13 Physics of Biological Systems

C. Andreoli (until October 2007), C. Escher, H.-W. Fink, M. Germann (since September 2007), Tatiana Latychevskaia, J.-N. Longchamp (since October 2007), Elvira Steinwand.

in collaboration with:
Eugen Ermantraut, Clondiag Chip Technologies (Germany); Dr. Pierre Sudraud, Orsay Physics (France); Prof. John Miao, University of California at Los Angeles (USA); Prof. Dieter Pohl, University of Basel; Prof. Andre Geim, Manchester Centre for Mesoscience and Nanotechnology, University of Manchester, Dr. Ilona Müllerová, Institute of Scientific Instruments (Czech Republic), Dr. Heinz Gross, ETH Zurich, Dr. Petr Formanek, University of Dresden (Germany).

In connection with the structural investigation of biological objects by Low Energy Electron Point Source (LEEPS) microscopy we established a sample preparation technique based on DNA. The molecules are stretched over holes in a structured thin film by a laminar flow followed by rapid quenching in under-cooled liquid nitrogen to embed DNA in amorphous ice. The final step, before transfer to the LEEPS microscope, is the freeze drying at low temperature in vacuum. The DNA molecule serves us as a template for presenting proteins to the coherent electron wave. DNA modifications for attaching proteins are designed by Clondiag Chip Technologies in Jena.

Free standing graphene membranes shall serve as transparent support for holography with low energy electrons in connection with the EU project SIBMAR. We are newly assisted in this endeavour by a group in Brno. They carry out Scanning Low Energy Electron Microscopy (SLEEM) studies on free-standing graphene membranes. A second approach towards structural biology on a single molecule level is based on phase retrieval from an over sampled coherent electron diffraction pattern of a single molecule. The instrument for that is now operational and preliminary data show that it is possible to generate a coherent parallel beam by employing a micro-structured electron lens. Some recent achievements towards our goal to obtain structural information from a single molecule are illustrated in some detail below.

13.1 Numerical hologram reconstruction

The recent solution of the long standing twin image problem in holography (1) has meanwhile also been applied to the reconstruction of holograms taken with 200 keV electrons in the holography laboratory of the University of Dresden. It turns out that the twin image removal technique leads to reconstructed in-line holograms comparable to those from off-axis holography where the object and reference wave are propagating in different direction. For low energy electrons, however, their strong forward scattering still needs to be build into the reconstruction routine.

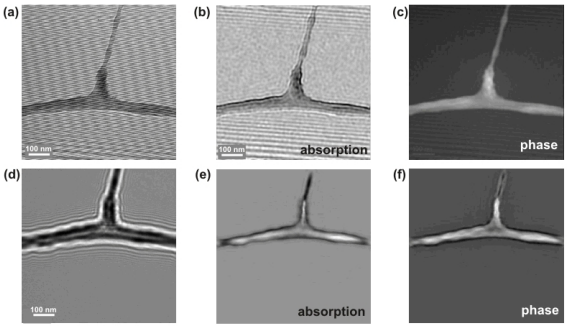

Figure 13.1: (a) High-energy electron off-axis hologram of a carbon net taken taken in the Holography laboratory at the University of Dresden. (b) and (c) Absorption and phase reconstructed from the off-axis hologram. (d) High-energy electron in-line hologram of a carbon net. (e) and (f) Absorption and phase reconstructed from the in-line hologram.

13.2 Pulsed holography with low energy electrons

Restrictions to the resolution in low-energy electron holograms are associated with residual vibrations and ac magnetic fields. Both limit the coherence of the beam and with this the interference resolution in the holograms. In order to circumvent these resolution limiting factors we now do acquire holograms in a pulsed mode. The pulse duration times have to be well below typical vibration periods and ac-magnetic field changes. However, short exposure times lead to holograms with a poor signal to noise ratio. This problem has been overcome by acquiring a large set of pulsed holograms of the same molecule and using cross-correlation to account and correct for the shift between subsequently taken holographic records of the same molecule.

Initial experiments show that this strategy of pulsed holography works and leads to improved interference resolution as evident from Fig. 13.2. The set of superimposed holograms of a DNA molecule shows the disturbing effect of vibrations which smear out high order interference fringes. If cross correlation is employed to align subsequently taken holograms, a significantly improved signal to noise ratio and visibility of high order fringes, already evident by visual inspection, is apparent.

