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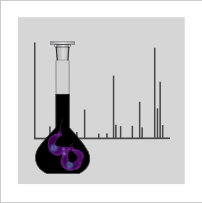

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Ultrastructure Expansion Microscopy to Uncover Novel Features of the Parasite *Trypanosoma brucei*

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Trypanosoma brucei is a single-celled eukaryotic parasite causing human African sleeping sickness and nagana in cattle. The parasite is transmitted by the tsetse fly in sub-Saharan Africa and infections in humans are fatal if left untreated. Aside from the medical and also veterinarian relevance, *T. brucei* has been developed into a model system for a number of basic biological questions including flagellum- and mitochondrial biogenesis.

The flagellum is required for the propulsion of the cell and attaches alongside the cell body. Biogenesis of the highly conserved flagellar structure initiates at the basal body inside the cell, which itself is also a very conserved organelle in biology. In *T. brucei* the basal body is in proximity to the mitochondrial genome, which is termed kinetoplast or kDNA. In fact, the kDNA is physically linked to the basal body *via* the unique protein-based tripartite attachment complex. Since this structure is essential for the parasite and is not present in the human or animal host it provides a potential avenue for therapeutic interventions.

In order to better characterize this ~200 nm wide structure inside the cell we require high-resolution microscopy techniques. Ultrastructure Expansion Microscopy (U-ExM) is a powerful novel approach to overcome resolution limits in microscopic imaging by increasing the size of the sample rather than increasing the resolution of the microscope.[1] We recently adapted the previously developed approach to trypanosomes.[2,3] For this the *T. brucei* cells are incubated in an anchoring solution to link all cell components to a gel polymer at nanometer scale. After the polymerization of the gel, the sample is heated to 95 °C in a solution with detergent to prepare the cell for the expansion step, which is followed by standard immunofluorescence staining to identify the molecular components of interest. The U-ExM method is compatible with epifluorescence or confocal microscopy, resulting in ~ 50 nm to 10 nm spatial resolution. In this our study the cells were isotropically expanded to 4.5 times their original size, which allowed us to closely observe the mitochondrial kDNA and its associated protein factors.

U-ExM enables the localization of individual cell components at nanometer scale, thus bridging the gap between light- and electron microscopy.

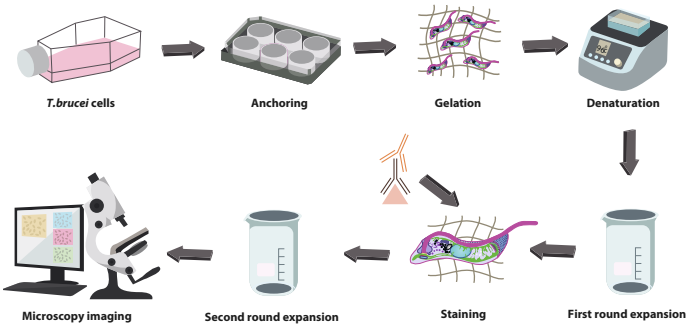

Fig. 1. U-ExM workflow in *T. brucei*.

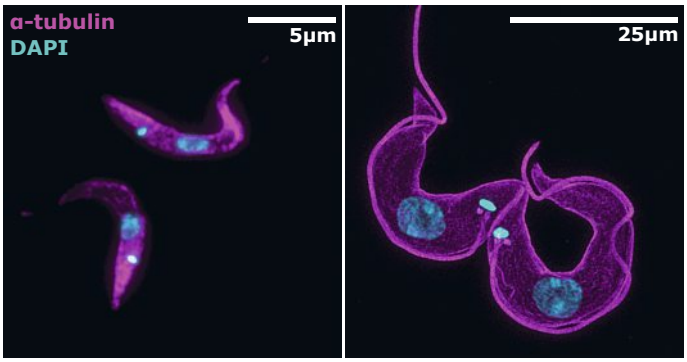

Fig. 2. Representative images of non-expanded and expanded *T. brucei*. Cells stained with anti alpha-tubulin antibodies (magenta; Alexa 647) and DAPI (cyan; kDNA and nucleus).

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