13 Physics of Biological Systems

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in collaboration with:
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The structural investigation of individual biological objects by employing coherent low energy electrons is the primary goal of our research. It involves holography with low energy electrons as well as coherent diffraction and is assisted by micro-structuring techniques using a focused gallium ion beam device.

Our current activities are divided in the following interconnected individual projects:

- **SIBMAR**
  Structural Information of Biological Molecules at Atomic Resolution is part of the EU "New Emerging Science and Technology" Programme. SIBMAR aims at high resolution structural information of individual biological molecules by employing coherent low energy electron waves. Partners from the Institute of Scientific Instruments Academy of Sciences of the Czech Republic in Brno, the Physics Department of the University of Manchester and the Physics-Institute of the University of Zurich are involved in SIBMAR. The overall idea is to apply holography with low energy electrons to investigate the structure of individual biological molecules. Major experimental challenges are to improve the interference resolution in electron holograms, establish methods for creating free standing thin films transparent for low energy electrons as well as appropriate techniques to present a single protein to the coherent electron wave front. Next to these experimental issues, a second, equally important aspect for achieving high resolution structural information is the reconstruction of the electron holograms. This is achieved by back-propagating the object wave information, recorded in the hologram plane, by employing a numerical algorithm to solve the integrals governing this coherent optics problem.

- **Electron and Ion Point Sources**
  We employ Field Ion Microscopy and related techniques for fabricating and using novel electron and ion point sources. Recently we have established collaboration with the PSI to characterize field emitter arrays produced by electron beam lithography and intended to be used as bright electron sources for the XFEL (X-Ray Free Electron Laser) project.

- **Fluorescent Microscopy**
  The aim of this project is to directly observe the dynamics of single DNA molecules in liquids by video fluorescent microscopy. In combination with molecular anchoring techniques, adopted from Clondiag, we address the energetics of a single DNA molecule. Appropriate DNA modifications for attaching fluorescent proteins to are designed by Clondiag Chip Technologies in Jena and shall serve us in our efforts to obtain structural information about proteins by electron holography.

- **Coherent Low-Energy Electron Diffraction Microscopy**
  This is a second approach, next to electron holography, of using a coherent electron wave front for structural biology at the single molecule level. It is in collaboration with John Miao from the University of California at Los Angeles and supported by the Swiss National Science Foundation and shall be described in some more detail below.

13.1 Summary

Most of the protein structural information data available today have been obtained from crystallography experiments by means of averaging over many molecules assembled into a crystal. While this has led to an impressive data base, a strong desire to gain structural data from just a single molecule is emerging for several reasons. Most of the biological relevant molecules exhibit different conformations; thus averaging over many of them smears out their fine structure. This need for averaging also limits cryo-TEM studies to particularly rigid species, like viruses (1) and radiation damage limits the obtainable resolution to $\approx 10\mathring{A}$ (2) The second motivation for the desire to image just one individual molecule is associated with the wish to drop the need to force proteins to assemble into crystals. If just one single protein could be analyzed in sufficient detail, the important class of membrane proteins that have a tendency to not crystallize would finally be accessible. Due to the strong inelastic scattering of X-rays and high energy electrons as employed in conventional electron microscopes, there is little hope for obtaining structural information from just one single molecule. The worldwide developments of X-ray Free Electron Lasers [6] did rise hope to circumvent the undesirable ratio between elastic and inelastic scattering by employing extremely short and bright pulses which do not leave enough time for the molecule to decompose owing to radiation damage.

The objective of this project is to derive atomic structure information from experiments carried out on just one individual molecule subject to the interaction with a coherent low energy electron wave. Evidence that there is no respectively undetectable radiation damage on a single molecule, combined with the fact that the de Broglie wave length of the low energy electron wave is in the $1\mathring{A}$ regime provide the potential for atomic resolution imaging of a single biomolecule. It appears that electrons with kinetic energies below 200 eV are the only known radiation today where elastic scattering dominates and radiation damage is minimal respectively not present at all (3). This in turn allows the acquisition of a large enough data-set from just one individual molecule without damaging it. This will be done by recording oversampled coherent low energy electron diffraction patterns of individual biological molecules. From these, the structure of the molecule can be recovered at a resolution of $2\mathring{A}$, which is sufficient to show the location of individual atoms in the molecule. The principle of iterative phase retrieval by oversampling pioneered by J. Miao (4), in which unique phase information about the wave front is recovered from a diffraction pattern, has already been demonstrated experimentally using X-rays and high energy electrons, limited however to robust inorganic samples.

