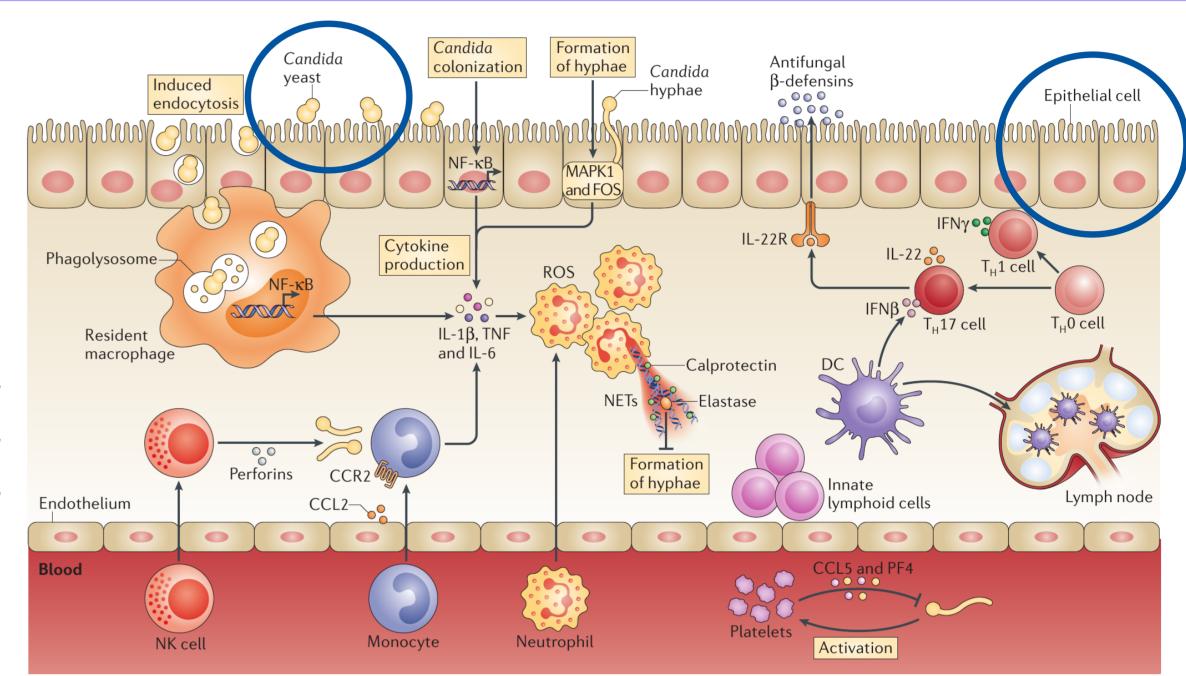
Automated image analysis methods for the quantification of cell damage and adherent fungal cells

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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, but the exact mechanisms on host and pathogen side are still unknown. Therefore, experiments were conducted by the research group Infection Biology to study the interplay between Aspergillus fumigatus aspf2 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.



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

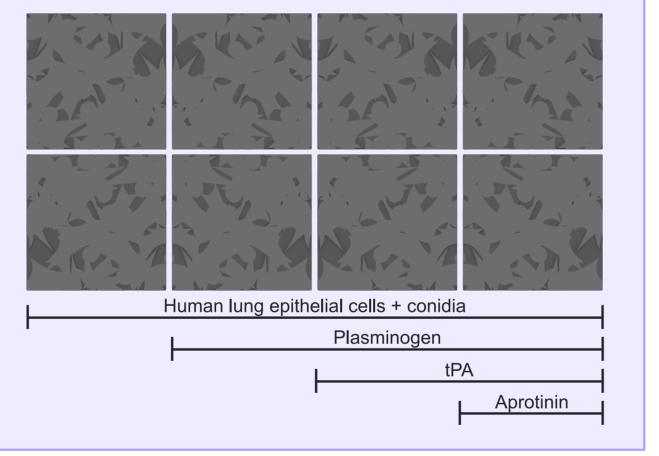
Experiments/Data

Adhesion assays C. albicans Human enterocytes (7 days, diff. oxygen (30 min) conditions) Constant oxygen

