Automated Characterization of Neutrophil Activation Phenotypes for Human *Candida* Bloodstream Infections

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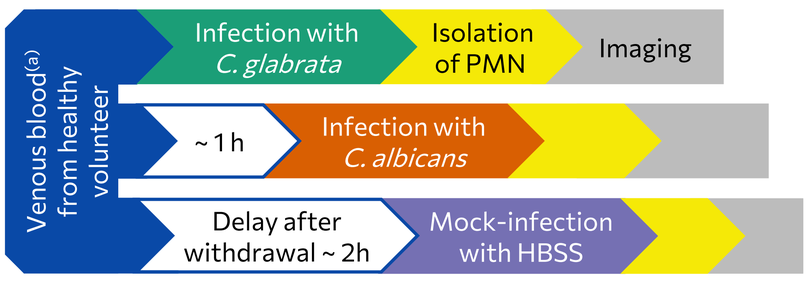
Objectives

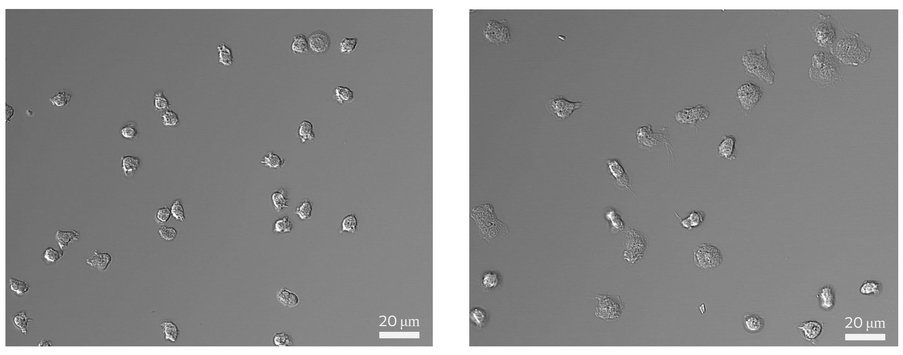
We hypothesize that polymorphonuclear neutrophils (PMN) after confrontation with fungal pathogens may exhibit a characteristic behavior in terms of cell morphology that allows (i) identifying the type of pathogen indirectly and (ii) providing information on therapeutic efficacy. In this feasibility study, we propose a method for the quantitative assessment of static and morphodynamic features of PMN based on label-free time-lapse imaging data from a human whole blood infection (WBI) assay with *C. albicans* and *C. glabrata* as the major causes of candidaemia.

Ex vivo human whole-blood infection assay, PMN isolation and live-cell imaging

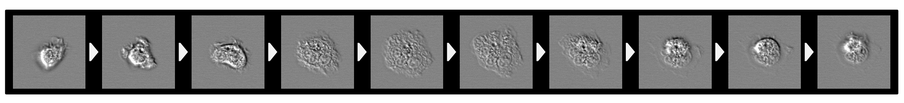
(I)



(II)



(III)

