Project B4

Pathogenic fungi and their human host: Networks of interaction
Collaborative Research Center / Transregio

Automated Image Analysis for Quantifying Fungus-host Interactions

Kaswara Kraibooj [1,2], Hanno Schoeler [2,5], Hea-Reung Park [2,3], Hans-Martin Dahse [4], Christine Skerka [2,4], Kerstin Voigt [2,3], Axel Brakhage [2,5] and Marc Thilo Figge [1,2]

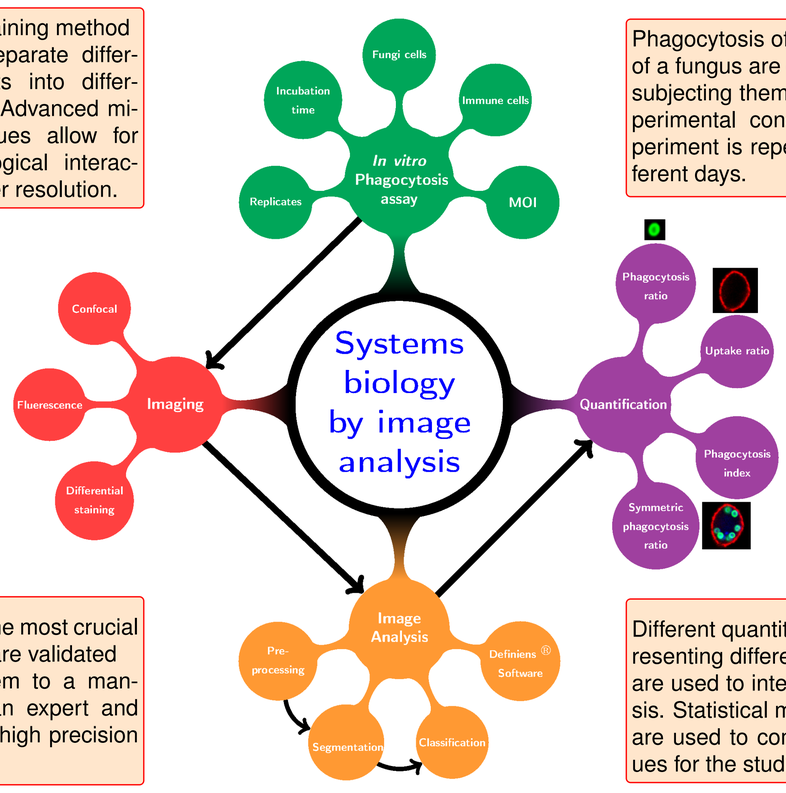
[1]Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany
[2]Friedrich Schiller University Jena, Jena, Germany
[3]Jena Microbial Resource Collection, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany
[4]Infection Biology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany
[5]Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany

1. Introduction

Fungus-host interactions have gained a lot of interest in the last decade. This is due to the increasing number of life-threatening infections caused by different human-pathogenic fungi in immunocompromised patients, and due to the limitation of available therapies. Therefore, inspecting the pathobiology of these fungi is of key importance for ultimately possible treatments. As the last advances of microscopy techniques allow for viewing the pathogen-host interactions (micrometer resolution) and consequently for generating a lot of images, automated image analysis raises as the most adequate approach for quantifying these interactions. We here show how we exploit automated image analysis to quantify the interaction between the fungi *Lichtheimia corymbifera* and *Aspergillus fumigatus* confronted with murine alveolar macrophages [1,2]. As the results are objective, it is expected that this kind of studies will play a crucial role in exploring the different strategies used by fungi during an infection process.

2. Methods

The differential staining method is exploited to separate different image agents into different image layers. Advanced microscopy techniques allow for viewing the biological interactions at micrometer resolution.



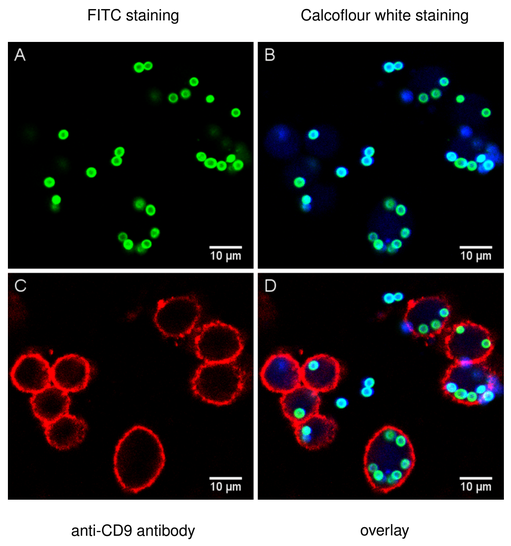
Phagocytosis of different strains of a fungus are compared after subjecting them to the same experimental conditions. The experiment is repeated on two different days.

