International Leibniz Research School
for Microbial and Biomolecular Interactions

IMAGE SEGMENTATION METHODS FOR THE QUANTIFICATION OF *CANDIDA* CELLS IN FLUORESCENCE IMAGES

Dietrich S.[1,4], Brandes S.[1,4], Hünniger K.[2], Engert N.[3,4], Jacobsen I.[3,4], Kurzai O.[2,4], Figge T.[1,4]
Applied Systems Biology[1], Fungal Septomics[2], Microbial Immunology[3]
Leibniz Institute for Natural Product Reserach and Infection Biology - Hans Knöll Institute, Jena
Friedrich Schiller University, Jena[4]

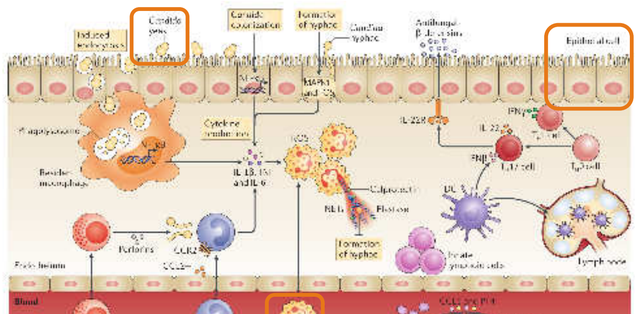
Abstract

Candida species are ubiquitous and can lead to severe infections. Our body possesses several defence mechanisms against infections, like cells of the innate immune system. To study the interplay of body cells and pathogens, different biological assays can be used, wherein fungal cells are labeled and imaged. We use automated image segmentation methods and machine learning to quantify *Candida* cells, thereby characterizing the cell interactions.

Introduction

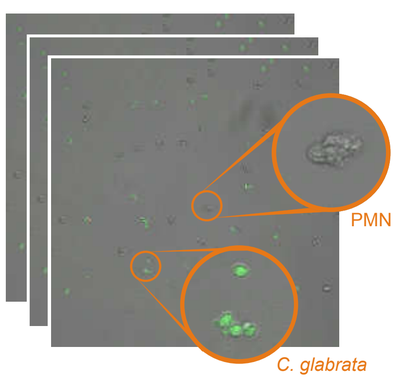
Candida species are commensals in the human body and do no damage under normal circumstances. However, in immunocompromised patients they can lead to severe infections. Our body posseses several defence mechanisms against these infections. For example polymorphonuclear neutrophils (PMNs) that are recruited to the site of infection and phagocytose and kill *Candida* cells or our natural skin barrier that builds the first line of defence against any infection.

To study the interplay of PMNs or epithelial cells and fungal cells, phagocytosis assays or adhesion assays can be used. Fungal cells are fluorescently labeled and imaged. Manual analysis of these images can be time consuming. Therefore, we use automated image segmentation methods for the quantification of *Candida* cells.

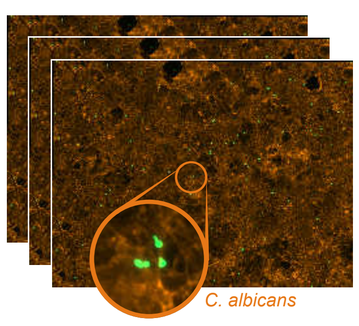


Overview of immune defence mechanisms against *Candida*. Adapted from [1]

Image data



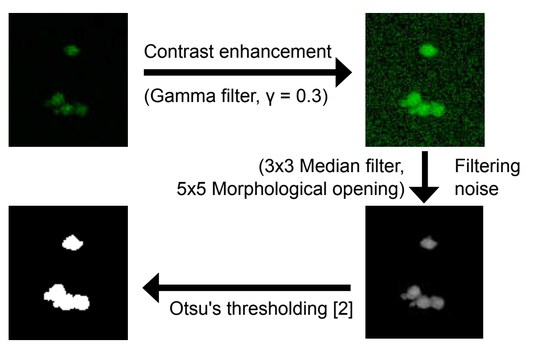
Images from phagocytosis assays with PMNs in gray and *Candida glabrata* cells labeled in green.

