Virtual Phagocytosis Assays –
From Observations via Quantifications to Mechanisms

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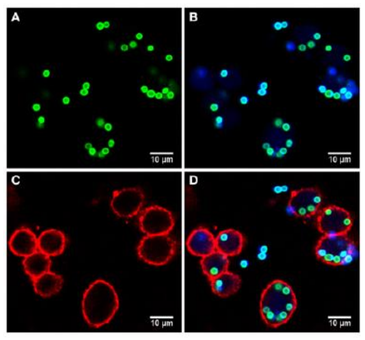
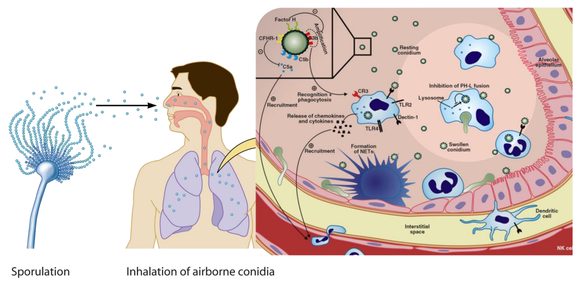
Phagocytosis Assays

- Commonly used in infection research
- Comparison of various conditions like:
  - Capability of immune cells to phagocytose different strains/mutants
  - Efficiency of vaccines
  - Various factors that activate immune cells
- Characterization via phagocytosis measures



Example: *Aspergillus fumigatus* Lung Infections



- Ubiquitous human-pathogenic fungus
- Small spores (conidia) distributed via the air:
  - Inhalation of several hundred per day
  - Phagocytosis by alveolar macrophages (AM)
- Immunocompromised patients:
  - Severe infections like aspergillosis
  - Mortality rates: 30 - 90%

Phagocytosis Measures: Relative Endpoint Measures

Phagocytosis ratio
Viewpoint of conidia

$$\varphi_p = \frac{N_c^{phag}}{N_c^{phag} + N_c^{adh}} \in [0,1]$$

Mech *et al. PLOS ONE* 2011

Uptake ratio
Viewpoint of macrophages

$$\varphi_u = \frac{N_m^{phag}}{N_m} \in [0,1]$$

Sano *et al. J Clin Invest* 2003

Phagocytic index

$$\varphi_i = \frac{N_c^{phag}}{N_m} \times \varphi_u \in [0, \mathrm{MOI}]$$

Sano *et al. J Clin Invest* 2003

Symmetrized phagocytic index

$$\varphi_s = \varphi_p \times \varphi_u \in [0,1]$$

Kraibooj *et al. Front Microbiol* 2015

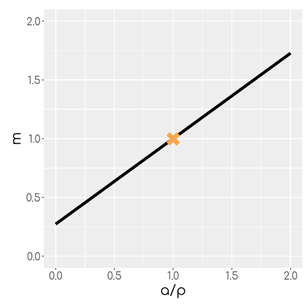
For 2 independent experiments E and E':

$$\varphi_s = \varphi_s' \implies \varphi_p \cdot \varphi_u = \varphi_p' \cdot \varphi_u'$$

$$\frac{N_c^{phag}}{N_c^{phag} + N_c^{adh}} \cdot \frac{N_m^{phag}}{N_m} = \frac{N_c^{phag}}{N_c^{phag} + \frac{a}{p} N_c^{adh}} \cdot \frac{m \cdot N_m^{phag}}{N_m}$$

$$m = \frac{N_c^{phag}}{N_c^{phag} + N_c^{adh}} \cdot \left(1 + \frac{a}{p} \frac{N_c^{adh}}{N_c^{phag}}\right)$$

For $N_c^{phag} = 30$ and $N_c^{adh} = 80$:



Limitations:

- No process measures
- Population-based measures
- Can give contradictory results
- Are not unique

N_m	# macrophages
N_m^{phag}	# phagocytosing macrophages
$N_c^{total} = N_c^{adh} + N_c^{phag}$	# macrophage-associated conidia
N_c^{adh}	# adherent conidia
N_c^{phag}	# phagocytosed conidia
N_c^{free}	# non-associated conidia

Microscopy & Image Analysis

Fluorescence microscopy:

