

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

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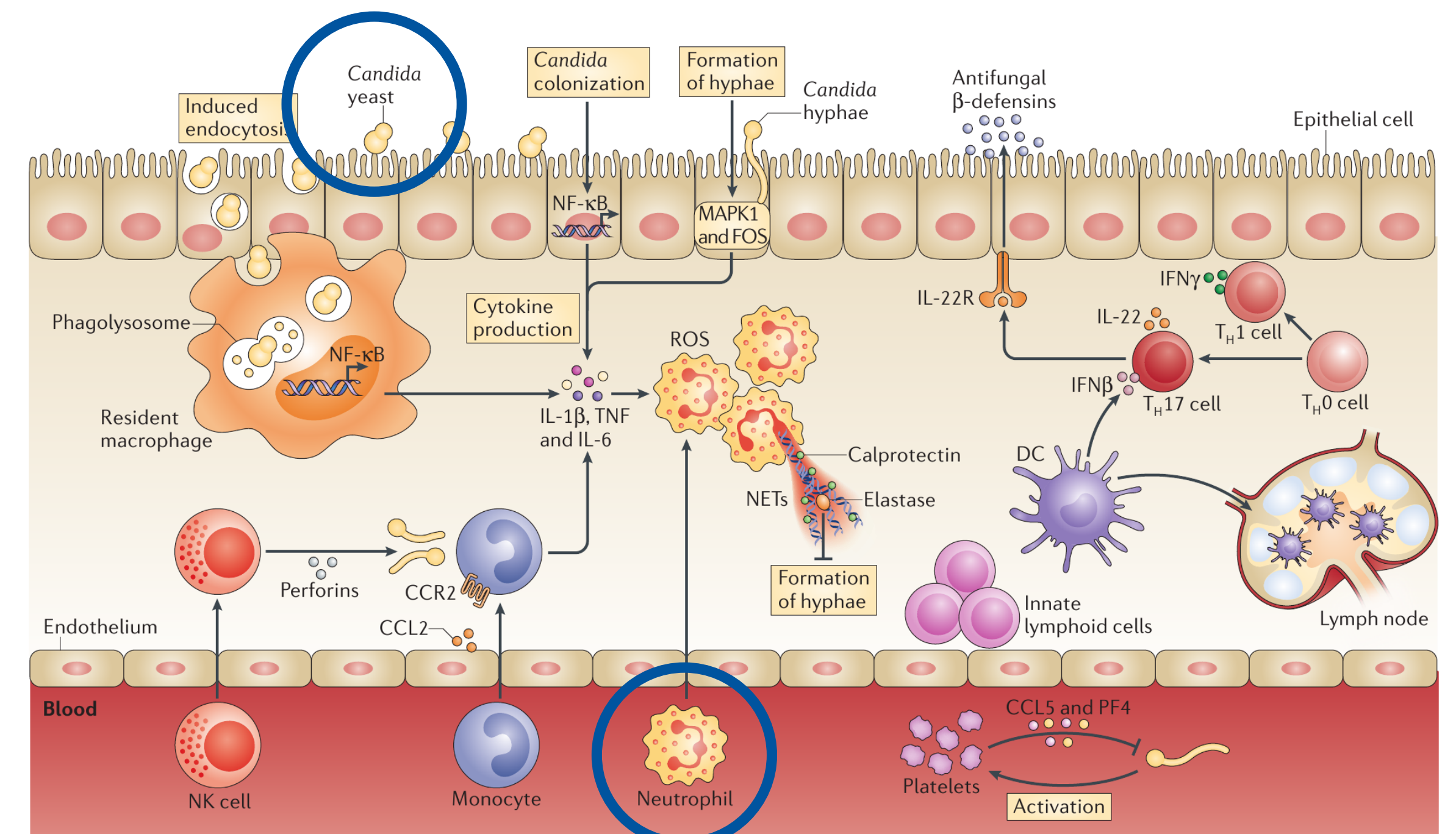
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Introduction

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 phagocytes like polymorphonuclear neutrophils (PMNs), which engulf and kill pathogens, to the site of infection.

