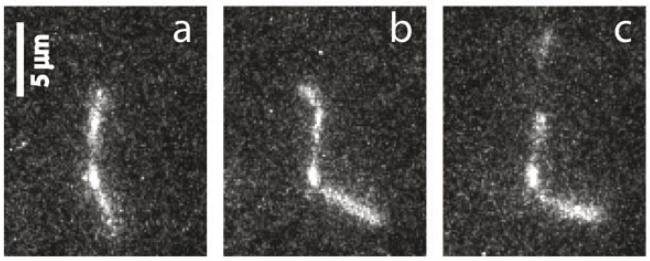
Scheme of the experimental setup. An actin filament (blue) is suspended between two beads held in the optical traps (yellow gradient and springs). The lower bead's image on a photo quadrant diode gives information about the applied tension that is used in a PC-controlled feedback-loop to adjust the position of the upper bead via an acusto-optical deflector (AOD).

Time-shared Optical Tweezers – Stretching Actin Filaments*

Biotinylated actin filaments stabilized by rhodamine-labeled phalloidin were suspended between two neutravidin-coated polystyrene beads. To apply a defined tension to the filament, one bead was moved via a feedback-control, while the other bead was imaged onto a photo quadrant diode to measure the force. Simultaneously, the whole process could be observed via fluorescence microscopy.
One goal of the experiment was to test the stability of the attachment of the actin filament to the beads. All our stretching experiments were finished prematurely by the rupturing of the filament itself. This means that the stability of the actin filaments itself is limiting the strength of the bead-filament-bead system.



Example trace. When the feedback is started, the position of the upper bead (black) is changed to apply a constant tension of ≈2pN (red). When the filament ruptures, the upper bead jumps to a maximum position.



Sequence of fluorescence images corresponding to the upper example trace. The images illustrate the slack (a), tensioned (b) and ruptured filament (c).

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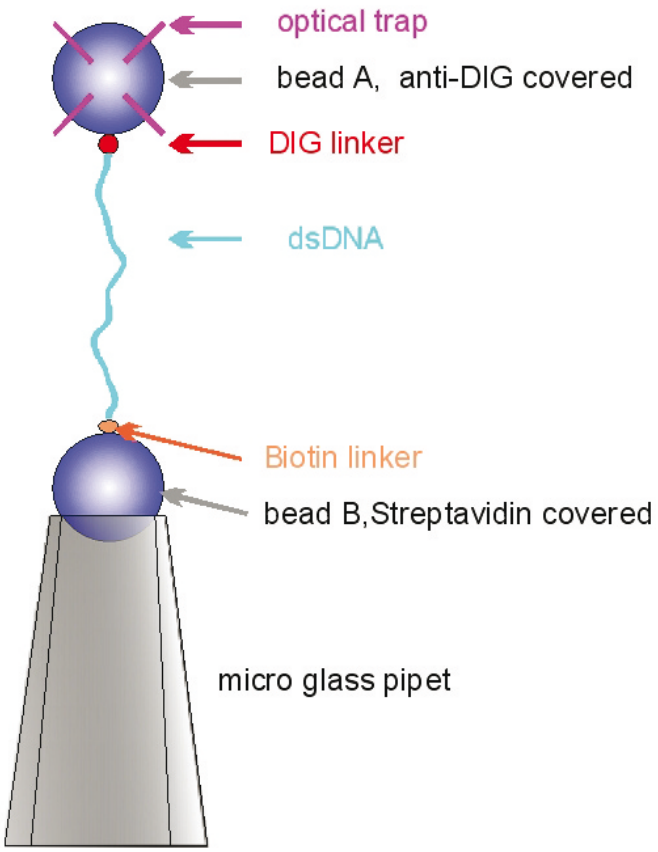
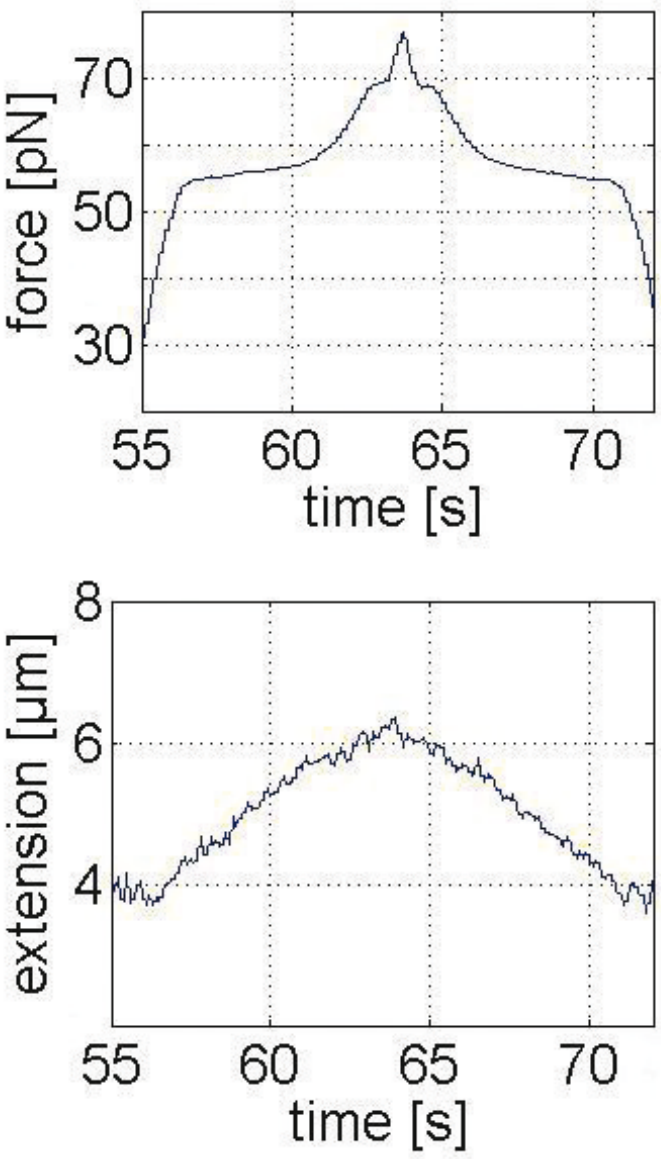
Dual Beam Optical Tweezers – Overstretching DNA**

