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AMIT: a novel algorithm for migration and interaction tracking for high-throughput analysis of phagocytosis assays

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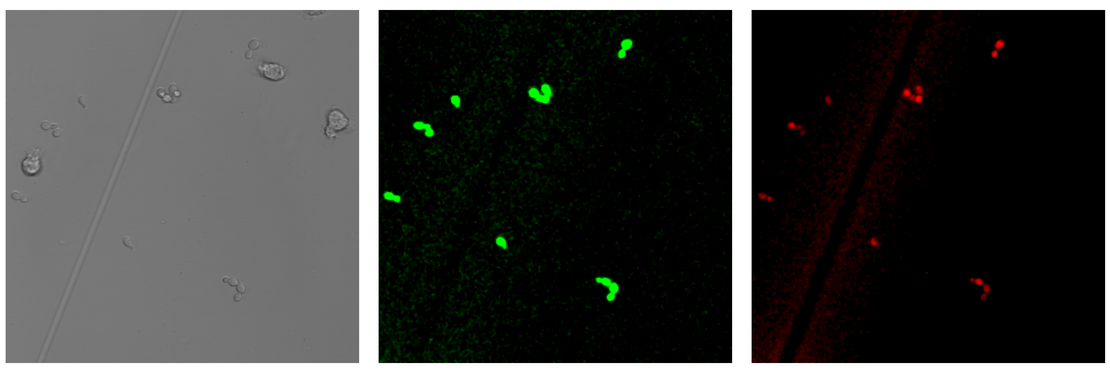
Time-lapse microscopy is an important technique to study the dynamics of various biological processes. Imaging of biological systems combined with the analysis for functional, dynamical, and morphological aspects is required to increase our understanding of complex processes. In the context of interactions between human innate immune cells and pathogens, algorithms are required for automated high-throughput analysis of time-lapse microscopy videos of confrontation assays. We present an automated segmentation and tracking framework for analyzing phagocytosis assays of polymorphonuclear neutrophils (PMNs) confronted with *C. glabrata*. The algorithm is based on our previously developed framework for tracking of non-rigid cells in brightfield microscopy [1] and was extended by (i) a segmentation approach for fluorescently-labeled pathogens and (ii) a state-transition-model and a cross-linking procedure for track interactions between PMNs and fungal cells. The PMN segmentation approach yields a sensitivity of 99% and a precision of 95% in object detection and the segmentation approach for fluorescently-labeled fungal cells yields a sensitivity of 84% and a precision of 93%. The phagocytic activity of PMNs is quantified in terms of percentage of phagocytically active PMNs, number of phagocytosis events per PMN, frequency of phagocytosis events, and killing rate of phagocytosed fungal cells. The findings of our automated analysis approach are directly compared with manual and experimental analyses [2, 3] and yield high consistency. AMIT comprises an automated segmentation and tracking framework and paves the way towards quantitative high-throughput analysis of time-lapse microscopy videos of confrontation assays.

Live-cell Imaging Data

Multi-channel time-lapse microscopy videos of confrontation assays of PMNs and *Candida glabrata*.



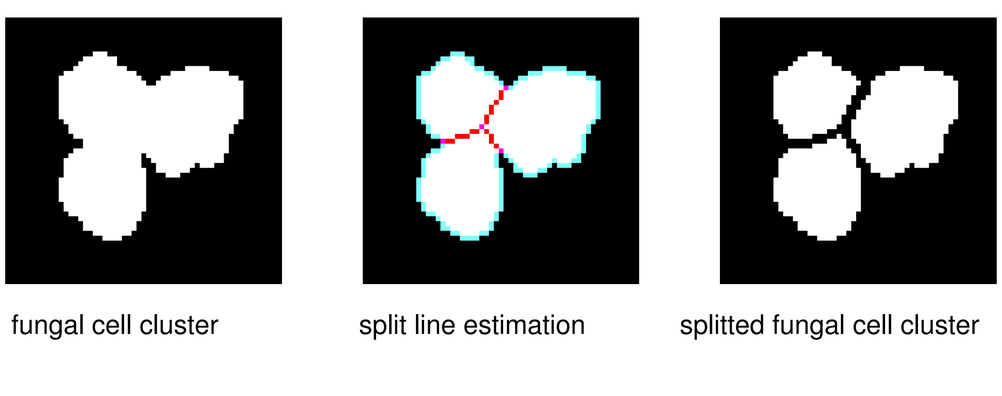
brightfield channel | green fluorescence channel | red fluorescence channel

Segmentation

Individual segmentation of different channel images.

