

# Automated image analysis methods for the quantification of cell damage and adherent fungal cells

S. Dietrich, P. Dasari, N. Engert-Ellenberger, I. Jacobsen,  
P. Zipfel, M.T. Figge

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**Automated image analysis methods for the quantification of cell damage and adherent fungal cells**

Stefanie Dietrich<sup>1,2</sup>, Prasad Dasari<sup>1</sup>, Nicole Engert-Ellenberger<sup>1,4</sup>, Ilse Jacobsen<sup>1,4</sup>, Peter Zipfel<sup>1,3</sup>, Marc Thilo Figge<sup>1,3</sup>

<sup>1</sup>Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany  
<sup>2</sup>Faculty of Biological Science, Friedrich Schiller University, Jena, Germany  
<sup>3</sup>Infection Biology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany  
<sup>4</sup>Microbial Immunology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany

**Introduction**  
Epithelial tissues are the first line of defence against many microbial invaders like human pathogenic fungi. An impaired functionality of these barriers can lead to invasion and infection. We developed image analysis methods and machine learning algorithms. Therefore, experiments were conducted by the research group Infection Biology to study the interaction between *Aspergillus fumigatus* *aspf* mutants and wildtype and human lung epithelial cells. The effect of mesenteric ischemia on the barrier function of enterocytes and adhesion of *Candida albicans* was studied by the group Microbial Immunology. Microscopic images of both assays were generated in order to quantify damage of the cell layer and the number of adherent fungal cells. We developed tailored image analysis algorithms to automatically analyse the image data in a fast and objective manner. We combine standard image analysis techniques with machine learning and advanced segmentation methods.

**Experiments/Data**  
**Adhesion assays**  
Human enterocytes (3 day old) + *C. albicans* (30 min)  
Constant oxygen: 0.2%, 1%, 2%, 5%  
Hypoxic shock: 21%, 0.2%, 21%, 1%, 21%, 2%, 21%, 5%  
Reoxygenation: 21%, 21%, 1%, 21%, 2%, 21%, 5%  
**Cell retraction assays**  
Human lung epithelial cells (A549)  
*Candida* WT + *A. fumigatus* or *A. fumigatus* *aspf*  
Plasminogen affected to retraction  
Plasminogen activated PA (inactive PA)  
PA inhibitor Argatroban  
**Segmentation and quantification**  
**Adhesion assays**  
Gamma transformation (constant hypoxia)  
Otsu's thresholding (constant hypoxia)  
Classification of fungal cells  
**Cell retraction assays**  
Adaptive thresholding  
Otsu's thresholding (morphological opening)  
Morphological opening (morphological, cluster analysis)  
**Quantification of void area and adherent fungal cells**  
Quantification of void areas in the cell layer  
Quantification of cell damage  
*aspf* Knock out mutant with plasminogen and IPA  
**References**  
[1] Netea, M.G. et al., *Nat. Rev. Immunol.* 15, 630-642 (2015)[2]  
[2] Otsu, N., *Automatica* 1(1), 62-66 (1975)[3] Alvarado, J. *Image Process.* 46 (3), 741-751 (2015)[4] Bräuer, S., Dietrich, S., Höppner, A., Kürzel, O., Figge, M.T. *Medical Image Analysis* (2016)[5] Dasari, P. et al. [under revision]

Contact: stefanie.dietrich@leibniz-hki.de