ESRF	**Experiment title:** Fine Inner Structures of Human Red Blood Cells Probed by X-ray Diffraction Microscopy	**Experiment number**: SC-3543
Beamline: ID10B	**Date of experiment**: from: 31.01.2013 to: 05.02.2013	**Date of report**: 18.02.2011
Shifts: 18	**Local contact(s)**: Dr. Yuriy Chushkin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

V. Ganza* (Univ. Heidelberg)

A. Burk* (Univ. Heidelberg)

F. Rossetti* (Univ. Heidelberg)

A. Yamamoto* (Univ. Heidelberg)

H. Rieger (Univ. Heidelberg)

M. Tanaka (Univ. Heidelberg)

Report:

During the allocated beamtime at ID10C we were able to optimize the sample preparation for the coherent diffraction X-ray microscopy (CDXM) under cryo-stream, and obtained very good quality images from human red blood cells (both healthy cells and cells infected by malaria parasites) in a cryo-loop. Prior to the beamtime, H. Rieger (PhD student in Tanaka Lab) optimized the preparation of malaria-infected red blood cells, at the Institute of Parasitology, Univ. Heidelberg (Prof. M. Lanzer). H. Rieger successfully upscaled the red blood cell culture for the CDXM and prepared "ghost cells" (cells without cytoplasm). In particular, the removal of iron-containing hemoglobin helped a lot to minimize the background scattering.

The ultimate goal of our project is to combine our expertise in "polymer-supported membranes" and CDXM and visualize the fine structures of the cytoplasmic surface of cells (Fig. 1, Fig. 2A). This is achieved by spreading erythrocyte ghosts on cellulose-coated Si_3N_4 electron microscopy grids. The challenges of the cryo-fixation of these samples (including the risk for the Si_3N_4 membrane to rip during plunging into liquid ethane), together with the good quality of the prepared cryo-loops, encouraged us to first concentrate on the loops. Specifically, the absence of hexagonal ice our samples, coupled with their flatness, made it possible to perform not only 2D scans (where the irradiation occurs at a fixed angle), but also several 3D scans, where the cryo-loops were rotated in 2° steps over a range of up to 120°. To the best of our knowledge, this was the first time that cells were imaged with CDXM in the 3D mode. Our 3D data looks very promising, since we were able to obtain images without signs of radiation damage (as inferred from the speckles pattern).

As presented in Fig. 2B, through treatment of our data with the fitting routine developed at ID10, we succeeded in obtaining real space images from both healthy and infected red blood cells, showing already clear qualitative differences. The reconstructed images from healthy red blood cell ghost show a round sphere with homogeneous content, while the image from the infected red blood cell ghost some structures are visible. Our local contact (Dr. Yuriy Chushkin) is now working on the reconstruction of the 3D data. For the next beam time, we have already started the discussion with the local contacts (Dr. Chushkin, Dr. Zontone) about the practical improvements.

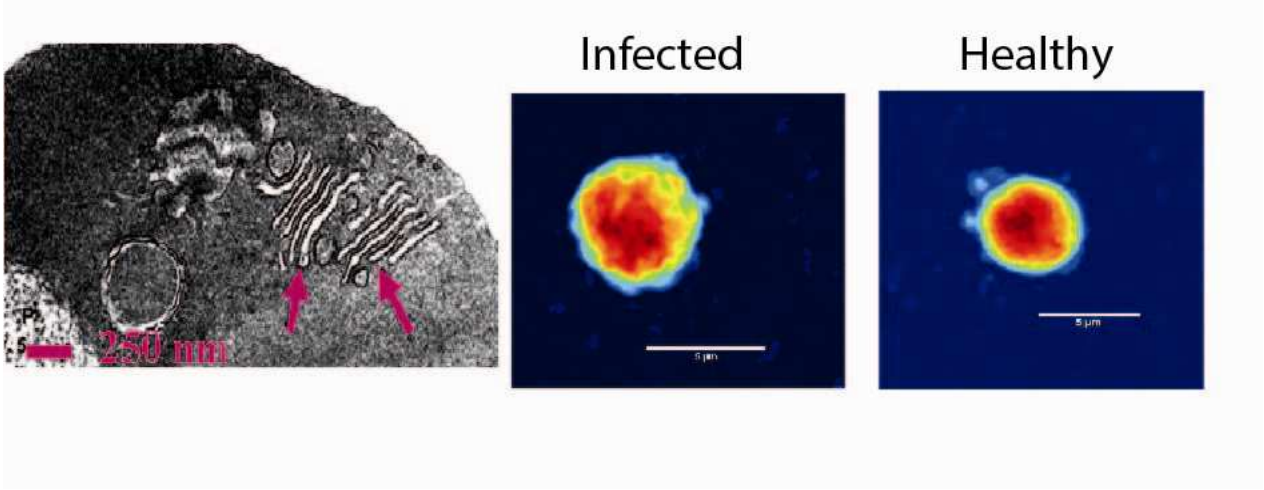

Fig. 1: (A) The target structure for the current project: Maurer's cleft (indicated with red arrows) bound to the cytoplasmic surface of red blood cells infected by malaria parasite. (B) Reconstructed real space images from infected (left) and healthy (right) red blood ghost cells.

We performed several test experiments towards the end of the beam time focusing on the spreading of erythrocyte ghosts on the Si_3N_4 membrane, but the time was unfortunately too tight to optimize the local environment in order to obtain evaluable data. Based on these very positive results, we are currently developing fitting routines for the 3D data and further optimizing the experimental conditions to visualize with CDXM the fine structures of cytoplasmic surface of the cell membranes spread on Si_3N_4.