

Modelling and Identification of Immune Cell Migration during the Inflammatory Response

PhD Viva (extended version)

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Outline

- 1 Background & Motivation
- 2 Environment inference: homogeneous cell behaviour
- 3 Environment inference: heterogeneous cell behaviour
- 4 Estimating cell morphodynamics
- 5 Conclusion



- The process of inflammation is driven by rapid migration of neutrophils towards and away from the infectious area.
- Chemotaxis - directed migration in response to chemical attractants
 - is the dominant mechanism of neutrophil recruitment.
- Neutrophil reverse migration hypotheses: driven away by repellents vs. random redistribution.
- Cell locomotion is linked to gradient sensing by changes in subcellular concentrations of various messengers.
- Majority of therapeutic treatments of inflammation rely on manipulating cells' presentation of their environment.

Mathematical modelling is a standard method for characterisation of the experimental data.



General problem: abundance of mathematical models without predictive capability.

Specific gaps in literature:

- Chemoattractant concentrations must be inferred from *in vivo* experiments.
- Random walk models do not provide insight into global environment influence.
- No studies contemplating the possibility heterogeneous cell behaviour during the resolution stage.
- PIP₃ is often assumed as the primary subcellular regulator without experimental evidence.

Common concept:
Complicated model → Realistic simulations.



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Systematic approach:

Simplified models → Linking to data → Meaningful predictions



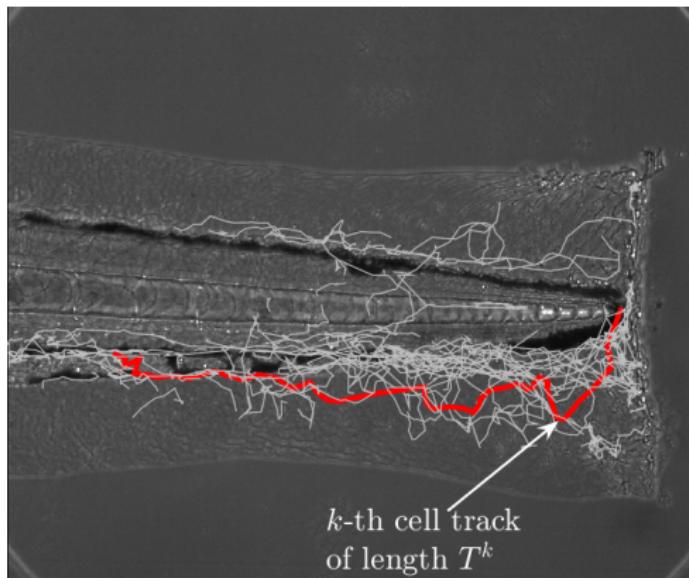
Objectives:

- Develop a dynamical model that describes cell interaction with the acting environment.
- Data-driven estimation of global chemoattractant concentration field and cell behavioural modes.
- Parameter estimation of neutrophil morphodynamics model.

Restrictions:

- Minimise prior assumptions about the shape of the environment.
- Minimise prior assumptions about the subcellular regulators.
- Ensure identifiability of models by keeping the number of unknown parameters low.



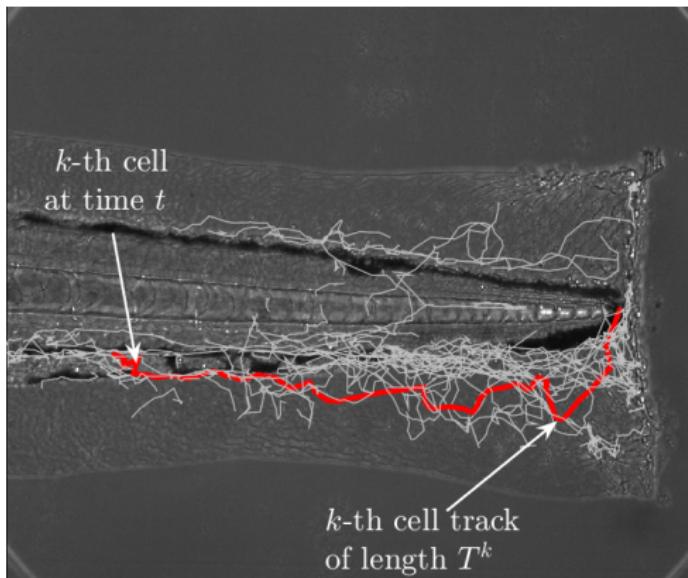


Time series data:

- K tracks: $\mathcal{Y} = \{\mathbf{y}^k\}_{k=1}^K$
- Single track:
 $\mathbf{y}^k = \{y_t^k\}_{t=1}^{T^k}$
- Single data point:
 $y_t^k = [\bar{s}_x, \bar{s}_y]^\top$
- Full state:
 $\mathbf{x}_t^k = [s_x, s_y, v_x, v_y]^\top$
- Environment influence:
 $u_t^k = u_t^k(s) = \nabla \mathcal{U}(s)$.

1. Develop a parametrised finite-order model of global $\mathcal{U}(s)$.
2. Estimate hidden global $\mathcal{U}(s)$ from localised data \mathcal{Y} .



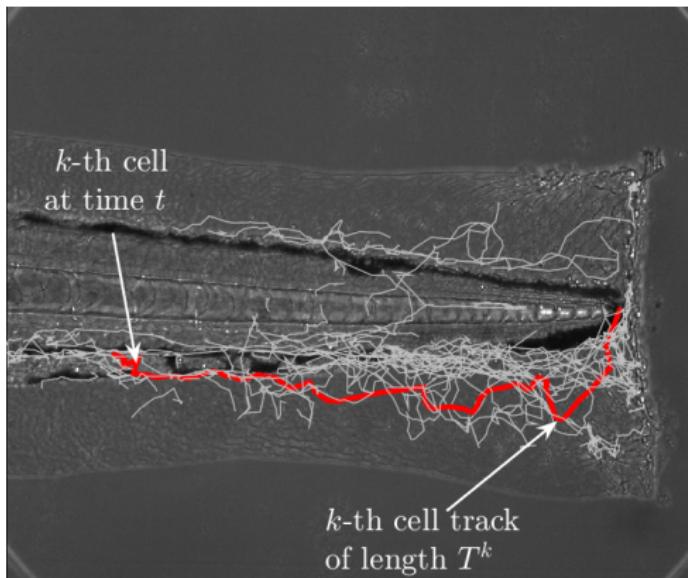


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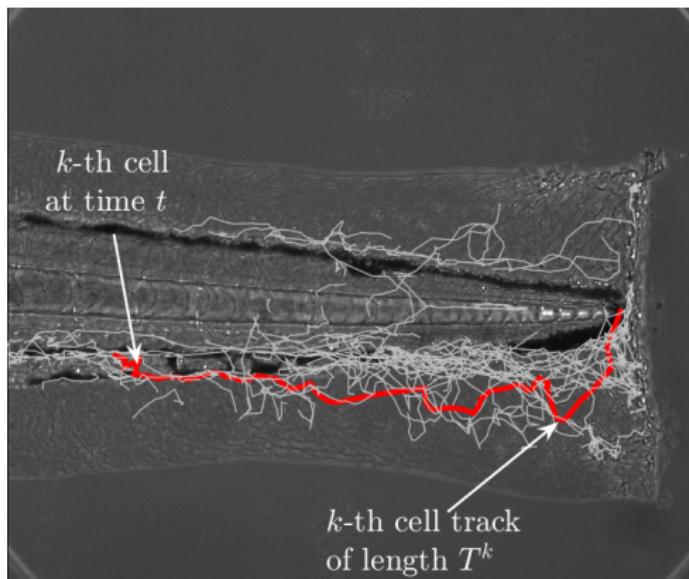


