

Modelling and Identification of Immune Cell Migration during the Inflammatory Response

PhD Viva

A. Kadochnikova¹

¹Department of Automatic Control and Systems Engineering
The University of Sheffield

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Neutrophils drive inflammatory response

Experimental studies

Recruitment
via chemotaxis

in vivo microscopy
on zebrafish larvae

Resolution via
reverse migration

Sensing to motion
via subcellular signals

Mathematical models

RDS models
for populations

Random walk models
for single cells

RDS models for
subcellular species

Morphodynamics as
intermediate level



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Models not
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Common concept:
Complicated model → Realistic simulations.



Systematic approach:
Simplified models → Linking to data → Meaningful inferences.



Systematic approach:

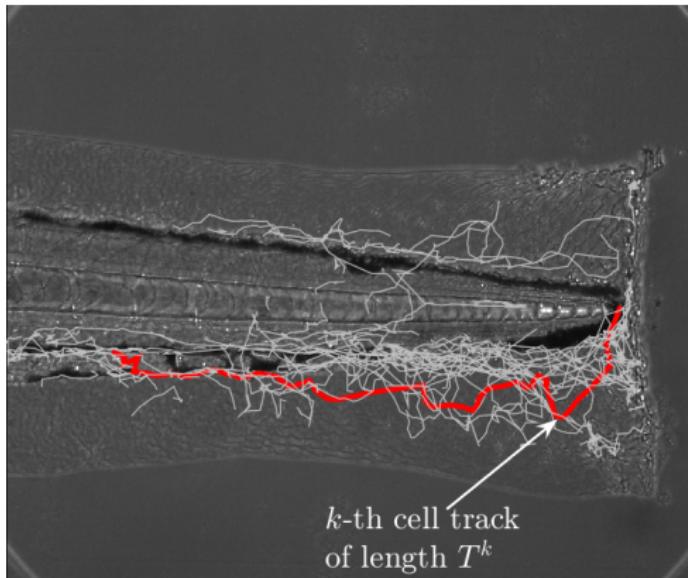
Simplified models → Linking to data → Meaningful inferences.

Objectives:

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



Hidden chemoattractant

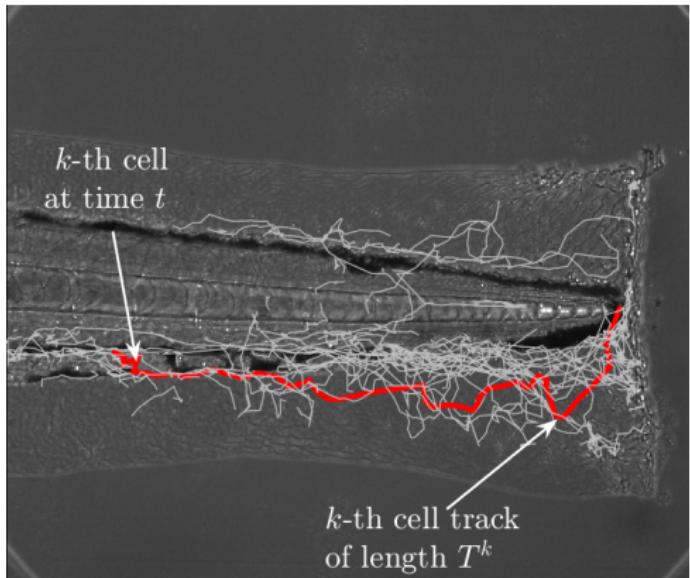


Time series data:

- K tracks: $\mathcal{Y} = \{\mathbf{y}^k\}_{k=1}^K$
- Single track:
 $\mathbf{y}^k = \{\mathbf{y}_t^k\}_{t=1}^{T^k}$
- Single data point:
 $\mathbf{y}_t^k = [\bar{s}_x, \bar{s}_y]^\top$
- Environment influence:
 $\mathbf{u}_t^k = \mathbf{u}_t^k(s) = \nabla \mathcal{U}(s)$.



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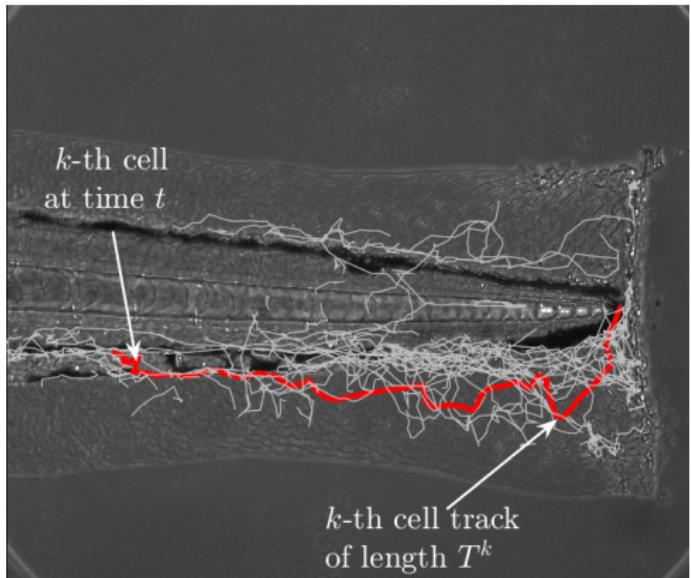


