# 10 Physics of Biological Systems

Cornel Andreoli, Conrad Escher, Hans-Werner Fink, Michael Krüger,
Tatiana Latychevskaia (since March 2004), Hiroshi Okamoto, Thomas Rusterholz,
Gregory Stevens (since November 2003), Sandra Pascale Thomann (since March 2004).

*in collaboration with:*
Jevgeni Ermantraut, Clondiag Chip Technologies (Germany), Pierre Sudraud, Orsay Physics (France), Roger Morin, CNRS Marseille (France), John Miao, Stanford University (USA), Dieter Pohl, University of Basel, Andreas Plückthun, Peter Lindner, Biochemistry Institute, University of Zurich, NCCR - National Center of Competence in Research Nano-Scale Science

A fair amount of dedicated equipment has been completed in the course of the past year and the group is now focusing on its scientific goals which remain to be in the exploration of nanometer-sized objects. The nanometer-scale is a unique scale for condensed matter physics in as much as it represents the transition between classical and quantum mechanical physics. At this scale, the transport of charges now relates to the transmission of waves in quantum wires. The de Broglie wavelength, respectively the value of Planck's constant and that of the electron mass, identify this unique length scale. Single electron effects become apparent only at the nanometer-scale which is directly related to the discrete nature and distinct value of the elementary charge of the electron. By using techniques like field ion microscopy and holography with low energy electrons, in combination with other more established tools, we are now in a position to address the above issues. A special emphasis of the group's efforts is related to biological systems. We have recently been able to quantitatively explore the energetics of individual DNA molecules in liquids as illustrated in some detail below. In our efforts to establish the LEEPS (Low Energy Electron Point Source) microscopy as a new tool for structural biology, we have made progress in fitting bacteriophages, that will eventually act as templates for imaging single proteins, onto nano-structured thin films. A focused ion beam machine, delivered in summer 2003, enables us to employ a $10$ nm gallium ion beam for milling structures in silicon devices or other thin-film materials to create suitable structures for interfacing molecules.

## 10.1 Studies of single DNA molecule in liquids

The activation free energy governing the transition from the stretched to the random coil configuration of individual DNA molecules has quantitatively been determined. From the temperature dependence of the transition times it is apparent that the formation of the DNA random coil is a thermally activated process. A total of 420 observations have been made, which were carried out on 80 different individual DNA molecules covering the temperature range from $-5$ C$^\circ$ to $+60$ C$^\circ$. The data indicate that the observed transitions are in fact thermally activated, which is evident from an Arrhenius type behavior. From the Arrhenius plot an activation barrier of a good fraction of $1$ eV has been determined. With this, the first direct determination of the energetics governing an individual DNA molecule in its natural liquid environment has been made.

The experiments have been performed using fluorescent video microscopy with a time resolution of $40$ ms. This technique, pioneered by Steven Chu of Stanford University, is meanwhile employed by a few laboratories around the world to study the dynamics of single DNA molecules. However, to study the energetics of single molecules, some technical challenges had to be mastered. The main problem was the realization of thermal equilibrium conditions free from disturbances of the observation process itself, as well as the ability to change and measure the equilibrium temperature. Furthermore,

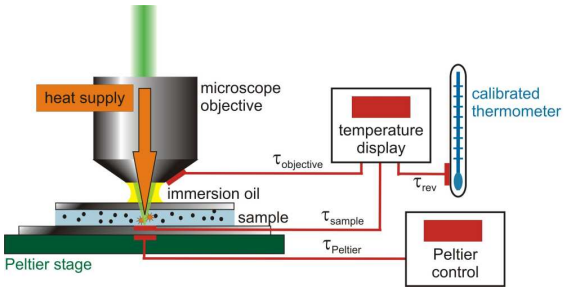


Figure 10.1: *Schematic of the set-up for measuring the energetics of single DNA molecules. Except for the objective lens, most of the optical- and data acquisition-system is not shown here. Four temperature sensors are employed to establish equilibrium in a thin water film. The temperature of the peltier element is adjusted to a fixed value by a PID controller. The temperature at the place where the scenario occurs is then calibrated against the peltier stage temperature, the heat sink, made up of the objective lens and the immersion oil drop, and the temperature of the surrounding air. - Conrad Escher*

