Report to the beam time SC-3793:

Coherent Diffraction Imaging of Maurer's Cleft on Two-Dimensional Erythrocyte Membranes

During the allocated beamtime at ID10 coherent X-ray diffraction imaging technique was used to investigate the internal structures of healthy and malaria infected erythrocyte ghosts in 2D and 3D tomography. The membranous structure of malaria-infected erythrocytes (Maurer´s clefts) was chosen as a target system to probe the potential of the new imaging technique for biological samples. In order to minimize the interference of iron containing hemoglobin with x-rays the erythrocytes were lysed for the CDI experiments. The formation of correct out-side-out ghosts was verified by specific outside and inside fluorescent staining.

For the coherent diffraction imaging the preparation of samples and experimental conditions were systematically optimized. During this beamtime the cryo-fixation method, which is commonly used in electron microscopy to keep the sample under almost native conditions, was adapted to the experimental setting of CDI experiment. For the visualization of internal membranous structures the cytoplasm of healthy and malaria-infected erythrocytes was removed by hypertonic lyses. The ghost cells were captured in “cryo-loops” prior to plunging into liquid ethane. After plunging loops were kept under liquid nitrogen, for storage, or subjected to CDI experiment.

Prior to image reconstruction data pre-analysis had to be developed and conducted on the measured speckle patterns. The raw data was subjected to the following corrections (a) detector dead-time correction, (b) flat field correction, (c) masking and (d) background subtraction. After the data pre-analysis was finished the obtained full-speckle pattern was used for the reconstruction of the real-space image. For the real space image reconstruction, the RAR algorithm with a loose support was used.

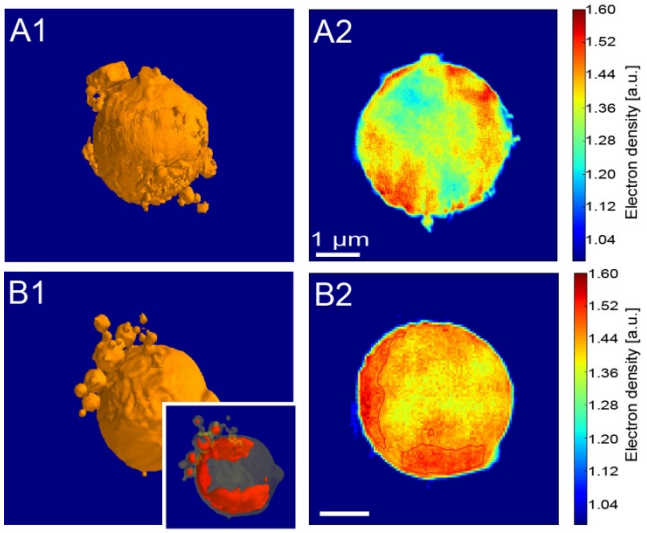

Figure 1: *Reconstructed 3D images (A) healthy human erythrocyte ghost and (B) ghost from human erythrocyte infected by P. falciparum (t = 17 h). 1; integrated electron densities, 2; ghost obtained from the coherent diffraction X-ray tomography. The high electron density regions found inside the infected cell are highlighted in red in inset of panel B1.*

The obtained results clearly demonstrate the difference in shape and electron density between the healthy and *P. falciparum* infected erythrocyte ghost (Figure 1). While the reconstructed real space image of healthy erythrocyte ghost shows a smooth surface and an almost homogeneous distribution in electron density (Figure 1A), the infected erythrocyte ghosts reveals a rough surface, assumed to come from the formation of knobs, and regions of high electron density, located near the inner surface of erythrocytes membrane. (Figure 1B) This coincides with the localization of Maurer´s clefts, which are supposed to be connected to the spectrin cytoskeleton.

The obtained results demonstrate the unique potential of CDI in imaging biological samples in 2D and 3D under almost native environmental conditions.