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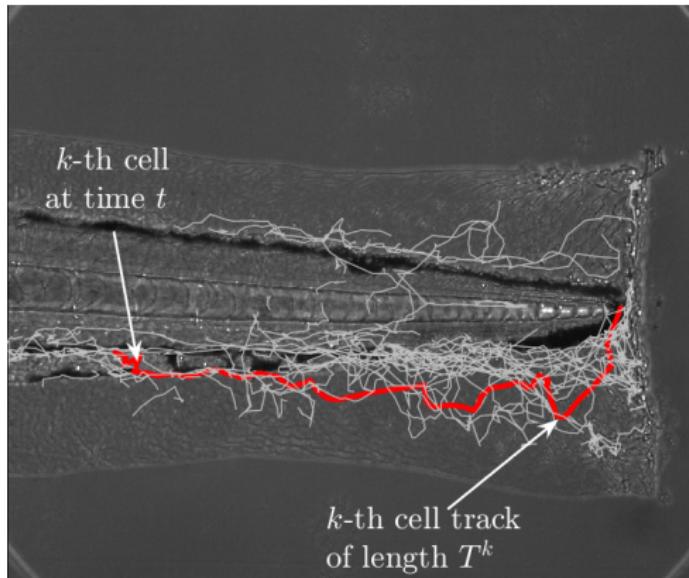


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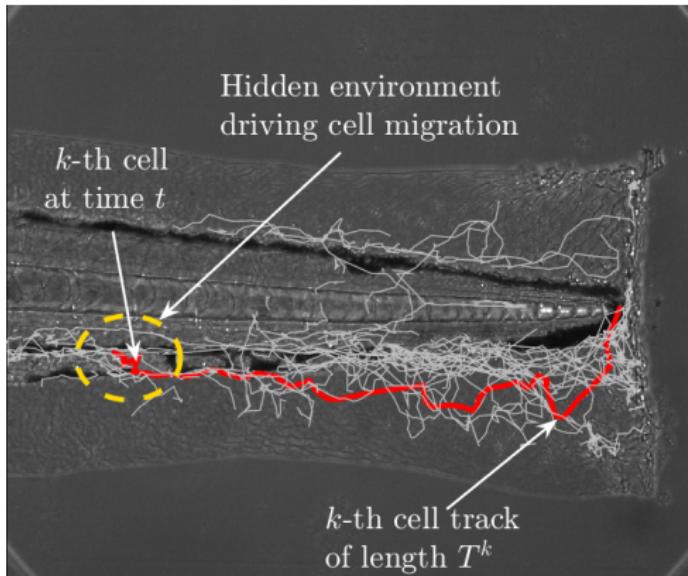


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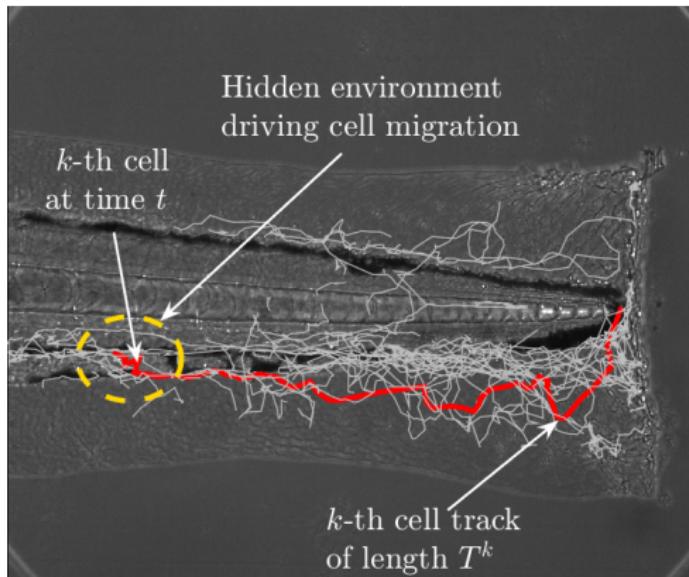