The participants accomplished single molecule measurements on double stranded DNA. More precisely, they overstretched a 10kbp long Lambda DNA which was attached to specific beads at both ends. For details see the schematic drawing below. It is known that overstretching of double stranded DNA results in a conformational change of the secondary DNA structure from B- to S-DNA. This transition takes place at a tension force of about 65pN. Therefore, a force plateau at 65pN was expected.
The main parts for accomplishing the experiment were to prepare a microfluidic chamber, a micro glass pipet, labeled dsDNA and functionalized beads. After aligning both trapping laser foci on one point, two types of beads had to be loaded, one bead of kind B had to be fixed on the glass pipet and one of kind A in the trap. Stretching experiments could be started by continuously increasing the distance between both beads, after the hookup between both beads had been found.



Experimental setup.



The upper graph shows the force in pN between the two beads versus time. The lower graph shows the bead extension in µm versus time. For the time range of e.g. 55 to 57s the force raises simultaneously with extension. At forces around 55pN a plateau occurs. Continuing the extension, a second - though smaller - plateau at about 70pN is visible. After 63 seconds the bead displacement was reduced continuously. Thus, the corresponding force curve shows inverse behavior.

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Friction Force Microscopy: Ultra Thin Films of KBr on Cu(100) [Tobin Filleter]

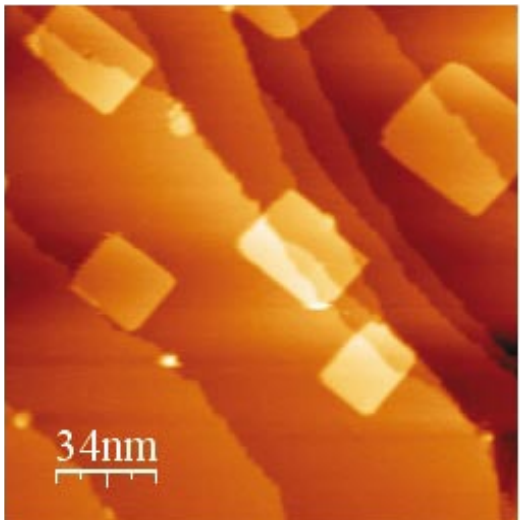
The Atomic Force Microscope (AFM) has been used in a lateral force mode know as Friction Force Microscopy (FFM) to investigate friction on KBr crystals. Dragging the tip of the AFM across the salt yields a steady stick-slip action as it travels across the atoms on the surface. This is observed in the variation of the lateral force measured between the tip and the sample with periodicity of the length scale of the atomic lattice.

