ESRF	Simultaneous high magnification phase imaging and scanning X-ray fluorescence imaging of human erythrocytes: influence of malaria infection and hemoglobin abnormality	**Experiment number**: **SC-4468**
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Shifts: 15	**Local contact(s)**: Dr. Yang YANG	*Received at ESRF:*

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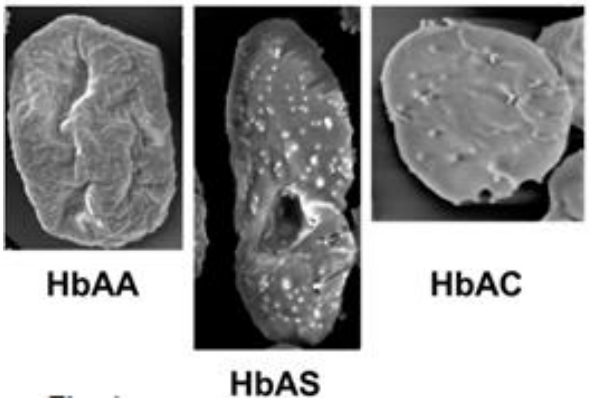

Malaria still remains as a serious health issue, but not every infected person develops the same symptoms. Some people are naturally protected from severe malaria due to genetic modifications in their ß-globin chain, such as haemoglobin S (HbAS) and haemoglobin C (HbAC). As presented in Fig. 1, clear differences in shape, distribution of protein "knobs", and degree of cytoskeletal remodelling have been reported, but little is understood about the mechanism.

Fig. 1: SEM images of human erythrocytes with different hemoglobin types: HbAA, HbAS, and HbAC.

The goal of our experiment was to unravel how the hemoglobin abnormality influences the fine structures on/inside plasma membranes and the accumulation of hemozoin (crystallites of heme enriched with Fe). The unique combination of magnified phase imaging and scanning X-ray fluorescence at ID16A is a powerful combination to achieve this goal, as the hard x-ray nanotomography allows reconstructing the elemental distribution over a wide range of atomic number and offers excellent depth resolution.

During our recent beam time allocation, we focused on normal HbAA, and compared with two types hemoglobin-abnormal cells, HbAS and HbAC (i) with and (ii) without malaria infection. We used a monochromatic beam of 17 keV to gain sufficient penetration depth and to excite the fluorescence from elements of interests, such as K, Ca, P, S, and Fe.

Fig. 2 represents a 3D tomogram of malaria-infected erythrocytes with HbAS. In all samples, we confirmed that the cryo-fixation was successful, therefore preserving the "native" feature of cells under frozen-hydrated conditions.

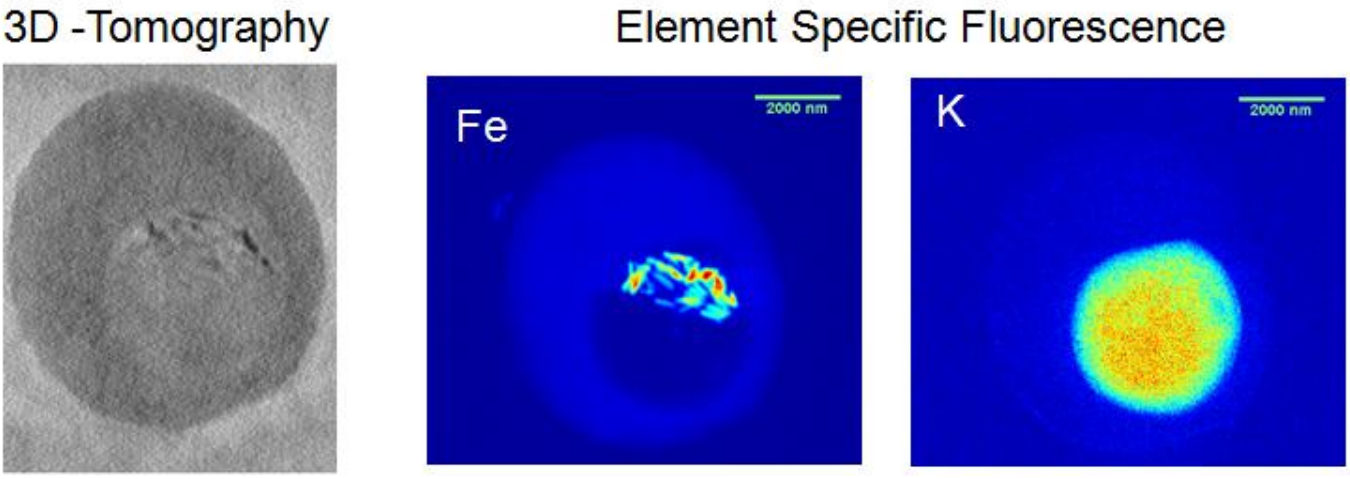

Fig. 2: (left) 3D tomogram and (right) scanning X-ray fluorescence images (Fe Kα and K Kα) of human erythrocytes at trophozoite stage (~ 24 h after malaria infection). These data coincide with an abnormal hemglobin types of HbAS (sickle cell gene).

Following the 2D phase imaging and 3D tomography, we performed the scanning X-ray fluorescece imaging with a beam size of 25x25 nm. Fig. 2 shows the X-ray fluorescence from Fe Kα (middle) and K Kα (right). Hemozoin crystals grown in the parasite vacuole could be identified from Fe signal, while K signal strongly suggested the codensation of K+ ions inside the vacuole membrane compartment.

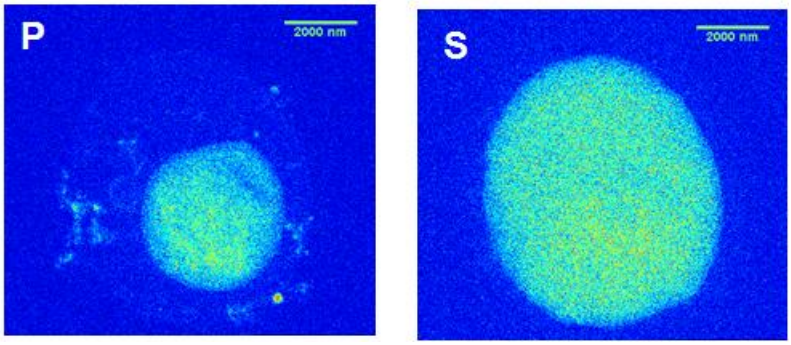

Fig. 3: Scanning X-ray fluorescence images (P Kα and S Kα) of human erythrocytes at trophozoite stage (~ 24 h after malaria infection). These data coincide with an abnormal hemglobin types of HbAS (sickle cell gene).

The signals from other elements, such as P and S are presented in Fig. 3. Though S signal (mostly from cysteine) shows no specific features, we observed a distinctly higher P signal from the vacuole, which can be attributed to the higher concentration of phospholipids in the parasite compartment. Moreover, the "corona-like" features observed here may conincide with the membranous superstructures, called Maurer's clefts, which play key roles in protein sorting inside infected erythrocytes. Currently, the quatitative analysis of the fluorescence signals is ongoing under collaboration with Dr. Y. Yang.