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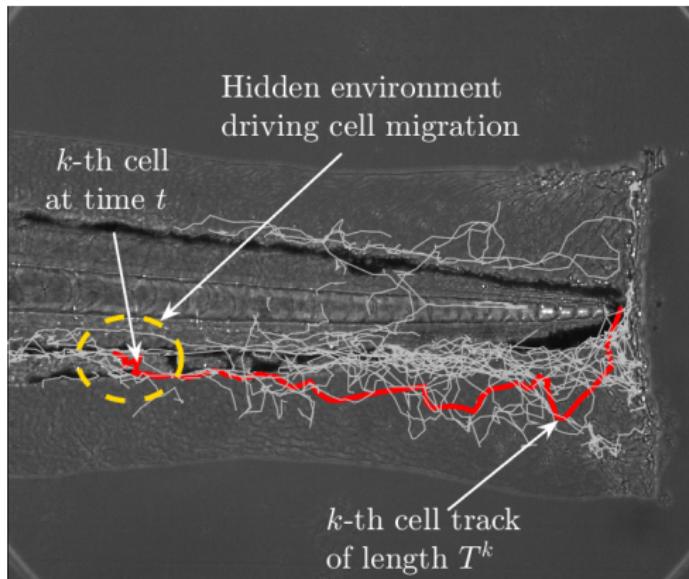
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1. Develop a parametrised finite-order model of global $\mathcal{U}(\mathbf{s})$.



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



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:

$$\mathcal{U}(s(t)) = \mathcal{U}(s).$$



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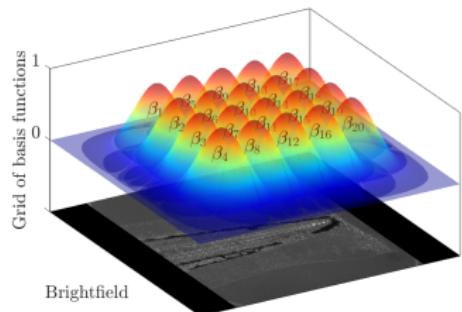
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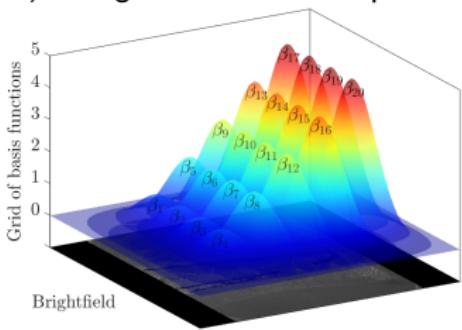
$$\mathcal{U}(\mathbf{s}(t)) = \mathcal{U}(\mathbf{s}).$$



Decomposition of the environment



a) 5x4 grid of tensor B-splines



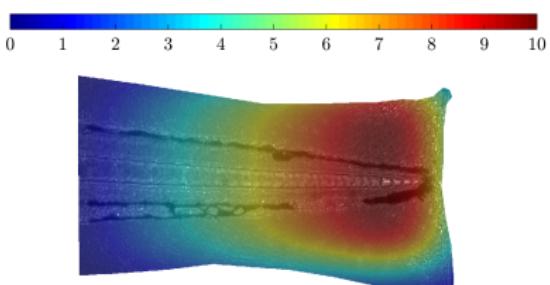
b) θ_h defines magnitude of $\beta_h(s_x, s_y)$

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

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

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

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



Example of the resultant field.



Model of neutrophil dynamics

Discrete time SSM of the k-th cell :

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

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

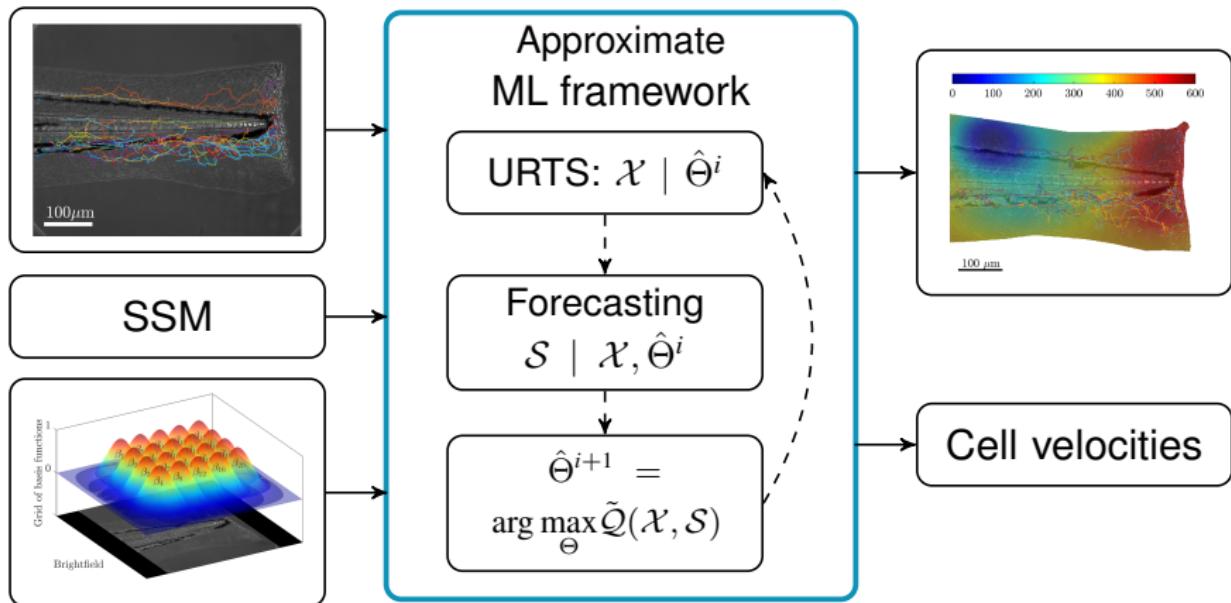
where $\boldsymbol{x}_t^k = [s_x, s_y, v_x, v_y]^\top$,