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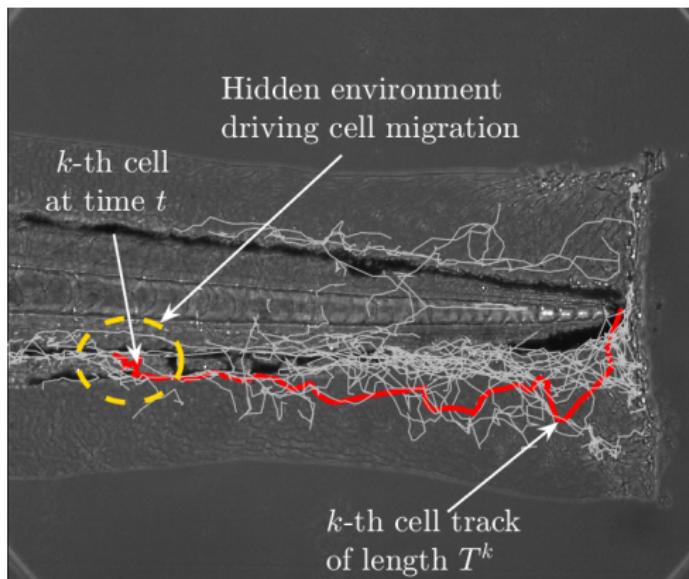


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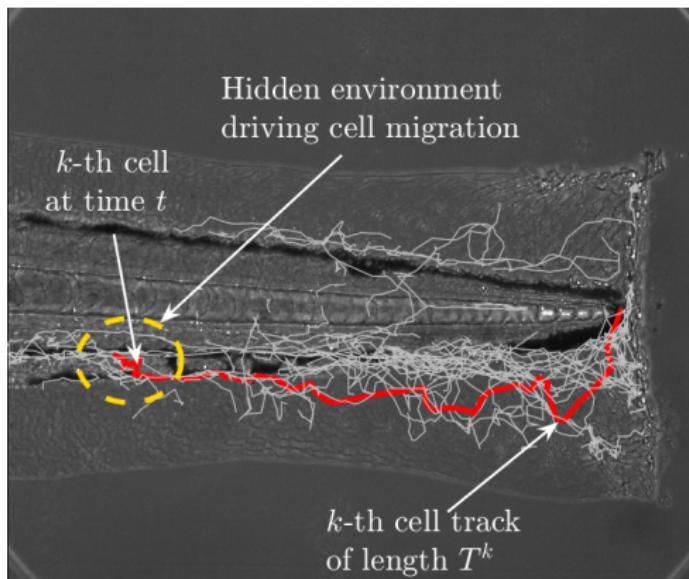


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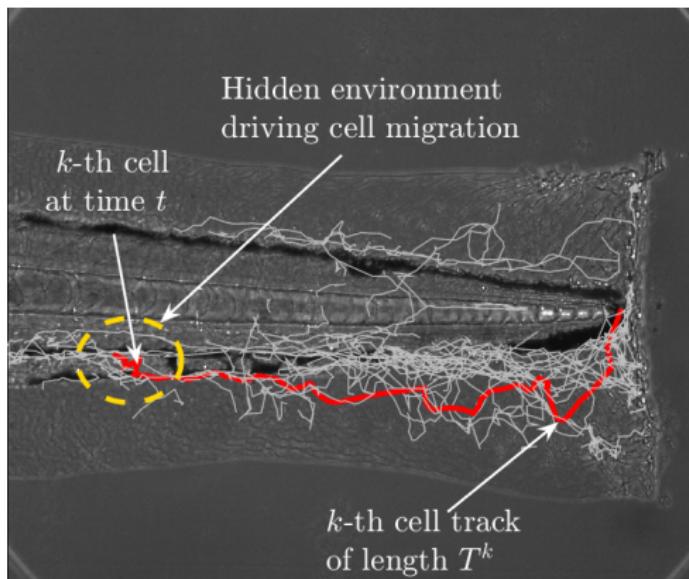


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Defining assumptions

- A migrating cell is moving as a massive Brownian particle:

$$\dot{v}(t) = -\rho v(t) + \sqrt{\sigma} \mathbf{W}(t).$$

- Each cell at each time is moving in response to the acting environment:

$$\dot{v}(t) = -\rho v(t) + \sqrt{\sigma} \mathbf{W}(t) + \psi(t).$$

- Hidden chemoattractant environment is acting on cells as a potential field:

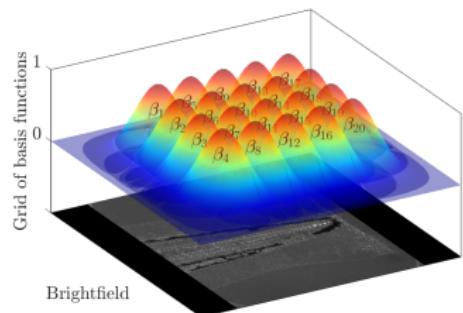
$$\dot{v}(t) = -\rho v(t) + \sqrt{\sigma} \mathbf{W}(t) + \nabla \mathcal{U}(s(t)).$$

- Hidden chemoattractant environment is time-invariant:

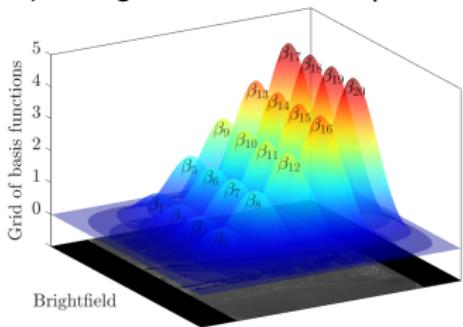
$$\mathcal{U}(t) = \text{const.}$$



Decomposition of the environment



a) 5x4 grid of tensor B-splines



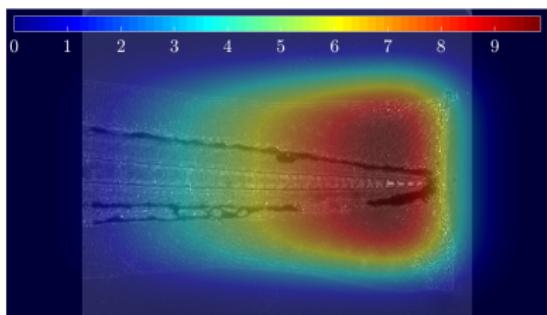
b) θ_h defines magnitude of $\beta_h(s_x, s_y)$

$$\mathcal{U}(s_x, s_y) = \mathcal{B}\Theta = \sum_{h=1}^{N_b} \beta_h(s_x, s_y)\theta_h,$$

$$\Theta = [\theta_1, \dots, \theta_h, \dots, \theta_{N_b}]^\top,$$

$$\mathcal{B} = [\beta_1, \dots, \beta_h, \dots, \beta_{N_b}],$$

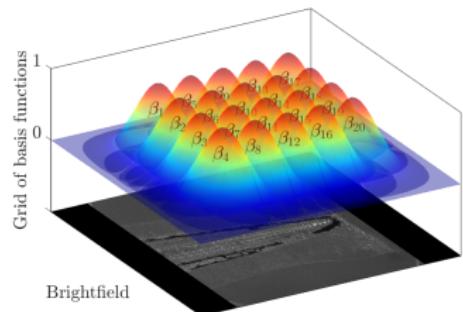
$$\beta_h(s_x, s_y) = \beta_l^4(s_x)\beta_m^4(s_y).$$



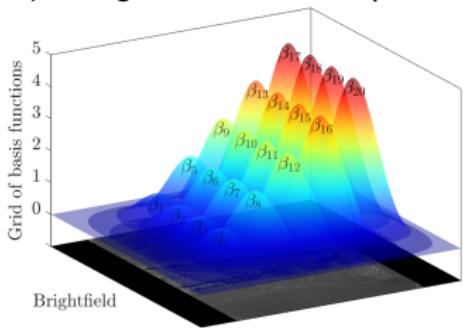
Example of the resultant field.



Decomposition of the environment



a) 5x4 grid of tensor B-splines



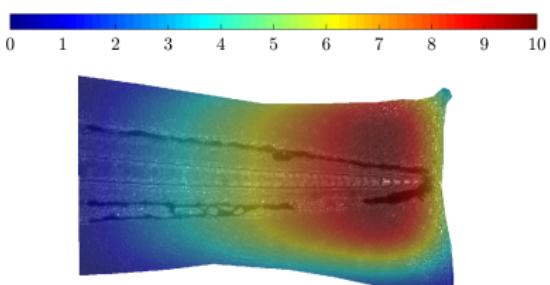
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$$\beta_h(s_x, s_y) = \beta_l^4(s_x)\beta_m^4(s_y).$$



Example of the resultant field.