Noncontact AFM Mode

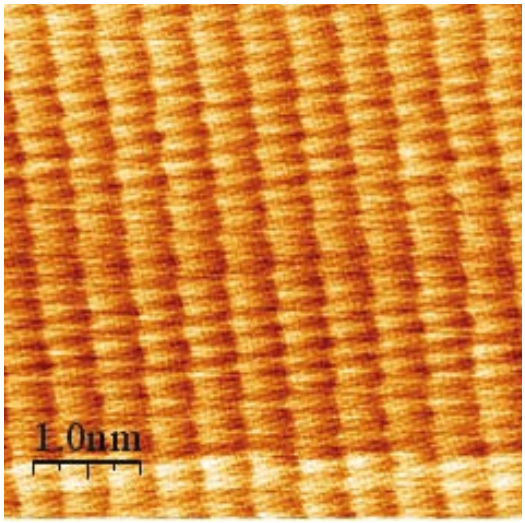
Performing the same experiment on a sample of Cu(100) does not result in regular stick-slip motion. Instead, the measured lateral force on the Cu(100) is irregular, with stick-slip jumps that are far less periodic; the lateral friction is not supported in the soft metal in the same way that it is in KBr. The project involves evaporating a few atomic layers of KBr salt onto the surface of the Cu(100) providing an ideal solid lubricant system. Noncontact AFM combined with FFM will then be used to characterize both the thin films topographic and frictional properties. The goal of the experiment is to determine how many atomic layers of the KBr coating are needed to support the lateral friction force on the surface.

Characterizing Surfaces

During the experiments conducted with the JNN group we were successful in characterizing the surface topography of a thin film with first and second monolayer coverage using NC-AFM imaging. This is demonstrated in the upper figure on the right which shows a high resolution topography image of second monolayer islands of KBr on Cu(100). In subsequent measurements we were also able to measure lateral force maps of the first monolayer of the same film using the FFM technique. This yielded a regular atomic stick-slip variation (lower figure) similar to that observed on bulk KBr. This finding suggests that one monolayer is sufficient to support the lateral friction force on the surface.



NC-AFM topography image showing first and second monolayer coverage of KBr on Cu(100).



FFM image showing the lateral force map on the first monolayer of the KBr film.

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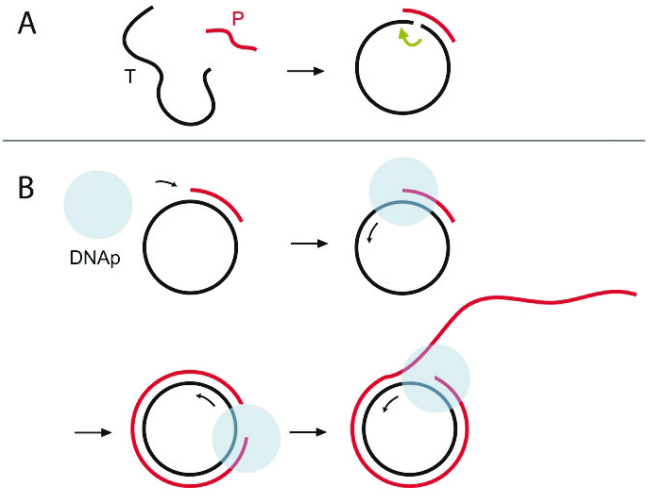
Fluorescence Correlation Spectroscopy on DNA [Eike Friedrichs]

