Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute

Automated image analysis of the host-pathogen interaction between immune cells and human-pathogenic fungi

– FungiNet –

seit 1558

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1. Introduction

- **Motivation:** Immunocompromised patients are susceptible for opportunistic infections of human-pathogenic fungi.
- **Goal:** Quantification of host-pathogen interactions in comparative studies of different mutants.
- **Methods:**

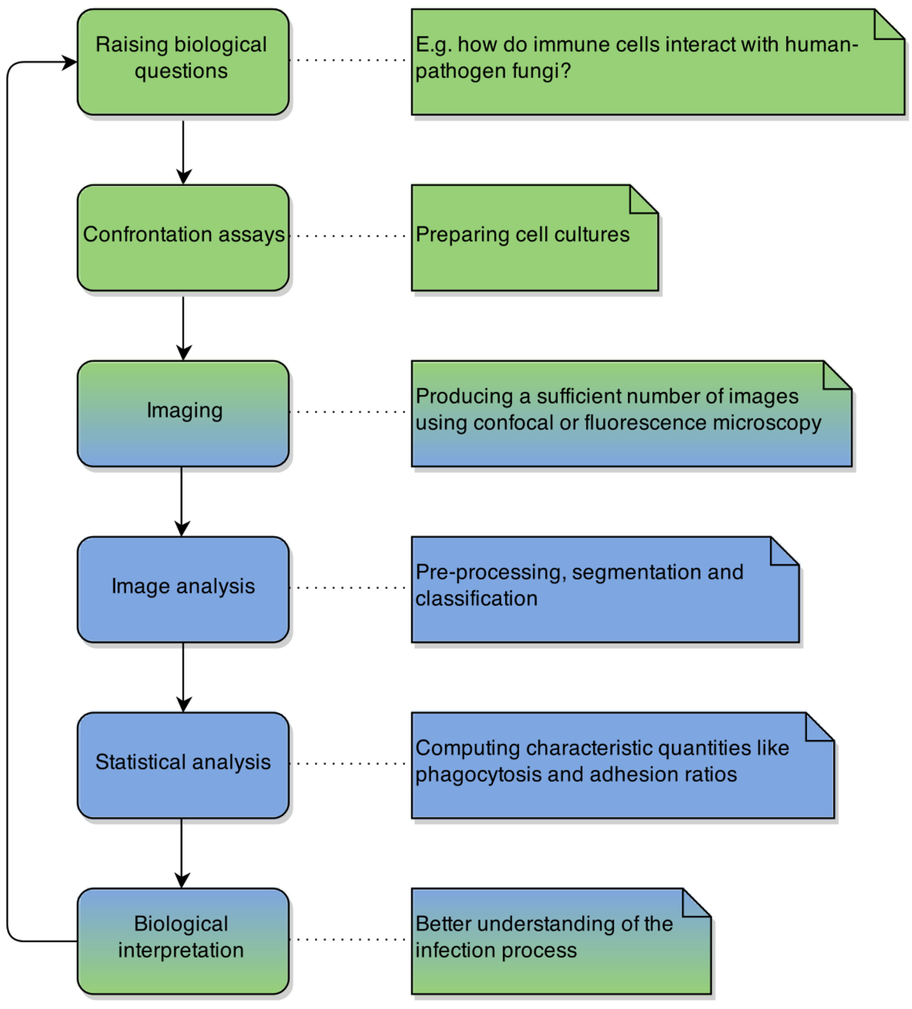

Figure 1 General schematic representation of the iterative cycle between experiment (green) and theory (blue).

2. Example: Phagocytosis Assay

- An automated analysis of 360 fluorescence microscopy images is performed [1]. The analysis is based on the method applied by Mech *et al.* [2]. The images depict the interaction between *Lichtheimia corymbifera* and murine alveolar macrophages. A virulent (JMRC:FSU:9682) and an attenuated (JMRC:FSU:10164) strain of this fungus are studied under three conditions: resting, swollen and opsonized spores.
- The algorithm is developed using the software environment Definiens ®.
- We computed characteristic quantities:
  - Phagocytosis ratio: $p_r = \frac{N_{pha}}{N_{pha}+N_{adh}}$; N_{pha} = number of phagocytosed spores, N_{adh} = number of adherent spores
  - Phagocyte-adhesion ratio: $a_p = \frac{N_{adh}}{N_{adh}+N_{non}}$; N_{non} = number of non-phagocytosed spores