Model of neutrophil dynamics

Discrete time SSM of the k-th cell :

$$\boldsymbol{x}_t^k = A\boldsymbol{x}_{t-1}^k + B\phi_{t-1}^k(s_x, s_y)\Theta + G\boldsymbol{w}_{t-1}^k, \quad \boldsymbol{w}_t^k \sim \mathcal{N}(0, Q)$$

$$\boldsymbol{y}_t^k = C\boldsymbol{x}_t^k + \boldsymbol{v}_t^k, \quad \boldsymbol{v}_t^k \sim \mathcal{N}(0, R)$$

where

$$\phi_t^k(s_x, s_y) = \nabla \mathcal{B}(s_x, s_y) = \begin{bmatrix} \frac{\partial \beta_1(s_x, s_y)}{\partial s_x} & \dots & \frac{\partial \beta_h(s_x, s_y)}{\partial s_x} & \dots & \frac{\partial \beta_{N_b}(s_x, s_y)}{\partial s_x} \\ \frac{\partial \beta_1(s_x, s_y)}{\partial s_y} & \dots & \frac{\partial \beta_h(s_x, s_y)}{\partial s_y} & \dots & \frac{\partial \beta_{N_b}(s_x, s_y)}{\partial s_y} \end{bmatrix}.$$

$$A = \begin{bmatrix} \mathbb{I} & T\mathbb{I} \\ \mathbb{O} & (1 - T\rho)\mathbb{I} \end{bmatrix}; B = \begin{bmatrix} \mathbb{O} \\ T\mathbb{I} \end{bmatrix}; G = \begin{bmatrix} \mathbb{O} \\ T\mathbb{I} \end{bmatrix}; C = [\mathbb{I} \quad \mathbb{O}].$$

SSM is linear in Θ and non-linear in x .



Approximate EM framework

E-step:

$$\mathcal{Q}(\Theta, \hat{\Theta}^i) = \mathbb{E} [\log p(\Theta | \mathcal{Y})] = \mathbb{E} \left[\sum_{k=1}^K \sum_{t=1}^{T_k} \log p(\mathbf{x}_t^k | \mathbf{x}_{t-1}^k, \Theta) | \mathcal{Y}, \hat{\Theta}^i \right] + c.$$

$$p(\mathbf{x}_t^k | \mathbf{x}_{t-1}^k, \Theta) = \mathcal{N}\left((G)^\dagger \left\{ \mathbf{x}_t^k - A\mathbf{x}_{t-1}^k - B\phi(C\mathbf{x}_{t-1}^k)\Theta \right\}, \Sigma_w^{-1}\right).$$

Forecasting step:

$$\mathbf{s}_t^k = C\hat{\mathbf{x}}_{t|T^k}^k, \quad t = 1, \dots, T^k, k = 1, \dots, K.$$

$$\begin{aligned} p(\mathbf{x}_t^k | \mathbf{x}_{t-1}^k, \Theta) &\approx p(\mathbf{x}_t^k | \mathbf{x}_{t-1}^k, \mathbf{s}_{t-1}^k, \Theta) \\ &= \mathcal{N}\left((G)^\dagger \left\{ \mathbf{x}_t^k - A\mathbf{x}_{t-1}^k - B\phi(\mathbf{s}_{t-1}^k)\Theta \right\}, \Sigma_w\right). \end{aligned}$$

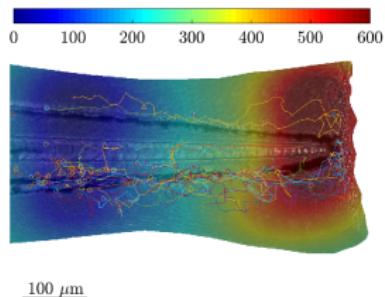
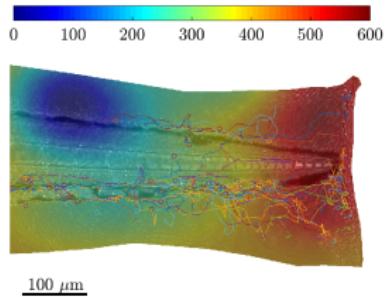
M-step:

$$\hat{\Theta}^{i+1} = \arg \max_{\Theta} \tilde{\mathcal{Q}}(\Theta, \hat{\Theta}^i).$$

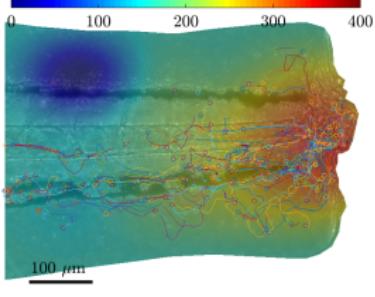
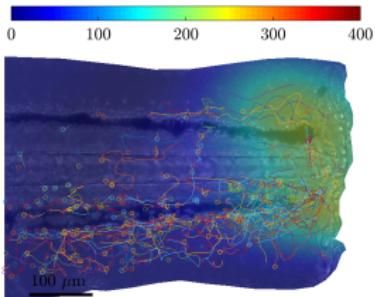
$${}^1\Sigma_w \triangleq \{(G)^\dagger\}^\top (Q_\omega)^{-1}(G)^\dagger$$



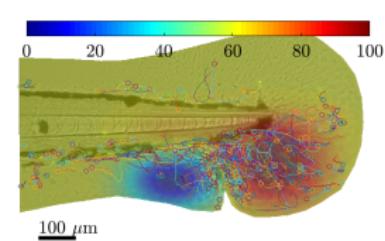
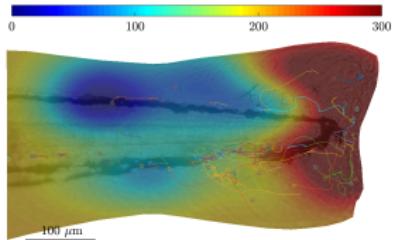
Inferred chemoattractant concentration



a) normal injury
(6 datasets)



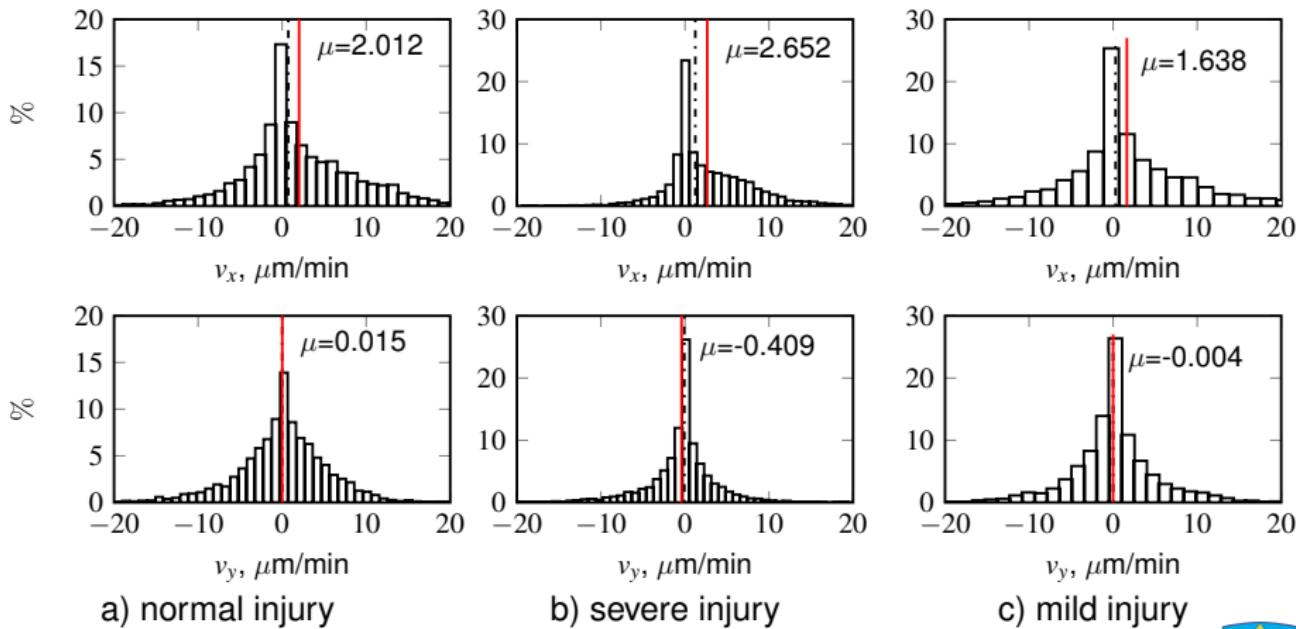
b) severe injury
(2 datasets)