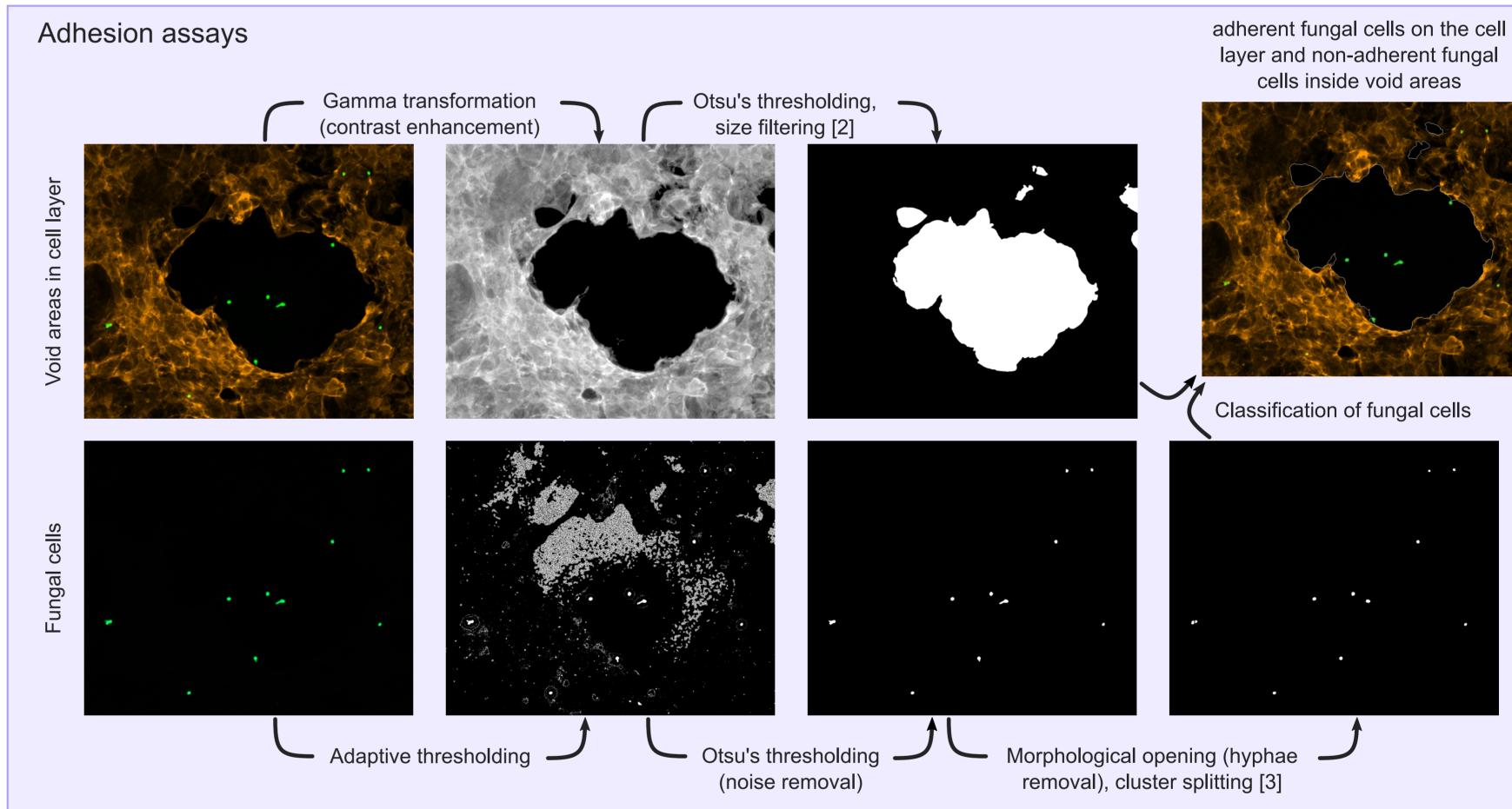
21% ▶ 0.2% | 21% ▶ 1% | 21% ▶ 2% | 21% ▶ 5% Hypoxic shock 0.2% ► 21% | 1% ► 21% | 2% ► 21% | 5% ► 21% Reperfusion