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

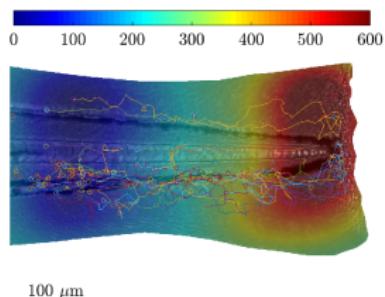
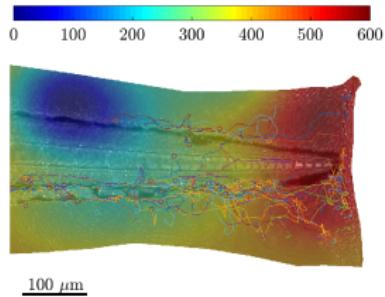
$$A = \begin{bmatrix} \mathbb{I} & T\mathbb{I} \\ \mathbb{O} & (1 - T\rho)\mathbb{I} \end{bmatrix}; B = \begin{bmatrix} \mathbb{O} \\ T\mathbb{I} \end{bmatrix}; G = \begin{bmatrix} \mathbb{O} \\ T\mathbb{I} \end{bmatrix}; C = [\mathbb{I} \quad \mathbb{O}].$$



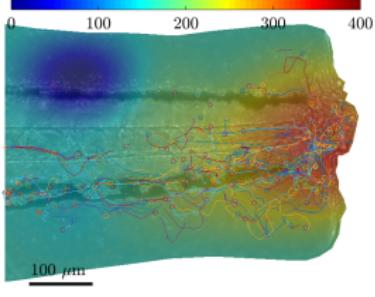
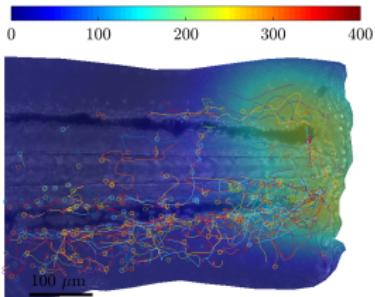
ML estimation with missing data



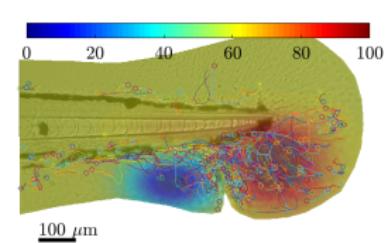
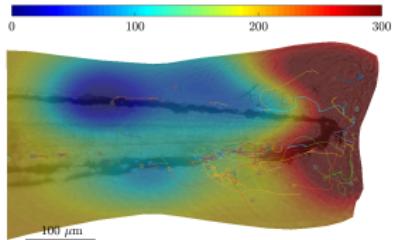
Inferred chemoattractant concentration



a) normal injury
(6 datasets)



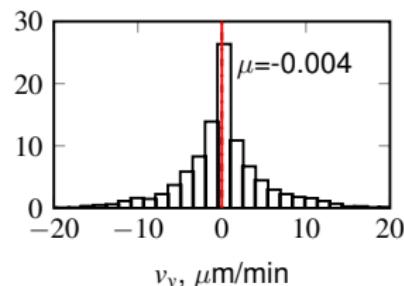
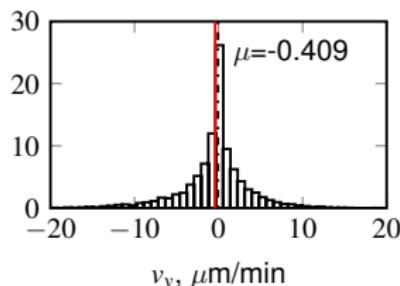
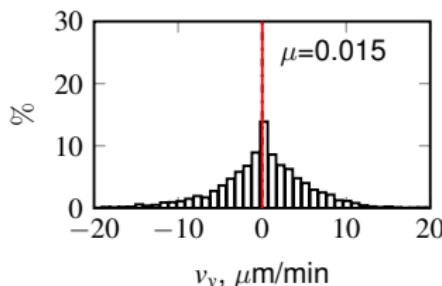
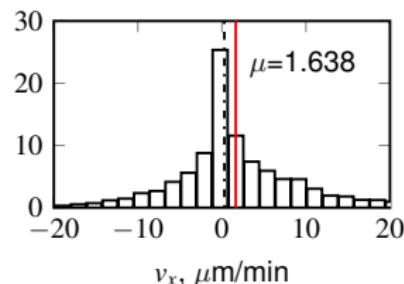
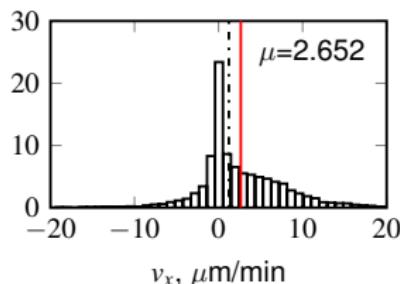
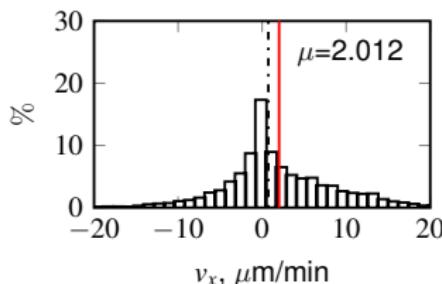
b) severe injury
(2 datasets)



