ESRF	Experiment title: **A freely suspended lipid bilayer facing asymmetric aqueous solutions: the effect of a E-field on the bilayer structure**	**Experiment number**: SC 2380
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Shifts: 18	**Local contact(s)**: Lutz Wiegert Anders Madsen	*Received at ESRF:*

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The main goal of this experiment was to apply x-ray reflectometry and phase contrast imaging to freely suspended lipid bilayers, called black lipid membranes (BLMs) in an aqueous environment. Here the influence of electric fields onto the bilayer structure, more precisely the electron density distribution of the membrane system, shall be probed. This technique will allow for simultaneous measurement of electrophysiological and structural properties of a reconstituted biological interface. A previous experiment at BM05 showed that hard x-ray phase contrast images in the propagation imaging mode can be obtained on the above membrane system. A bulged membrane spanning an aperture in a solid substrate in a coherent parallel x-ray beam was imaged 1-3 m downstream from the sample.

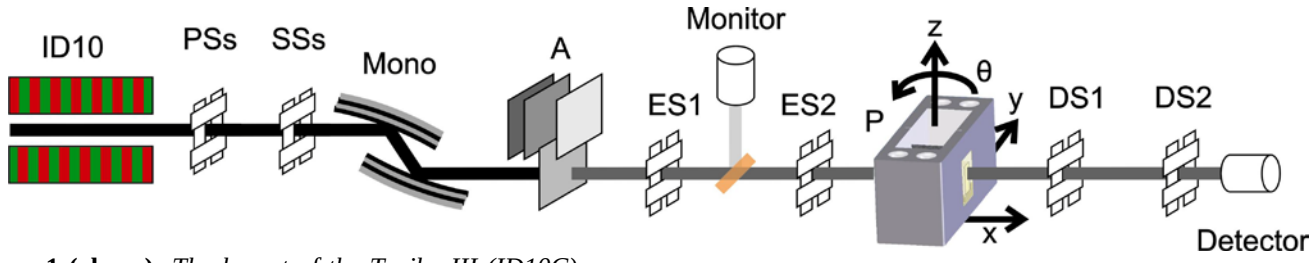

Figure 1 (above): *The layout of the Troika III (ID10C) insertion device shows the setup of both types of experiments. The primary (PS) and the secondary slits (SS) are placed in front of the monochromator (Diamond(111)), that is adjusted to a working energy of 20.9 keV. Photon flux at the sample (P) was controlled by a set of copper absorbers (A) to minimize radiation damage. In the first part of the experiment sample chamber and detector were mounted onto a two circle Huber goniometer. The beam size is controlled by two entrance slits (ES) and the detection volume is precisely defined by two detector slits (DS). For the second part DS is removed and the detector is replaced by a 2-d CCD camera far behind the sample on a separate table.*

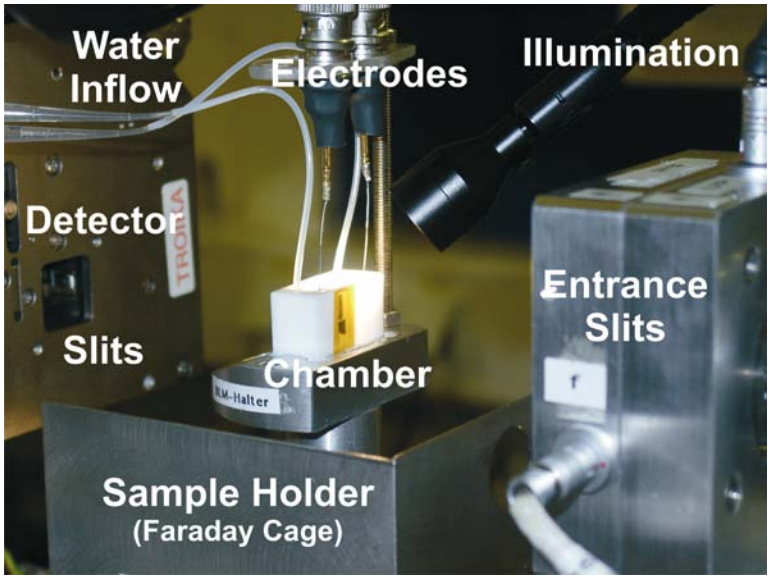

Figure 2 (right): The *Tefon chamber with the silicon substrate including a 900 µm x 2.5 mm aperture spanned by the lipid membrane. It is shielded by a Faraday cage to minimize external noise during electrophysiological measurements. The water levels inside the chamber are controlled from outside by two tubings leading into the two aqueous compartments separated by the lipid bilayer.*

Black lipid membranes (BLMs) were prepared by the painting method of Müller and Rudin. A silicon substrate including a micro structured aperture was fixed in a Teflon chamber, see Figure 2, with two reservoirs acting as cis- and trans-side of the final interface. The lipid-solvent solution, consisting of DPhyPC

(Avanti Polar Lipids, Alabaster, USA) dissolved in n-decane (Sigma-Aldrich, Germany) (5 mg/ml), was painted across the aperture of the silicon substrate. Subsequently the electronic response of the system was recorded with a modified Port-a-Patch NPC-1 system (Nanion, Germany) equipped with an EPC-10 amplifier (HEKA, Germany). An increase in the capacity showed up, that was caused by diffusion of the solvent towards the rim of the aperture, forming a Plateau-Gibbs-border (PGB) and finally led to a thinning of the lipid membrane.