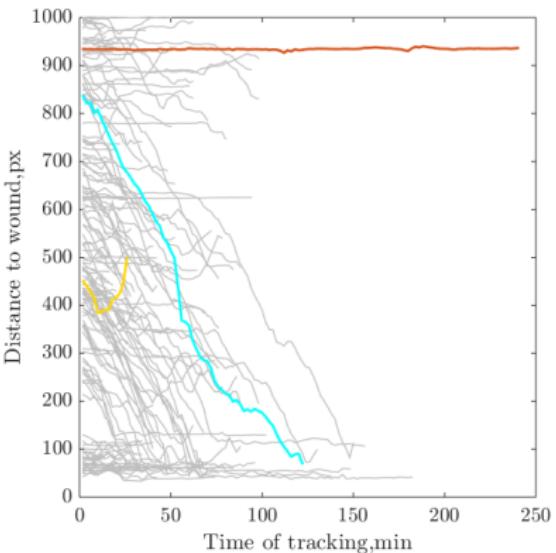
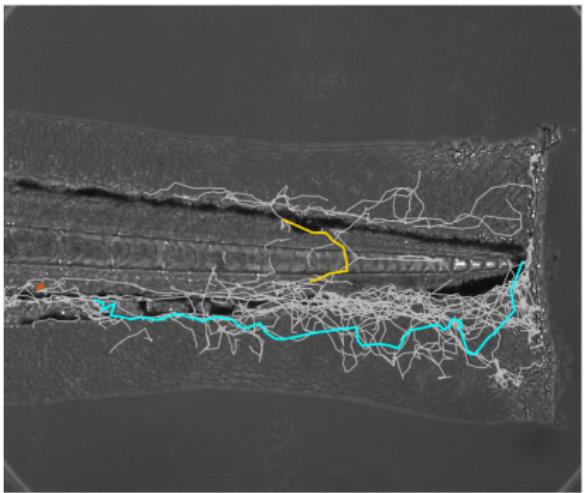


c) mild injury
(6 datasets)



Estimated cell velocities

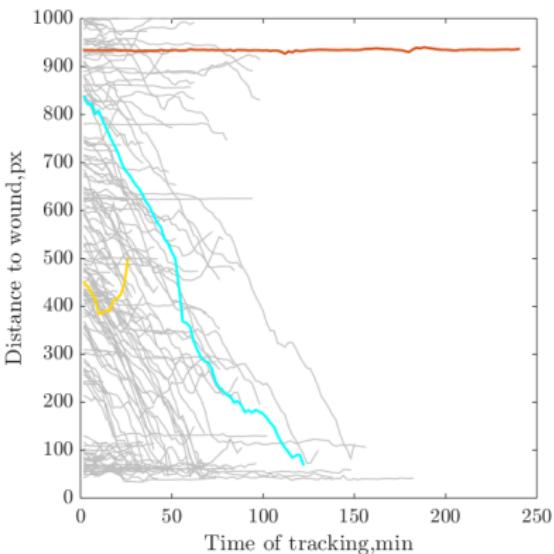
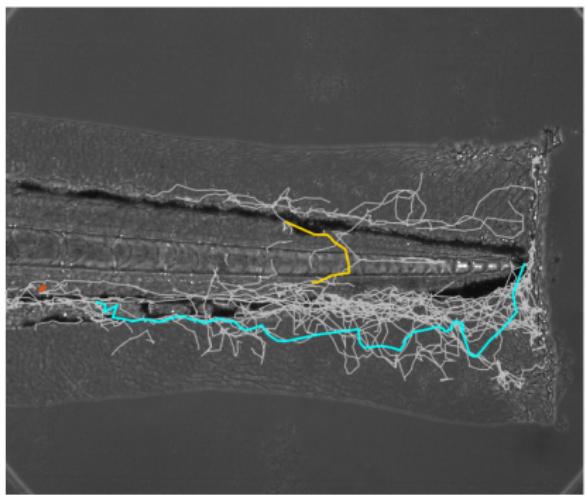




Neutrophil behaviour is not uniform.

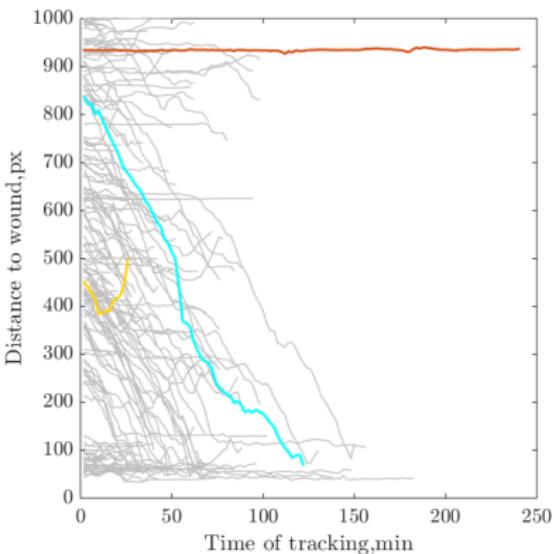
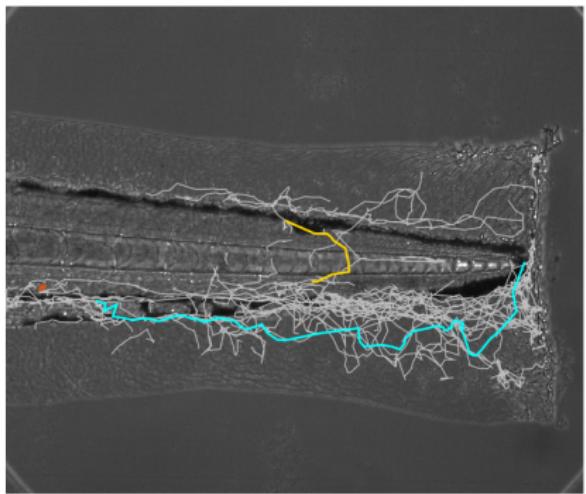
1. Does a cell at a given time interact with the environment $\mathcal{U}(s)$?
2. Estimate hidden global $\mathcal{U}(s)$ from the interaction with responsive cells.





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Defining assumptions (upd.)

Remain the same:

- Each migrating cell is moving as a massive Brownian particle.
- Hidden chemoattractant environment is acting on migrating cells as a potential field.
- Hidden chemoattractant environment is time-invariant.

Relaxed assumptions:

- Each migrating cell at any time can be in one of free modes: stationary, responsive or non-responsive.
- Switching between modes happens randomly.
- Each behavioural mode can be reached from any other mode.