[6] The Linac Coherent Light Source (LCLS) in the USA, http://www-ssrl.slac.stanford.edu/lcls/, to be completed in 2009, will be the world's first X-ray free electron laser for the creation and study of exotic states of matter, imaging structures and dynamics of biological and chemical molecules on an atomic scale and probing the fundamental aspects of atomic structure. A similar project, DESY in Germany, http://www.desy.de/html/home/index.html is expected to be operational by 2012. More recently, a Swiss XFEL project has been initiated at the PSI, http://fel.web.psi.ch.

13.2 The coherent electron diffraction microscope

The overall setup of the coherent electron diffraction imaging experiment is sketched in Fig. 13.1. A sharp W-tip acts as a field emitting electron point source of a coherent spherical electron wave of energies between 20 and 300 eV. A micro-lens placed a few microns away from the electron emitter forms a coherent parallel wave that impinges onto a molecule attached to a micro-structure some distance behind the lens in a field-free region. At a distant detector, the amplitude of the diffraction pattern, which corresponds to the Fourier transform of the diffractive object, is recorded with high spatial resolution. In order to be able to sample this pattern with sufficiently high frequencies to match the oversampling requirement, the object must be surrounded by a no-density region.

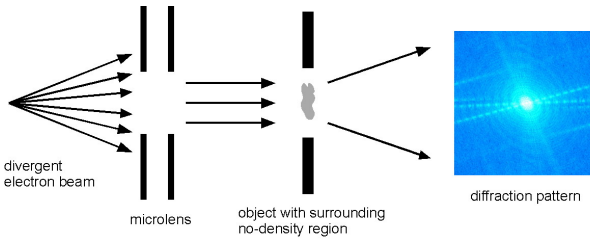

Figure 13.1: Schematic representation of the coherent electron diffraction microscope.

13.2.1 Experimental setup

A new diffraction microscope has been designed and built featuring a dedicated detector system for obtaining oversampled diffraction patterns (see Fig. 13.2). It comprises a micro-channel plate, a small grain phosphor screen directly deposited onto a fibre optic plate and a high resolution CCD camera. An electrochemically etched single tungsten (111) crystal, as used in LEEPS microscopy, serves as the source of coherent low energy electrons. It is mounted on a three-axis manipulator so that the tungsten tip can be positioned with a precision of 50 nm. The sample is mounted on a separate four-axis manipulator to enable centering in the beam. An additional degree of freedom for rotating the sample has been included so that diffraction patterns at various tilt angles can be acquired.