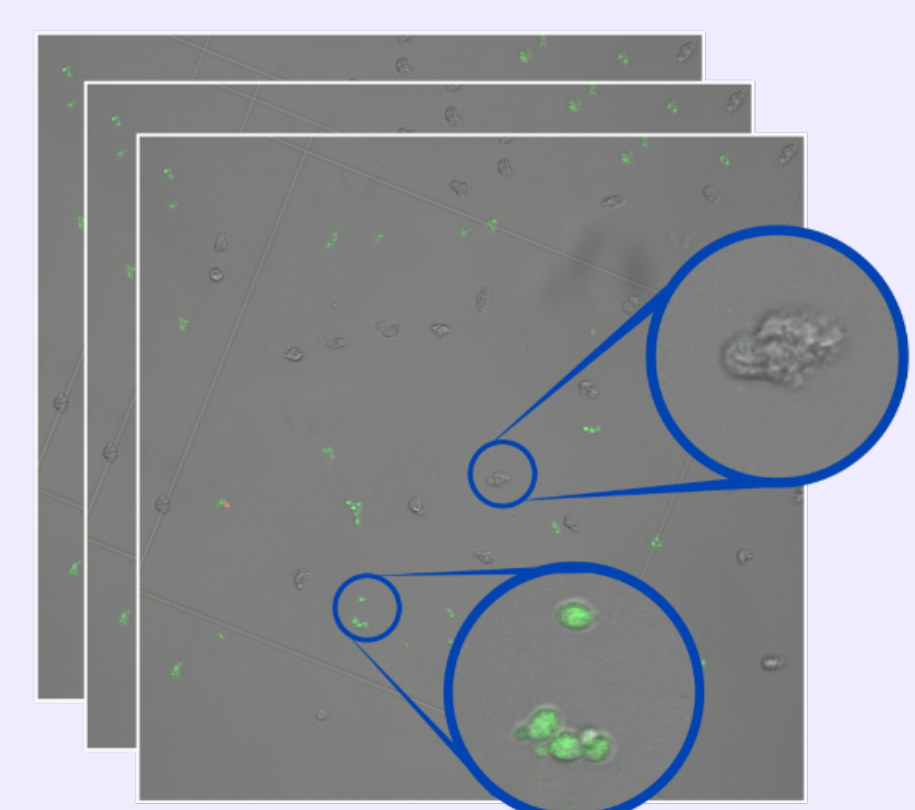
To gain a quantitative understanding of the interplay between phagocytes and fungal pathogens, live-cell imaging was applied to monitor the dynamic process of phagocytosis in time and space. We used our previously developed frame work AMIT (algorithm for migration and interaction tracking [1,2]) for 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]

