

4D IMAGING AND RENDERING OF MALARIA PARASITE MIDGUT INVASION IN LIVING *ANOPHELES* MOSQUITOS

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Malaria is a serious disease caused by protozoan parasites of the genus *Plasmodium* (Protozoa/Apicomplexa). It is the most common tropical disease with more than 300 million human infections and over 1 million fatalities per year. The life cycle of *Plasmodium* takes place in two hosts: humans and bloodsucking mosquitos of the genus *Anopheles*.

Using advanced imaging methods we have studied a crucial phase of the mosquito infection cycle: the transition of malaria parasites (ookinete stage) from the bloodmeal through the *Anopheles* midgut. Newly designed GFP expressing *P. berghei* parasites allowed to visualize the movement of the pathogens during midgut invasion *in vivo*. Two complementary approaches were used for imaging.

- 4D measurements of invading parasites were made inside living mosquitos using a spinning disk confocal microscope. Thus the movement patterns of *P.berghei* parasites could be observed under realistic *in vivo* conditions.
- Complementary high resolution 4D measurements of explanted midguts were made on a single beam scanning confocal. The *in-vivo* data of parasite movement were thus expanded by a detailed study of the characteristics and the location of the observed movement types in explanted midguts that were counterstained with the lipophilic stain FM 4-64

The analysis of the movement data yielded the following results:

P. berghei parasites in the ookinete stage are highly motile during midgut invasion. The *in vivo* movements are of a gliding type. They can be separated into three categories:

Stationary rotation (curved cell shape), translocational spiraling (curved cell shape) and straight segment movements (straight cell shape)

Stationary rotation as observed for isolated Apicomplexa in many *in vitro* studies also takes place *in vivo*. It is the most common and also most persistent movement. Translocational spiraling is related to the rotational movement, but consists of a novel biphasic movement. It includes transient stopping of the posterior end while the anterior end continues in a tethered rotation. In straight segment movements the parasite glides with a straight body shape.

Parasite movements are highly variable and all movement types can be interconverted. Movement phases are interrupted by transient phases of immobility. Translocational spiraling in contact with intracellular membranes was observed inside midgut cells and therefore seems to be part of the invasive movement.

The parasite movement during midgut invasion is determined by the cell shape and by the distribution of gliding properties along the cell body.