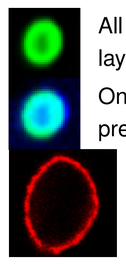
Segmentation is the most crucial step. The results are validated by comparing them to a manual analysis by an expert and accordingly show high precision and sensitivity.

Different quantitative values representing different point of views are used to interpret phagocytosis. Statistical measurements are used to compare these values for the studied strains.

3. Image Analysis

Raw data



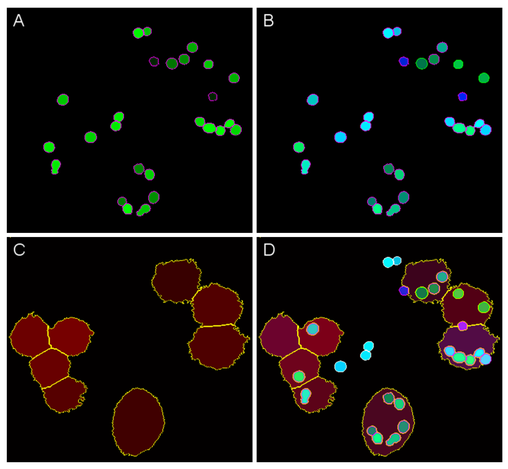


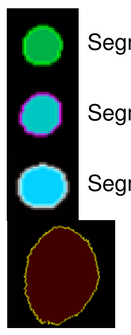
All fungal spores are present in the green layer

Only non-phagocytosed spores are present in the blue layer

All macrophages are present in the red layer

Processed data





Segmented phagocytosed spore

Segmented adherent spore

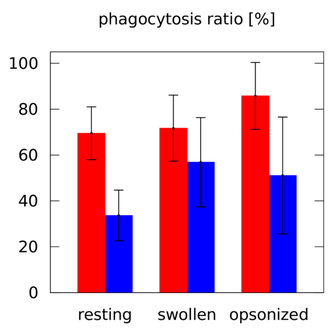
Segmented non-adherent spore

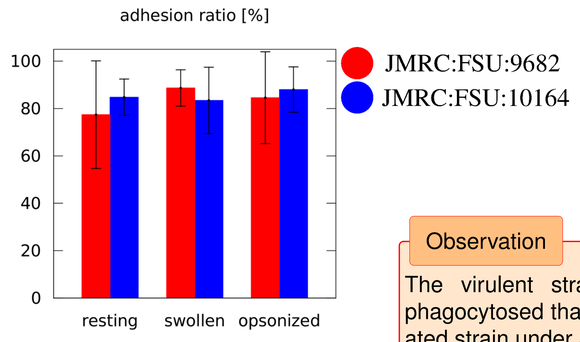
Segmented macrophage

The automated image analysis algorithm uses a combination of filtering processes, thresholding, watershed segmentation and feature-based object classification. The algorithm allows for the segmentation of individual macrophages in the images and this enables us to compute the distribution of phagocytosed and macrophage-adherent conidia over all macrophages. The novel automated image-based analysis provides access to all cell-cell interactions in the assay and thereby represents a framework that enables comprehensive computation of diverse characteristic parameters and comparative investigation for different strains.

4. Results

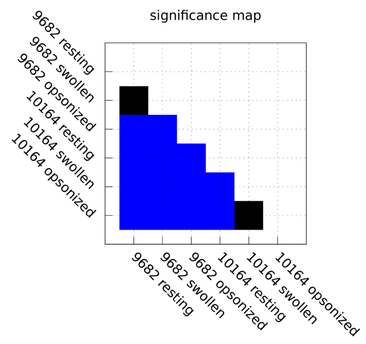
Lichtheimia corymbifera

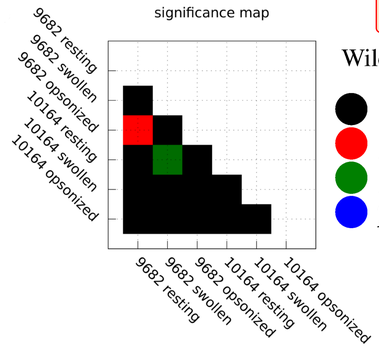




Observation

The virulent strain is more phagocytosed than the attenuated strain under all conditions whereas both strains adhere in the same way.