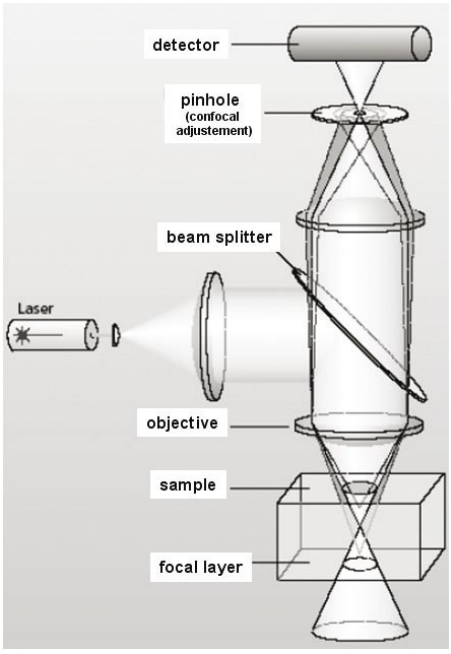
The aim of this research project is to use fluorescence correlation spectroscopy (FCS) to monitor rolling circle amplification (RCA) in real time. FCS is a technique that is used to determine diffusion properties of single (macro-)molecules in solution. Molecules of interest must be labeled with an adequate fluorescence dye.

Rolling Circle Amplification

To produce ssDNA with a repeated base sequence Rolling Circle Amplification (RCA) is a novel method supplementing well-established Polymerase Chain Reaction (PCR). For RCA initiation one needs a template (T) and a primer strand (P), just as with PCR. The template strand must be complementary to the base sequence that is to be repeated. One end of the primer has to be complementary to the beginning of the template strand while the other has to be complementary to the template's end so that both form a circle together after being hybridized. A ligase has to be added that removes the nick between the first and last base of the template strand. After that, a polymerase elongates the primer by adding single nucleotids (dNTPs) so that a full double stranded circle emerges. After one round of transcription the polymerase reaches the beginning of the primer again, displaces it from the template strand, and transcribes the template strand for a second time. Thus, an ssDNA with repeated template complementary base sequence results.



(A) Hybridizing primer (P) and template strand (T) is followed by enzymatically ligating both to a circular template (green arrow).
(B) Phi29 polymerase (DNAp) binds and begins transcribing the circular template resulting in a transcript with a periodically recurring base sequence complementary to the template's strand sequence.



Schematic FCS setup.

Monitoring RCA with FCS

Fluorescence Correlation Spectroscopy (FCS) is based on exciting fluorescently labeled macromolecules by a laser and recording their photon emission. Laser light illuminates an extremely small volume (realized by a confocal illuminating setup) of a solution of these molecules. The combination of such small illumination volumes (femtoliters) together with very low concentrations (nanomolar) makes sure that only a small number of labeled molecules are expected to be in the focal volume at the same time. Molecules diffuse in and out of the volume, and they continuously emit photons as long as they stay in the focal volume. Photons are registered by a photodiode. By measuring fluorescence signal over a long time and calculating an autocorrelation function, it is possible to derive the diffusion coefficient of the labeled molecule. Elongation of a DNA strand will alter its diffusion coefficient and can therefore be monitored by FCS.

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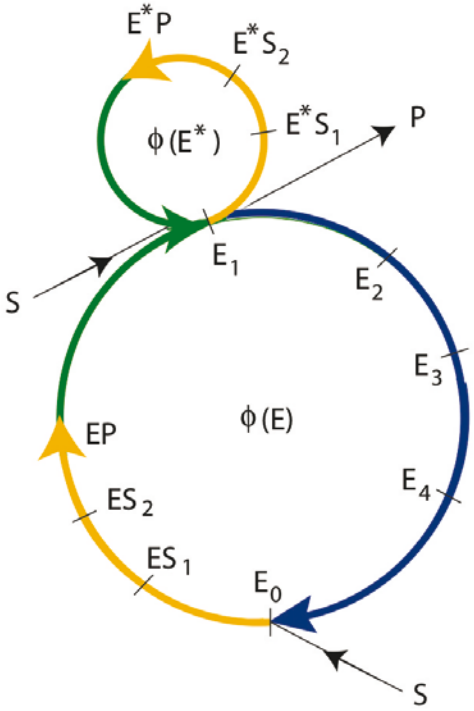
Observing and Controlling Single-Molecule Enzyme Kinetics [Hermann Gumpp]

Understanding the mechanisms underlying enzymatic catalysis on the single-molecule level is a key issue on the way to creating artificial nanobiomachines. Lipases and esterases are exoenzymes that belong to the group of hydrolases, which are generally extremely stable and therefore widely used in industrial applications.