Jump Markov system

$$\boldsymbol{x}_t^k = A(M^j)\boldsymbol{x}_{t-1}^k + B(M^j)\phi_{t-1}^k(s_x, s_y)\Theta + G(M^j)\boldsymbol{w}_{t-1}^k, \quad \boldsymbol{w}_t^k \sim \mathcal{N}(0, Q(M^j)).$$

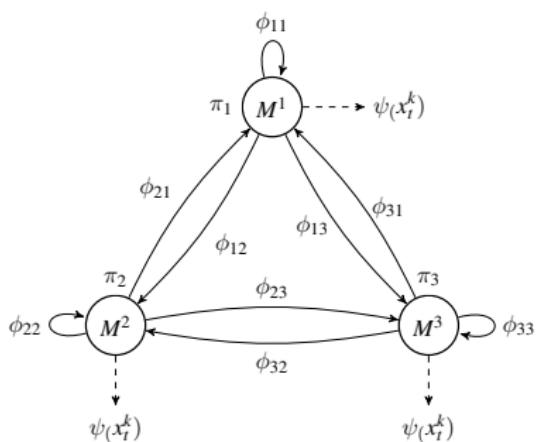
Cell modes:

$$M^1 : A = \begin{bmatrix} \mathbb{I} & T\mathbb{I} \\ \mathbb{O} & (1 - T\rho(M^1))\mathbb{I} \end{bmatrix} B = \begin{bmatrix} \mathbb{O} \\ T\mathbb{I} \end{bmatrix}$$

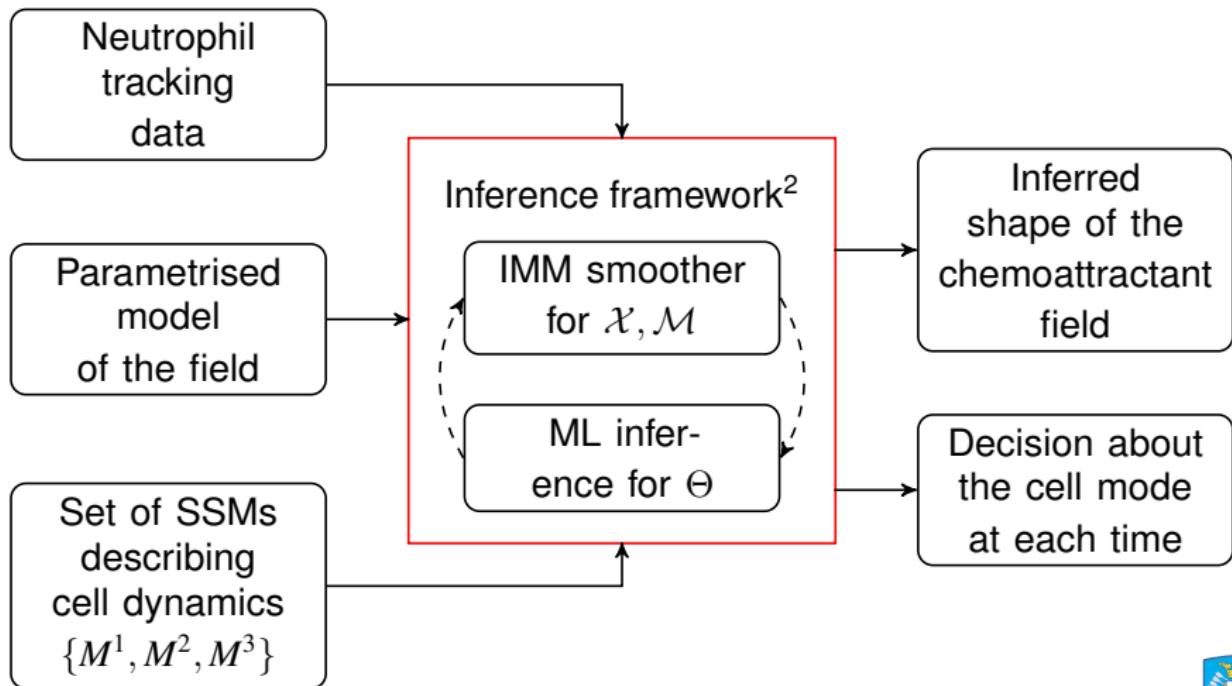
$$M^2 : A = \begin{bmatrix} \mathbb{I} & T\mathbb{I} \\ \mathbb{O} & (1 - T\rho(M^2))\mathbb{I} \end{bmatrix} B = \begin{bmatrix} \mathbb{O} \\ \mathbb{O} \end{bmatrix}$$

$$M^3 : A = \begin{bmatrix} \mathbb{I} & T\mathbb{I} \\ \mathbb{O} & (1 - T\rho(M^3))\mathbb{I} \end{bmatrix} B = \begin{bmatrix} \mathbb{O} \\ \mathbb{O} \end{bmatrix}$$

