Virtual Phagocytosis Assays Resolve Ambiguities in Traditional Phagocytosis Measurements

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Fungal Infections

- ***Lichtheimia corymbifera***
  - Ubiquitous soilborne zygomycete fungus
  - Opportunistic human pathogen in immunocompromised patients
  - Can cause life-threatening diseases (e.g. mucormycosis)
- ***Aspergillus fumigatus***
  - Ubiquitous mold
  - Opportunistic pathogen with mortality rates up to 90% in immunocompromised patients
  - Can cause life-threatening invasive aspergillosis

Phagocytosis Assays

- Used to study the phagocytic ability of macrophages
- Enable comparison of various conditions
- Characterized via **phagocytosis measures:**

PHAGOCYTOSIS RATIO
VIEWPOINT OF CONIDIA

$$\varphi_p = \frac{C_p}{C_p + C_a} \in [0,1]$$ [1]

UPTAKE RATIO
VIEWPOINT OF MACROPHAGES

$$\varphi_u = \frac{M_p}{M} \in [0,1]$$ [2]

PHAGOCYTIC INDEX

$$\varphi_i = \frac{C_p}{M} \cdot \frac{M_p}{M} = \frac{C_p}{M} \cdot \varphi_u \in [0, MOI]$$ [2]

SYMMETRIZED PHAGOCYTIC INDEX

$$\varphi_s = \frac{C_p}{C_p + C_a} \cdot \frac{M_p}{M} = \varphi_p \cdot \varphi_u \in [0,1]$$ [3]

$C = C_a + C_p + C_{na}$	# conidia	C_{na}	# non-associated conidia
C_a	# associated conidia	M	# macrophages
C_p	# phagocytosed conidia	M_p	# phagocytosing macrophages

Limitations of Phagocytosis Measures

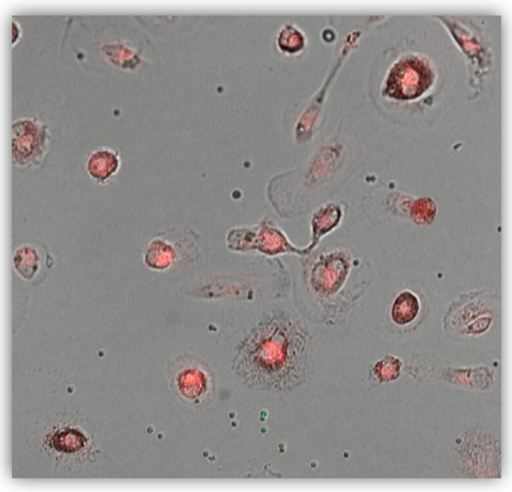
i. Do not provide microscopic parameters
ii. Are not unique
iii. Can give contradictory results

Goals of the Study

- Estimate microscopic parameters
- Resolve ambiguities of phagocytosis measures
- Assist in experimental design (e.g. determine the number of macrophages or images required)

Experimental Setup

- Co-incubation:
  Macrophages incubated with either *A. fumigatus* or *L. corymbifera* for one hour
- Multiplicity of infection: 1, 3 or 5
- Label:
  - Green fluorescent protein (GFP) for *Aspergillus fumigatus GnoA-eGFP*
  - Calcofluor-white (CFW) for *Lichtheimia corymbifera 9682*
  - CytoPainter DeepRed for macrophages



Microscopy

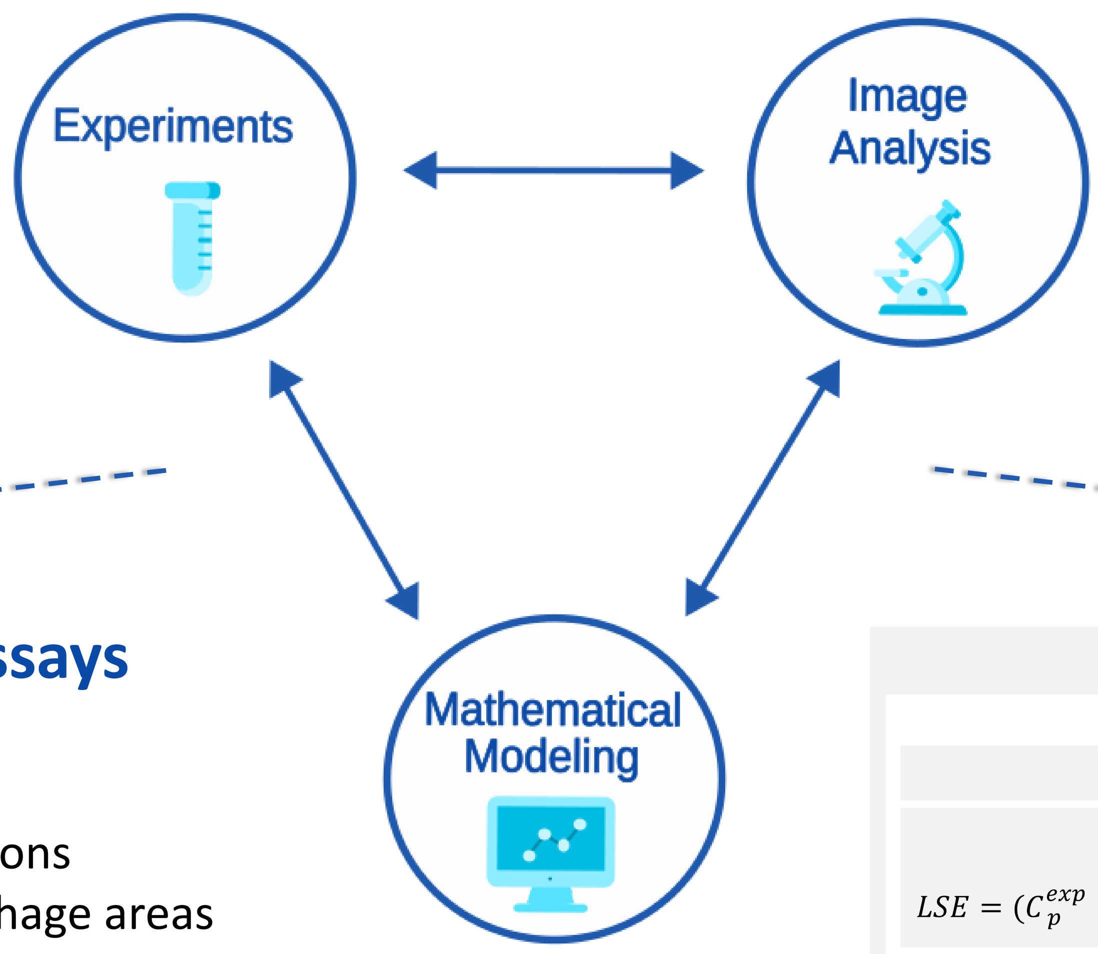
- Endpoint images and live-cell imaging with spinning disc confocal microscope

