

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

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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

Step 2: Live-cell imaging of primary human neutrophils

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 MACSplex Beads, yielding enriched target cells of high purity.

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 96-well plates containing 1% heat-inactivated human serum and 2% IgG⁺ of propidium iodide (PI). PI is widely used as a vital dye that labels the nucleus in living cells, which lack an intact plasma membrane. Cells were incubated in an environmental control chamber at 37°C and 5% CO₂. Images were acquired every 30s with an LSM 780 confocal microscope.

Morphokinetics analysis of neutrophils from whole-blood infection assays

From images to diagnosis: the workflow

Imaging → Cell detection → Tracking → Population analysis

Descriptor extraction → Semi-supervised classification → Population analysis

Non-flat or flat? Two points of view.

Method I: Kinetic feature vector → Cells classification → Cells feature distribution

Method II: Single cells descriptors → Cells classification → Tracking → Distributions comparison

Single cell segmentation and characterization

Area, Perimeter, Minimal Feret Diameter, Equivalent diameter = 2 × Area / Perimeter, Extent = Area / Feret

Track data

Kinetic feature vector

Method I: results

Method II: results

The example of results of two control experiments. The profiles shown include the distribution density, the solid points - means of distributions, used - histograms to represent distributions for "infected" and samples. The distance is based on minimal distance.

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