$$Q(M^3) \ll Q(M^1) < Q(M^2).$$



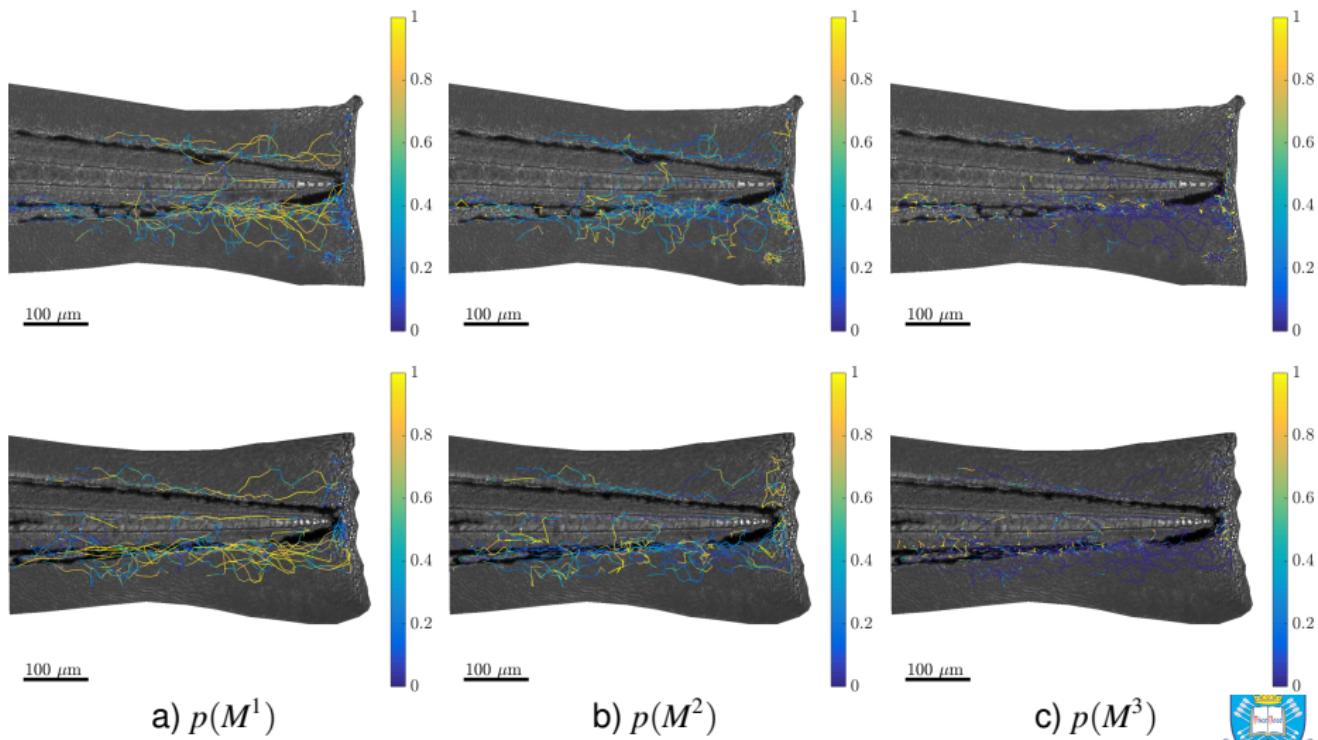
Inference framework



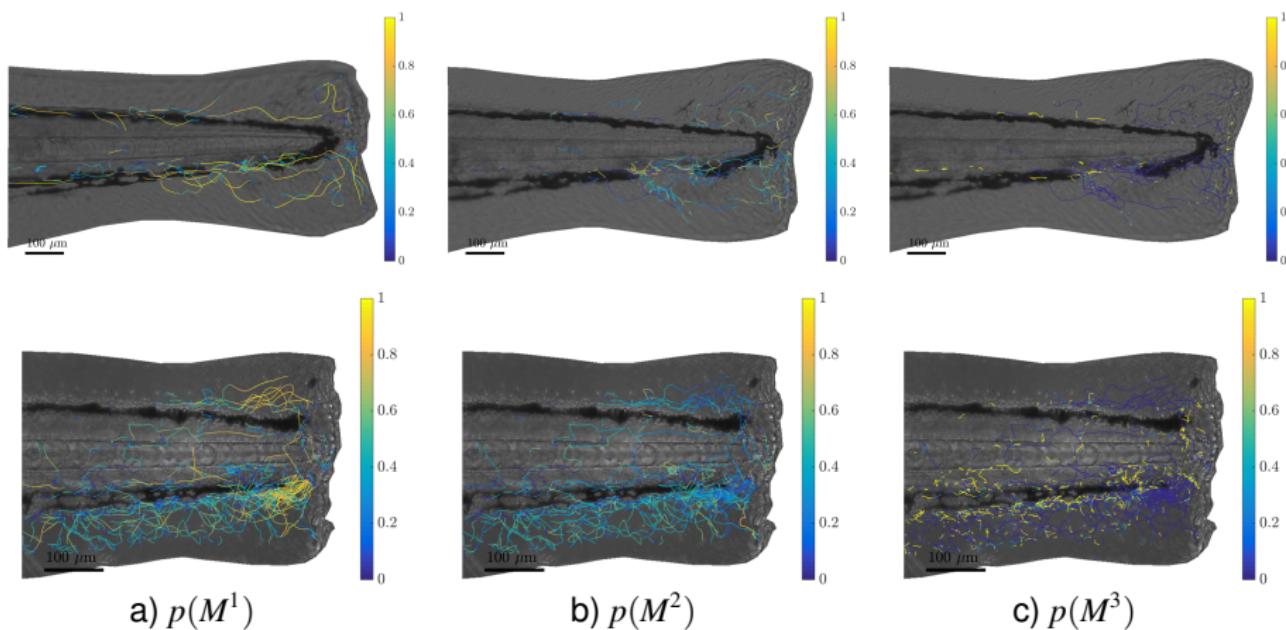
²The framework is derived and validated on MC simulations in Chapter 4.



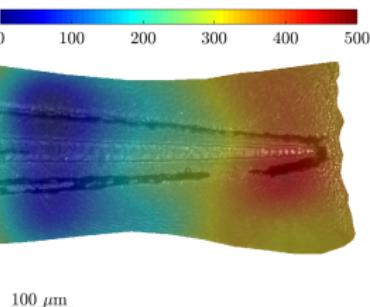
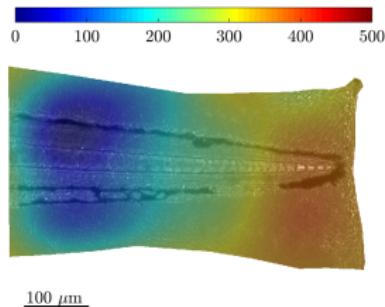
Migratory modes - normal injury



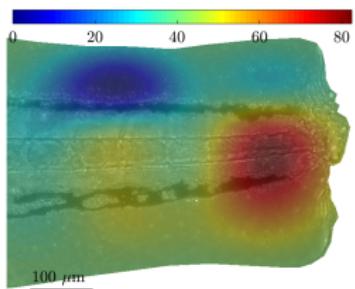
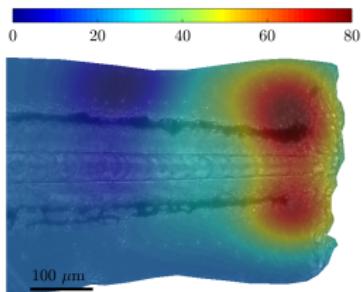
Migratory modes - severe and mild injury



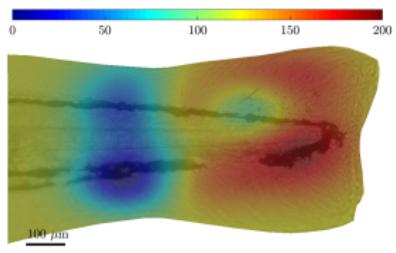
Inferred chemoattractant environment



a) normal injury



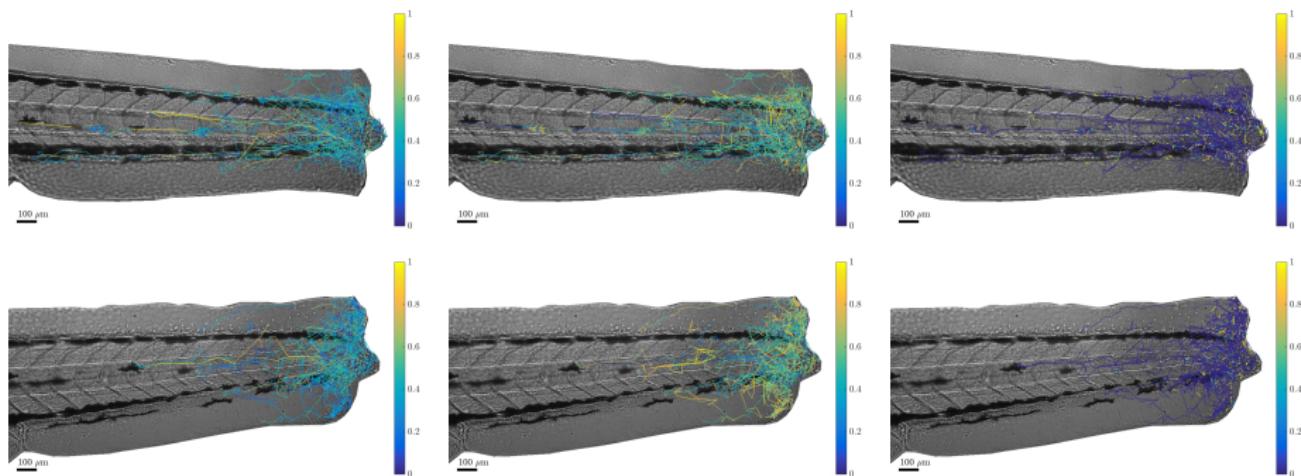
b) severe injury



c) mild injury

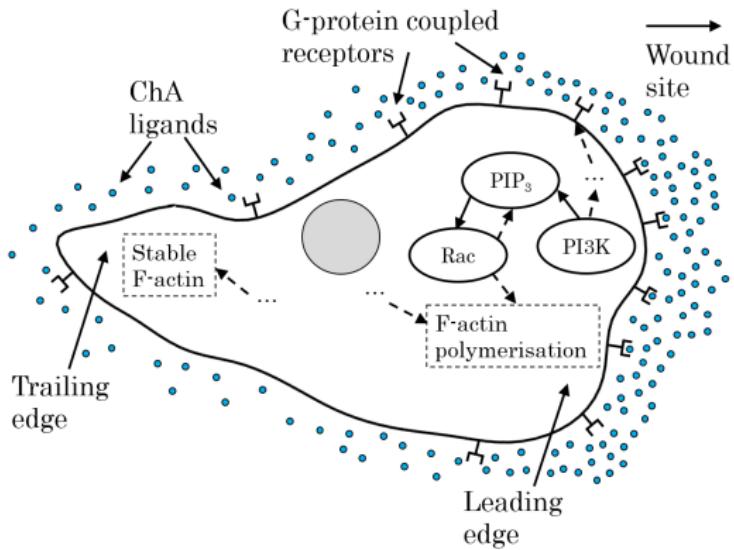


Reverse migration

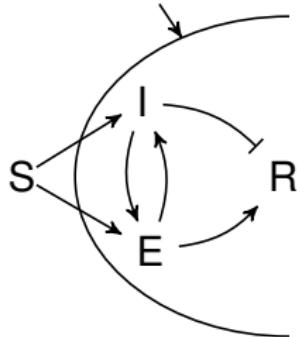


For 4 datasets there is higher probability of neutrophils diffusing away from the wound.