c) mild injury
(6 datasets)



Estimated cell velocities



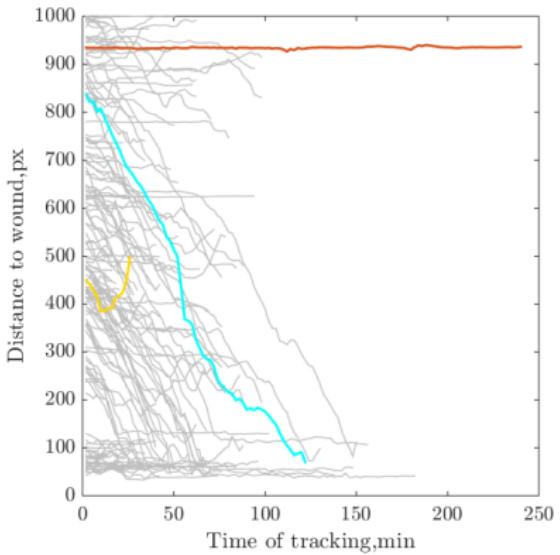
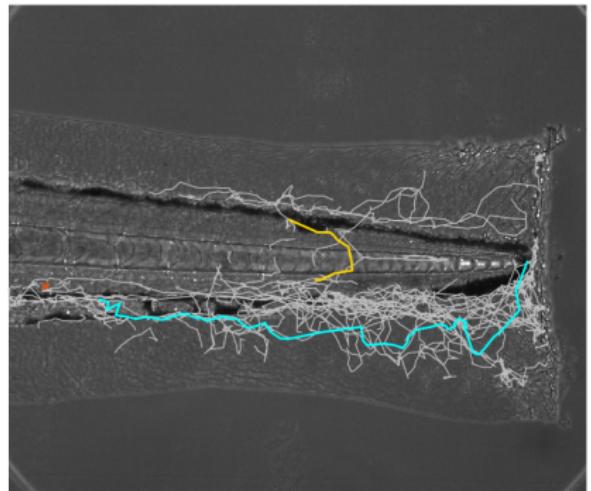
a) normal injury

b) severe injury

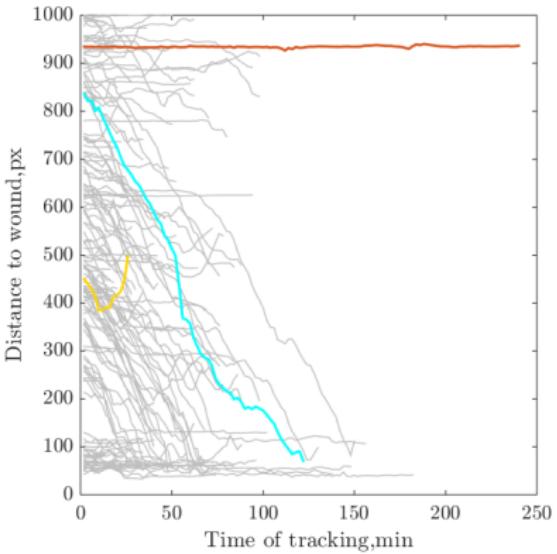
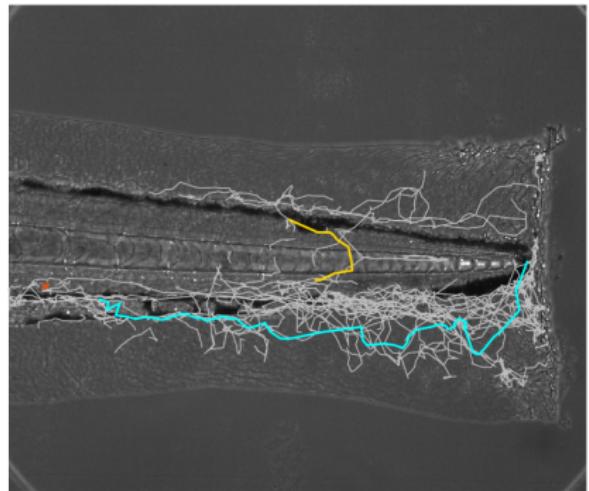
c) mild injury