in providing a proper distance reference, stretching the molecules in an electric field prior to re-establishing equilibrium and routine methods for anchoring the 16 $\mu$m long and 2 nm thick molecules at one end had to be developed. Attachment of the DNA at one end to a fixed position was essential for being able to derive sufficient statistics to obtain quantitative energy values. This has been achieved in a close collaboration with Clondiag Chip Technologies that provided us with state of the art molecular biology techniques for binding just one end of the molecule to a silicon oxide substrate. The experiment takes place in a liquid environment in which a single DNA molecule is embedded in just a few pico-liters of ultra pure water. It is part of a 10 $\mu$m thick water film which is sealed from the environment to avoid evaporation and associated erratic liquid flow which would perturb the equilibrium conditions, essential for deriving quantitative free energy data. A schematic of part of the apparatus is illustrated in Fig. 10.1.

## 10.2 Towards the limits of holography with low energy electrons

The use of a coherent single-atom electron source for low energy electrons has developed into a tool that appears to be particularly useful for investigating individual objects of nanometer-sized dimensions. The absence of radiation damage due to the low energy of the electrons and the high contrast in scattering by light atoms holds promise as a new tool for structural biology of individual molecules. The sensitivity to small electric and magnetic fields, combined with the high spatial and time resolution of the technique, will also be used to explore the properties of mesoscopic physics objects, such as quantum wires and single electron devices. This project aims at reaching the experimental resolution limit of this technique, which will then be made available to other projects, both inside and outside of our research group. The ultimate limit is given by the spatial and temporal coherence of the source. It relates to the size of the source and the energy spread of the emitted electrons, which is fundamentally limited only by the Heisenberg uncertainty relationship. In order to come close to this fundamental limit, which is associated with atomic resolution in 3 dimensions, a number of experimental issues need to be addressed and new technologies developed. They include: shielding the coherent electron source from external mechanical and electronic noise, developing a new two-dimensional detector for low energy electrons and improving the angular spread of the coherent electron wave-fronts by adsorbing $C_{60}$ clusters onto the atomic point-source. The numerical work of Tatiana Latychevskaia is directed towards the important second step in holography, namely the reconstruction. This implies employing appropriate coherent optics schemes for reconstructing the 3-dimensional structure of the nanometer-sized object from its experimental electron hologram record.

## 10.3 Cryogenic LEEPS microscopy

This project primarily aims at improving the resolution of room-temperature LEEPS-microscopy, taking advantage of the extraordinary mechanical stability of cryogenic instruments and the possibility of utilizing novel schemes to effectively reduce the energy spread of the electron point source.

The project began in February 2002, and we have completed the construction of the instrument in June 2003. Since then we have been testing the microscope. The specially designed piezo stepping motor system, together with the computer control system have been thoroughly tested at room temperature as well as at cryogenic temperatures down to $4.2$ K. The difficulty of using a micro-channel plate (MCP) at cryogenic temperatures made it necessary to search for an alternative detector. A post-acceleration detection system utilizing a micron-scale metallic mesh has been successfully developed in collaboration with diploma student Thomas Rusterholz[1]. With this detection system we obtained reasonable image quality at an emission current of $10$ nA. We have also observed electron interference fringes near carbon fibers at room temperature, and we expect to see interference fringes at cryogenic temperatures in the near future. The instrumental testing phase will be completed within a few months, and we can then move on to the scientific investigations of novel methods in electron microscopy and its application to biology.

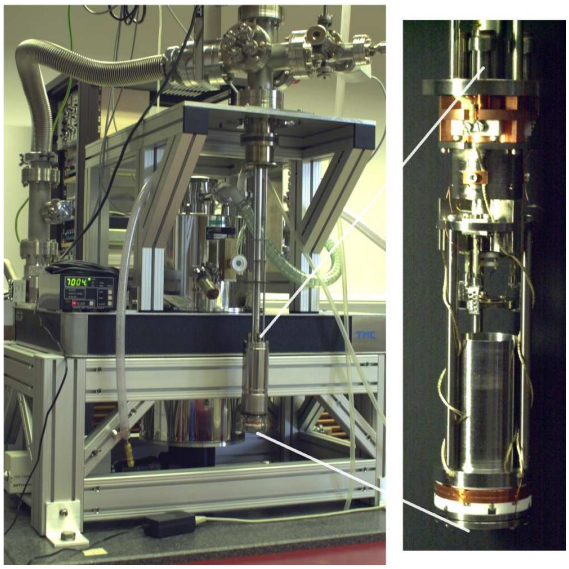

Figure 10.2: *The Cryogenic LEEPS microscope. - Hiroshi Okamoto*

[1] Thomas Rusterholz, *Ein neuer Detektor für Holographie mit langsamen Elektronen*, diploma thesis Physik-Institut der Universität Zürich, January 2004.