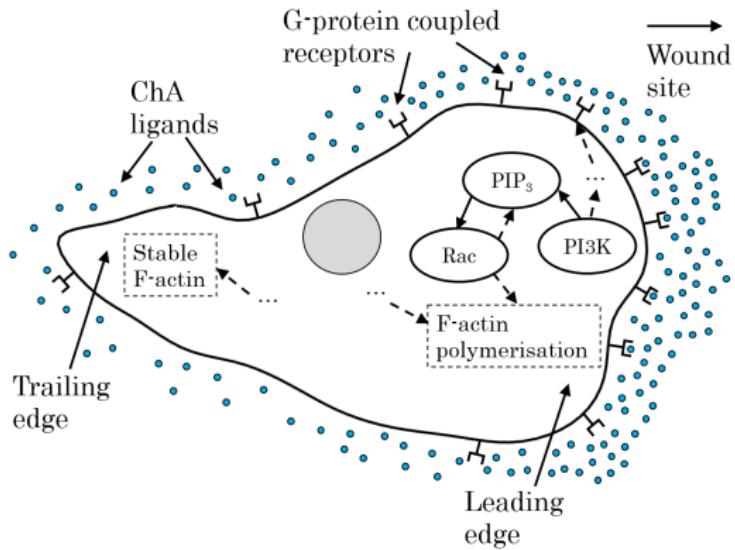


Cell membrane

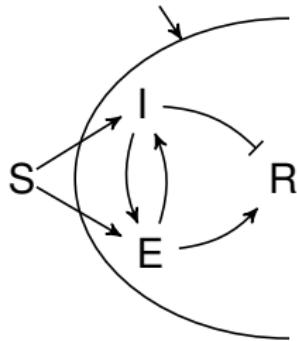


Does PIP₃ activate pseudopod growth?





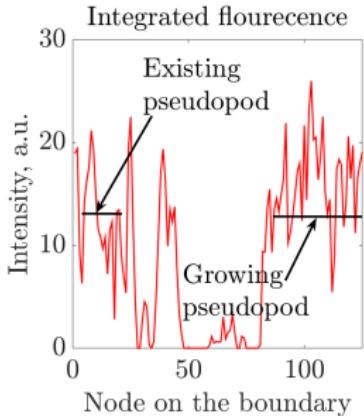
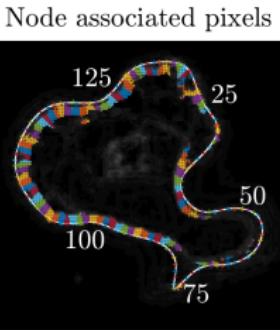
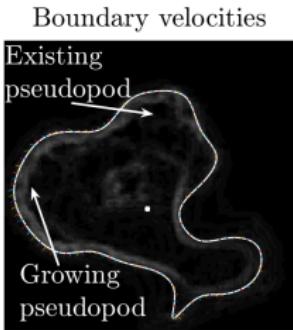
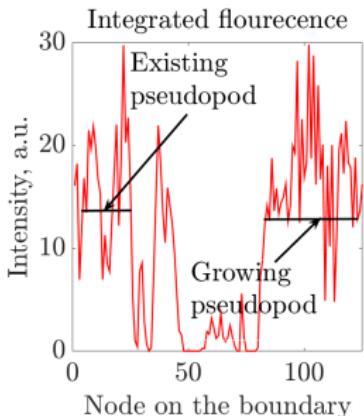
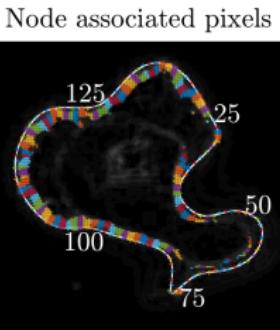
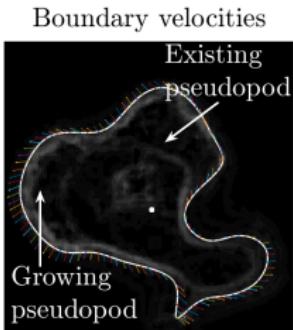
Cell membrane



Does PIP₃ activate pseudopod growth?



Data



Defining assumptions

- PIP_3 is the only activator regulating the cell membrane protrusion.
- The integrated fluorescence intensity obtained from the imaging data is proportional to the local PIP_3 concentration.
- Local shape change is fully described by normal velocity.
- The cell is flat.



Forces acting on cell boundary

$$\mathcal{F} = (\mathcal{F}_{\text{visc}} + \mathcal{F}_{\text{pro}} + \mathcal{F}_{\text{ten}} + \mathcal{F}_{\text{vol}})\nu,$$

- **Protrusive force** proportional to concentration of species active along the cell boundary:

$$\mathcal{F}_{\text{pro}} = \alpha_{\text{pro}} a_t^k.$$

- **Surface tension** corresponds to surface energy that prevents cell membrane from stretching:

$$\mathcal{F}_{\text{ten}} = \alpha_{\text{ten}} \kappa_t^k.$$

- **Volume conservation** balances small volume changes:

$$\mathcal{F}_{\text{vol}} = \alpha_{\text{vol}} \Delta A_t.$$

- **Viscous force** opposes cell motion:

$$\mathcal{F}_{\text{visc}} = -\alpha_{\text{vv}} v_t^k.$$



State space model

Cell boundary is represented by a discrete polygon with K vertexes.
Local evolution for each vertex:

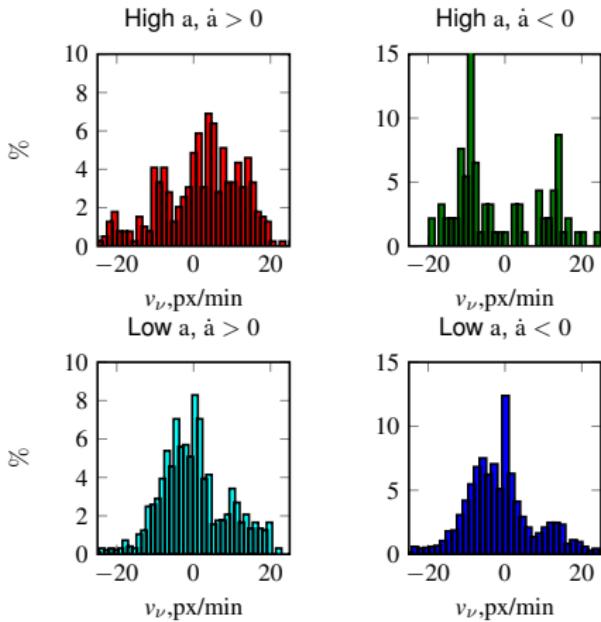
$$\mathbf{v}_{t+1}^k = A\mathbf{v}_t^k + B\mathbf{u}_t^k + \mathbf{w}_t^k, \quad \mathbf{w}_t^k \sim \mathcal{N}(0, Q).$$

$$\mathbf{y}_t^k = C\mathbf{v}_t^k.$$

- $A = 1 - \alpha_{vv}$; $B = [\alpha_{pro}, \alpha_{ten}, \alpha_{vol}]$;
- $\mathbf{u}_t^k = [a_t^k, \kappa_t^k, \Delta\mathcal{A}_t]^\top$, where
 - a_t^k - local concentration of PIP₃;
 - κ_t^k - local curvature;
 - $\Delta\mathcal{A}_t = \mathcal{A}_t - \mathcal{A}_0$ - change in cell shape.
- $\Theta = \{A, B, Q, \mathbf{v}_0, P_0\}$ - estimated via classic EM algorithm.



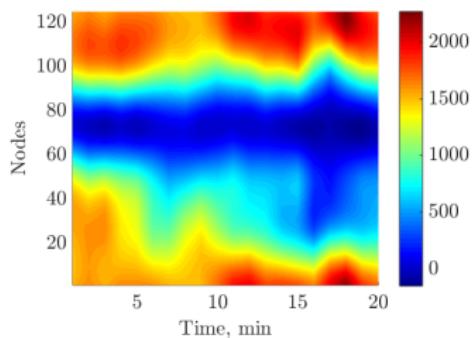
Motile cells observed in vivo



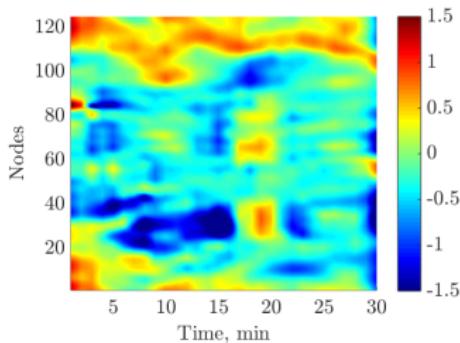
- Very weak correlation between a_{t-1}^k and v_t^k ;
- Correlation coefficients are inconsistent for different cells in the same set;
- Mann-Whitney test results: on average, higher concentration of PIP₃ accelerates protrusion growth, but does not activate it.