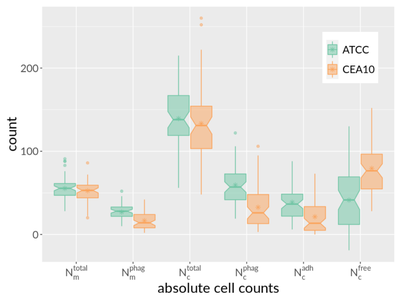
- Co-incubation: AM and *A. fumigatus* for 1 h
- Two *A. fumigatus* strains: ATCC, CEA10
- Differential staining

- **conidia:** FITC
- **macrophages:** anti-CD9 antibody
- **non-phagocytosed conidia** after incubation: calcofluor white staining

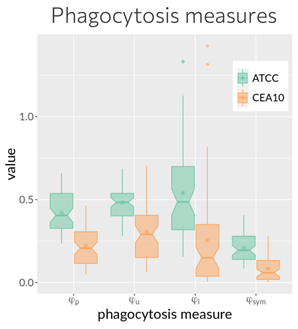
Analysis of endpoint images [1,2]

- Segmentation using CellPose [3]
- Cluster splitting: Watershed algorithm
- Classification based on morphology and color

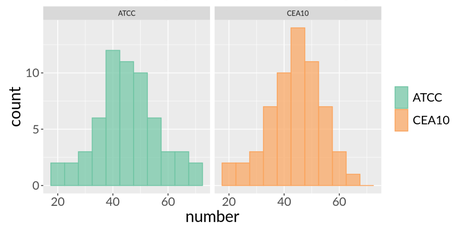
Absolute cell counts



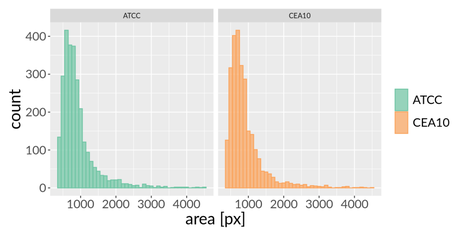
Phagocytosis measures



Macrophage number per image

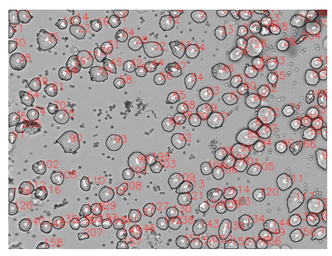


Macrophage area

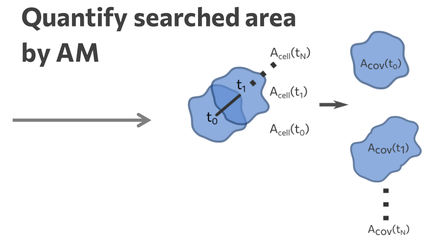


Analysis of live cell imaging

- Segmentation using CellPose [3]
- Tracking using AMIT [4]



Quantify searched area by AM



Virtual Phagocytosis Assays (VPA): Absolute Process Measures

C++ simulation framework CellRain:

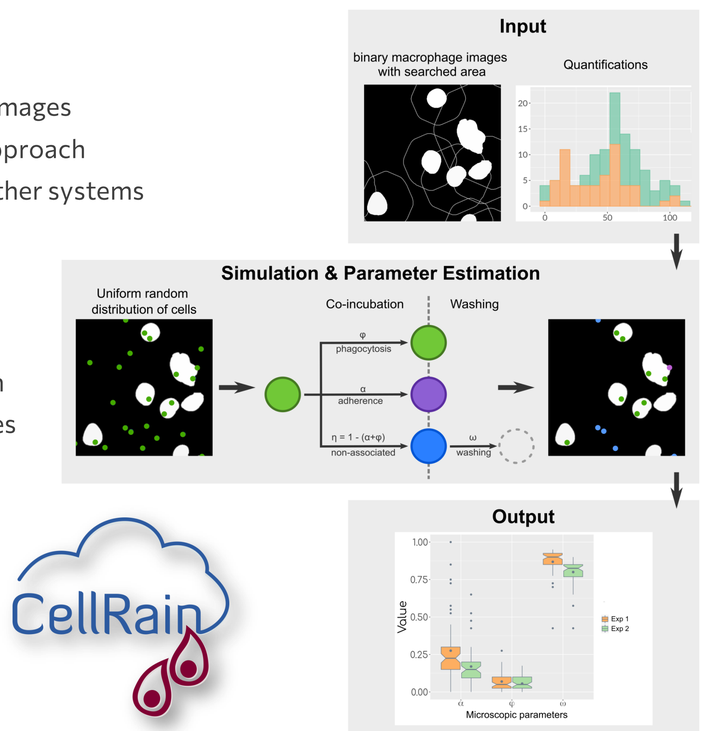
- Monte-Carlo simulations on endpoint images
- Parameter estimation via grid-based approach
- Generalized framework adaptable to other systems