## 10.4 Structure of individual bio molecules

The purpose of this project is to develop tools and methods to image individual bio-molecules with a coherent low energy electron beam to obtain structural information. As a first step, novel methods need to be developed to attach individual macromolecules to surfaces. These investigations have begun with a filamentous phage called M13, which is to be attached to a carbon support film so that individual phage particles are suspended over holes in the film so they can be imaged using the low energy electron beam. The distribution of attached phage particles is being characterized using a transmission electron microscope (TEM).

Since beginning the project in November 2003, we have prepared a homogeneous suspension of M13 phage using a standard protocol and have developed a method for making holes of a desired size and shape in carbon support films using a focused ion beam. We have investigated ways of pre-treating the carbon film by glow discharge in air so as to impart a hydrophilic property to the surface that is necessary for attachment of M13 and other bio-molecules suspended in aqueous media.

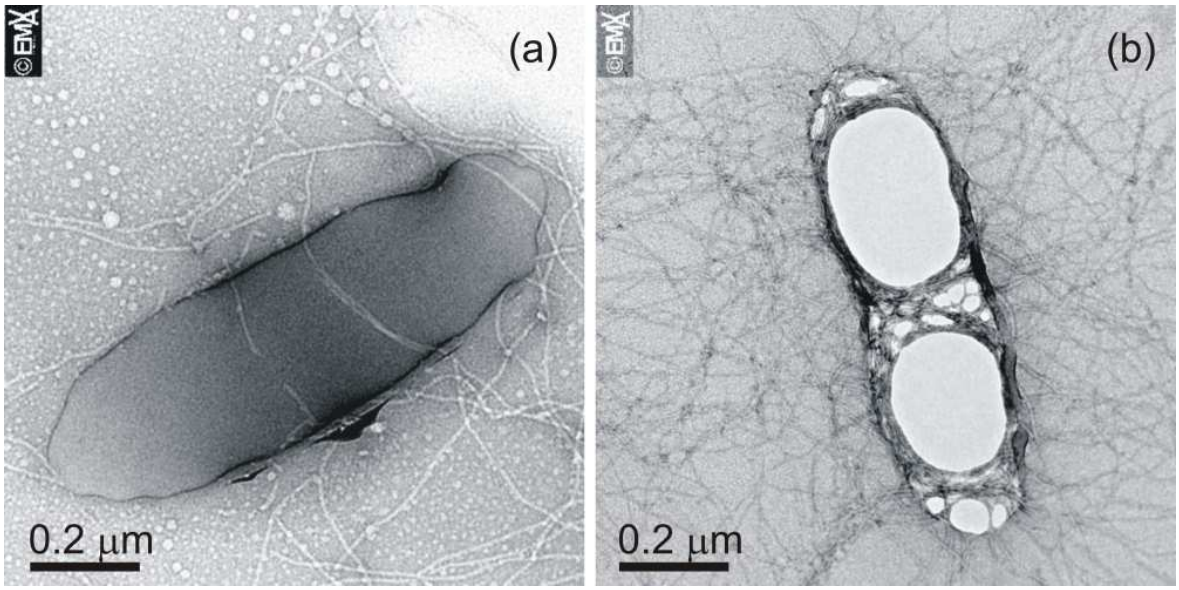


Figure 10.3: *TEM (Transmission Electron Microscopy) images of individual M13 phage particles suspended across holes in a carbon film. The samples were negatively stained with uranyl acetate, which has formedinto a film across the hole (a). In the case where the negative stain did not form a film across the hole (b), the M13 filaments were found to lie in bundles across and around the edge of the hole* [3]*. - Gregory Stevens.*