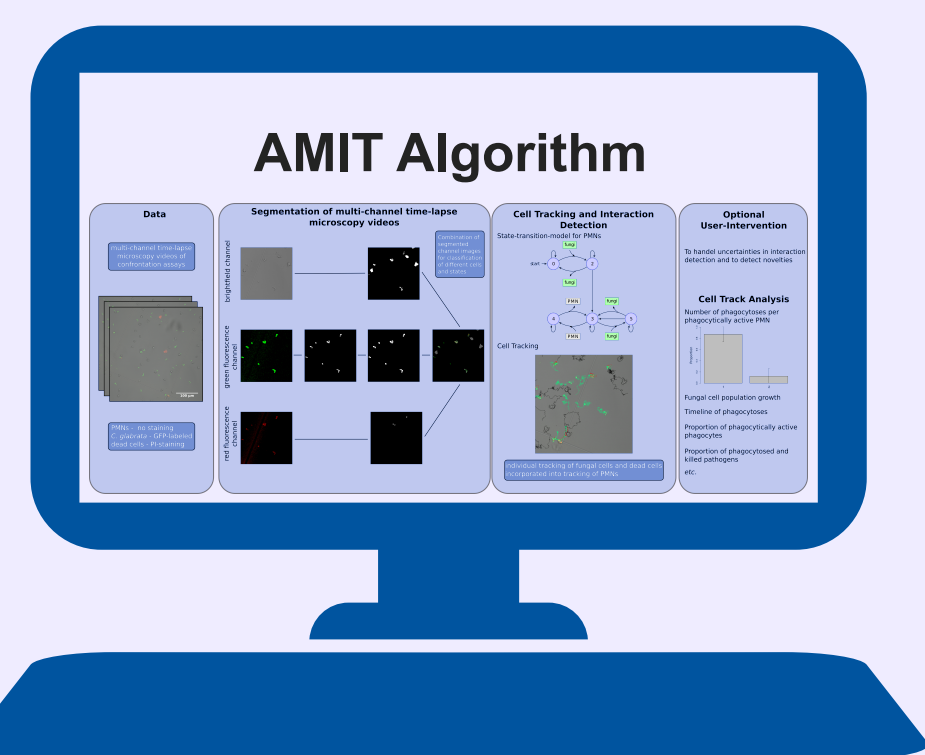
Experiments/Data and Video Analysis

Confrontation Assays



- multi-channel time lapse microscopy
- 3 channels:
 - unstained PMNs
 - GFP-labeled *C. glabrata*
 - PI-stained dead cells
- 1 hour
- 360 frames/video (6 frames/min)

Automated Segmentation and Tracking



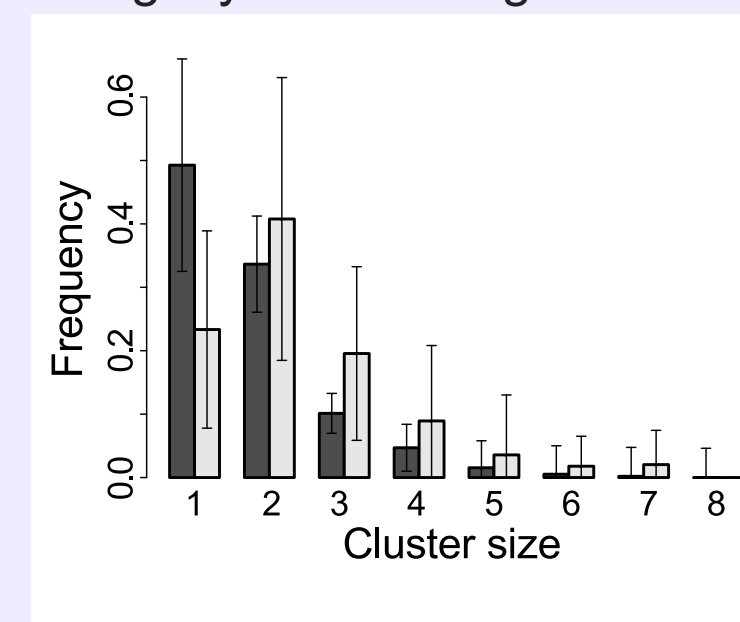
- segmentation of PMNs and fungal cells
- classification of cells:
 - free PMNs/fungal cells
 - touching PMNs (contact to fungal cells)
 - phagocytosing PMNs (overlap with fungal cells)
 - interacting PMNs
- tracking of PMNs and fungal cells separately

Quantitative Analysis

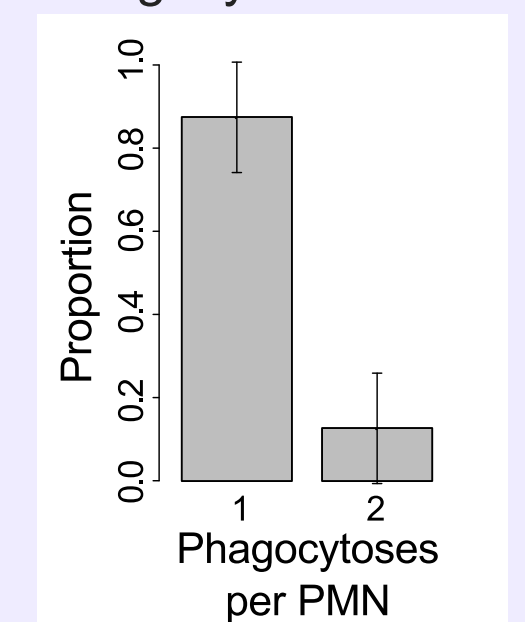
Track file for PMN with ID 66 for frames 149 to 161

frame	x	y	ID	A	S	nPh	Is	Fids	DFids	Phids
149	271.304	27.6576	66	1320	0	0	0	0	0	0
150	261.101	56.9184	66	1972	0	0	0	0	0	0
151	258.752	60.2615	66	1866	0	0	0	0	0	0
152	252.855	62.034	66	2116	0	0	0	0	0	0
153	246.056	71.8832	66	2004	0	0	0	0	0	0
154	239.56	61.2318	66	1773	0	0	0	0	0	0
155	227.428	55.508	66	1557	0	0	0	0	0	0
156	194.537	61.7262	66	3323	2	0	0	3	0	0
157	197.788	55.013	66	3841	2	0	0	3	0	0
158	194.054	50.9078	66	3188	2	0	0	3	0	0
159	196.397	53.9732	66	3439	2	0	0	3	0	0
160	191.358	58.841	66	3340	3	1	0	3	0	3
161	194.655	59.4663	66	3506	3	1	0	3	0	3

