LIFE MEETS LIGHT

Second Scientific Conference of the Leibniz ScienceCampus InfectoOptics

Infecto Optics

Combining whole-blood infection assays with live-cell imaging to identify morphokinetic parameters for infection classification

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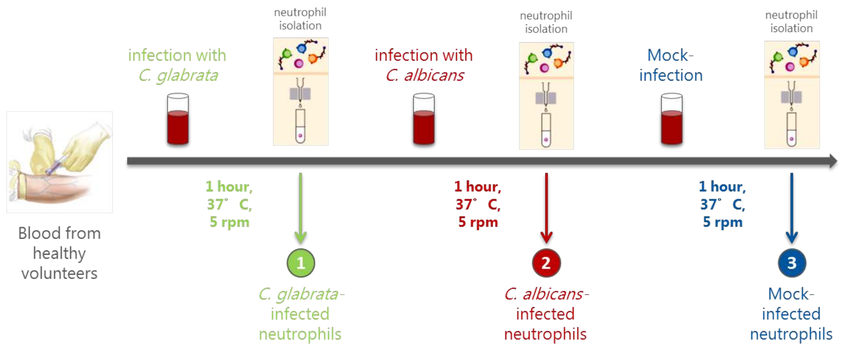
Introduction

Candida albicans and *Candida glabrata* are the two most prevalent pathogens in the genus *Candida* and account for the majority of cases of candidiasis worldwide. Several lines of evidence suggest that neutrophils are of outstanding importance in the response against invasive *Candida* infections. In line with this, results from our *ex vivo* performed whole-blood infection assays clearly demonstrated a predominant, but differential role of neutrophils during *C. albicans* and *C. glabrata* infection, mediated by phagocytosis and secretion of cytokines upon activation. The aim of this project is to establish a dynamic hemogram from whole blood infection assays that goes beyond standard blood count examination by integration of information on migration and interaction of blood cells, especially neutrophils.

Combining whole-blood infection assay with live-cell imaging

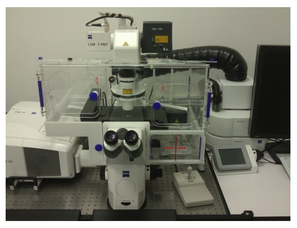
To identify changes in neutrophil behavior induced by *C. albicans* and *C. glabrata*, respectively, during whole-blood infection in comparison to mock-infected control samples, neutrophils were isolated following a one-hour confrontation in human whole blood for separate analysis by time-lapse microscopy to visualize their dynamic features:

Step 1: *Ex vivo* human whole-blood infection assay and neutrophil isolation



Human whole blood from healthy volunteers was either infected with *C. glabrata* or *C. albicans* and compared to mock-infected control samples. Neutrophils are isolated from whole blood of each condition. Non-target cells are removed by immunomagnetic depletion using MACSxpress Beads, yielding untouched target cells of high purity.

Step 2: Live-cell imaging of primary human neutrophils



ZEISS LSM780

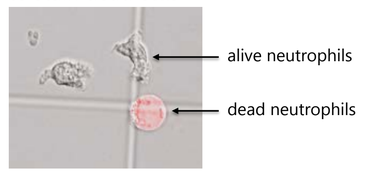
with incubation @

temperature: **37°C**

CO_2 conc.: **5%**

LSM 780 confocal microscope

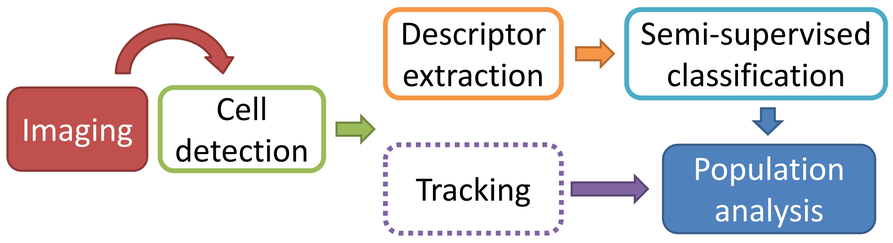
Propidiumiodide (PI) to stain dead cells



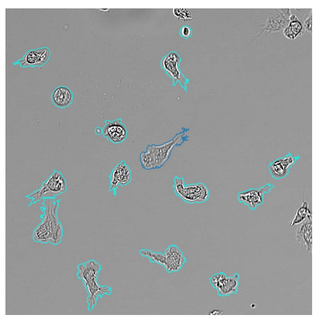
For live-cell imaging, neutrophils isolated from mock-treated whole blood or infected with either GFP-expressing *C. albicans* or *C. glabrata* were incubated in RPMI1640 medium containing 5% heat-inactivated human serum and 2.5 ng ml^{-1} of propidium iodide (PI). PI is widely used as a vital dye that labels the nucleus in dying cells, which lack an intact plasma membrane. Cells were incubated in an environmental control chamber at 37°C and 5% CO_2. Images were acquired every 10s with an LSM 780 confocal microscope.