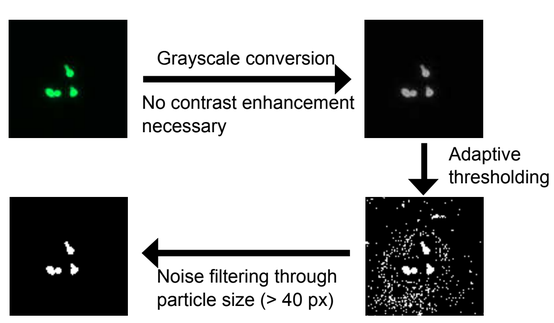


Adhesion assays with *Candida albicans* cells labeled in green on epithelial cells

Basic image segmentation

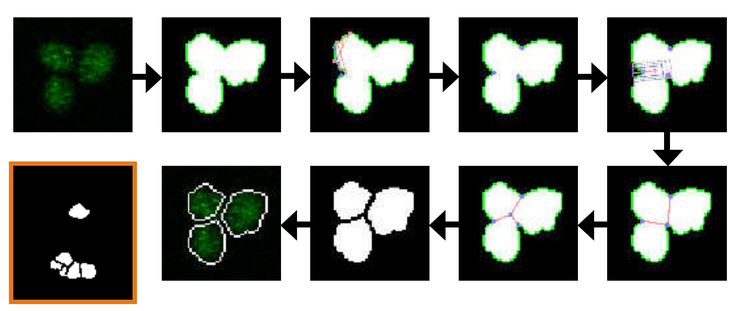
Input: green fluorescence channel images converted to grayscale
Output: segmented single cells and cell clusters



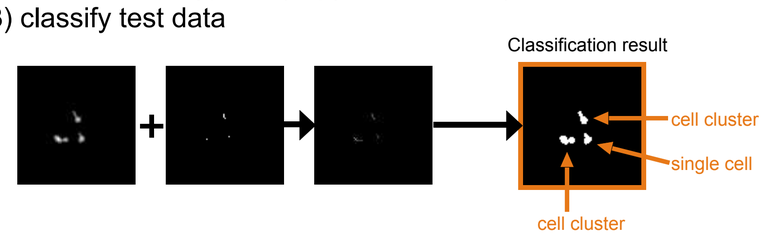


Advanced segmentation and classification

Cluster splitting using concavity points [3]:
1) find concavity points in cell cluster
2) find corresponding point pairs for formation of cut lines
3) postprocess cut lines



Cell classification using cluster skeleton
1) compute skeleton and distance transform
2) train SVM on labeled data with 4 classes (noise, single cells, cells with hyphae (chp), cell clusters)
3) classify test data



Segmentation evaluation

- cluster splitting works good on small clumps, worse on large/occluded clumps
- evaluation using manual segmentation of 30 images with 2045 cells:

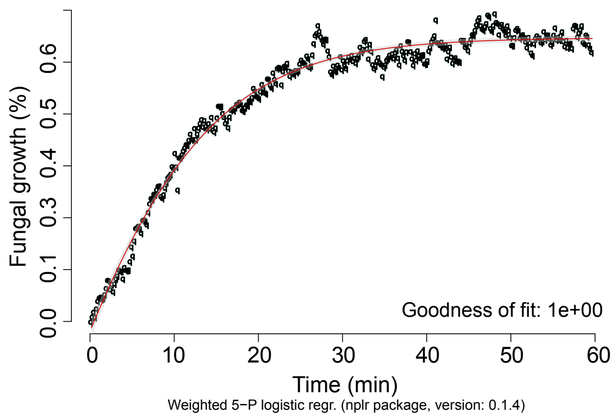
# cells (man. Seg.)	# cells (aut. Seg.)	TP	FP	FN	Recall	Precision
2045	1927	1894	78	151	0.93	0.96

- classification can distinguish well between noise and single cells
- classification of hyphae and clusters needs to be improved
- classification on 176 objects using cross validation (k=4, 10 runs)
- chp = cells with hyphae

		Identified class			
True class	%	noise	cell	chp	cluster
	noise	0.908	0.068	0	0.022
	cell	0	0.976	0	0.024
	chp	0	0.1	0	0.9
	cluster	0.053	0.111	0	0.836

Quantification

The image segmentation allows the automated measurement of fungal growth over time. In combination with segmentation and tracking of PMNs, phagocytosis rates can be measured [4]. In combination with segmentation of epithelial cells, the number of adherent fungal cells can be counted.
The classification of cells with hyphae is the first step in measurement of hyphal growth and quantification of invasion behaviour under different environmental



Relative fungal growth over one hour. Taken from [4]

References

[1] M. G. Netea *et al*., Immune defence against *Candida* fungal infections, *Nat.Rev.Immunol*. 15, 630-642 (2015)
[2] N. Otsu, A threshold selection method from gray-level histograms, *Automatica C* (1), 62-66 (1975)
[3] M. Farhan *et al*., A novel method for splitting clumps of convex objects incorporating image intensity and using rectangular window-based concavity point-pair search, *Pattern Recognit.* 46 (3), 741-751 (2015)
[4] S. Brandes *et al*., Migration and Interaction Tracking for Quantitative Analysis of Phagocyte-Pathogen Confrontation Assays (submitted)

Contact: stefanie.dietrich@leibniz-hki.de

Acknowledgement

This work was supported by the Leibniz Association.