

## Image Segmentation Methods for the Quantification of *C. albicans* cells in Fluorescence Images

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### Image Segmentation Methods for the Quantification of *Candida* cells in Fluorescence Images

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#### 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 possesses 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.

#### Image data

Images from phagocytosis assays with PMNs in grey and *Candida* glabrate 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

Contrast enhancement  
Median filter:  $\mu = 2.5$   
(3x) Median filter  
Filtering noise  
(5x) Morphological opening  
Otsu's thresholding [2]

#### 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. S.)	# 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

True class	Identified class			
	% noise	cell	chp	clust.
noise	0.908	0.068	0	0.022
cell	0	0.978	0	0.024
chp	0	0.1	0	0.9
clust.	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 conditions.

#### References

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