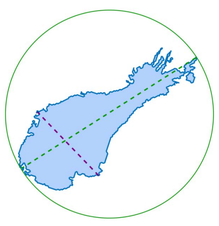
Morphokinetics analysis of neutrophils from whole-blood infection assays

From images to diagnosis: the workflow



Single cell segmentation and characterisation





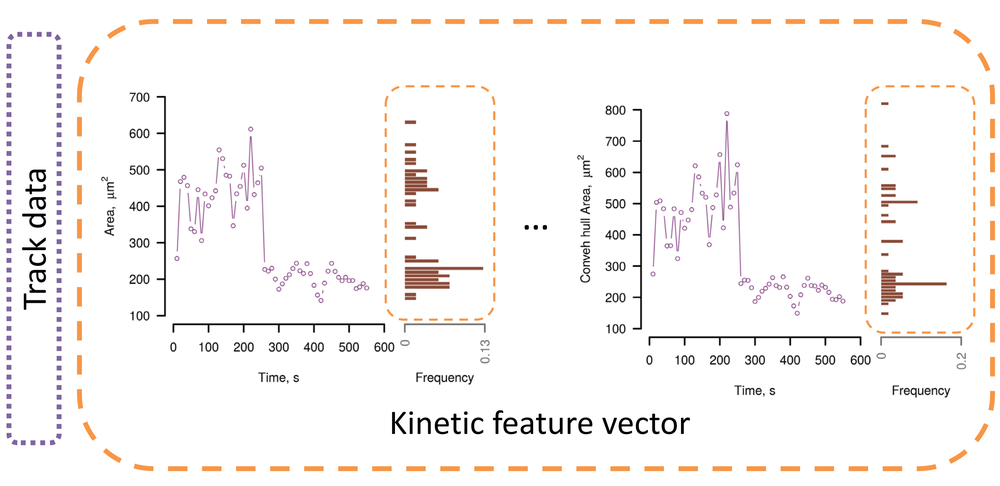
Area

Perimeter

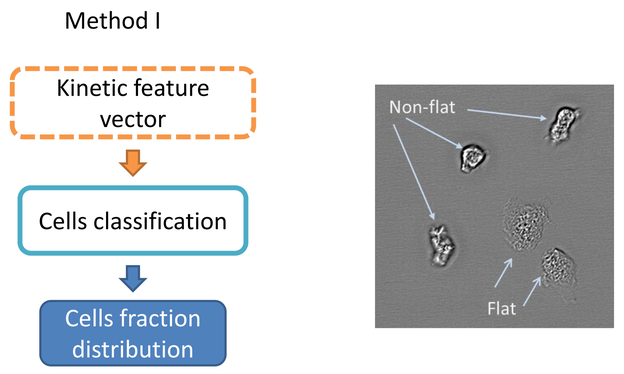
Minimal Feret Diameter

$$\text{Equivalent diameter} = 2 * \sqrt{\frac{Area}{\pi}}$$

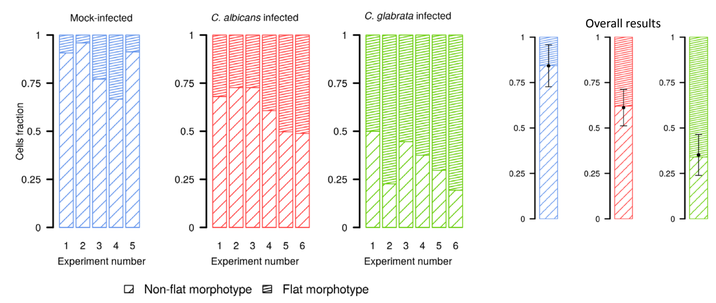
$$\text{Extent} = \frac{Area}{\pi R_{max}^2}$$



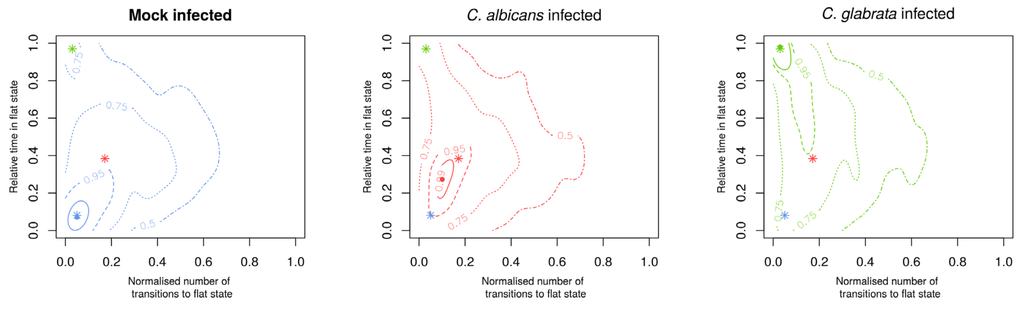
Non-flat or flat? Two points of view.



Method I: results



Method II: results



The example of results of live-one-out experiments. The geodesic curves indicate the distribution density, the solid points – maxima of distributions, mark * correspond to maxima of distributions for “unknown” test samples. The decision is based on minimal distance.

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