Heterogeneous cell behaviour



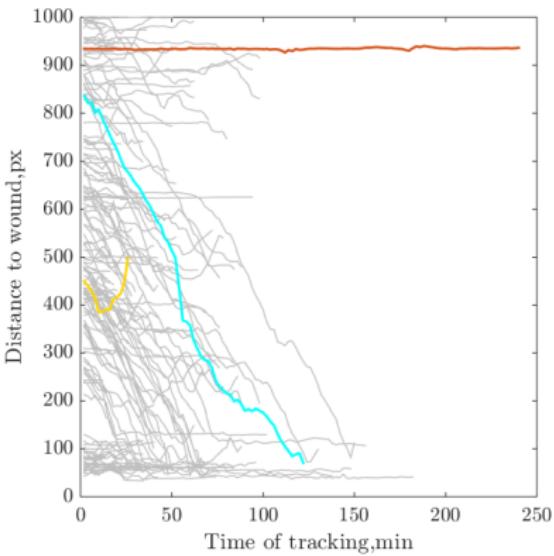
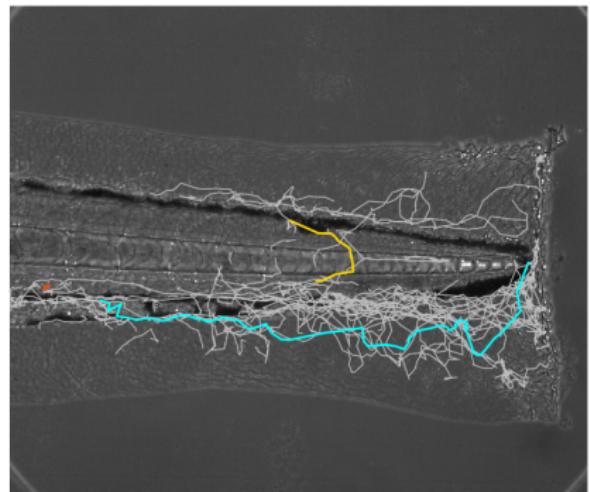
Heterogeneous cell behaviour



1. Determine whether a cell at a given time interacts with the environment $\mathcal{U}(s)$.



Heterogeneous cell behaviour



1. Determine whether a cell at a given time interacts with the environment $\mathcal{U}(s)$.
2. Estimate unobserved $\mathcal{U}(s)$ from the interaction with responsive cells



Defining assumptions (upd.)

Previous assumption:

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

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.



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Jump Markov system

$$\begin{aligned} \mathbf{x}_t^k &= A(\mathbf{m}_t^k) \mathbf{x}_{t-1}^k + B(\mathbf{m}_t^k) \phi_{t-1}^k(s_x, s_y) \Theta + G(\mathbf{m}_t^k) \mathbf{w}_{t-1}^k, \\ \mathbf{w}_t^k &\sim \mathcal{N}(0, Q(\mathbf{m}_t^k)), \\ \mathbf{m}_t^k &\in \{M^1, M^2, M^3\}. \end{aligned}$$

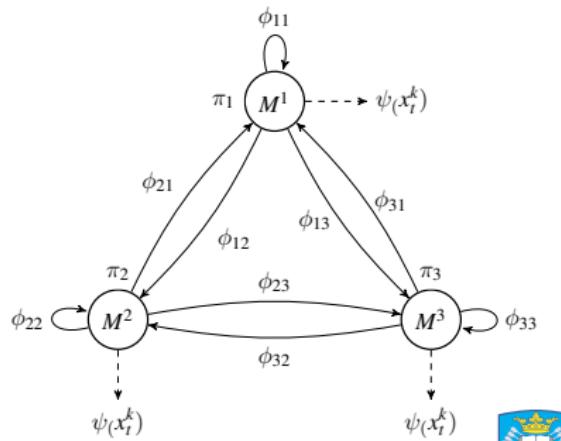
Cell modes:

