

# FLUORESCENCE TECHNIQUES FOR THE INVESTIGATION OF THE HOMEOSTASIS OF MALARIA INFECTED RED BLOOD CELLS

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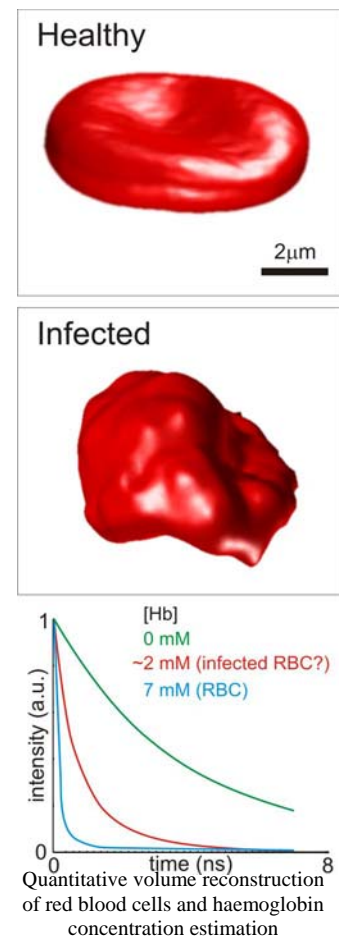
## 1. BACKGROUND

*Plasmodium falciparum* (*Pf*) causes the most lethal form of malaria in humans. Early research exposed two perplexing facts: 1) that during its 48 h intraerythrocytic cycle the parasite permeabilizes the host cell so much that a comparably permeabilized uninfected red blood cell (RBC) would lyse prematurely, and 2) that *Pf* digests far more haemoglobin than needed for its metabolism. A model of the homeostasis of a *Pf* infected RBC suggested a common explanation of both puzzles [1]: excess haemoglobin digestion is required to reduce the colloid osmotic pressure within the host cell thus ensuring its osmotic stability to the end of the *Pf* asexual cycle (the *colloid osmotic hypothesis*).

## 2. RESULTS AND DISCUSSION

Early osmotic fragility measurements supported the *colloid osmotic hypothesis*; however, other critical model predictions, on stage-related volume and haemoglobin concentration changes, remain untested. Methodological developments were necessary to quantify these parameters. Here we present preliminary results with calcein-loaded and Qdots-tagged infected RBCs imaged by confocal deconvolution microscopy and fluorescence lifetime sensing.

Surface reconstruction insensitive to photobleaching and to the arbitrary selection of a global threshold [2] have been implemented, providing high 3D resolution and robustness of the volumetric and morphological estimators. Furthermore, lifetime imaging was shown to be a valuable tool to map haemoglobin concentrations in the RBC cytoplasm by the analysis of energy transfer induced by molecular crowding [3]. Application of these tools is providing a deeper understanding of the homeostasis of the intraerythrocytic stage of *Pf*.



[1] Lew, V.L., *Packaged merozoite release without immediate host cell lysis*. Trends Parasitol., 2001. **17**(9): p. 401-403.

[2] Yim, J.P.S., M.R. *Analytic surface reconstruction by local threshold estimation in the case of simple intensity contrasts*. in *Medical Imaging 1998*. 1999: SPIE.

[3] Bennett, R.G., *Radiationless intermolecular energy transfer. I. Singlet -> Singlet transfer*. The Journal of Chemical Physics, 1964. **41**(10): p. 3037-3040.