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

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Introduction

- 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.

Validation

strain	N_{sp}	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_{sp} = the total number of spores, FP (false positive) = number of spores which are incorrectly segmented and classified, FN (false negative) = number of spores which are incorrectly segmented or classified as spores (surface or non-segmentation), FN (false negative) = number of spores which are incorrectly not recognized

Methods

- Image analysis [1]**
 - Pre-processing – contrast enhancement and noise reduction
 - Segmentation – separating regions of interest (ROIs) from background
 - Classification – identify ROIs by features, e.g. size, color, morphology.
- Validation based on a manual analysis of a subset of images**
 - Sensitivity
 - Precision
- Characteristic quantiles**
 - Phagocytosis ratio: $p_i = \frac{N_{phag}}{N_{sp}} ; N_{phag}$ = number of phagozytized spores, N_{sp} = number of adherent spores
 - Phagocyte-adhesion ratio: $a_j = \frac{N_{ad}}{N_{mac}} ; N_{ad}$ = number of non-phagozytized spores
 - Fungal aggregation ratio: $ag_k = \frac{N_{agg}}{N_{sp}} ; N_{agg}$ = number of aggregated spores
- Statistical analysis and significance tests**
- Biological interpretation**

Image Analysis

Results

• 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-throughput screening for different strains to perform comparative studies in an automated fashion

References

[1] Meich F, Thyeßen A, Guthe R, Braßage 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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