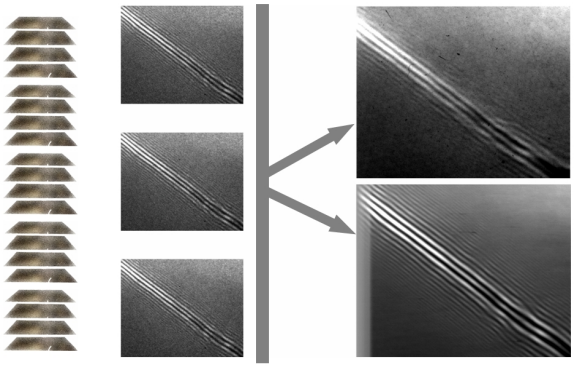

Figure 13.2: A set of 400 electron holograms has been taken in the pulsed mode at 140 eV kinetic energy of the electrons. The MCP was held at a dc bias of 670 V and a pulse of 500V was applied to the MCP to increase the gain for 50 microsecond duration. Three out of 400 noisy data sets are shown. At the top right, the superposition of all 400 records is shown, at the bottom right, the superposition after cross- correlation alignment is displayed.

Apart from improvements in the detector system, our major strategy for improving interference resolution relies now on pulsed holography. While the first data seem promising, improving the short time electronics is a major challenge. In order to avoid unnecessary exposure of the molecule during the time in between pulses, it will be necessary to also pulse the electron source with a precision of a few 10 mV in order to not sacrifice temporal coherence.

13.3 Radiation damage by low energy electrons

Radiation damage is one of the major obstacles in using radiation with sufficiently short wave length, like x-rays or energetic electrons, for structural determination of single molecules. In x-ray diffraction, only every thousandth photon is scattered elastically and carries information about the molecules structure; all the rest just causes damage to the fragile object. For low energy electrons it is so far only qualitatively known that the amount of damage is much less. We have started a detailed study on determining the extent of radiation damage and contrast transfer by low-energy electrons in the range between 10 and 500 eV. The permissible dose will ultimately determine the signal-to-noise ratio for the high-resolution terms incorporated in the holographic reconstruction and will allow optimal set-up of imaging conditions in LEEPS microcopy. We hope to discover energy windows in which essentially no damage to proteins occurs. This would then make low energy electrons the only known

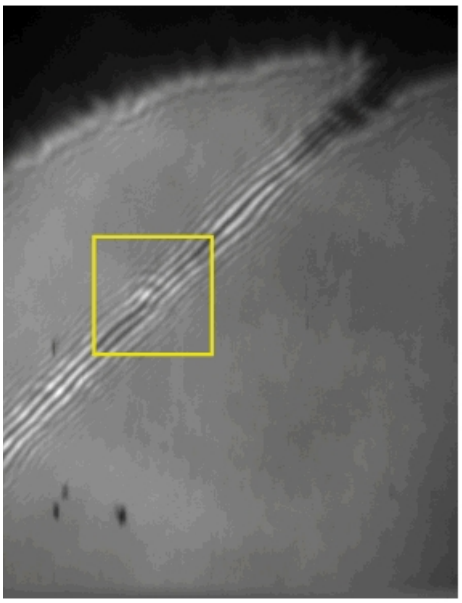

Figure 13.3: Electron hologram of a DNA. Marked in yellow is the area that is used for computing the cross-correlation between subsequent hologram.

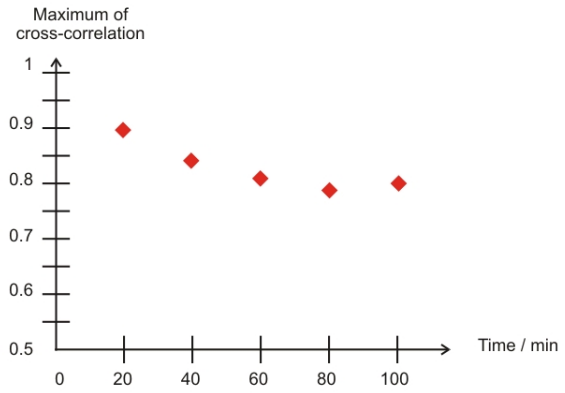

Figure 13.4: Maximum of the cross-correlation between the first hologram and the later recorded holograms as a function of time.

radiation with potential atomic resolution not plagued by radiation damage. A first indication that such windows might indeed exist is displayed in Figs. 13.3 and 13.4. A DNA molecule has continuously been imaged for 100 minutes with coherent electrons of 107 eV. The total emission current of 100 nA corresponds to an estimated dose of 108 electrons/nm^2. Despite this massive dose the hologram of the molecule has not altered significantly as evident from the cross correlation coefficient between subsequent images which maintains a high value throughout the 100 minutes of observation.

[1] T. Latychevskaia and H.-W. Fink, Phys. Rev. Lett. 98, 233901 (2007).