Aim of the first part of the experiment was to obtain reflectivity curves from a BLM. Due to the high coherence of the x-ray beam there was a spurious reflection of entrance slit scattering from the silicon substrate. A scattered signal of the water-membrane-water interface could not be extracted from such curves because it was dominated by the parasitic scattering. We can conclude from such results that additional focusing optics have to be used to avoid an over illumination of the aperture spanned by the membrane patch.

For phase contrast experiments the detector was changed to a Sensicam QE 12-bit CCD camera (PCO Imaging, Germany) with 1376 (h) x 1040 (v) pixels and a pixel size of 6.54 µm x 6.54 µm. The beam was cut to a size of 1 mm x 1 mm and the detector slits were removed. The CCD was placed at three different positions z (0.06 m, 2.18 m and 3.63 m) behind the sample in the unfocused, parallel synchrotron beam. A thin scintillation foil (9.9 µm Europium doped Lutetium Aluminum Garnet (LAG:Eu) on 170 µm undoped YAG), which is imaged by a 10x magnification objective, was used to convert x-ray into visible-light photons.

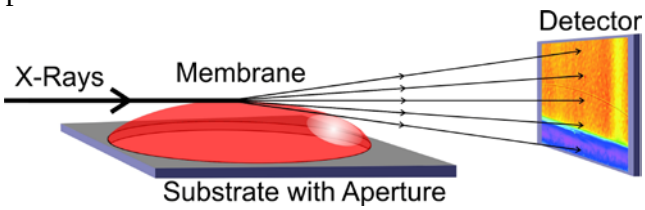

Figure 3 (above): *Schematic view of the imaging setup. The parallel x-ray beam goes through the BLM tangentially and projects the phase contrast image onto the detector.*

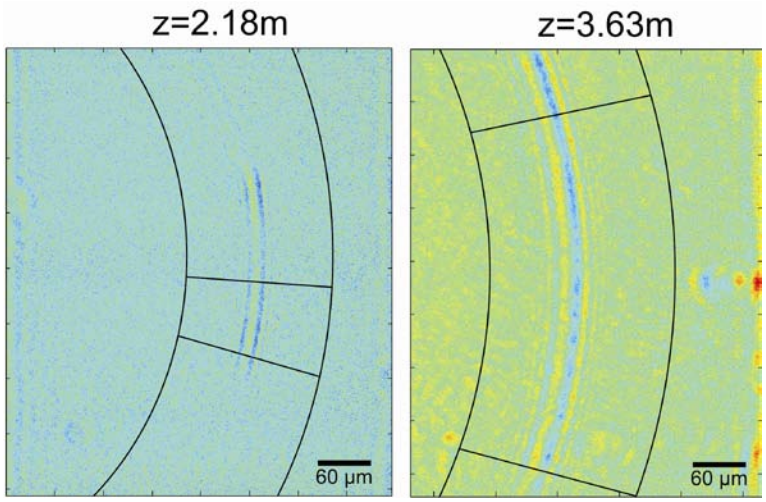

Figure 4 (upper right): *Hard x-ray phase contrast images of bulged BLMs at different propagation distances z. The images clearly show the development of Fresnel fringes for larger sample to detector distances. Black lines indicate regions of interest where cross sections are taken. Subsequently all radial line cuts are averaged to reduce the signal to noise ratio, see Fig. 5.*

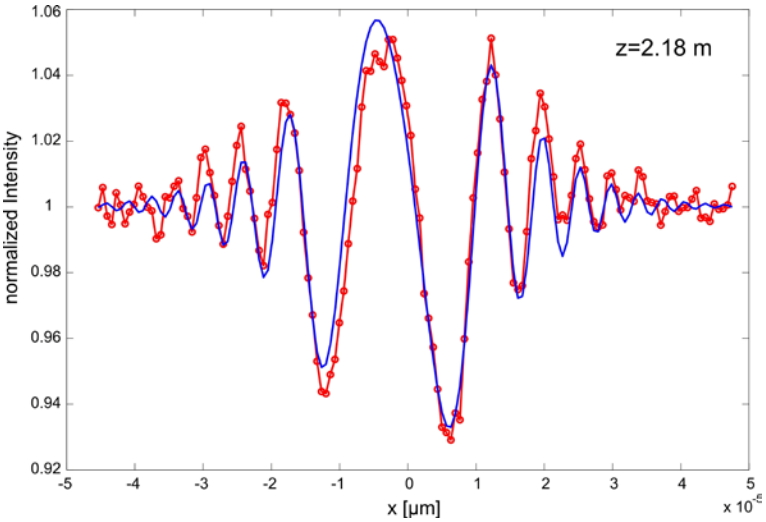

Figure 5 (lower right): *By Fourier propagation algorithms the cross sections from the 2-d images (here z=2.18 m, see Figure 3) can be modeled (straight blue) and finally fitted to the experimental data (dotted red).*

The bulged membrane, spanning the aperture in the silicon substrate is aligned tangentially to the parallel x-ray beam, see Figure. 3. The phase contrast images, see Figure 4, are dark count and background corrected subsequently. In the following step the membrane is located by a gradient based contour tracking algorithm, assuming that it is perfectly spherical and radial cuts are taken. To increase the signal to noise ratio the cross sections are averaged along a region of interest (ROI), see black lines in Figure 4, where the contrast of the signal is supposed to be homogeneous. The loss of contrast in the contour indicates a thinning or completely thinned membrane, which is below the detection threshold. Image analysis and determination of the density profile with the structural membrane parameters is ongoing work, see Figure 5. It was clearly shown that even a very weakly scattering biological object can be imaged and structures much smaller than the physical pixel size of the detector system can be resolved. The open question is now how far the resolution limit can be improved towards the goal of resolving the details in the electron density profile.