ESRF	**Experiment title:** Fine Inner Structures of Human Red Blood Cells Probed by X-ray Diffraction Microscopy	**Experiment number**: SC-3166
Beamline: ID10B	**Date of experiment**: from: 15.06.2011 to: 18.06.2011 from: 06.07.2011 to: 09.07.2011	**Date of report**: 29.08.2011
Shifts: 18	**Local contact(s)**: Dr. Yuriy Chuskin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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A. Burk* (Univ. Heidelberg, Internship at ESRF June – August 2011)

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Report:

During the allocated beamtime at ID10C we were able to optimize the sample environments for the coherent diffraction X-ray microscopy (CDXM) under cryo-stream, and obtained some very promising images from human red blood cells (healthy cells and cells infected by malaria parasites) in a cryo-loop. Prior to the beamtime, we carried out cryo-TEM (transmission electron microscopy) experiments at Electron Microscopy Center in Heidelberg (R. Schröder). This enabled the main experimentalist (V. Ganza) to deposit sufficient experiences with cryo-fixation, which is the most crucial step in the sample preparation. In addition, another PhD student in Tanaka Lab (H. Rieger) optimized the preparation of malaria-infected red blood cells, at the Institute of Parasitology, Univ. Heidelberg (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 cytoplasmic surface of cells (Fig. 1). For the allocated beam time, we decided to split the beam time in two periods for testing and improving the experimental procedure. The first period was mainly dedicated to optimize many practical steps, such as (a) the transfer of samples from the cryo-dewer to the sample stages, (b) the optimization of the sample mounting under the cryo-stream, etc. With

an aid of the local contact (Dr. Chuskin), we were able to get the first speckle patterns from red blood cells fixed in a cryo-loop.

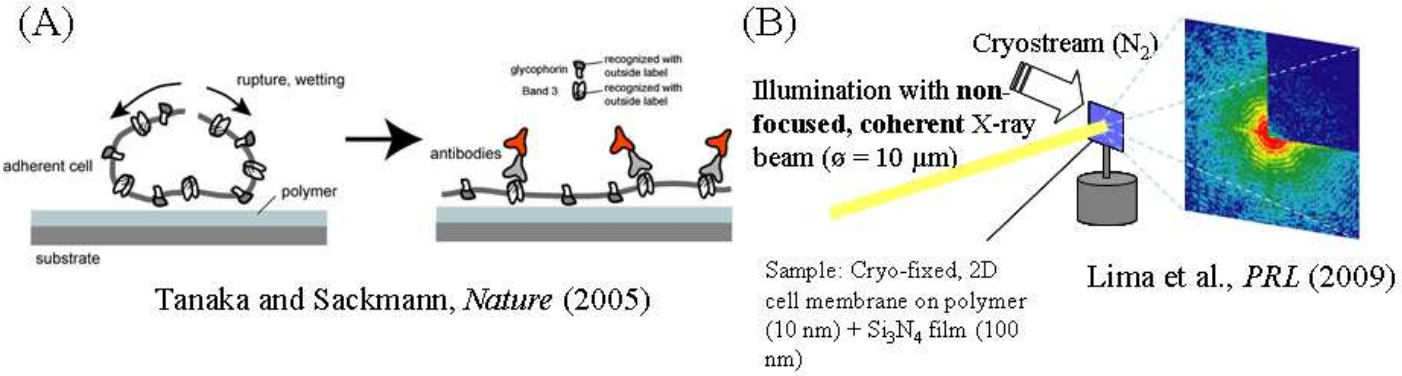

Fig. 1: (A) Two-dimensional cell membranes on polymer supports developed by Tanaka Lab, and (B) CDXM set up at ID10, ESRF.

Based on the preliminary results from the first half of the beam time, we focused on getting the CDXM images with sufficiently high statistics. As presented in Fig. 2, we succeeded in obtaining very high speckle patterns from healthy and infected red blood cells, showing already clear qualitative differences. Unfortunately, we had to invest very long time to obtain the high quality images because (i) the intensity was approx. 3 times weaker than the full operation (1 undulator), and (ii) it was necessary to carry out 9 measurements at 2 detector positions. For the next beam time, we have already started the discussion with the local contacts (Dr. Chuskin, Dr. Zontone) about the practical improvements.

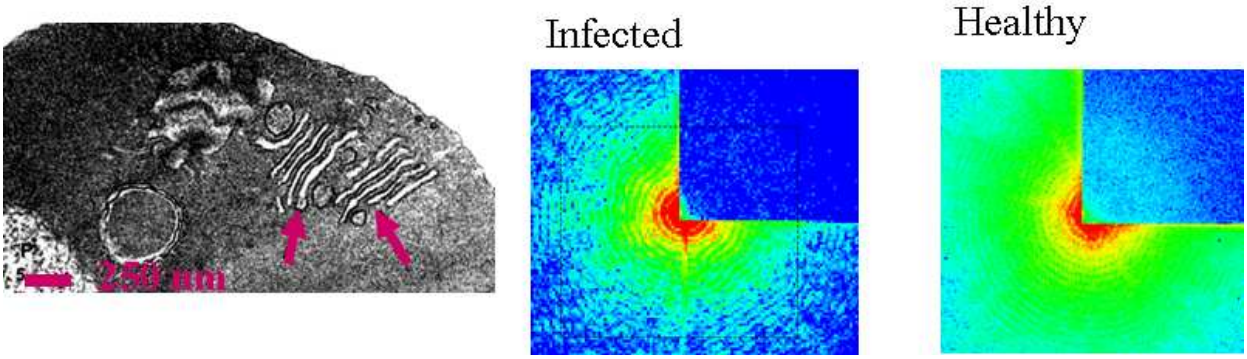

Fig. 2: (Left) The target structure for the current project: Maurer's cleft (indicated with red arrows) bound to the cytoplasmic surface of red blood cells infected by malaria parasite. (Right) First, high quality CDXM images from infected and healthy red blood ghost cells.

Between the two beam times, V. Ganza also performed cryo-TEM experiments with 2D cell membranes on polymer supports. We performed several experiments towards the end of the beam time, but the time was unfortunately too tight to optimize the local environments for the Si_3N_4 membrane samples. Based on these very positive results, we are currently developing the fitting routines and further optimizing the experimental conditions to visualize the fine structures of cytoplasmic surface of the cell membranes with CDXM.