Morphokinetic analysis of live-cell imaging data

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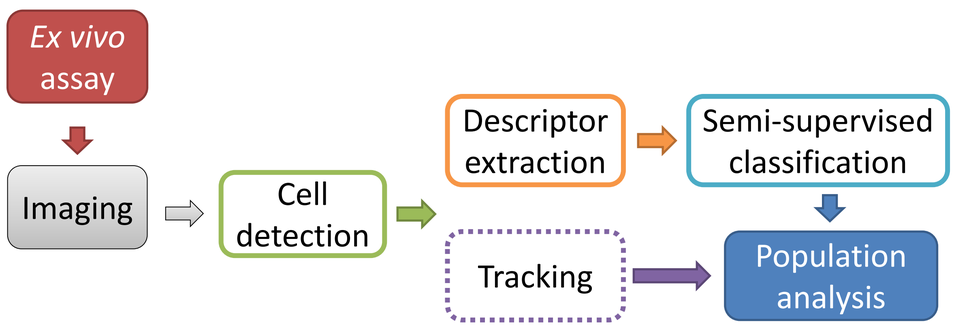
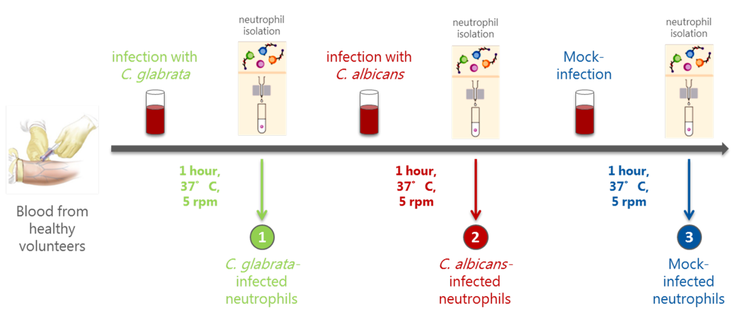
1. Project aim

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

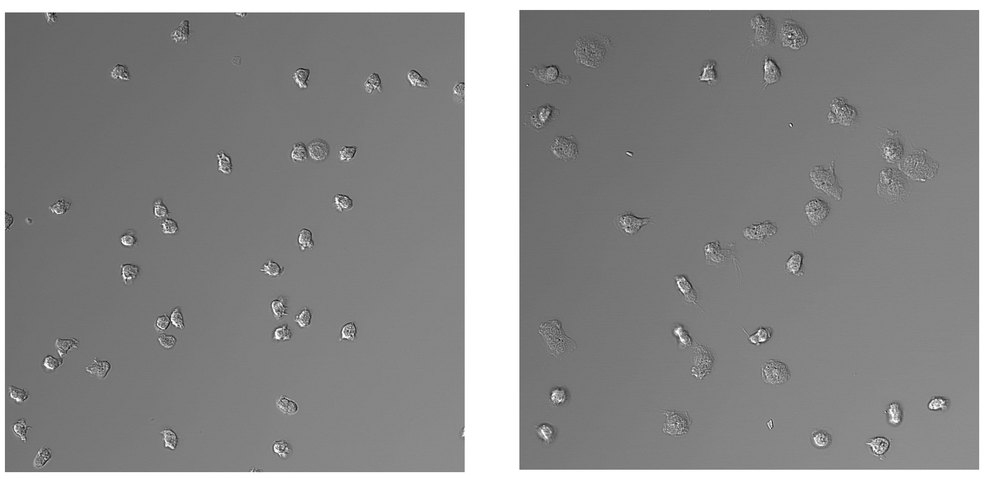


3. *Ex vivo* human whole-blood infection assay and neutrophil isolation



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 MACSxpress 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% CO2. Imaging: Zeiss LSM 780, DIC, time-lapse, 7 sec, ~0.2 µm/px.