We have examined the dependence of phage concentration on the distribution of particles deposited onto carbon films and can obtain even distributions of particles at a preferred density. As shown in Fig. 10.3a, we have obtained TEM images of individual phage particles suspended across holes in a carbon film that were subsequently embedded in a film of negative stain. In cases where the negative stain film was absent, phage particles were found to adhere to the edge of the hole after drying, as shown in Fig. 10.3b. We have shown how surface tension in a water film is responsible for rearrangement of filaments that were previously suspended over holes, after part of the water film breaks during drying. In order to overcome such rearrangement during drying, we are currently investigating methods for rapidly freezing the specimen and then sublimating the surrounding ice under vacuum so that phage particles remain undisturbed.

We have begun to address concerns that bio-molecules may undergo significant conformational changes when placed in the vacuum environment of a LEEPS microscope by showing that Green Fluorescent Protein (GFP) retains its ability to fluoresce after several days in a vacuum. In future, GFP may be used for investigating the extent of radiation damage to biological material in the LEEPS microscope. We are currently examining the possibility of attaching lysine residues on the exterior of the phage coat to a gold surface using N-hydroxy-succinimide (NHS) chemistry. To this end, we have obtained a mutant of a related filamentous phage f1, which has been genetically engineered to have extra lysine residues on its surface. This might be useful for attaching gold nanoclusters of known size to the surface of the phage for purposes of calibrating images, or to prevent rearrangement of phages by strongly attaching them to a gold substrate film.

[3]We gratefully acknowledge the Institutes of Veterinary Anatomy and Virology, University of Zürich, for the use of their facilities in making these images.

## 10.5 Nanometer-sized structures for creating and investigating mesoscopic physics devices

This effort is directed towards directly observing the dynamics of individual charges in mesoscopic devices, like quantum dots, wires or single electron transistors. Single charges and their associated fields are expected to be visualized by holography with low energy electrons. What is needed for such a program is a quantum dot weakly coupled to at least two electrical leads by a tunnel barrier. Our focused ion beam appears to be invaluable in achieving that. Fig. 10.4 shows a carbon nanotube that has been cut using the focused gallium ion beam. The next step will be to trap an appropriate small metal cluster in this gap and to make sure that it is weakly coupled via two tunnel-barriers to the two nanotubes electrodes.
Another approach is to use highly oriented pyrolytic graphite (HOPG) sheets and the focused ion beam to in-situ fabricate quantum wires and measure their properties in particular in relation to ballistic electron transport. An example of this effort is shown in Fig. 10.5.

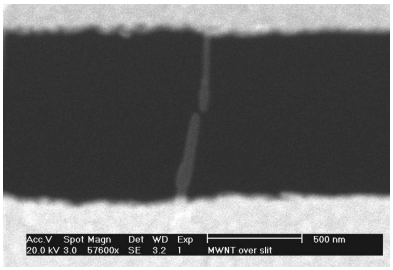


Figure 10.4:
*A freestanding multi-wall carbon nanotube has been deposited over a slit in a SiN-membrane and subsequently been cut by a focused ion-beam.*
*- Michael Krüger.*

While the preparation of the structure as well as the transfer to the microstructure with the integrated electrodes is now routinely possible, no current has yet be detected up to a bias of 70 V. Unfortunately, just as with molecular electronics structures, the contact resistances appear to be a particularly puzzling problem at this scale, which needs to be solved.

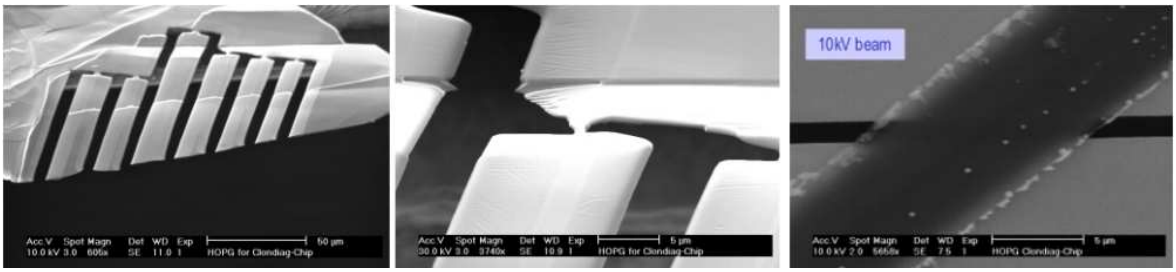


Figure 10.5: *HOPG sheets structured by a focused ion beam, removed by a precision manipulator and subsequently deposited onto a silicon device for electrical measurements.*

