

# Visualizing Plasmodium Falciparum Ultrastructure at Subnanometer Resolution Across the Asexual Blood Stages Using in Situ CryoET

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Malaria is a mosquito-borne disease that puts almost half of the world's population at risk, causing 619,000 deaths in 2021. The rapid development of drug resistance by the malaria parasite, *Plasmodium falciparum*, poses an urgent need for the discovery of new treatments to combat it. However, our current knowledge of the parasite's pathogenesis is limited.

Light microscopy does not provide enough resolution to study the detailed molecular mechanisms of these parasites. Specifically, structural changes at the molecular level between different life cycle stages of the parasite are not well understood. To address this, in-situ cryo electron tomography (cryo-ET) is a promising approach to study these parasites in a near-native environment with high resolution.

We generated thinned sections of vitrified parasite-infected human erythrocytes (lamella) using cryo focused ion beam scanning electron microscope (cryoFIB-SEM) prior to tomographic tilt series collection in a 300kV cryo transmission electron microscope (cryoTEM). We then reconstructed the tilt series into 3D volumes (tomograms) to visualize parasite proteins and organelles at subnanometer resolution.

We then used CNN based segmentation tools to generate surface representations of the ultrastructural features of the cells, allowing us to interpret the 3D volumes more easily. We are able to visualize the important organelles in the parasite, such as the mitochondrion, apicoplast, nucleus, and ER, among others. Combined with a mapped back high-resolution map of ribosomes, we can identify membrane-bound ribosomes on the rough ER surface.

These high-resolution details of the parasite in a near-native environment provide insights into how malaria parasites function, and this will further aid the development of new approaches to combat drug resistance.