Phagocytosis of fungal clusters



Phagocytosis events



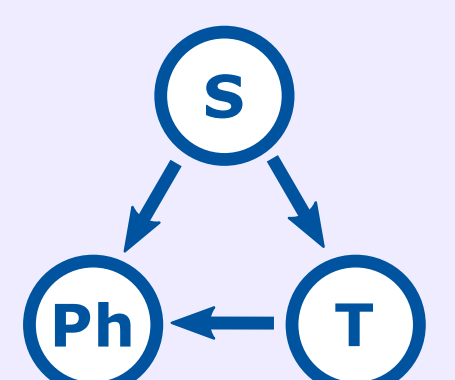
Bayesian Network

Bayesian Network

- probabilistic graphical model represented by directed acyclic graph
- **nodes:** variables under investigation
- **directed edges:** conditional dependencies
- **missing edges:** conditional independence
- **conditional probability distribution (CPD)** for every node

Example

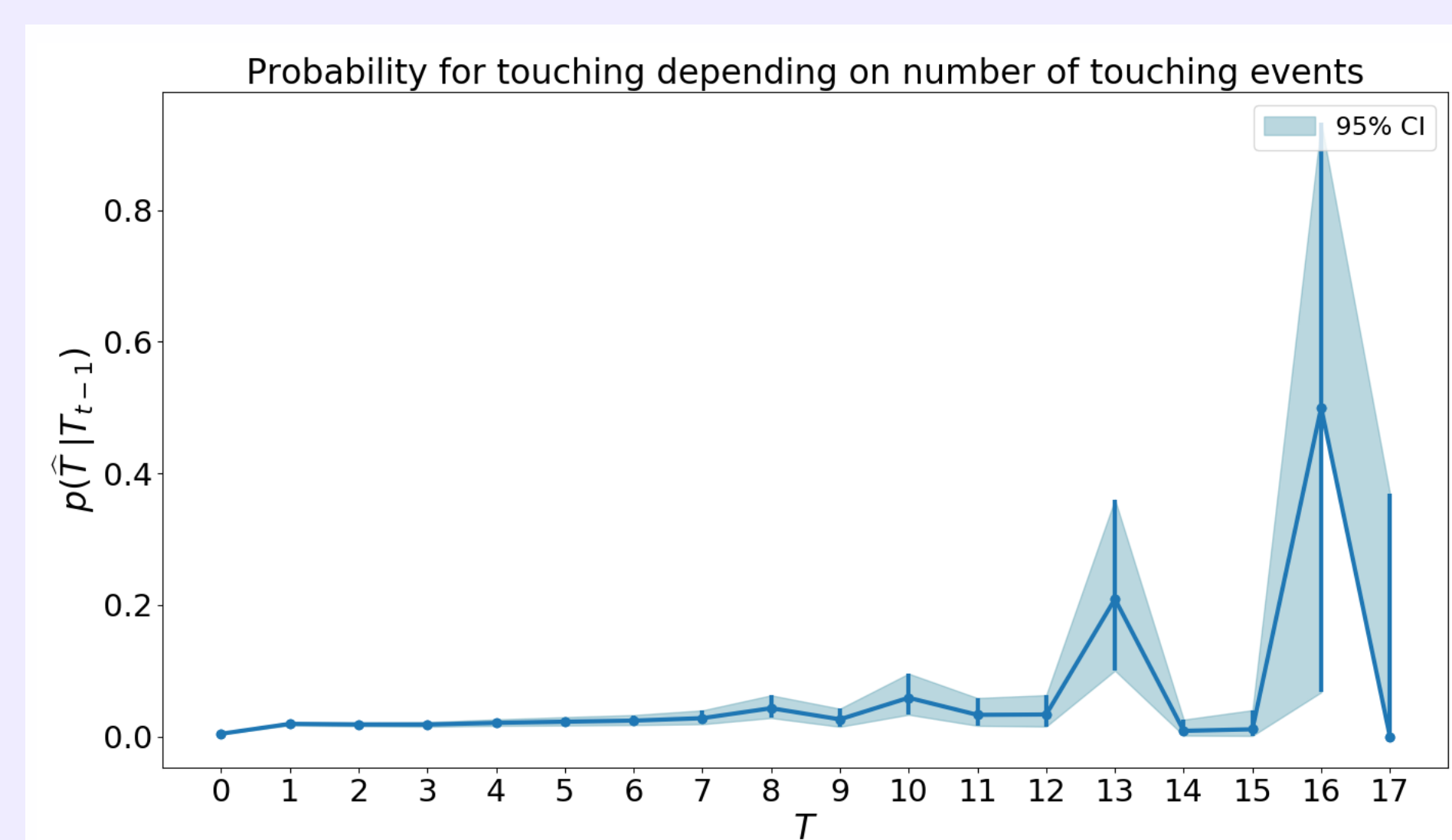
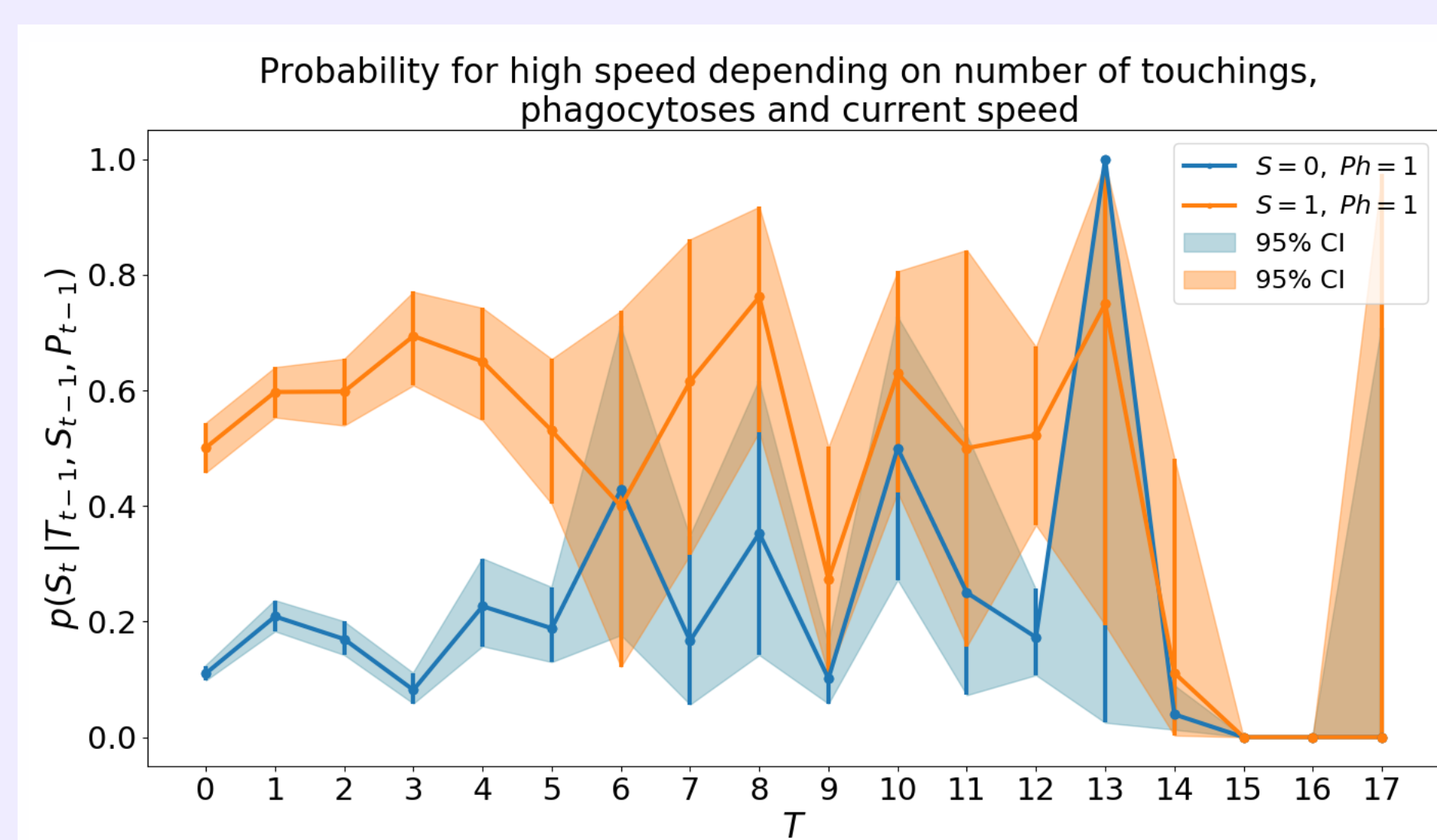
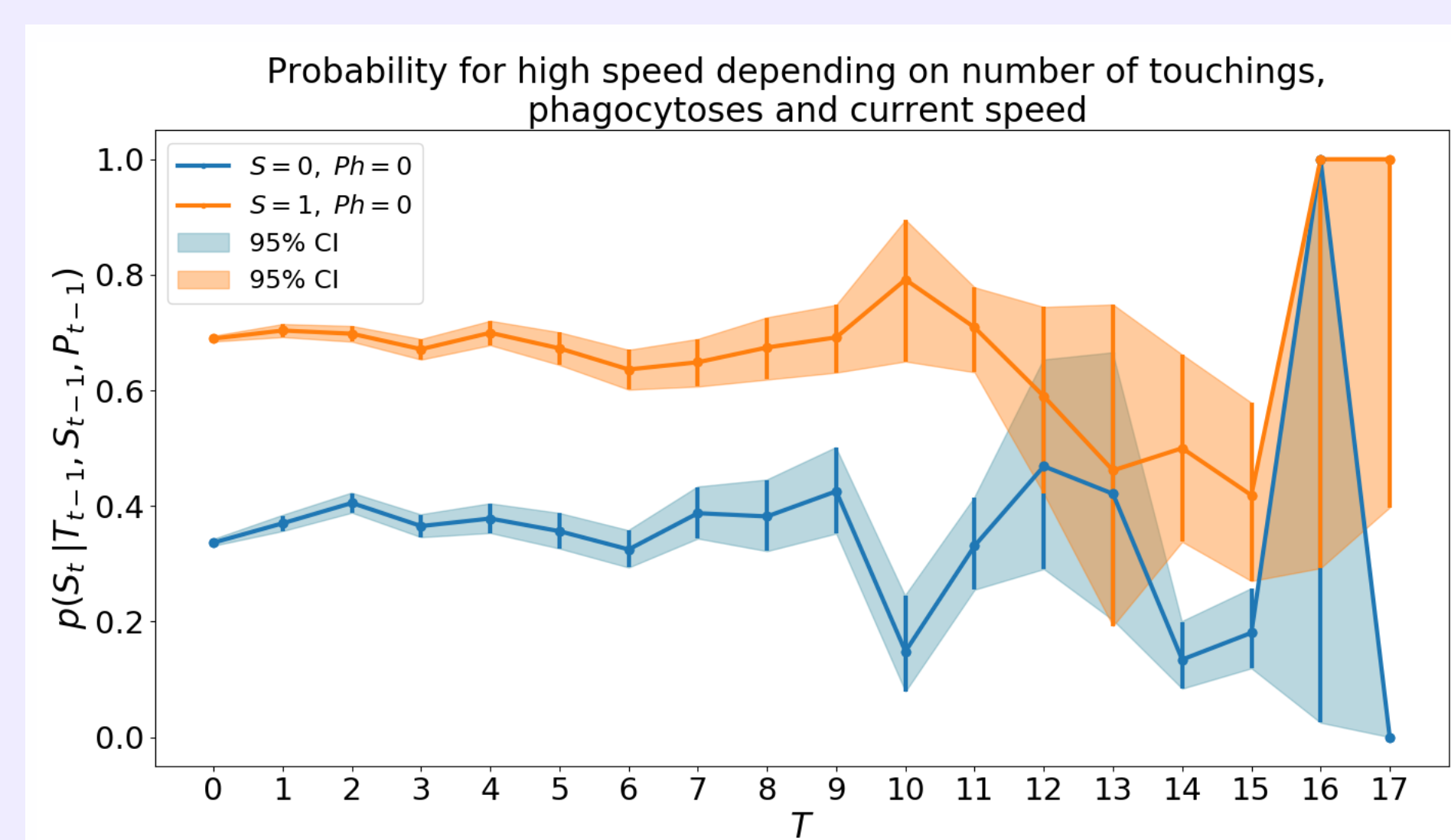
- **nodes:**
 - speed (binary: high or low)
 - phagocytosis (binary: yes or no)
 - touching (binary: yes or no)
- **edges:**
 - speed is independent
 - speed affects phagocytosis and touching
 - touching affects phagocytosis
 - phagocytosis is affected by touching and speed