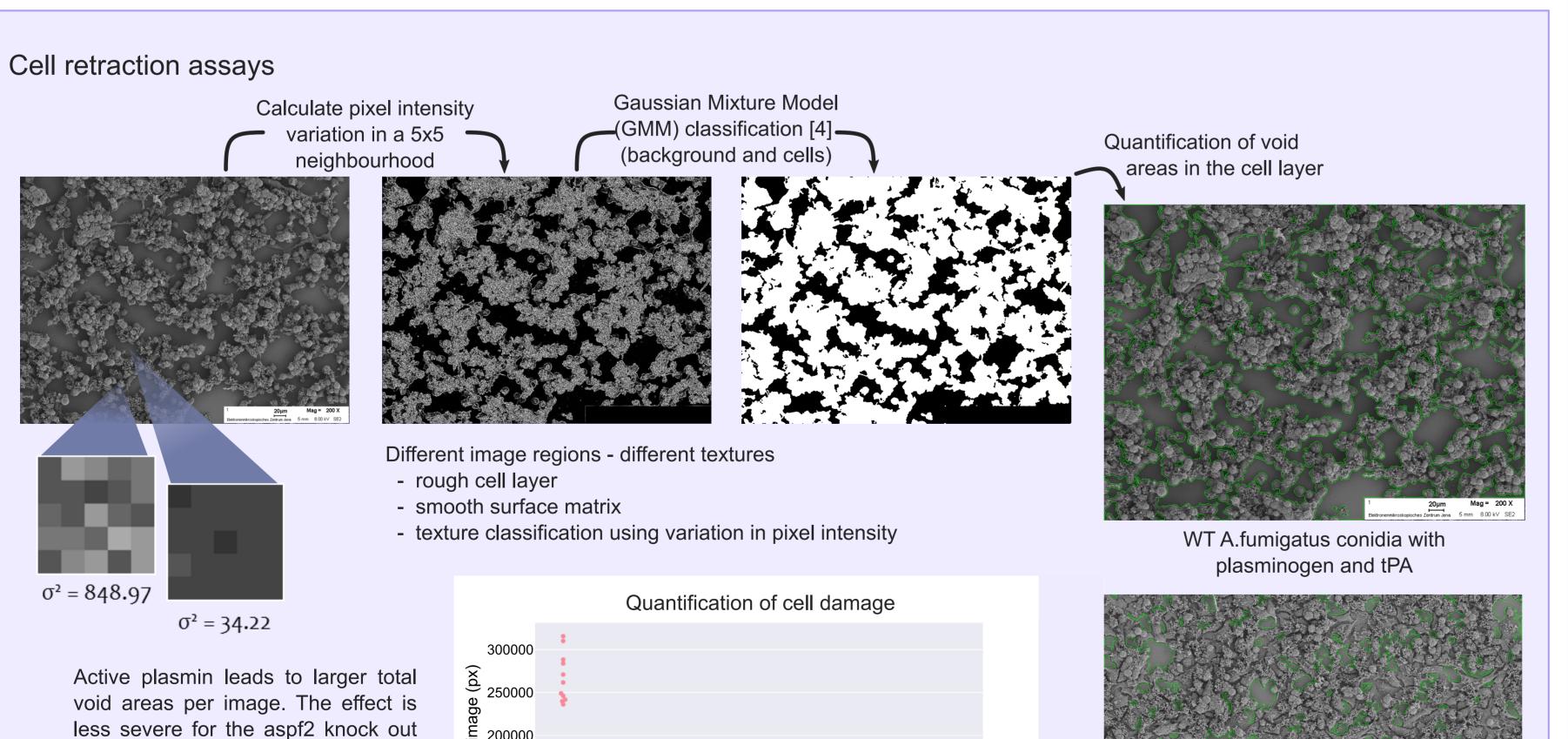
Cell retraction assays

- Human lung epithelial cells (A549)
- Conidia: WT A. fumigatus or
- aspf2 knock out mutant - Plasminogen attached to
- conidia - Plasminogen activator tPA
- (incubated 2hours, 37°C) - Plasmin inhibitor Aprotinin



