

B4: Cause - Effect Relationships in Confrontation Assays with *C. glabrata* and Human Neutrophils using Bayesian Networks

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Introduction

Invasive fungal infections are emerging as a significant health risk for humans. The innate immune system is the first line of defence against invading microorganisms and involves the recruitment of polymorphonuclear neutrophils (PMNs), which engulf and kill pathogens, to the site of infection.

To gain a quantitative understanding of the interplay between phagocytes and fungal pathogens, live-cell imaging was applied to monitor the dynamic process of phagocytosis in time and space. We used our previously developed frame work AMIT (algorithm for migration and interaction tracking [1,2]) for the automated high-throughput analysis of multi-channel time-lapse microscopy videos of phagocyte-pathogen confrontation assays. Thereby, we are able to quantify phagocytosis, touching and interaction events between cells. We now aim to find cause - effect relationships between events using Bayesian networks to gain deeper insights into the chronology and dependence of events.

Overview of immune defence mechanisms against Candida. Adapted from [3]

Experiments/Data and Video Analysis

Confrontation Assays

- multi-channel time lapse microscopy
- 3 channels
- GFP-labeled PMNs
- GFP-labeled *C. glabrata*
- GFP-labeled dead cells
- 1 hour
- 360 frames/video (8 frames/min)
- tracking of PMNs and fungal cells separately

Automated Segmentation and Tracking

- segmentation of PMNs and fungal cells
- classification of cells
- touching PMNs (contact to fungal cells)
- overlapping PMNs (overlap with fungal cells)
- interacting PMNs

AMIT Algorithm

- segmentation of PMNs and fungal cells
- classification of cells
- touching PMNs (contact to fungal cells)
- overlapping PMNs (overlap with fungal cells)
- interacting PMNs

Quantitative Analysis

Track file for PMN with ID 66 for frames 149 to 161

Probability distributions for speed, touching and phagocytosis events

Bayesian Network

probabilistic graphical model represented by directed acyclic graph

- nodes: variables
- edges: directed edges
- directed edges: conditional dependencies
- missing edges: conditional independencies
- each node has probability distribution ($P(\cdot | \text{do}(\text{pa}))$) for every node

Example

- nodes:
 - speed (binary: high or low)
 - touching (binary: yes or no)
 - phagocytosis (binary: yes or no)
- edges:
 - speed is independent
 - touching affects speed and touching
 - phagocytosis is affected by touching and speed

Jointed probability distribution:

$$P(S, P, T) = P(S|P, T) \cdot P(T|L, P) \cdot P(P|S, T)$$

$$= P(S) \cdot P(T|S) \cdot P(P|T, S)$$

Conditional Probability Distributions

Probability for high speed depending on number of touchings, phagocytosis and current speed

Probability for high speed depending on number of touchings, phagocytosis and current speed

Probability for touching depending on number of touching events

There is a strong connection between speed and touching. Nodes P_{t+1} and T_{t+1} also carry information about the connections between the number of phagocytoses in the current frame and the next frame and also between speed and touching. There are almost never found higher p-values.

Resulting Network

Dynamic Bayesian Network

- variables for current and next time step frame
- A dependency graph
- time resolved
- temporally directed edges
- variables:
 - speed
 - touching
 - phagocytosis
- number of touchings in current and next frame $P_{t, t+1}$
- number of touchings in current and next frame $P_{t, t+1}$
- speed in current and next frame $P_{s, s+1}$
- adding of a touching event in next frame P_{t+1}

Find connections between variables among all possible connections

• pairwise mutual information between variables

• keep edges with $p < 0.005$

• calculations based on track data from 10 confrontation assay videos

References

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[3] Netea, MG, Joosten LA, van der Meer JW, Kullberg BJ. Nature Reviews Immunology (2015)

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FungiNet

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