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

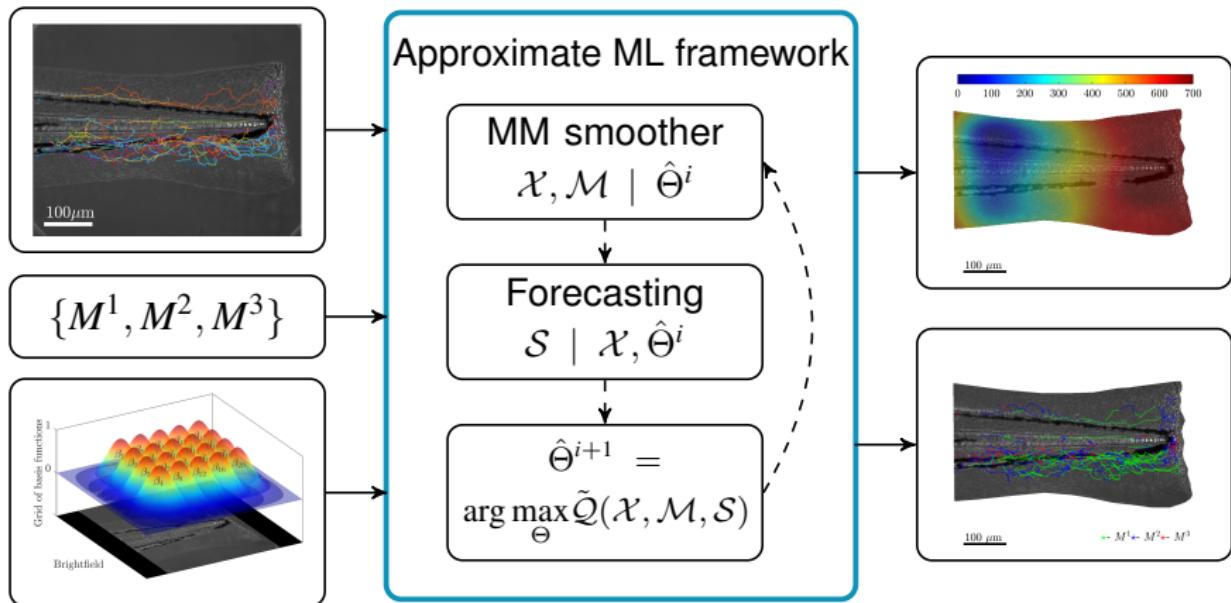
$$M^2 : A = \begin{bmatrix} \mathbb{I} & T\mathbb{I} \\ \mathbb{O} & (1 - T\rho(M^2))\mathbb{I} \end{bmatrix} B = \begin{bmatrix} \mathbb{O} \\ \mathbb{O} \end{bmatrix}$$

