


## Morphokinetic analysis of live-cell imaging data

I. Belyaev, A. Marolda, A. Medyukhina, K. Hünninger, O. Kurzai, M. T. Figge


26/09/2019

**Morphokinetic analysis of live-cell imaging data**  
 Belyaev I.<sup>1,2</sup>, Marolda A.<sup>3</sup>, A. Medyukhina<sup>4</sup>, K. Hünninger<sup>5</sup>, O. Kurzai<sup>6</sup>, M. T. Figge<sup>1,3</sup>  
<sup>1</sup>Research Group Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany  
<sup>2</sup>Research Group Fungal Septicemia, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany  
<sup>3</sup>Institute of Microbiology, Faculty of Biological Science, Friedrich-Schiller-University Jena, Germany  
<sup>4</sup>University of Würzburg, Würzburg, Germany

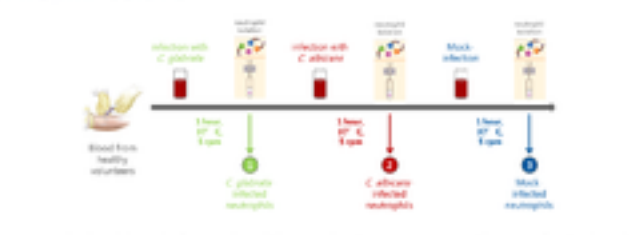


**1. Project aim**  
 Automated characterisation of cells based on interpretable features, in order to establish a dynamic hemogram from whole blood infection assays that goes beyond standard blood count examination by integration of information on migration and interaction of blood cells, especially neutrophils.

**2. From blood sample to diagnose: the workflow**

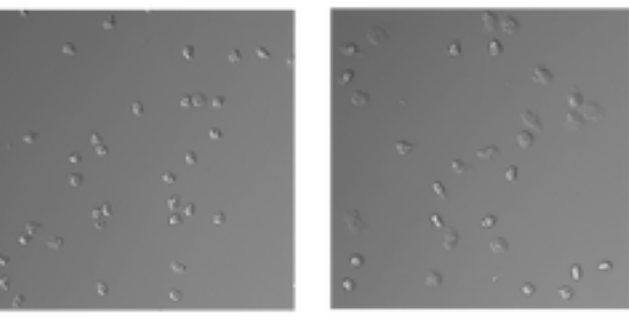


**3. Ex vivo human whole-blood infection assay and neutrophil isolation**



Human whole blood from healthy volunteers was either infected with *C. glabrata* or *C. albicans* and compared to mock-infected control samples. Polymorphonuclear neutrophils (PMNs) are isolated from whole blood for each condition. Non-target cells are removed by immuno-magnetic depletion using MACSexpress Beads, yielding untouched target cells of high purity.

**4. Live-cell imaging of primary human neutrophils**

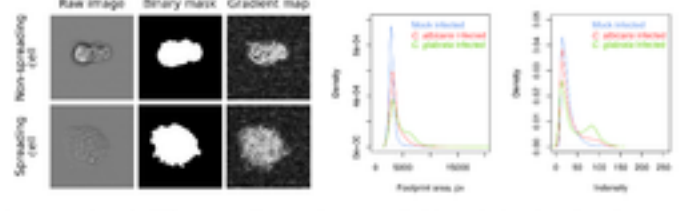


Examples of single frames for mock-infected (left) and infected (right) samples. It is possible to see two types of morphological appearances: spreading (S) and non-spreading (N) PMNs. For the majority of PMNs in the *C. albicans* infection scenario the typical duration of cell spreading episodes is shorter than in the *C. glabrata* infection case. Medium: RPMI1640 with 5% heat-inactivated human serum. Vital dye: propidium iodide (PI), 2.5 ng/ml. Environment conditions: 37°C, 5% CO<sub>2</sub>. Imaging: Zeiss LSM 780, DIC, time-lapse, 7 sec, ~0.2 μm/px.

**5. Hypothesis**  
 A cell population in any specimen can be described as a mixed distribution:  

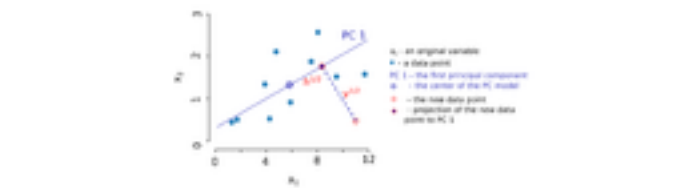
$$M_{mix} = (1 - \mu)N + \mu S,$$
 where  $0 \leq \mu < 1$  is the fraction of S-cells. It was observed that S-cells were rarely present in mock-infected samples, which allows creating a soft model of N-cells.

**6. Single cell characterisation**



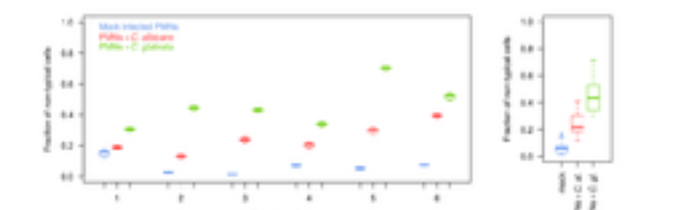
An example of different cell morphotypes (left) and density distributions plots for whole populations for feature descriptors (right). We used cell footprint area as descriptor of size and gradient-based features as a roughness measure. Bimodality is found in characteristics of infected populations.

**7. Non-spreading cells modelling**



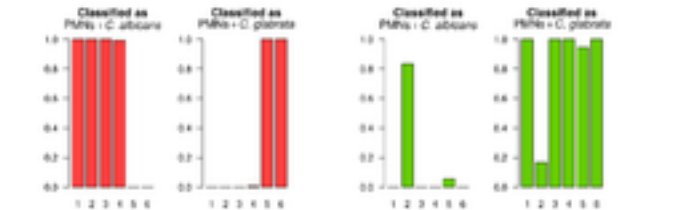
We used Data-driven SIMCA (Pomerantsev, Rodionova, 2014) for static cell classification. This method utilized PCA to approximate any regular behavior within the class. The score distance  $h$  and orthogonal distance  $v$  are used for model interpretation and new objects classification.

**8. Analysis of static images**



Box diagrams of the estimated fraction of non-typical cells for each movie (left) and the pooled data sets for the three infection scenarios (right). Bootstrapping was performed with 10<sup>5</sup> iterations.

**9. Analysis of semi-kinetic data**



The ratio of correct/erroneous classification for each movie over all iterations for *C. albicans* infected (red) and *C. glabrata* infected (green) blood samples. Classification was done using SVM with leave-one-out sampling.

**10. Summary**  
 A visual inspection of misclassified sets revealed similar PMNs behavior in different infection scenarios. It indicates, that our approach works properly, but it also means that the hypothesis about infection-specific time pattern of spreading regime was not confirmed. There are at least two contributing factors, which we can speculate about: the donor specificity and PMNs population microheterogeneity (Hong, 2017).

**References:**  
 1. Pomerantsev A. L., Rodionova O. N. Concept and role of extreme objects in PCA-SIMCA. *Journal of Chemometrics*, vol. 18, 2014.  
 2. Hong C.-W. Current Understanding in Neutrophil Differentiation and Heterogeneity. *Immune Netw.*, 2017, 17(3): 238-256. Review article.

**Contact:** Ivan.Belyaev@leibniz-hkn.de  
 Research Group Applied Systems Biology, IKI, Jena, Germany