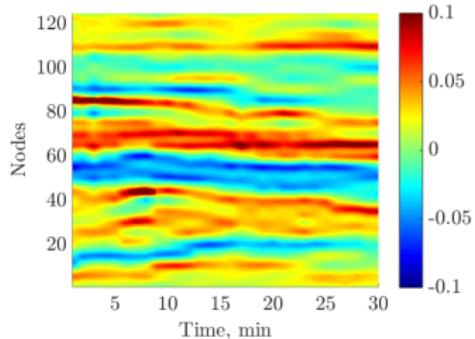
Motile cells observed in vivo



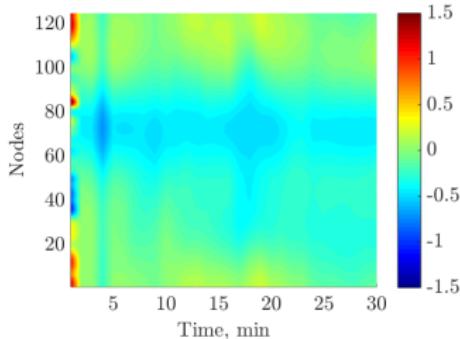
a) Smoothed intensity.



c) Estimated velocity.



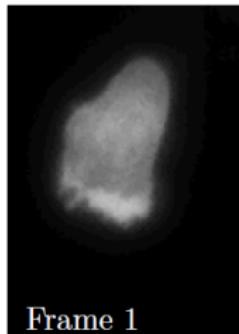
a) Local curvature.



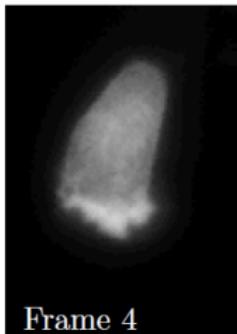
d) Predicted velocity.



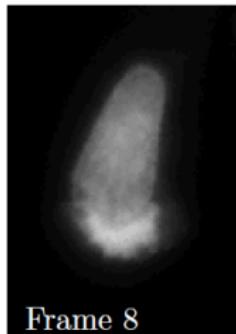
Polarised cell observed in vitro



Frame 1



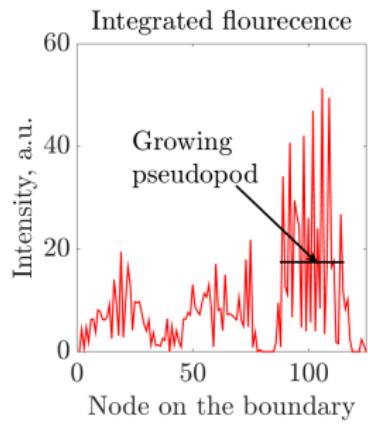
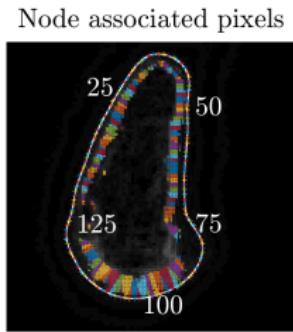
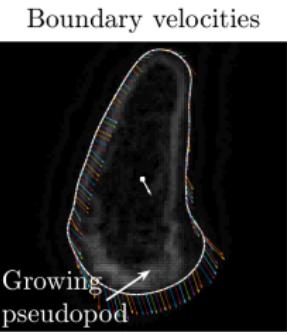
Frame 4



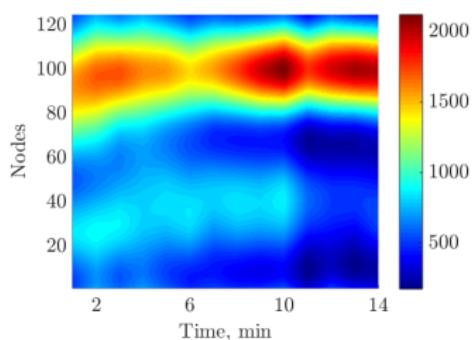
Frame 8



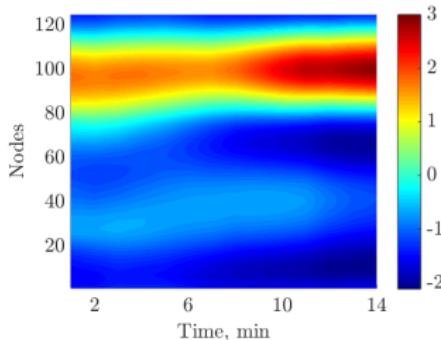
Frame 12



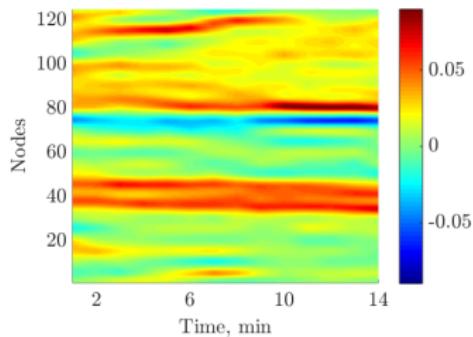
Polarised cell observed in vitro



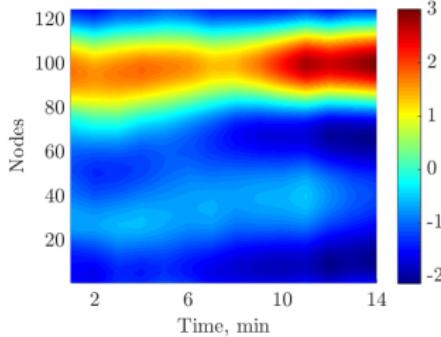
a) Smoothed intensity.



c) Estimated velocity.



a) Local curvature.



d) Predicted velocity.



Technical contributions

- A reconfigurable hybrid model of individual cell dynamics that incorporates the influence of the global chemoattractant environment.
- A statistical framework for simultaneous inference of the global chemoattractant environment and cell behavioural modes from cell tracking data.
- An image processing and estimation framework that links local cell boundary evolution to observed subcellular concentrations.



Contributions in field of application

- Investigation of cell-environment interaction on different stages of inflammation.
- Quantitative evidence that the dominant mode of neutrophil reverse migration is random walk.
- Quantitative evidence that PIP_3 does not activate protrusions but accelerates existing leading edges in cells performing chemotaxis.



Future work

- Utilising hierarchical/multi-resolution basis functions in environment decomposition.
- Introducing priors for the field parameters and Bayesian inference.
- Considering time-varying environment for recruitment stage of inflammation.
- Considering competing gradients for resolution stage of inflammation.
- Shorten this presentation.



Disseminated results

- A. Kadochnikova, H.M. Isles, S.A. Renshaw, V. Kadirkamanathan. "Estimation of Hidden Chemoattractant Field from Observed Cell Migration Patterns". A peer-reviewed paper in *Proceedings of 18th IFAC Symposium on System Identification SYSID 2018*.
- H.M. Isles, C. Muir, A. Kadochnikova, C.A. Loynes, V. Kadirkamanathan, P.M. Elks, S.A. Renshaw. "Non-apoptotic pioneer neutrophils initiate a swarming response in a zebrafish tissue injury model" under review in eLife Reports, 2019.

In preparation:

- A. Kadochnikova, V. Kadirkamanathan. "An Approximate Maximum Likelihood Framework for Estimating the Environment Driving multiple objects with Hybrid Dynamics".
- A. Kadochnikova, H.M. Isles, S.A. Renshaw, V. Kadirkamanathan. "Inference of the External Stimuli Environments from Heterogeneous Behaviour of Migrating Neutrophils in Zebrafish Model of Inflammation".



Thank you!
Questions?