Segmentation and quantification





Experiments

for constant oxygen levels of 0.2%, 1% and 2% oxygen. This indicates a weakening of the epithelial barrier. Most distributions of the number of adher-

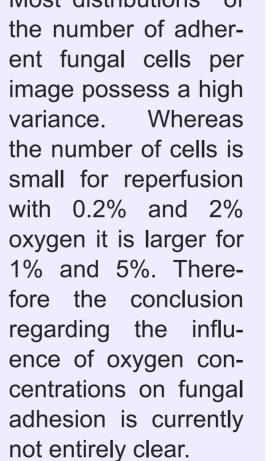
The void area per

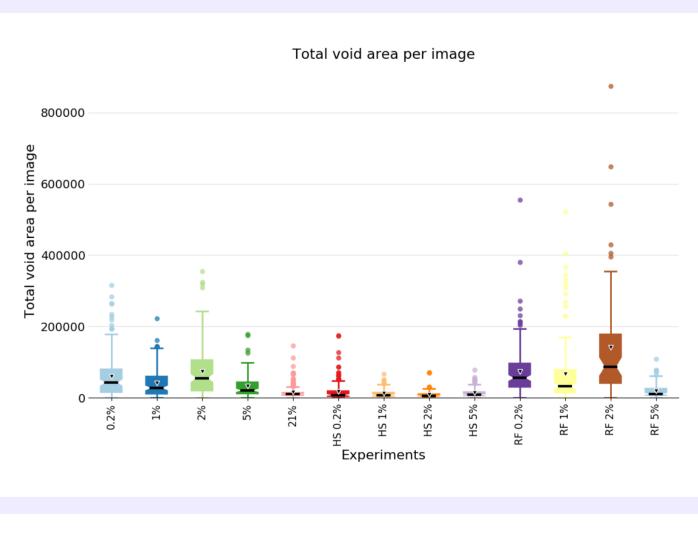
image is increased

for reperfusion con-

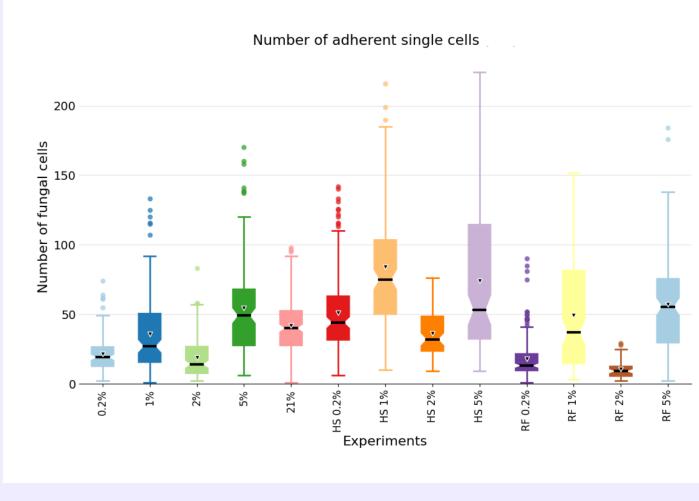
ditions with 0.2%, 1%

and 2% oxygen and





Quantification of void area and adherent fungal cells



References

[1] Netea, M.G. et al., Nat. Rev. Immunol 15, 630-642 (2015)[2]

200000

150000

100000

50000

[2] Otsu, N., Automatica C (1), 62-66 (1975)

mutant. This underlines the

importantce of A. fumigatus aspf2 for

the activation of plasminogen to

plasmin and shows another aspect

of its ability to modulate the host

immune response [5].

- [3] Farhan, M. et al., Pattern Recognit. 46 (3), 741-751 (2015)
- [4] Brandes, S., Dietrich, S., Hünniger, K., Kurzai, O., Figge, M.T. Medical Image Analysis (2016)





aspf2 Knock out mutant with

plasminogen and tPA