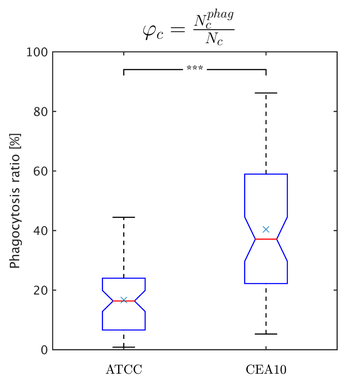


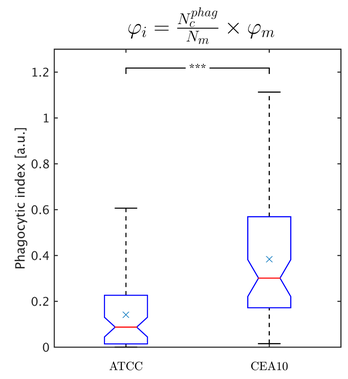


Wilcoxon rank-sum test:

- $p \geq 0.05$
- $p < 0.05$
- $p < 0.01$
- $p < 0.001$

Aspergillus fumigatus



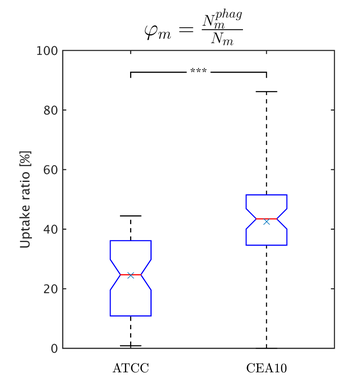


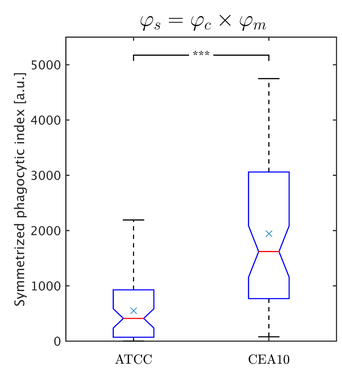
Notations

- Notched box plots
  - notches ⇔ 95% confedence interval
  - X mean value
  - — median
  - Boxes: 25% of the data
  - Outlires: 4% of the data
- Wilcoxon rank-sum test:
  - $***$ ⇔ $p < 0.001$
  - $**$ ⇔ $p < 0.01$
  - $*$ ⇔ $p < 0.05$
  - n.s. ⇔ $p \geq 0.05$

Observation

All measures point toward a higher degree of phagocytosis for the CEA10 strain compared to ATCC.





5. Discussion

- In both examples, the more virulent strain is significantly more phagocytosed. The question arises: is there a direct correlation between the observed difference in virulence and phagocytosis ratio? In case the spores are able to inhibit killing after being phagocytosed they could use macrophages as a survival niche and escape from the phagocyte by germination [3]. However, further experiments would have to be performed to prove this hypothesis.
- The algorithm here is generally applicable to assays of cells with close-to circular morphology and can be straightforwardly extended to assays for more than two different cell types. In contrast to techniques based on flow cytometry, approaches based on microscopy images do provide a richer amount of information, e.g., on spatial correlations and morphological properties of cells.

Outlook

- Quantitative answers to biological questions by an image-based systems biology approach
- High-throughput screening for different strains to perform comparative studies in an automated fashion
- A quantitative data base for the development of mathematical models that enable realistic simulations of biological processes on the computer

Acknowledgements

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References

[1] Kraibooj K, Park H-R, Dahse H-M, Skerka C, Voigt K, & Figge, MT (2014). Virulent strain of Lichtheimia corymbifera shows increased phagocytosis by macrophages as revealed by automated microscopy image analysis. *Mycoses, 57*(s3), 56-66.
[2] Kraibooj K, Schoeler H, Svensson CM, Brakhage AA, & Figge MT (2015). Automated quantification of the phagocytosis of Aspergillus fumigatus conidia by a novel image analysis algorithm. *Frontiers in Microbiology, 6*, 549.
[3] Amin S, Thywissen A, Heinekamp T, Saluz HP and Brakhage, AA (2014). Melanin dependent survival of Apergillus fumigatus conidia in lung epithelial cells. *Int. J. Med. Microbiol. 304,* 626–636. doi:10.1016/j.ijmm.2014.04.009

Friedrich-Schiller-Universität Jena
Julius-Maximilians-Universität Würzburg
HKI
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