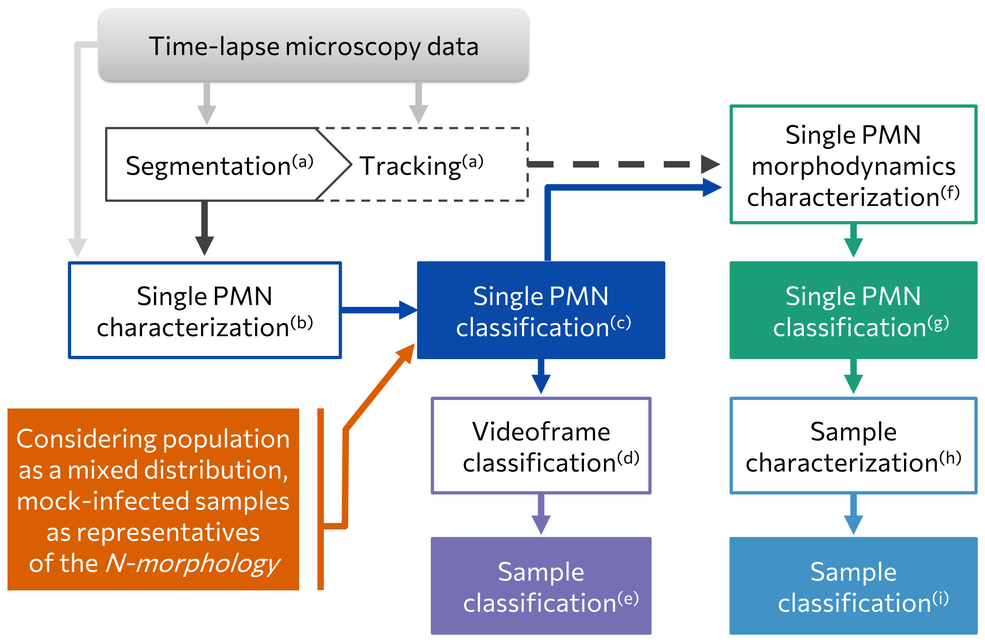


(I) Pipeline of *ex vivo* WBI assay [1] followed by live-cell imaging
(II) Examples of single frames for mock-infected (left) and infected (right) samples
(III) Example of dynamic PMN morphology change during 10 consecutive frames: PMN develops into spreading state (*S-morphology*) and back (*N-morphology*)

Visual observations from time-lapse microscopy videos:

- PMN morphology in the spreading state rarely present in mock-infected samples
- PMN spreading episodes for *C. albicans* infection scenarios are typically shorter than for PMN from infection scenarios with *C. glabrata*

Automated single PMN and population analysis



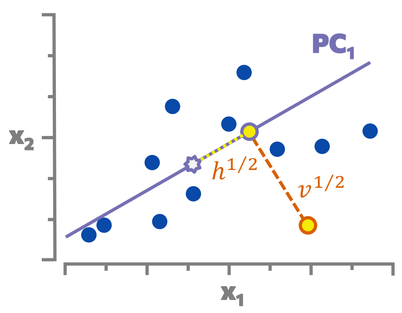
[a] Segmentation and tracking by our software AMIT [2, 3, 4, 5]
[b] Cell characterization via footprint area and percentiles of gradient amplitude distribution
[c] Classification with Data-driven Soft Independent Modelling of Class Analogy [6]
[d] Using a naïve Bayesian classifier based on fraction of PMN with S-morphology
[e] Based on majority voting for classes mock / *C. albicans* / *C. glabrata*
[f] Total time PMN spend in S-mode and maximal duration of spreading episodes for a PMN
[g] Bayesian classifier (in case of two descriptors a naïve Bayesian classifier was used)
[h] Fraction of PMN likely to follow either *C. albicans*- or *C. glabrata*-specific statistics
[i] Based on dominant class for individual PMN

N/S-morphology detection

Cell population as a mixture distribution: $M = [1-\mu]N + \mu S$, where $0 \le \mu < 1$ – the fraction of cells with *S-morphology,* for mock-infection $\mu \ll 1$.

Data-driven Soft Independent Modelling of Class Analogy

(I) PCA and objects characterization



x_i – an initial variable
● – a calibration data point
PC_1 – the 1st principal component
✿ – the centre of the PC model
● – new data point
● – projection to PC model
$h^{1/2}$ – score distance
$v^{1/2}$ – orthogonal distance

(II) Outliers detection :

$$O_\gamma = \left\{(h,v):\ N_h\frac{h}{h_0} + N_v\frac{v}{v_0} > \chi^{-2}((1-\gamma)^{1/I},\ N_h + N_v)\right\}$$

(III) Class acceptance rule:

$$H_\alpha = \left\{(h,v):\ N_h\frac{h}{h_0} + N_v\frac{v}{v_0} \le \chi^{-2}(1-\alpha,\ N_h + N_v)\right\}$$

h_0, v_0, N_h, N_v – parameters of SD and OD distributions
α – probability of type I errors in decision making
γ – outliers significance level
I – number of samples

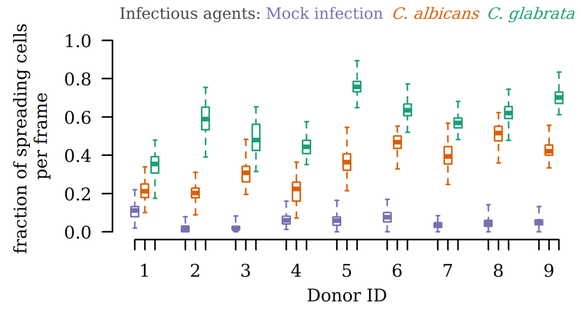
Estimation of SD and OD distribution parameters:

$$\begin{cases} M = \dfrac{u_0}{N}\chi^{-2}(0.5, N)\Big), \\ S = \dfrac{u_0}{N}[\chi^{-2}(0.75, N) - \chi^{-2}(0.25, N)] \end{cases} \Bigg| (N, u_0) \in \begin{Bmatrix}(N_h, h_0),\\ (N_v, v_0)\end{Bmatrix}$$