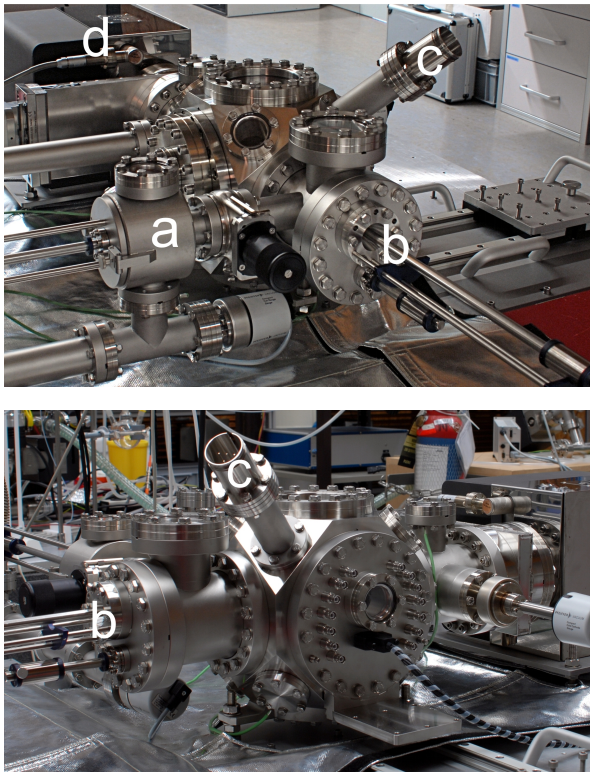

Figure 13.2: Vacuum chamber of the Coherent Diffraction Microscope. It comprises a load-lock (a) for rapid sample transfer, a wobble-stick (b) for flexible sample manipulation, a secondary-electron detector (c) for possible future applications and an elaborate pumping-system (d) to achieve UHV conditions.

The microscope has been designed to match the requirements of the oversampling phasing method and to optimise the

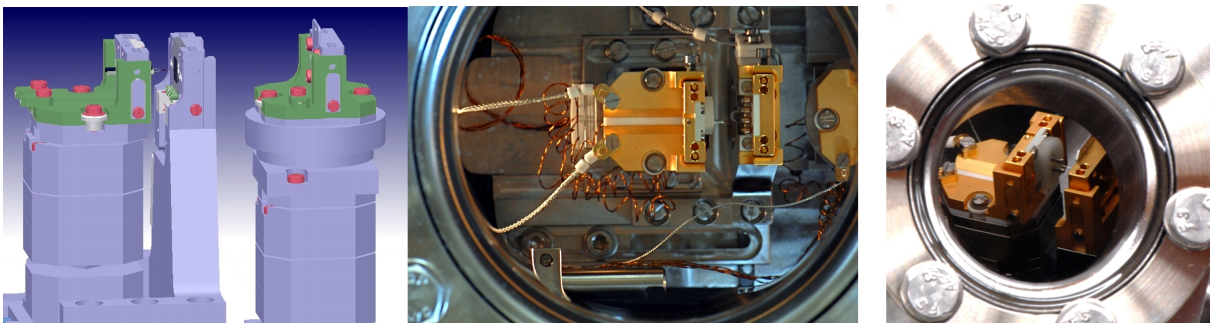

Figure 13.3: Electron source and sample are attached to manipulators featuring nm precision (schematic at left). A top (middle) and side view (right) into the Coherent Diffraction Microscope.

recording of diffraction patterns. These requirements on the experimental setup are summarized by:

$$\Delta \leq z\frac{\lambda}{Oa} \quad ,$$

where λ is the wavelength, a the object size, Δ the pixel size of the detector, z the object-screen distance and O the square root of the ratio of the size of the no-density region surrounding the sample and the sample size, the so called oversampling ratio. The resolution is limited by the size of the detector, the sample-detector distance, the wavelength of the electrons, the oversampling ratio and the temporal coherence of the electron wave. With a state of the art pixel detector and a temporal coherence of the electron wave which is approximately 0.0005 for 200 eV electrons, an ultimate resolution of 0.70 $\AA$ could eventually be reached.

13.2.2 Electrostatic micro-lens

In the coherent diffraction microscope the micro-lens shall collimate the divergent electron beam emitted from the tungsten tip to form a parallel beam which is directed onto the molecule. The overall idea of employing a micro-lens is associated with the fact the electron lenses suffer from intrinsic spherical aberrations, essentially since there exists no concave electron lens, as has been recognized by Scherzer (5) a long time ago. Our way of circumventing the Scherzer theorem is to scale down electron source and lens dimensions. Since our electron source exhibits already an ultimate size of atomic dimension it is thus a matter of scaling down the lens and placing it at a short distance from the source. The lens diameter is directly associated with the spherical aberration Cs, as illustrated in Fig. 13.4 for a two-electrode lens. A typical macroscopic lens has an inner bore exhibiting centimeter

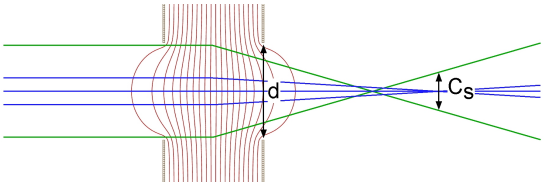

Figure 13.4: Schematic to illustrate the implication of lens dimensions d on the intrinsic spherical aberration Cs creating a disk of least confusion in the Gaussian focal plane of an electron lens.