Analyzing Catalysis Kinetics with a TIRF Setup

Here, the lipase B from Candida antarctica (CalB) is utilized for catalyzing a saponification reaction that converts the non-fluorescent substrate carboxyfluorescein ester (CFDA) into the highly fluorescent product carboxyfluorescein which can be observed, reporting the completion of one enzymatic turnover cycle. Single CalB molecules were covalently bound to a glass cover slip and imaged at approx. 200Hz by wide-field Total Internal Reflection Fluorescent (TIRF) microscopy using an Electron Multiplying Charge-Coupled Device (EM-CCD) camera. Hence the kinetics of catalysis can be derived from statistical analysis of the fluorescence timetraces of each individual molecule.

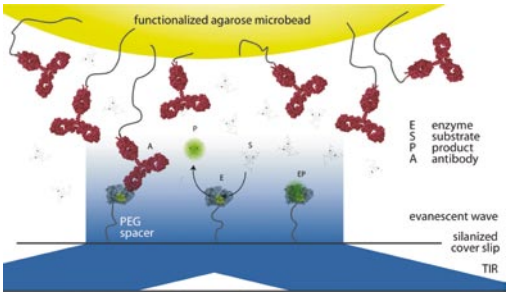


Proposed model of reaction coordinates Φ and substates of enzymatic catalysis in a slow (Φ(E)) and a fast (Φ(E)) cycle, where substrate molecules S are being converted into products P.*

Simultaneous manipulation of the system is achieved by a functionalized microbead bound to an AFM cantilever. Pulling enzymes using a specific antibody with known binding rate leads to conformational changes of the enzyme that influence the reaction coordinate of the catalytic reaction. CalB can be switched between two catalytic cycles by mechanical manipulation. Pulling the enzyme causes conformational changes and triggers oscillations between transient states in the single-enzyme system. The fluorescent on states EP and EP* (product P bound to enzyme E) can be observed and the kinetic constants can be recovered from auto-correlation and probability density functions of the digitized binary time traces. One aim of our research is finding intrinsic resonances in the fluctuating enzyme related to catalysis in order to reveal the network of interconnected transient states and control enzyme activity more specifically.

Imaging Blinking Quantum Dots

The TIRF setup can also be used for fast imaging of quantum dot blinking. Here, instead of enzymes polymer coated CdSe quantum dots are immobilized to the surface and the blinking statistics is analyzed. It turns out that quantum dots also exhibit a stretched exponential decay of the waiting times between on and off states. Moreover, the defined quantized luminosity of quantum dots can be used to distinguish if there is one, two or more individual quantum dots imaged on one pixel where cross-correlation shows if they are actually bound to each other.



Using TIRF microscopy and AFM techniques to visualize and manipulate single molecule enzymatics.

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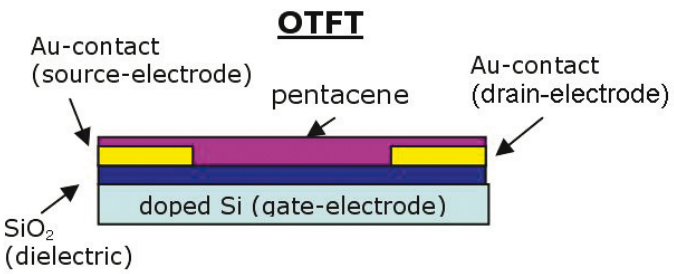
Organic Semiconductors [Martin Huth]

Aromatic hydrocarbons were subject to intense research in the last years as some of these molecules revealed semiconducting properties. Thin film transitors using pentacene - the most famous molecule for this application - as the semiconducting layer, exhibit charge carrier mobilities around typical values of amorphous silicon, making them highly interesting for applications in organic electronics. The different physical properties of the organic devices allow the development of completely new products, such as flexible displays.

