# 14 Physics of Biological Systems

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*in collaboration with:*
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In the past year the new laboratory has become available and the installation and operation of most of the scientific equipment was celebrated in October 2002. The overall goal of our efforts is directed towards an understanding of the unique properties of objects with nanometer-scale dimensions with a special emphasis on biological systems. The high brightness and coherence of our electron source in combination with high phase contrast in scattering off biological molecules and the lack of radiation damage are key issues that make us believe that LEEPS - Low Energy Electron Point Source - microscopy has the potential of becoming a novel tool for structural biology on a level of individual molecules. Our recently completed laboratory environment with its mechanical damping platforms and ac-magnetic field compensation has been designed in such a way as to preserve the coherence of the low energy electron wave fronts used for imaging.

Apart from the imaging aspects needed for structural information, the LEEPS technique also allows to mechanically and electronically manipulate individual objects at the nanometer scale. This possibility of in-situ imaging and manipulation makes it possible to also address issues related to the electrical conductivity in molecules or other mesosopic systems.

In order to be able to control single molecules already in the liquid phase we adopted the technique of optical fluorescent video microscopy in the frame of Conrad Escher's diploma thesis that was completed in early 2002. Meanwhile, video microscopy has become a powerful tool for us, not only for controlling single molecules in the liquid environment, but also for developing strategies to interface them to silicon structures. This is currently pursuit in a close collaboration with Clondiag Chip technologies, a biotechnology company in Jena.

## 14.1 Low energy electron point source - LEEPS- microcopy

The key to the LEEPS technology is a coherent electron source of atomic dimension that emits low energy electrons. This allows performing holography in a lens-less setup with the advantages of absence of lens aberrations and high contrast due to the low energy of the electrons. The lack of radiation damage, also due to the low kinetic energy, makes this technique particular appealing for applications in molecular biology. The latter is pursued in the frame of our involvement in the National Center of Competence in Research (NCCR) Nano-Scale Science. In collaboration with Andreas Plckthun's group of the Biochemistry Institute at the University of Zurich, techniques for preparing individual proteins to be investigated by low energy electron holography shall be developed.

An important issue, not only for structural biology, is the ultimate resolution that can be achieved. Preliminary holograms of carbon nanotubes and fibers, taken in late 2002, indicate already a significantly improved interference resolution as evident from the increased number of fringes in the holograms. A detailed distance calibration and a quantitative evaluation of the data are currently in progress. A state of the art CCD detection system has made it possible to gather holograms with a

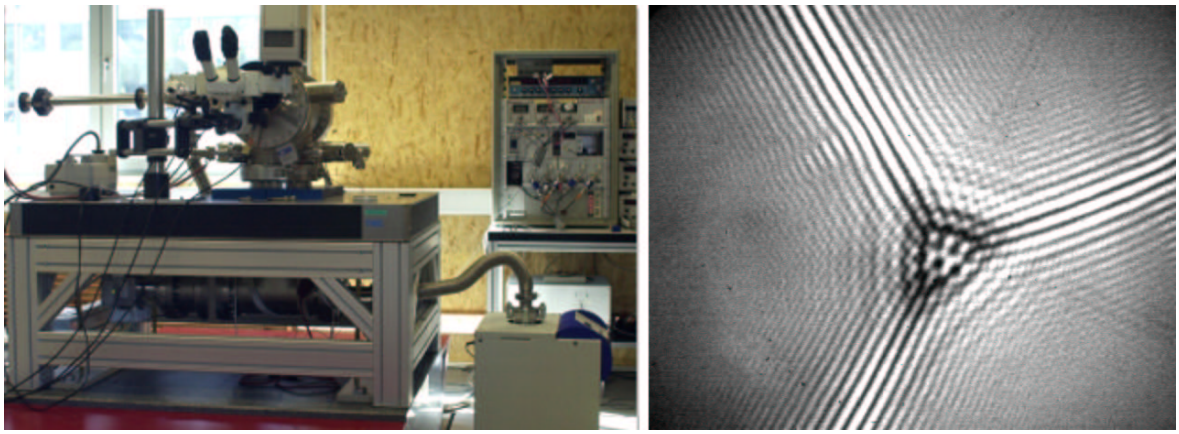

Figure 14.1: *Room temperature LEEPS microscope (left) and holograms of carbon nanotubes taken with 100 eV electrons.*

continuous variation of the kinetic energy by a factor of three. This is an important aspect to eliminate the twin image problem in the numerical reconstruction process and a prerequisite for reaching 3-dimensional real space information.