brightfield channel | green fluorescence channel | red fluorescence channel

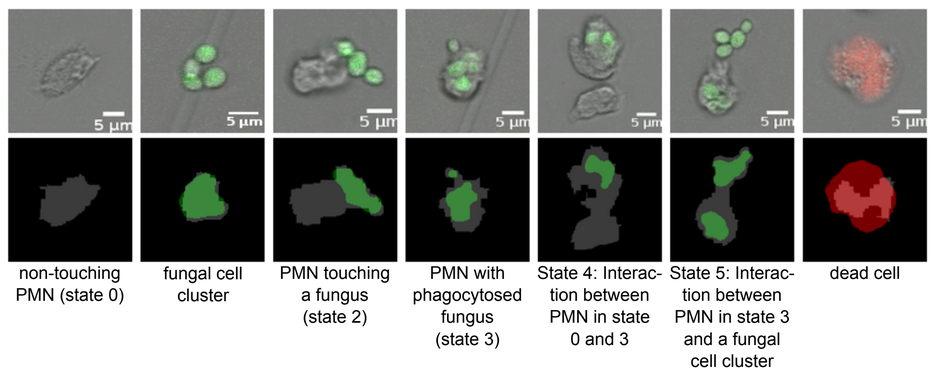
Brightfield images are segmented using a Gaussian-Mixture-Model (GMM) that is based on spatio-temporal image variances [1]. Fluorescence images are segmented using morphological operations and Canny edge detection (green fluorescence channel) or Ostu's thresholding (red fluorescence channel). Additionally, fungal cell clusters are separated into their constituent cells using a method developed by Farhan *et al.*, which detects concavity points in object contours [4].



fungal cell cluster | split line estimation | splitted fungal cell cluster

Classification

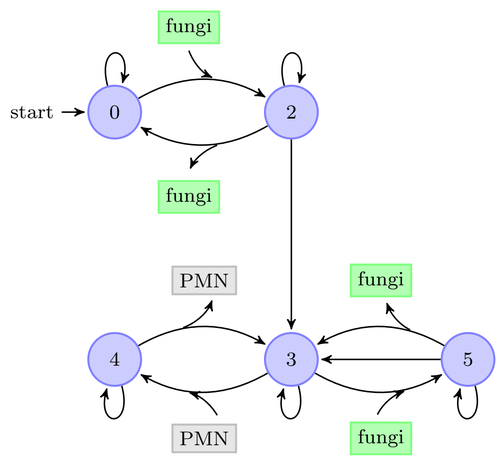
Identification of different cell types and PMN states.



non-touching PMN (state 0) | fungal cell cluster | PMN touching a fungus (state 2) | PMN with phagocytosed fungus (state 3) | State 4: Interaction between PMN in state 0 and 3 | State 5: Interaction between PMN in state 3 and a fungal cell cluster | dead cell

Interaction Tracking

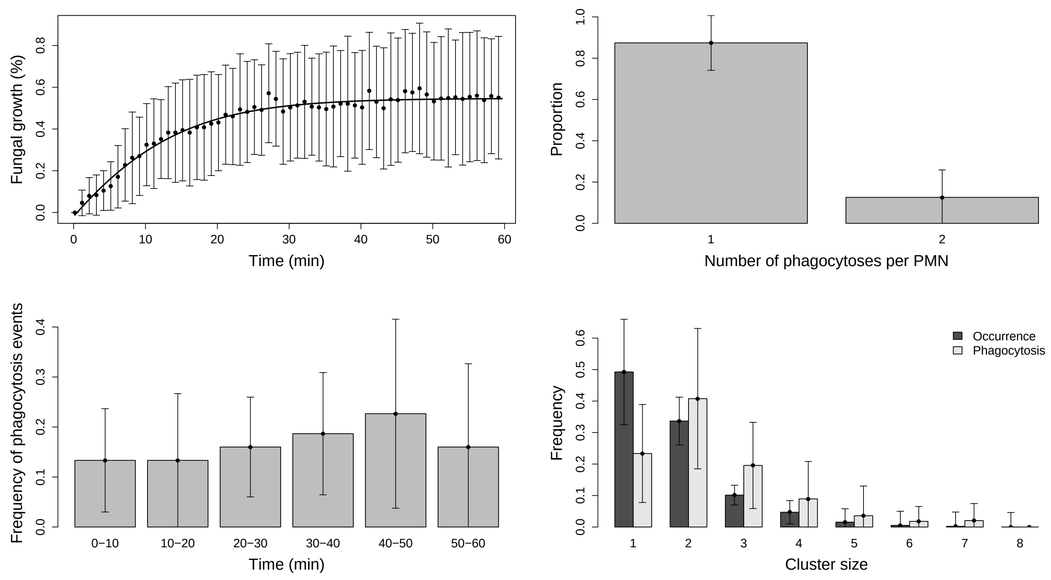
State-transition model.



The tracking of PMNs is based on the tracking algorithm developed in [1] and extended for the detection of interactions with fungal cells.
The state-transition model for PMN states represents the basic principles of pathogen–phagocyte interactions.

Results

Quantitative analysis of cell track data reveal information about the growth of the fungal cell population as well as the phagocytic behavior of PMNs.



References

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[4] M. Farhan, O. Yli-Harja, and A. Niemistö. A novel method for splitting clumps of convex objects incorporating image intensity and using rectangular window-based concavity point-pair search. *Pattern Recognition*, 46(3):741–751, 2013.

Acknowledgement

Leibniz Association

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