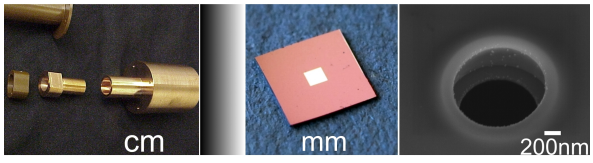

Figure 13.5: From left to right: macroscopically machined lens with centimeter dimension bore, silicon wafer with SiN window, electrostatic lens with 1 micron aperture.

dimension as illustrated in Fig. 13.5. We use a 5 mm by 5 mm silicon chip with a 1 mm by 1 mm SiN window of 1 micron thickness to form a micro-lens. With appropriate micro-machining techniques, explained in some detail below, we end up with a lens exhibiting an aperture of just 1 micron as shown in the SEM image at the right of Fig. 13.5. In comparison to conventional lenses, we have scaled lens dimension and thus spherical aberration by four orders of magnitude.

13.2.3 Micro-lens designs

During the past two years, we developed several procedures for fabricating micro-lenses. Principally, the lens consists of two carbon electrodes, separated by an insulating layer, with micrometer-sized apertures (see Fig. 13.6. Ray tracing calculations have shown that such design leads to lenses exhibiting the desired properties, in particular, the ability of the insulating layer to withstand the potential difference between the electrodes needed to collimate the electron beam. For the detailed fabrication of the lens there exist several approaches ranging from assembling the lens manually to more elaborate methods requiring several steps of evaporation and micro-structuring.

One of the methods considered as promising starts with the structuring of a 5 μm large hole in a 1 μm thick SiN membrane with the help of our focused ion beam (FIB) machine. Afterwards, two carbon flakes, previously deposited onto mica sheets, are transferred on

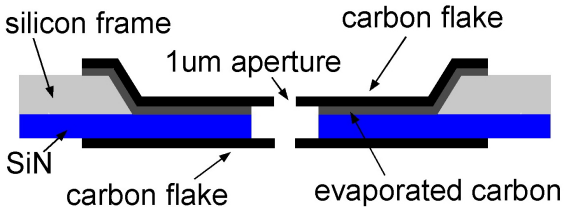

Figure 13.6: Schematic side-view of a micro lens.

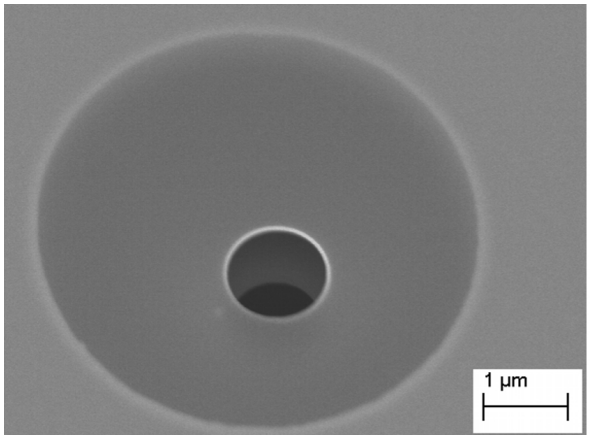

Figure 13.7: SEM image of a micro-lens taken at a tilt angle of 30^o. The 5 micron bore covered on each side by a thin carbon flake is still visible.

both sides of the structured membrane. Finally, the 1 μm lens aperture is structured in the free-standing carbon flakes deposited over the 5 μm hole. A SEM image of the final result is presented in Fig. 13.7.

The picture series displayed in Fig. 13.8 demonstrates clearly that we are able to produce a parallel electron beam. To further characterize the lens focusing properties, the dependence of the focal length on the applied voltage has been extracted from several series of projection images. This demonstrates that the fabricated lenses work as expected and that our ray-tracing simulations are a reliable tool for designing electrostatic optical elements on a micrometer scale and shall in the future serve us as a guide for more elaborate micro-lens systems.