Analysis of Endpoint Images

- Segmentation: JIPipe [4] and CellPose [5]
- Cluster splitting: Watershed algorithm

Analysis of Live Cell Imaging

- Segmentation: JIPipe [4] and CellPose [5]
- Mask and segment spores and macrophages: Imaris [6]
  - Classification of spores to distinguish associated, non-associated, and phagocytosed conidia
- Tracking: TrackMate [7]
  - Quantify observed area of macrophages



Virtual Phagocytosis Assays

Input

- Quantifications, e.g. cell counts and size distributions
- Binary macrophage images with observed macrophage areas
- Multiplicity of infection

Simulations and Parameter Estimation

- Monte-carlo simulations
- Individual-based model
- Event rates = absolute process measures
- Conidia uniformly distributed using multiplicity of infection from experiments

Output

- Optimal parameter set of absolute process measures φ, α, η and ω

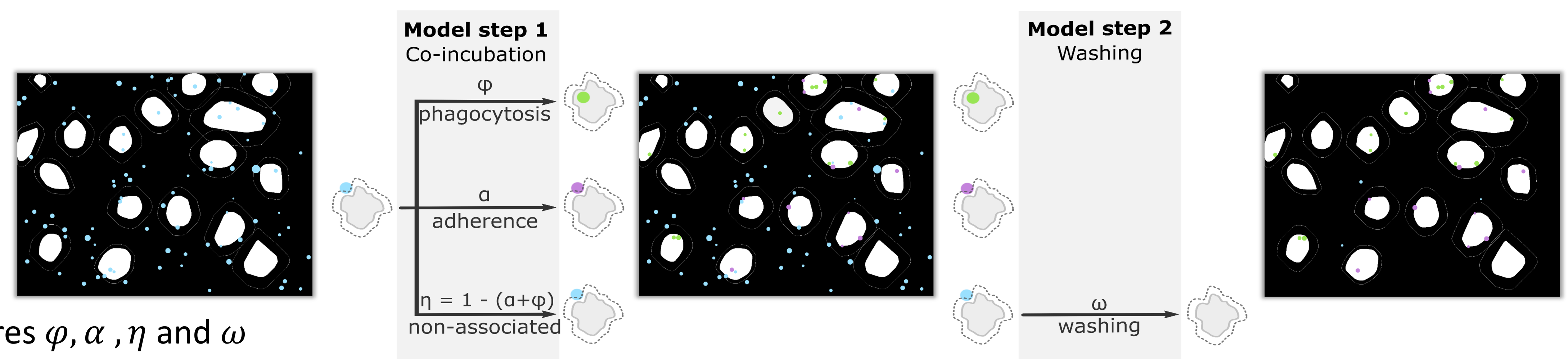
Parameter Estimation

For each parameter set $(\varphi, \alpha, \omega) \in (0,1)$
Perform n simulations for each image i
Calculate Least-Squared-Error (LSE)

$$LSE = (C_p^{exp} - C_p^{sim})^2 + (C_a^{exp} - C_a^{sim})^2 + (C_{na}^{exp} - C_{na}^{sim})^2 + (M_p^{exp} - M_p^{sim})^2$$

Optimal parameter set for each image
$P_i^{opt} = (\varphi, \alpha, \omega)$

Calculate average parameter set over all P_i^{opt}



Framework Allows to

- Estimate absolute process measures to resolve ambiguities of phagocytosis measures
- Perform and generate *in silico* experiments
- Assist in experimental design

Outlook

- Complete analysis with newly performed experiments
- Assist in experimental design, e.g. for providing the required number of images
- Investigate the macrophage phagocytosing behaviour, e.g. saturation or activation of macrophages

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References
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