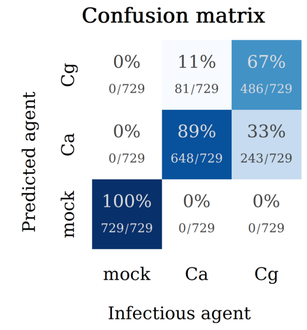
M, S – median and interquartile range of calibration data

Results

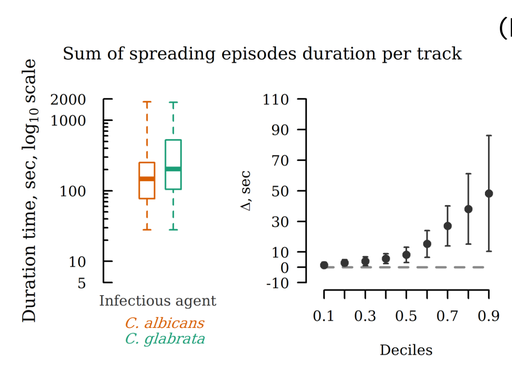
(I)



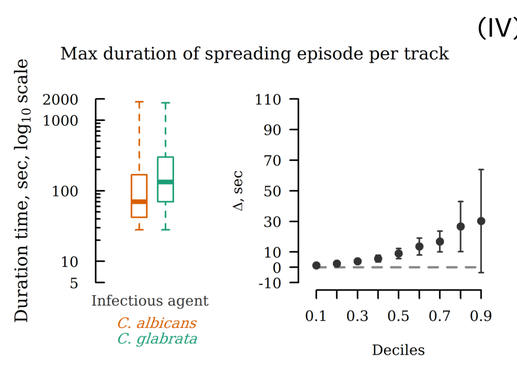
(II)



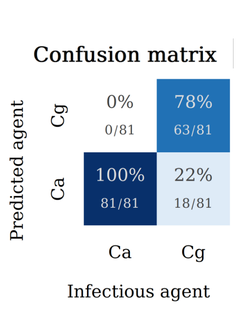
(III)



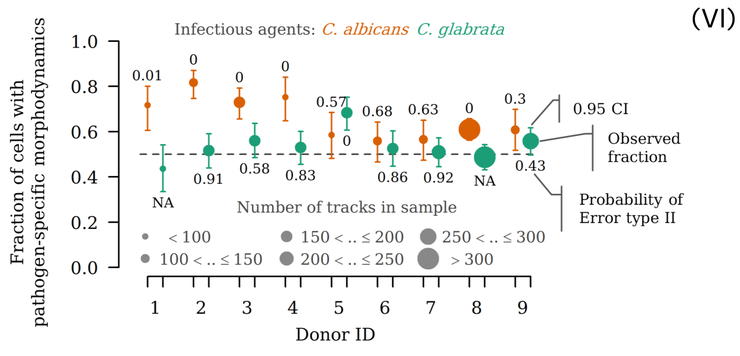
(IV)



(V)



(VI)



(I) Fraction of PMN with S-morphology exhibits differences between infection scenarios
(II) Identification of videos from mock-infected samples with 100% accuracy in experiments with leave-one-out cross-validation (LOOCV) sampling
(III) PNM tend remaining longer time in S-mode after confrontation with *C. glabrata* compared to *C. albicans*
(IV) Maximal duration of single episode in S-mode is longer for PMN confronted *C. glabrata*
(V) Identification of videos from *C. albicans*-infected samples with 100% accuracy using morphodynamic descriptors in experiments with LOOCV sampling
(VI) Existence of pathogen-specific morphodynamic needs to be confirmed through further experiments with larger donor cohort of ~ 10^3 participants

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References

[1] Hünniger *et al.* 2014. *PLoS Comput Biol.* 10(2):e1003479
[2] Brandes *et al.* 2015. *Med Image Anal* 20:34-51
[3] Brandes *et al.* 2017. *Med Image Anal* 36:172-183
[4] Al-Zaben et al. 2019. *Sci Rep* 9:3317
[5] Belyaev *et al.* 2021. *Cytom Part A.* cyto.a.24466
[6] Pomerantsev and Rodionova. 2014. *J Chemom.* 28(5): 429–438