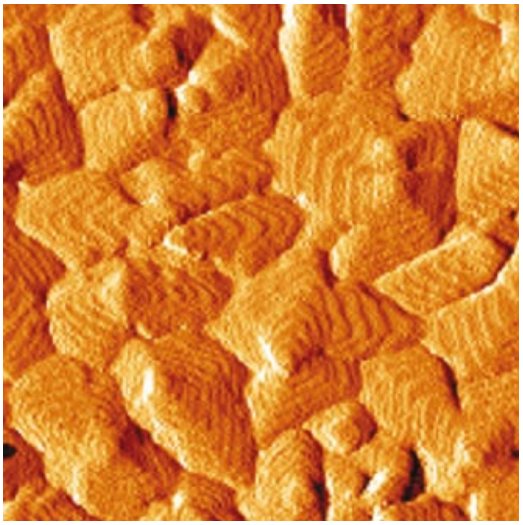
Fabrication of Organic Thin-Film Transistors

The lab course included the fabrication of gold (Au) transistor structures on silicon oxide, the vapor deposition of the organic semiconducting layer (crystalline pentacene), and the electronic characterization of the Organic Thin-Film Transistor (OTFT).

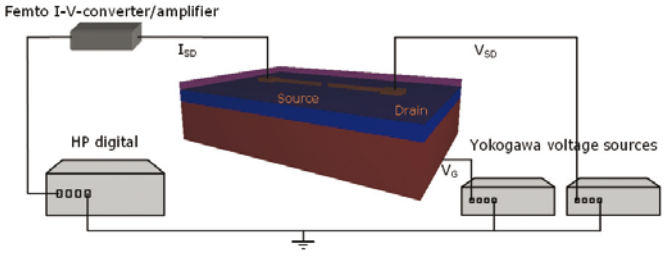


Sketch of a pentacene OTFT.

In detail, after a chemical cleaning procedure (RCA, piranha), source and drain contacts of the transistor structures were thermally evaporated on the silicon wafer which was structured using optical lithography. The samples, now containing the Au-structures, are mounted in a custom made vacuum chamber. The organic material is vaporized from a custom made Knudson cell using two quartz crystals as sensors for layer thickness monitoring. The substrate temperature can be regulated in the range from room temperature up to ~400°C with the help of halogen heating controlled by a Proportional-Integral-Derivative (PID) circuit. A pentacene layer of up to 40nm is vapor deposited on top of the Au contacts. These contacts are then connected using a wedge bonder. The characteristic I-V curves of the transistor are measured to determine the charge carrier mobility.



AFM picture (size: 2x2µm) of a 12nm thick pentacene-layer on SiO_2.



Setup for electronic characterization.

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Synthesis of Gold Nanoparticles [Adil Kassam]

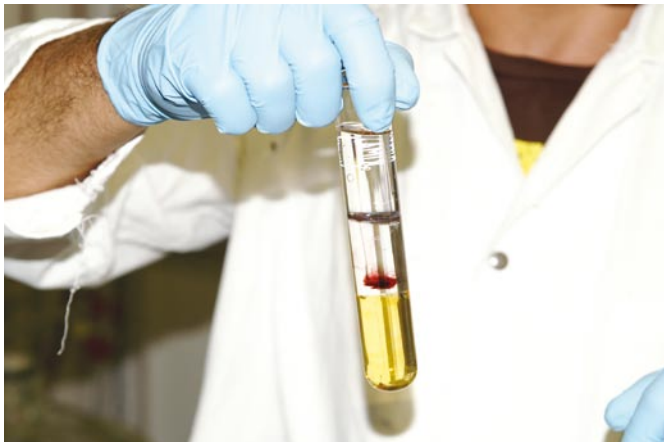
Gold nanoparticles are used in a variety of applications especially in biosensors and biotagging. In this lab course the exchange students were shown different methods of growing gold nanoparticles. Dependent on the growth method chosen (Citrate, Brust-Shifrin or DMAP), gold nanoparticles of sizes in a range of 2 to 50nm could be grown.

Characterization of the Samples

These nanoparticles were then characterized by absorption (UV-VIS) spectroscopy, Nuclear Magnetic Resonance (NMR) and Thermo-Gravimetric Analysis (TGA). In addition, Transmission Electron Microscope (TEM) images were recorded to determine their size distribution. Furthermore, the nanoparticles were handed over to Jonathan Belisle who demonstrated the technique of Third Harmonic Generation (THG) imaging on them.