## 14.2 Low temperature LEEPS microscopy

Once optimal protection of the coherent wave front is achieved and routinely implemented, intrinsic thermal vibrations will still be present. To freeze in the vibrational modes of molecules, Hiroshi Okamoto has initiated a cryogenic temperature LEEPS microscope project. He started the design and building of a low temperature system in February 2002. Meanwhile, all mechanical and electronic parts, built by our machine- respectively electronic-shop, are completed. Since channel-plates do not operate at low temperature, a new spatial detector for low energy electrons is being developed in the frame of the diploma thesis of Thomas Rusterholz. The testing and assembly of all parts of the low temperature LEEPS are in progress and first results are hoped to show up soon.

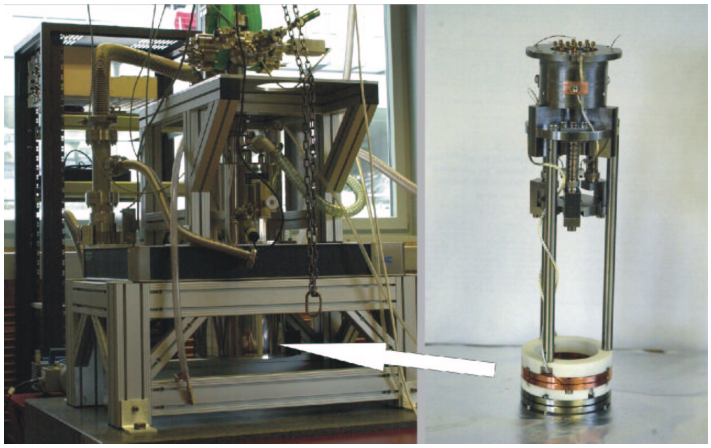

Figure 14.2:
*Low temperature (2.5K) LEEPS microscope - Hiroshi Okamoto.*

The scientific benefit of a low temperature holographic microscope is not just related to aspects of ultimate resolution. It is also expected to provide new insight into single electron quantum effects since individual elementary charges, respectively their surrounding electric field, can be visualized by coherent low energy electron wave fronts. Thus, at low enough temperature, the dynamics of individual charge transfer processes in single electron devices could be made visible.

## 14.3 Numerical hologram reconstruction

The second step in holography is concerned with the retrieval of the 3-dimensional shape of the object. Back-propagating the waves from the holographic record, which contains amplitude and phase information, does this. However, back propagating implies replacing the time (t) by (-t) which can -fortunately- not be achieved in real life, but by the use of a computer.

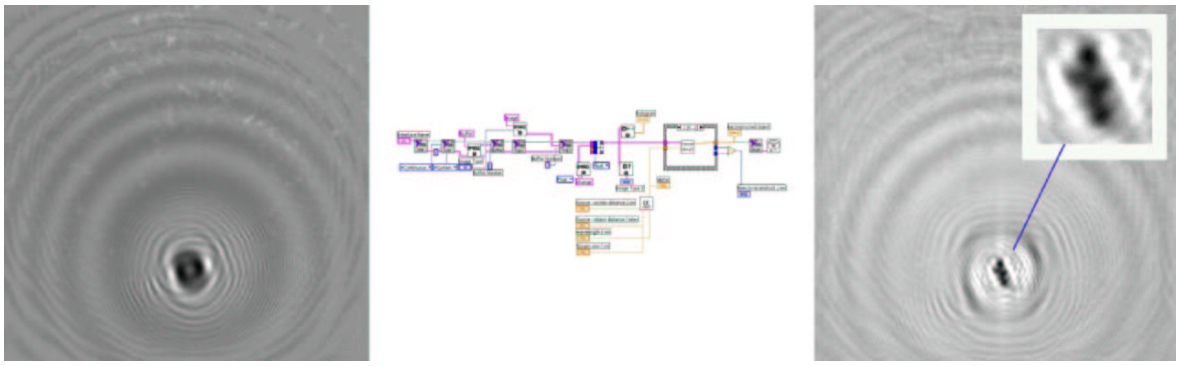

Figure 14.3: *Numerical on-line hologram reconstruction tested here with green Laser light and a sample consisting of 1-micron diameter Latex spheres on a glass plate. The hologram is shown at left. Data acquisition from a CCD system and the numerical reconstruction takes about three seconds before the reconstruction, shown at right is being displayed - Tatiana Latychevskaia.*

Tatiana Latychevskaia has started to work on a project concerning the numerical reconstruction of holograms. The goal is to reach a fast on-line reconstruction routine based on a numerical solution of the Fresnel Kirchhoff Integral. The numerical methods were tested with an optical set-up and it appears that the essential requirements for a fast on-line reconstruction routine can be met.