Monte-Carlo simulations:

- Individual-based model
- Events modelled in a rule-based fashion
- Event rates = absolute process measures

Simulation framework allows to:

- Estimate absolute process measures
- Perform *in silico* experiments
- Generate artificial images

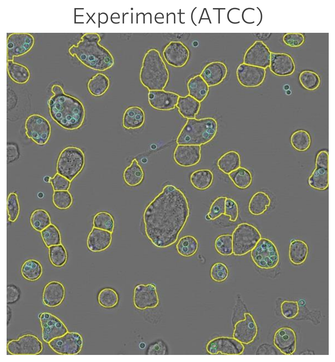


Aims of the study:

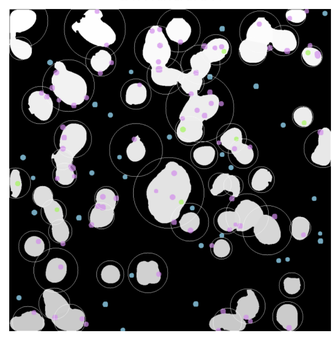
1. **Estimate microscopic parameters** (*e.g.* phagocytosis probability)
2. **Resolve ambiguities of phagocytosis measures**
3. **Assist in experimental design** (*e.g.* number of images required)

Results: Estimation of absolute process measures

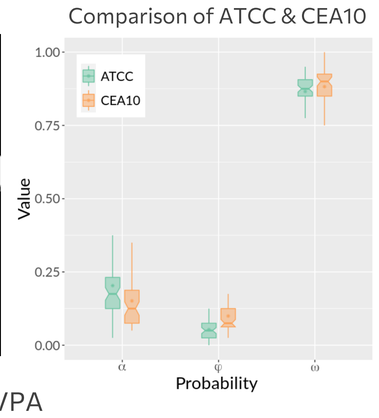
Experiment (ATCC)



Simulation



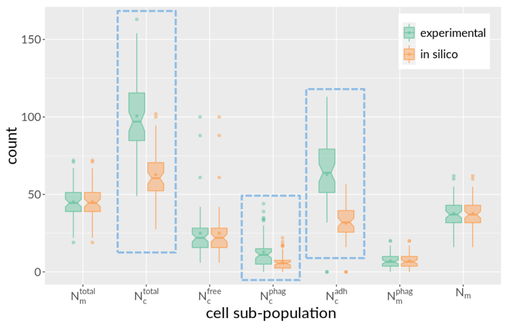
Comparison of ATCC & CEA10

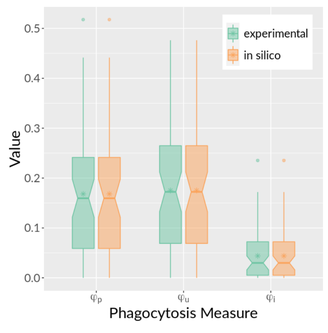


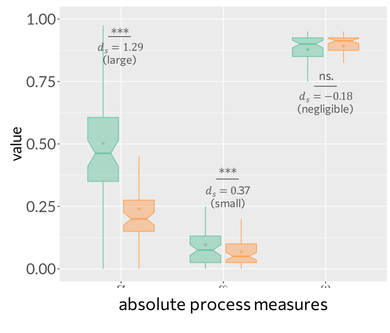
- Absolute process measures can be obtained from VPA

Results: Comparison of ATCC with *in silico* twin

- Generation of *in silico* data set with: $m = 1$ and $a = p = 0.5$
- Number of phagocytosed and adherent conidia different but equal phagocytosis measures
- VPA can identify significant differences in the absolute process measures







Outlook

- Augment macrophage images to:
  - Compare *in silico* twins with $m \neq 1$
  - Assist in experimental design: *e.g.* required number of images
- Apply to other pathogens/ immune cell types

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References
[1] Kraibooj *et al.* (2015) *Front Microbiol*
[2] Cseresnyes *et al.* (2018) *Cytometry A*
[3] Stringer *et al.* (2021) *Nat Methods*
[4] Belyaev, Praetorius *et al.* (2021) *Cytometry A*

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