The next step shall be to place a single molecule into the parallel beam generated by the micro-lens and record a diffraction patter with a high sampling ratio.

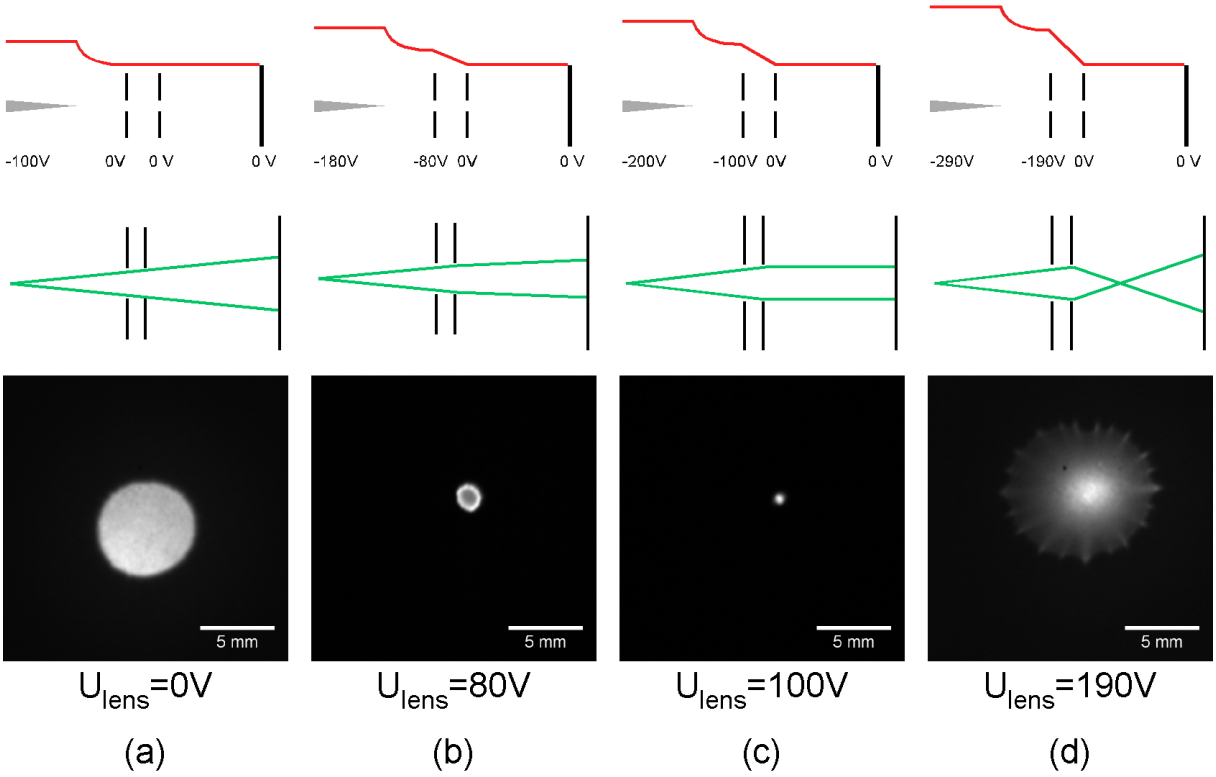

Figure 13.8: Series of projection images of a lens with varying negative voltage at the first lens electrode. For each projection image the corresponding potential energy of the electrons and the focusing situation are displayed. (a) Projection image of the lens aperture without applied voltage, (b) beam with a reduced divergence angle, (c) parallel beam impinging onto the detector, (d) cross-over point only a few microns behind the lens. In all situations, the distance between tip and lens amounts to approximately 8.5 μm and the lens to detector distance is fixed to 70 mm.

[1] M. van Heel et al., Quart. Rev. Biophys. 33 (2000) 307.

[2] J. Frank, Ann. Rev. Biophys. Biomol. Struct. 31 (2002) 303.

[3] M. Germann, T. Latychevskaia, C. Escher, H.-W. Fink, to be submitted.

[4] J. Miao et al., Nature 400 (1999) 342.

[5] O. Scherzer, Z. f. Physik 101 (1936).