## 10.6 Field ion microscopy

Our field ion microscope, previously being used only to characterize our electron point sources, has now two dedicated purposes. They are being pursued by Sandra Thomann and Cornel Andreoli. The first and major goal is the investigation of the adsorption of single $C_{60}$ clusters onto pyramidal tungsten tips, from which we expect to learn something about single molecule adsorption events as well as to obtain superior coherent electron sources. This work started in March 2004 and benefited from the expertise of the Institutes Surface Science group, in particular from Thomas Greber and Anna Tamai. Based on their experience in handling the sublimation of $C_{60}$ clusters,Cornel Andreoli and Kurt Bösiger designed and build a $C_{60}$ evaporator particular to our needs, namely in being free from disturbing light emission during sublimation of the clusters. The second project involving field

ion microscopy is the investigation of electrolyte tips for creating novel ion sources. Here the ion supply is provided by mobile bulk ions inside the electrolyte. This has distinct advantages over the ionisation from gas phase atoms. This work is carried out in collaboration with Dieter Pohl from the Physics Institute of Basel and had just started before the end of this report period. However, it already appears promising.

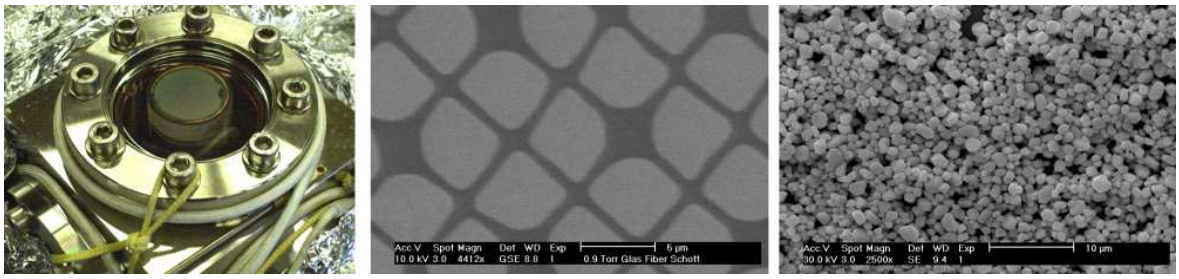


Figure 10.6: *Field emission microscope to study the diffusion of individual Cs atoms (left). Essential parts of the detector systems are a fiber optic plate (middle), donated to us by Shott-Glass with* $5$ *micron thick fibers, and a fine phosphorous powder (right) of about 1 micron grain size, donated to us by the medical science division of Siemens. - Hiroshi Okamoto, Cornel Andreoli, Chan Mei Po (summer-student in 2003).*

## 10.7 Field emission microscopy to study the diffusion of Cs adsorbed on tungsten surfaces.

A field emission microscope is dedicated for students' experiments in association with the lecture series entitled "Physics on the Nanometer-Scale". It allows the students to measure the field emission current from a region of some $10$ nm in diameter. The fluctuation of the current is directly related to the diffusion of Cs atoms entering or leaving the probe area. From the autocorrelation of this signal, the diffusion coefficient can be evaluated at different temperatures, starting at $80$ K. This project is under the supervision of Hiroshi Okamoto who benefited from occasional help of summer students. With the assistance of the University glass blower Daniel Schnarwiler, Cornel Andreoli managed to build a quite original detector for this experiment, in as much as each fibre of the plate transmits a signal corresponding to a sub-nanometer region of the surface on which the motion of just a few Cs atoms takes place. The students can thus define a $10$ nm diameter region to be probed by simply taking a black piece of paper with an appropriate hole of mm dimension at the atmospheric side of the detector. In this way they can probe the field emission current from a nanometer-sized region by using a photomultiplier to pick up the signal delivered from a few hundreds of these fibres.

The experiment is expected to become available to the students shortly after the first course on "Physics on the nanometer-scale" is completed, which is after the summer-semester 2004.