## 14.4 Field ion microcopy (FIM)

Atomically sharp tips, which we employ as electron point sources, are shaped by field ion microscopy techniques. Field evaporation is used to create the desired structures by removing one atom at a time in a controlled fashion. A Field Ion Microscope has been completed and became operational in fall of 2002.
At present, the FIM serves mainly as a tool to characterize our electron sources. Its design however is such as to be able to also carry out correlation experiments on a high phase space density ensemble of free electrons to explore their fermion statistics. This project has been pursuit for one year, unfortunately with no success. Thus, it has been stopped in summer 2002.

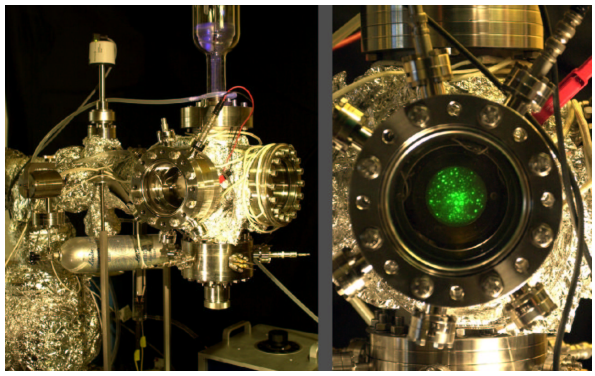

Figure 14.4: *Liquid nitrogen cooled Field Ion Microscope, designed and built by Cornel Andreoli. At right, a helium field ion image of a tungsten tip is shown with atomic resolution.*

## 14.5 Polymer dynamics

The possibility to control biological molecules in their natural liquid environment appears to be highly desirable for a number of our research goals including the open questions in regards to DNA conductivity. In the course of these efforts some basic questions in polymer physics are also being addressed. The theory of polymer physics, as put forth by Gilles de Gennes, predicts that a coil to stretch transition plays an important role in understanding the properties of these interesting materials. To actually observe this transition and to quantitatively measure its kinetics and energetics on an individual molecular level is an experimental challenge. In Fig.14.5 an experiment of this sort is illustrated. A single DNA molecule, a biopolymer, has been anchored to a surface at one end. Under ordinary thermal conditions, the Brownian agitation drives the system to assume its equilibrium state, a configuration of minimal free energy. In the case of a DNA this configuration is a random coil, the accepted model for this highly flexible polymer. By employing an external force, in our case realized by an electric field, a stretched DNA configuration can be arranged. This is a situation that would hardly ever be observed under equilibrium conditions. By switching off the electric field, the equilibrium situation is re-established almost instantaneously, considering the time-scales relevant for the molecular dynamics. Thus, the stretched polymer configuration evolves again towards its equilibrium configuration, the random coil. By using video microscopy it became possible to measure the relaxation time of a single DNA molecule. Further experiments for improving the data statistics to be carried out at various temperatures should reveal quantitative values for the random coil free energy, that is believed to be made up mainly of an entropy term.