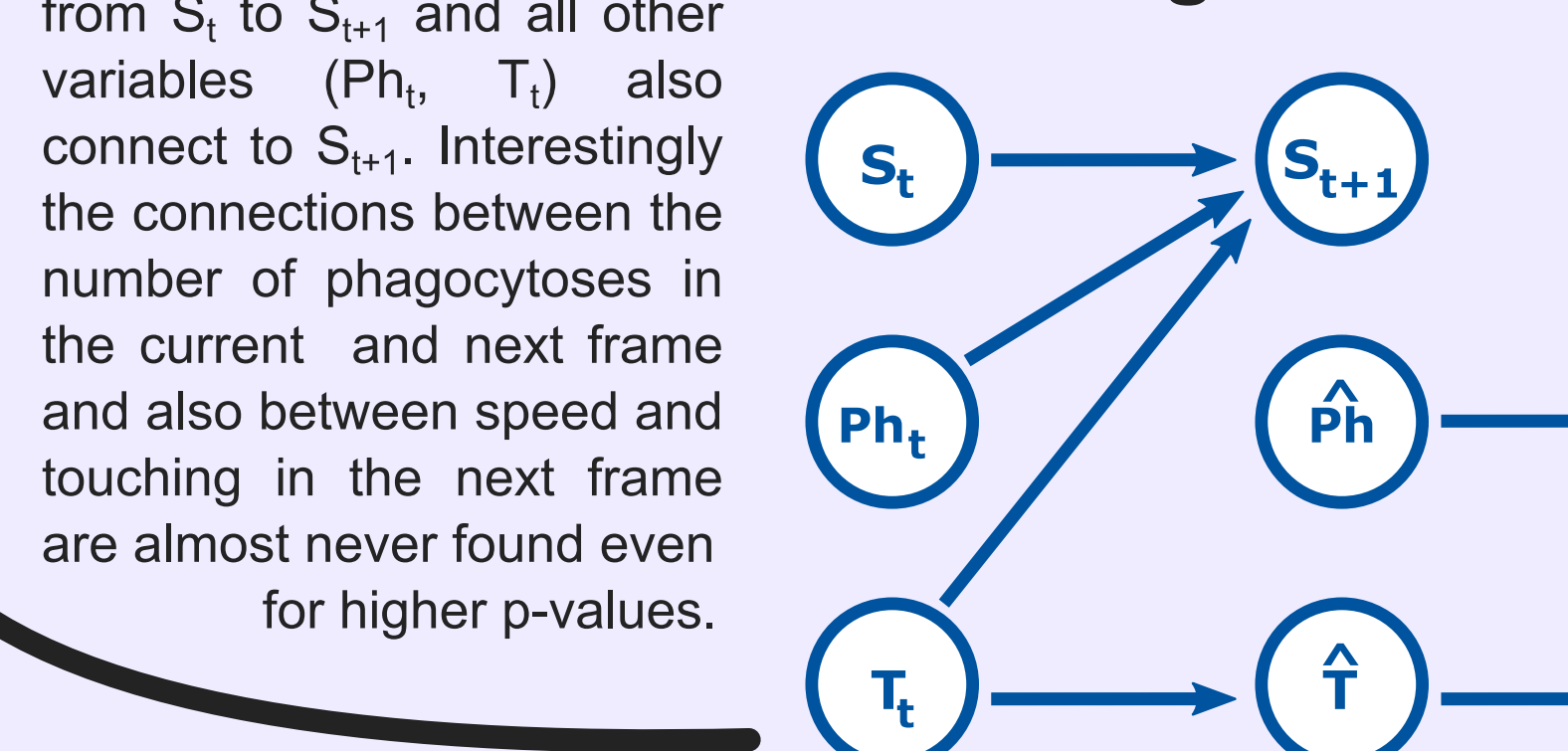
joined probability distribution:

