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Revealing Surface and Intracellular Membrane Morphologies of Host Pathogen Interactions Combining Chromatic Aberration Corrected SEM and STEM Imaging

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Toxoplasma gondii is the causative agent of toxoplasmosis, a cosmopolitan parasitic infection that can lead to blindness and miscarriage, among other health disorders. Recognition, invasion and some steps of the intracellular development of this parasite are events of high complexity that take place in a very short time, making their observation very rare, requiring multiple technical approaches.

A major challenge facing researchers is how to image morphologies of pathogens like *Toxoplasma gondii* in greater detail with minimal sample preparation. Sub-nanometer resolution and high detection sensitivity is desired for revealing such surface morphologies using scanning electron microscopy. Hence, lower beam energies need to be used. As beam energy decreases, image resolution in a typical SEM is compromised by chromatic aberration. Recent advancements in SEM technology have overcome this limitation to provide sub-nanometer resolution even at low accelerating voltages in the range at and below 1 kV [1, 2, and 3].

In addition to surface morphology the imaging of intracellular membranes of host pathogen interactions is relevant. Usually this requires TEM imaging. Recent advancement in detection technologies enables BF, DF and HAADF STEM imaging of membranes while resolving the lipid bi-layer using the standard TEM sample prep route for biological specimens. For the first time, complementary information from the surface and internal structure at the true nanometer level is obtained in the same SEM. We show morphological imaging of until recently inaccessible substructures of *Toxoplasma gondii* during cellular uptake and inside host cells, and describe the contrast mechanisms used to image the samples.

Figure 1 shows two parasitophorous vacuoles 24hpi. The image on the left was obtained from a cell that was gently scraped with scotch tape after critical point drying and before metal coating in order to expose the parasitophorous vacuole and the rosette of parasites inside it. The rosette is held together at the posterior end by a residual body (not seen) and a network of membranous nanotubules (white arrow). The right image is obtained from a 200 nm thick section of a plastic embedded sample in the same stage of development. Both the residual body and the nanotubular network are readily recognized. At higher magnification the unit membranes of these tubules are clearly distinguished, proving that the resolution of the STEM observation mode is, at least, equivalent of classical TEM. Combination of this mode of observation (STEM) and gold labeling may constitute a very useful tool in the near future, allying high resolution and operational convenience (easiness) to approach several issues.

References

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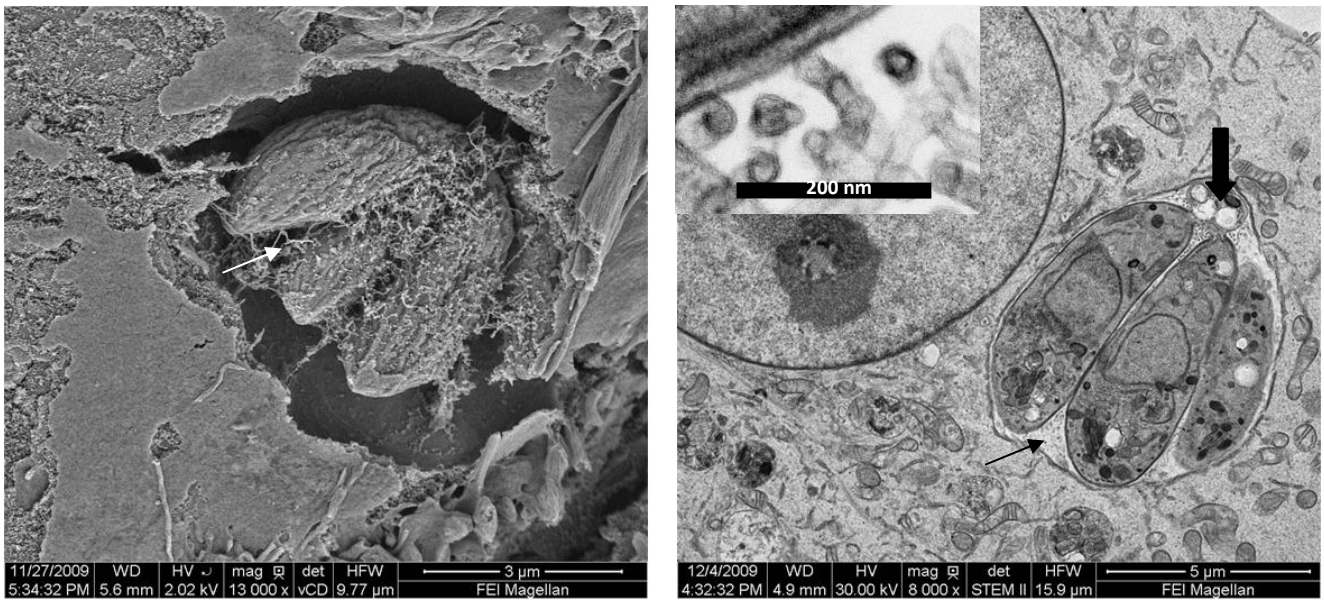

Fig.1: Surface (left) and intracellular image of a parasitophorous vacuole of *Toxoplasma gondii,* the inset (upper left corner, left image) reveals a nanotubular structure using STEM detection

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