3. Image Analysis

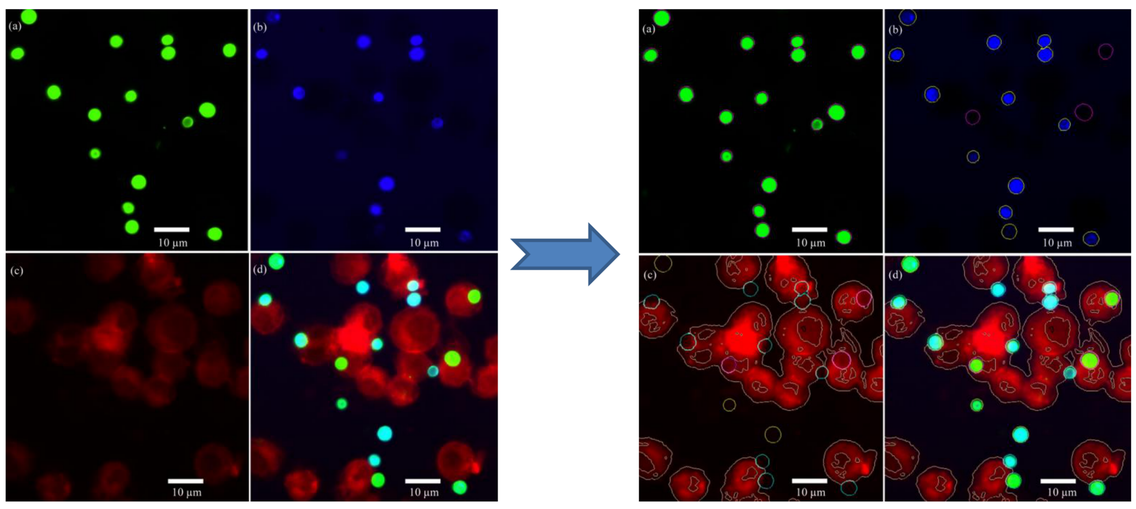

Figure 2 Raw data: Phagocytosis assay of *Lichtheimia corymbifera* by alveolar macrophages. Different layers are obtained using differential staining : (a) all fungal spores (green), (b) non-phagocytosed spores (blue), (c) macrophages (red) and (d) overlay. Processed data: Result of the automated image analysis separately applied on the RGB layers, i.e. (a) all spores with purple segmentation lines. (b) non-phagocytosed spores with yellow segmentation lines and (c) macrophages with white segmentation lines, and the overlay (d) adherent spores with cyan segmentation lines. Detecting spores reached sensitivity and precision values $\geq$ 95%.

4. Results

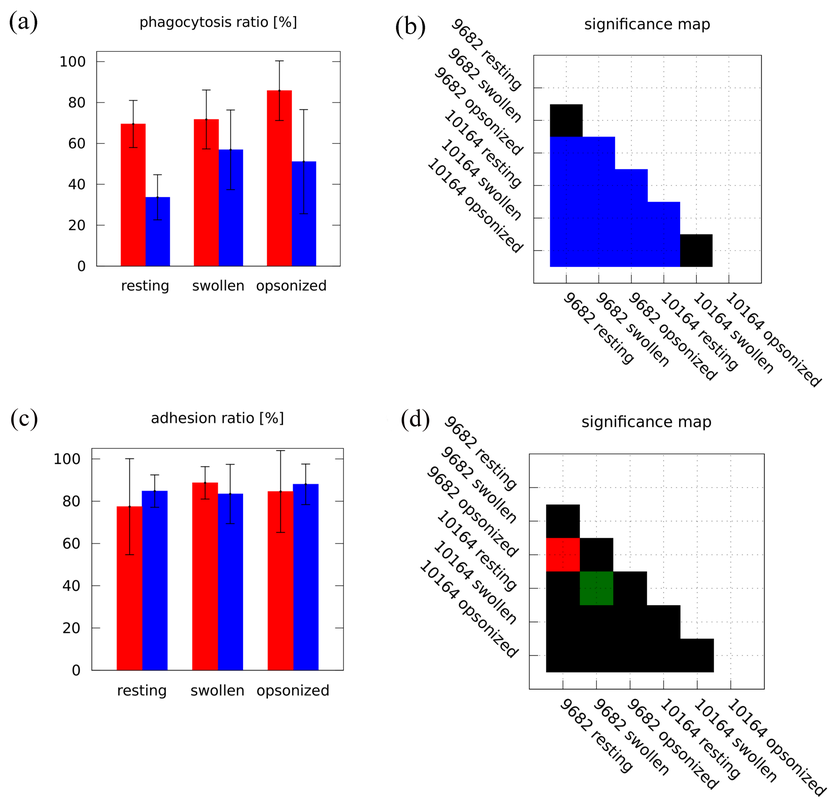

Figure 3 (a) Phagocytosis ratio for virulent *Lichtheimia corymbifera* strain JMRC:FSU:9682 (red) and attenuated *Lichtheimia corymbifera* strain JMRC:FSU 10164 (blue) under the three conditions: resting, swollen and opsonized spores. (b) Significance map for Phagocytosis ratio for both strains and the three conditions as obtained from the Wilcoxon rank-sum test with color coding according to P-value: black for P-value$\geq$ 0.05, red for P-value < 0.05, green for P-value < 0.01, blue for P-value < 0.001. (c) The same as in (a) but for the adhesion ratio. (d) The same as in (b) but for the adhesion ratio.

5. Discussion

- We found a significant increase of the phagocytosis ratio for the virulent strain of *Lichtheimia corymbifera* in comparison with the attenuated one.
- The virulent strain might survive in alveolar macrophages and use the phagocytes as vehicles for dissemination *via* the blood stream causing systemic infections.

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

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References

[1] Kraibooj K, Park H-R, Dahse H-M, Skerka C, Voigt K and Figge MT (2014) Virulent strain of *Lichtheimia corymbifera* shows increased phagocytosis by macrophages as revealed by automated microscopy image analysis. Mycoses 2014.
[2] Mech F, Thywißen A, Guthke R, Brakhage AA, Figge MT. Automated image analysis of the host-pathogen interaction between phagocytes and *Aspergillus fumigatus*. PLOS ONE 2011; 6: e19591.

Acknowledgements

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