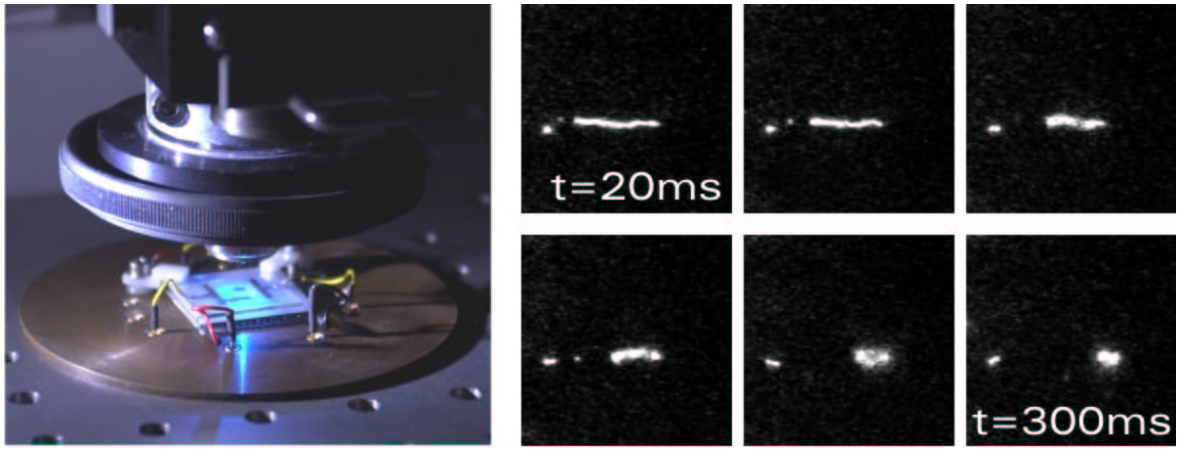


Figure 14.5: *Objective lens and cooling stage of a modified commercial fluorescent microscope are shown at left. The sequence at right shows a single DNA molecule, anchored to a surface at its right end and stretched by an electric field to its contour length of 16 micron. Once the external force is switched of, the molecule assumes its equilibrium configuration. As evident from the above sequence, it is possible to observe the transition from the stretched configuration to the random coil structure at 5 C and thus measure quantitatively the polymer relaxation time - Conrad Escher.*

## 14.6 Interfacing molecules to micro- and nano-structures

For combining molecules with silicon structures, an understanding of the liquid solid interface is needed. We employ a commercial ESEM microscope, modified by Cornel Andreoli to allow for sample cooling and electrical contacts to the samples, to investigate wet samples.

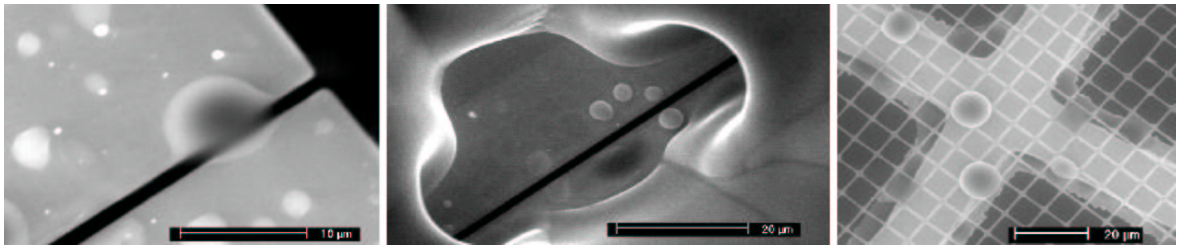


Figure 14.6: *Wetting behaviour of a slit-structure in a silicon nitride membrane, provided by Condiag chip technologies. At right: condensation of water on a micro-machined carbon grit (Quantifoil GmbH).*

The same scanning electron microscope is also used for creating sub-micron structures of freestanding objects. An example of which is shown in Fig.14.7. Gap structures of the order of 10 nm can be produced in this way.

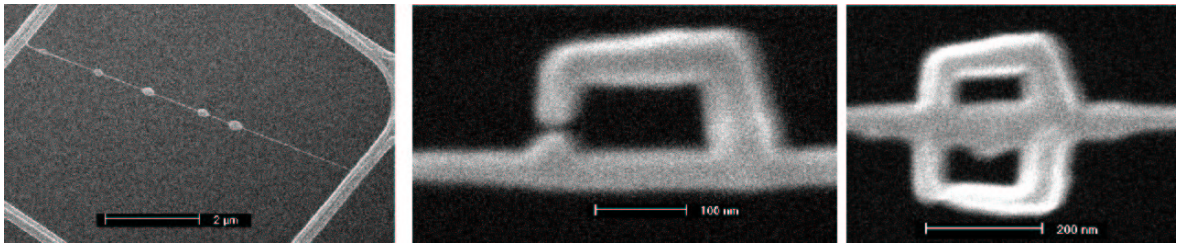


Figure 14.7: *In-situ growth of amorphous carbon structures onto a free-standing carbon single-wall nanotubes rope. Left: About 8 micron long carbon nanotube-rope, as stretched over a grid, and subsequent writing of a structure onto it.*