Colored Liquids

Probably the most enjoyable part of the experiment was that different sized particles show different optical characteristics (colors) in solution. One group decided to make a representation of the German flag with gold particles of three different sizes suspended in the appropriate solvents. The particles the students synthesized were then used in an optical laser setup for the second part of this experiment. Overall, all groups were able to make different particles and while some techniques afforded a greater range of sized particles, all the participants were able to successfully complete the laboratory work.



Suspending gold nanoparticles of different sizes in liquids of different densities.

Gold nanoparticles of different sizes (from bottom to top): 0.4nm (elemental), 15.0nm and 2.0nm, suspended in appropriate immiscible solvents.

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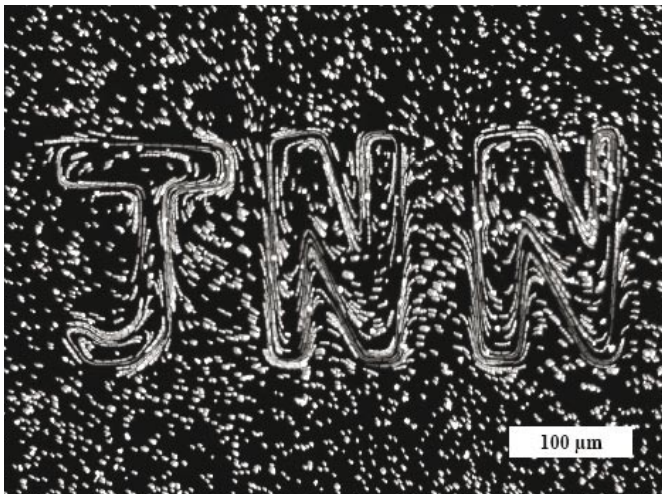
Light Driven Microfluidics [Franz M. Weinert]

Water is pumped along arbitrary patterns with a laser scanning microscope. The fluid follows the path of the focus in arbitrary flow patterns without the need for microfluidic tubings. The flow is driven by temperature dependent viscosity under fast thermal expansion. Pump speed rises quadratically for decreasing thickness, reaching 20μm/s in 2.5μm thin water films. Liquids of high viscosity are equally pumped. The technique has the potential to free micro- and nanofluidics from the overhead of channel lithography, delicate interfacing, and complex pump design.

Pumping Water Optically

Microfluidics have revolutionized complex liquid manipulation for a wide variety of biological and chemical applications in the life sciences. They allow faster analysis times with extremely small liquid volumes and offer high levels of throughput and parallelization. Optical fluid control will allow highly flexible and dynamic liquid handling, both in microfluidic channels and in free two-dimensional gels. By a mechanism of temperature dependent viscosity, the fluid follows the arbitrary shaped two-dimensional path of an infrared laser scanning microscope. Programming the fluid flow by the pathway of the laser focus is straightforward. For example, water can be pumped along letters, forming the words "laser pump" by writing it with the laser spot in reverse direction.

The result is full liquid control in both space and time at optical resolution with velocities of up to 20μm/s. Note that such velocities are faster than DNA migration in an electrophoresis gel, for example. After a theoretical introduction of optical pumping and microfluidics, the effect was shown by pumping water long various defined structures in a 10μm thin sheet of water (see figure). The research project contained the construction of a microfluidic blank consisting of a 5μm thin agarose gel between glass windows. Channels were melted into the agarose gel with the same infrared laser microscope used to pump fluid. The experiment was observed in situ with fluorescence microscopy. Flows and mixing have been visualized with fluorescent beads and fluorescent dyes.



Pumping water optically without walls along a freely defined pattern. Fluid flow is driven by a laser scanning microscope. Complex flow patterns are easily accomplished. Pumping is a result of small fluid movements for each passage of the laser focus. Water flow is visualized by overlaying the difference of successive pictures.

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Networking Beyond Science

Exploring the German Museum in Munich – From the Roots of Science to the Future of Nanotechnology

Being one of the world's leading science museums, an off-hour visit of the German Museum (Deutsches Museum) has been one of the highlights of the exchange program in Munich. Students were taken on a guided tour through central parts of the main exhibition by the museum's general director, Prof. Wolfgang Heckl.