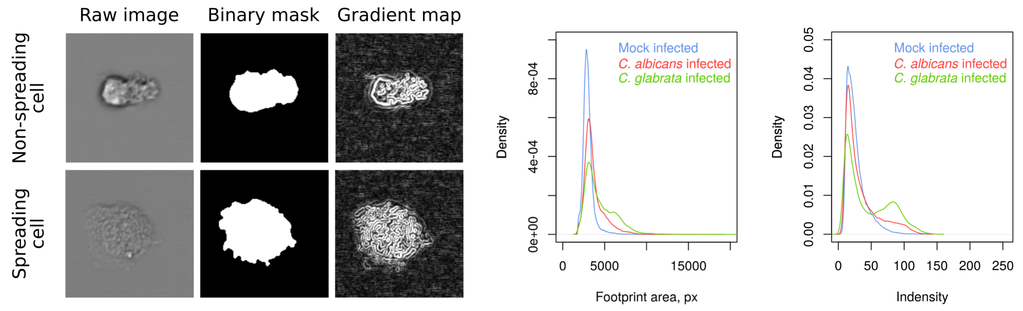
5. Hypothesis

A cell population in any specimen can be described as a mixed distribution:

$$M_{SN} = (1-\mu)N + \mu S,$$

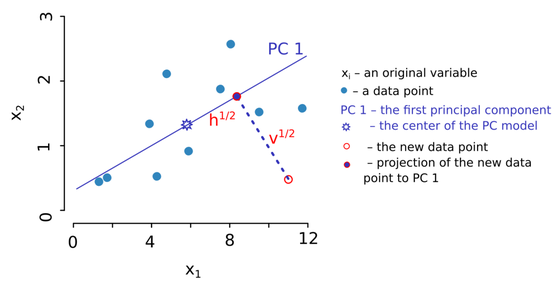
where $0 \leq \mu < 1$ is the fraction of S-cells. It was observed that S-cells were rarely present in mock-infected samples, which allows creating a soft model of N-cells .

6. Single cell characterisation



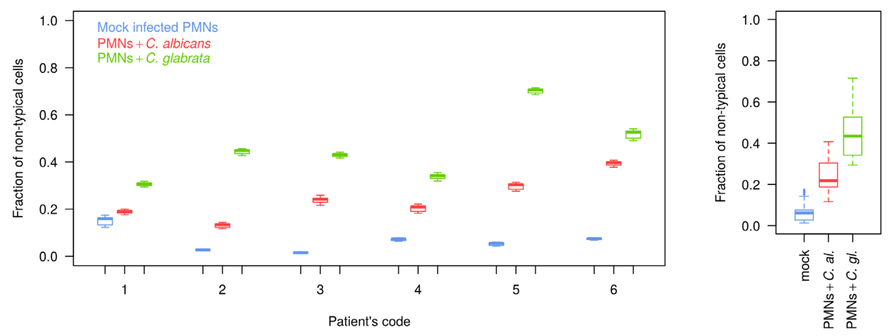
An example of different cell morphotypes (left) and density distributions plots for whole populations for feature descriptors (right). We used cell footprint area as descriptor of size and gradient-based features as a roughness measure. Bimodality is found in characteristics of infected populations.

7. Non-spreading cells modelling



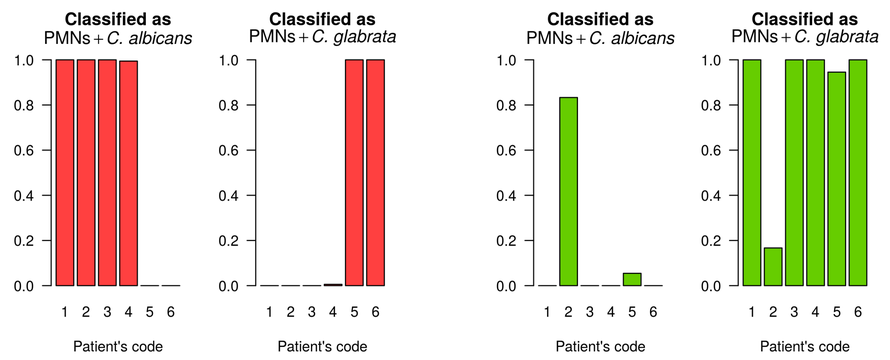
We used Data-driven SIMCA (Pomerantsev, Rodionova, 2014) for static cell classification. This method utilised PCA to approximate any regular behavior within the class. The score distance h and orthogonal distance v are used for model interpretation and new objects classification.

8. Analysis of static images



Box diagrams of the estimated fraction of non-typical cells for each movie (left) and the pulled data sets for the three infection scenarios (right). Bootstrapping was performed with 10^3 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 microheterogenity (Hong, 2017).

Reference:

1. Pomerantsev A. L., Rodionova O. Ye. Concept and role of extreme objects in PCA/SIMCA, Journal of Chemometrics, vol. 28, 2014.
2. Hong C.-W. Current Understanding in Neutrophil Differentiation and Heterogeneity, Immune Netw., 2017, 17(5):298-306. Review article..

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