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

# Comparative Study of Phagocytosis-assays for Lichtheimia Species by Automated Analysis of Fluorescence Microscopy Images



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- Motivation: the fungus Lichtheimia corymbifera can cause life-threatening diseases by attacking the human lung.
- Goal: understanding the mechanisms underlying pathogenicity of L. corymbifera.
- Method: automated analysis of 350 fluorescence microscopy images. The images depict the interaction between *L. corymbifera* and alveolar macrophages by differential staining. A virulent (JMRC:FSU:9682) and an attenuated (JMRC:FSU:10164) strain of this fungus are studied under three conditions: resting, swollen and opsonized.

#### strain $N_{tot}$ FP/FP rate (%) FN/FN rate (%) TP S (%) P (%)

9682 2130	87/ 4.08	80/ 3.75	2043	96.37	95.91
1016 1653	75/ 4.54	61/ 3.7	1578	96.28	95.46
Sum 3787	162/ 4.28	141/ 3.73	3621	96.25	95.72

 $N_{tot}$  = the total number of spores, TP (*true positive*) = number of spores which are correctly segmented and classified, FP (false positive) = number of image objects which are incorrectly segmented or classified as spores (artifacts or oversegmentation), FN (*false negative*) = number of spores which are incorrectly not recognized

### **Methods**

## . Image analysis [1]

(a) Pre-processing ~> contrast enhacement and noise reduction (b) Segmentation ~> separating regions of interest (ROIs) from background (c) Classification  $\rightsquigarrow$  identify ROIs by features, e.g. size, color, morphology.

## 2. Validation based on a manual analysis of a subset of images

- Sensitivity
- Precision

## 3. Characteristic quantities

- Phagocytosis ratio:  $p_r = \frac{N_{pha}}{N_{pha}+N_{adh}}$ ;  $N_{pha}$  = number of phagozytozed spores,  $N_{adh}$  = number of adherent spores • Phagocyte-adhesion ratio:  $a_p = \frac{N_{adh}}{N_{adh}+N_{non}}$ ;  $N_{non}$  = number of nonphagocytozed spores • Fungal aggregation ratio:  $a_f = \frac{N_{agg}}{N_{adb}+N_{non}}$ ;  $N_{agg}$  = number of aggregated spores

## **Results**



- 4. Statistical analysis and significance tests
- 5. **Biological interpretation**

## Image Analysis







before after

• Parameters: area, intensity, asymmetry and position of image objects

• A significant increase of phagocytosis ratio of the virulent strain in comparison to the attenuated one

#### Outlook

- Quantitative answers to biological questions by an image-based systems biology approach
- High-troughput screening for different strains to perform comparative studies in an automated fashion

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#### References

[1] 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.

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