Nanodialogue

Given the scientific background of the students, a special focus of the guided tour was the nanotechnology exhibition "Nanodialogue". This exhibitions aimed at providing information and raising awareness among general public on the latest research in nanotechnologies and nanosciences. The event continued with a welcome reception in the museum which offered the possibility for informal discussions with Prof. Heckl, general director of the museum, and Ms. Katrin Foldenauer, director of the Bavarian-French Cooperation Center (Bayerisch-Französisches Hochschulzentrum, BFHZ). Prof. Gaub, the German organizer of the JNN program, thanked Ms. Foldenauer for the financial support from the BFHZ.

Though spare time was generally limited for the visiting researchers due to a tight schedule consisting of terms of intensive lab courses as well as many social and cultural activities, most of the Canadian visitors tried to catch another glimpse of the technical marvels inside the museum on one of the following days.

From left to right: Dr. Klaus Molitoris (Bavarian State Ministry of Sciences, Research and the Arts), Ms. Katrin Foldenauer (BFHZ), Prof. Wolfgang Heckl (Deutsches Museum), and Prof. Hermann Gaub (CeNS).

Prof. Heckl explains the main highlights of the special exhibition on nanotechnology entitled "Nanodialogue".

View on the German Museum, located on an island in the middle of the river Isar which flows through Munich.

Getting to Know People and Culture

Establishing close relationships on the scientific and personal level within the group was a main objective of the JNN exchange program. The rich cultural program aimed at introducing the Canadian and German guest researches to the culture of Bavaria and Québec, respectively, and successfully stimulated lively discussions between the participants.

Housing Your Peer

Living with your counterparts across the ocean and being immersed into the local culture at a level not afforded by mere tourism surely was the most effective way to promote close relationships within the group even in the relatively short time of three weeks. Gaining this kind of insight into another country's culture was a rare and precious experience for all participants.

Spending a Day Out

The self-organized character of the JNN program gave all host students the opportunity to realize ideas in how to offer a rich social and cultural program to the visitors. Being supported in organization by CeNS and MIAM, as well as by Prof. Gaub and Prof. Grütter, helped the students to come up with a diverse range of cultural events and outdoor activities. To name only the most exciting ones, these events included a thrilling experience of wild water rafting in Canada, an excursion to the famous Bavarian castles of Neuschwanstein and Linderhof, a day out at Prof. Grütter's cottage in the beautiful surroundings of Montréal, and a mountain biking tour in the Bavarian Alps organized by Prof. Gaub.

CeNS Workshop in Venice

All JNN participants attended the CeNS workshop at the Venice International University on the beautiful island of San Servolo, located just in front of the old town of Venice. In addition to the breathtaking backdrop, this unique surrounding attracted internationally renowned speakers in the field of nanotechnology and put all the exchange partners "off-site". This special situation created a strong sense of community and gave the possibility for numerous interesting discoveries in the labyrinth of the small streets and canals of Venice.

Building Strong Ties for Future Collaborations

Thanks to the intensity of the JNN exchange project, a cross-linked network was formed between the participants. These established connections will serve as a support system throughout the scientific careers of the young researchers and provide a reliable base for future science projects.

Upcoming collaborations will focus on topics where different strengths of technical equipment and know-how can be combined in order to achieve new results in a variety of fields of NanoBioTechnology. Two prospective examples of planned collaborations between Canadian and German participants of the JNN exchange program are described in the following.

Novel Functionalized Surfaces

Chemistry student Adil Kassam (McGill University) is collaborating with the physics student Martin Huth (LMU) on the developement of novel functionalized surfaces. Adil Kassam´s expertise in nanoparticle synthesis combined with Martin Huth´s knowledge on the fabrication of nanopatterned surfaces will be combined to build organized nanostructures on micropatterned substrates. This collaboration would not have been made possible via the typical meetings at seminars and conferences but was only initiated by the intensive discussions offered during this exchange program.

New Method for the Characterization of Nanoparticles

Hermann Gumpp (LMU) and Samuel Clarke (McGill University) are discussing the possibility of combining their respective research specialities: Samuel Clarke's laboratory in Montréal synthesizes a variety of nanoparticles which could be studied in a new way with the single molecule fluorescence setup available in the group of Hermann Gumpp.

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