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

Conditional Probability Distributions



Resulting Network

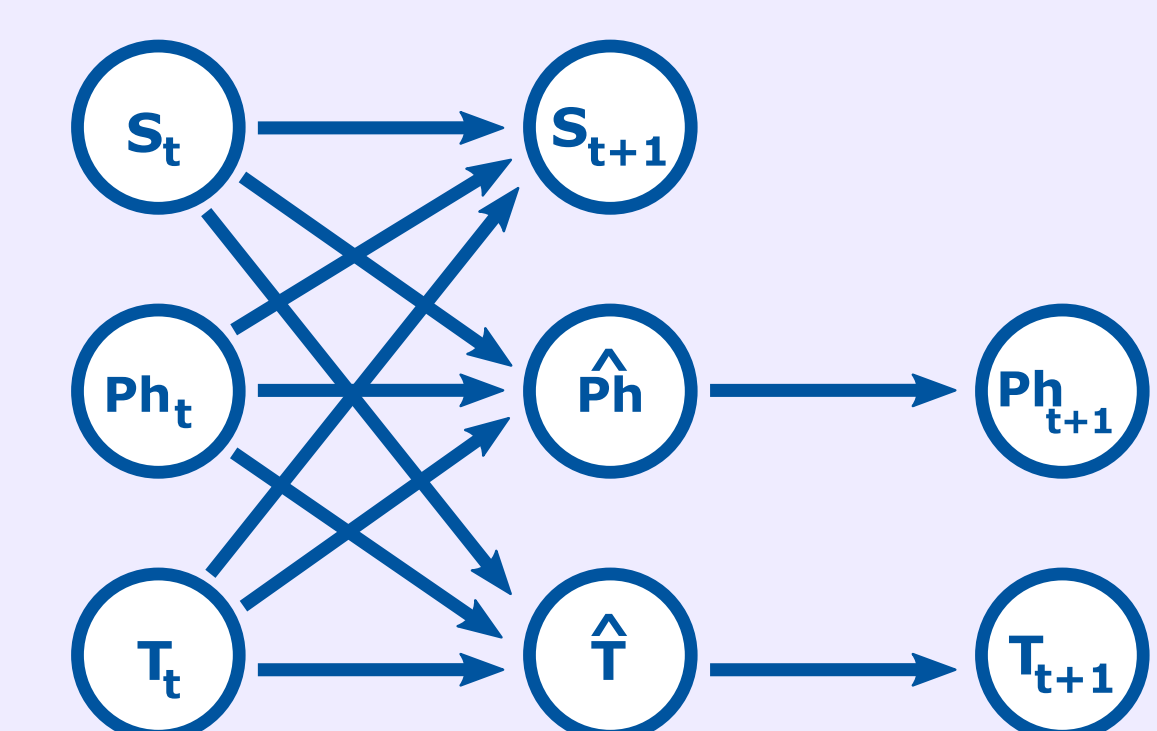


There is a strong connection from S_t to S_{t+1} and all other variables (Ph_t , T_t) also connect to S_{t+1} . Interestingly the connections between the number of phagocytoses in the current and next frame and also between speed and touching in the next frame are almost never found even for higher p-values.

Dynamic Bayesian Network

- variables for current and next time step/frame
- **Advantages:**
 - time resolved
 - automatically directed edges
- **variables:**
 - speed in current and next frame S_t, S_{t+1}
 - number of phagocytoses in current and next frame Ph_t, Ph_{t+1}
 - number of touchings in current and next frame T_t, T_{t+1}
 - adding of a phagocytosis event in next frame \hat{T}
 - adding of a touching event in next frame \hat{Ph}

find connections between variables among all possible connections



- pairwise mutual information between variables to find dependencies
- keep edges with a p-value > 0.005
- calculations based on track data from 10 confrontation assay videos

References

- [1] Brandes, S., Dietrich, S., Hünigler, K., Kurzai, O., Figge, M.T. *Medical Image Analysis* (2017)
- [2] Brandes, S., Mokhtari, Z., Essig, F., Hünigler, K., Kurzai, O., Figge, M.T. *Medical Image Analysis* (2015)
- [3] Netea, M.G., Joosten, L.A., van der Meer, J.W., Kullberg, B.J., van der Veerdonk, F.L. *Nature Reviews Immunology* (2015)

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