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

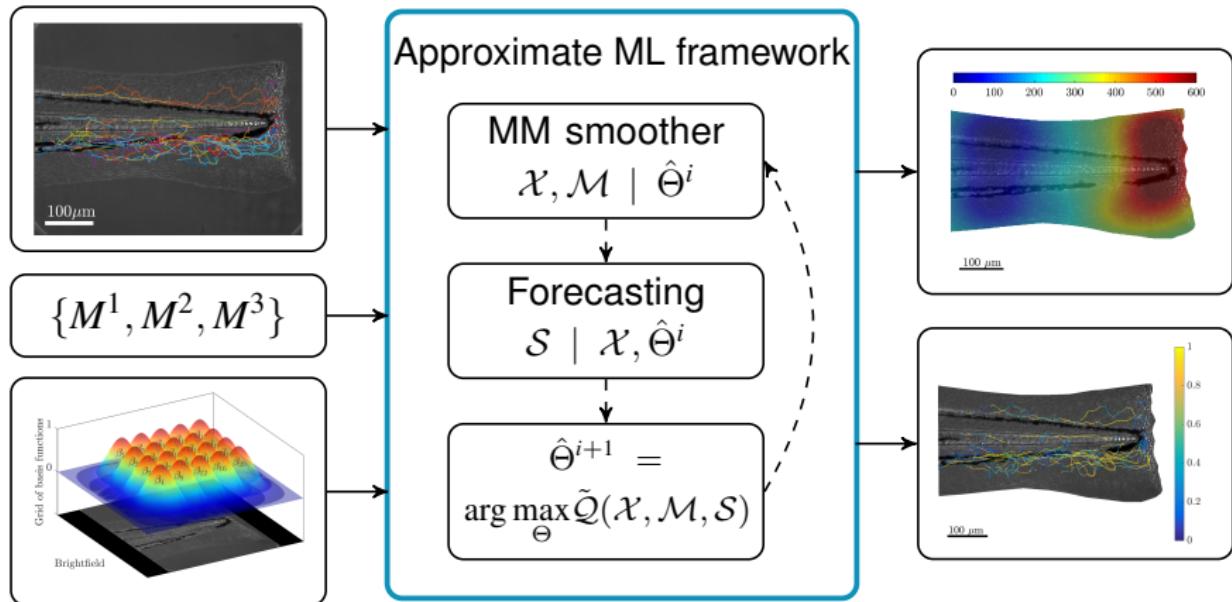
$$Q(M^3) \ll Q(M^1), Q(M^2).$$



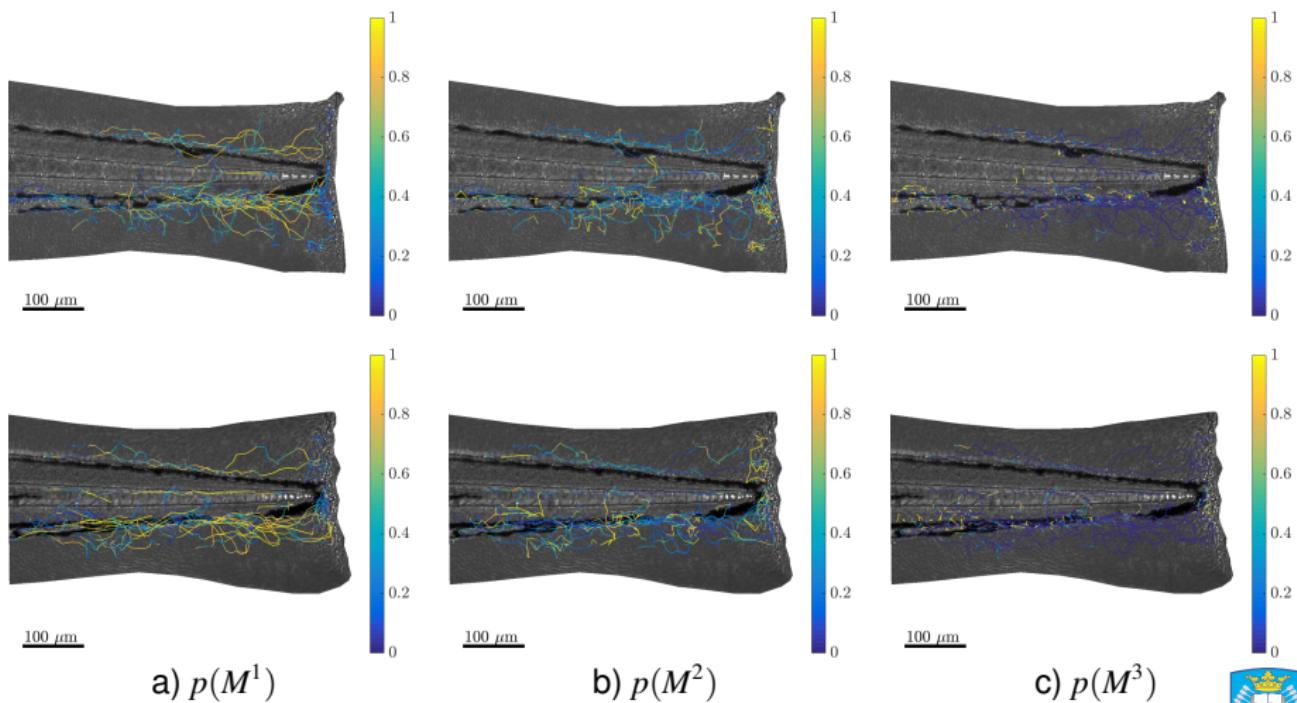
Inference framework



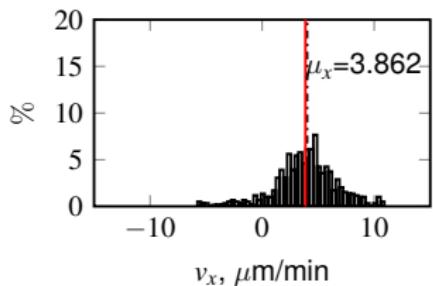
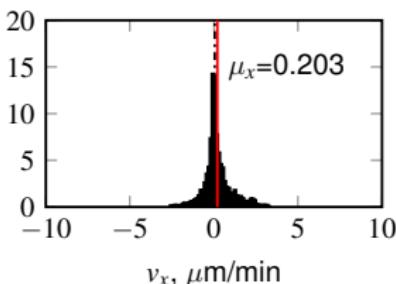
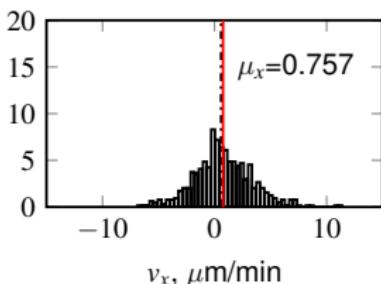
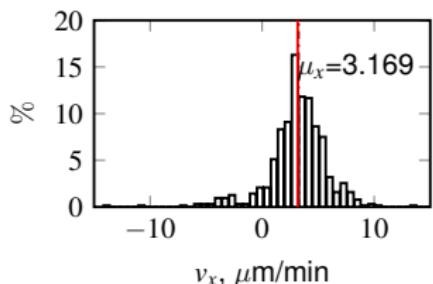
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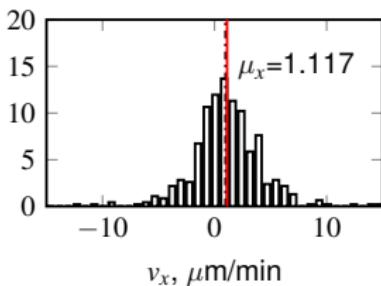
Migratory modes - normal injury



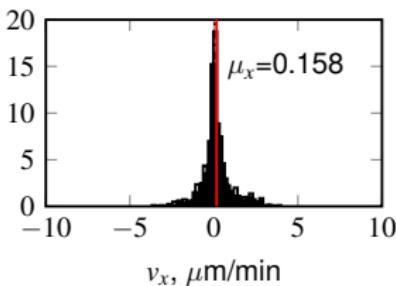
Estimated cell velocities



a) M^1



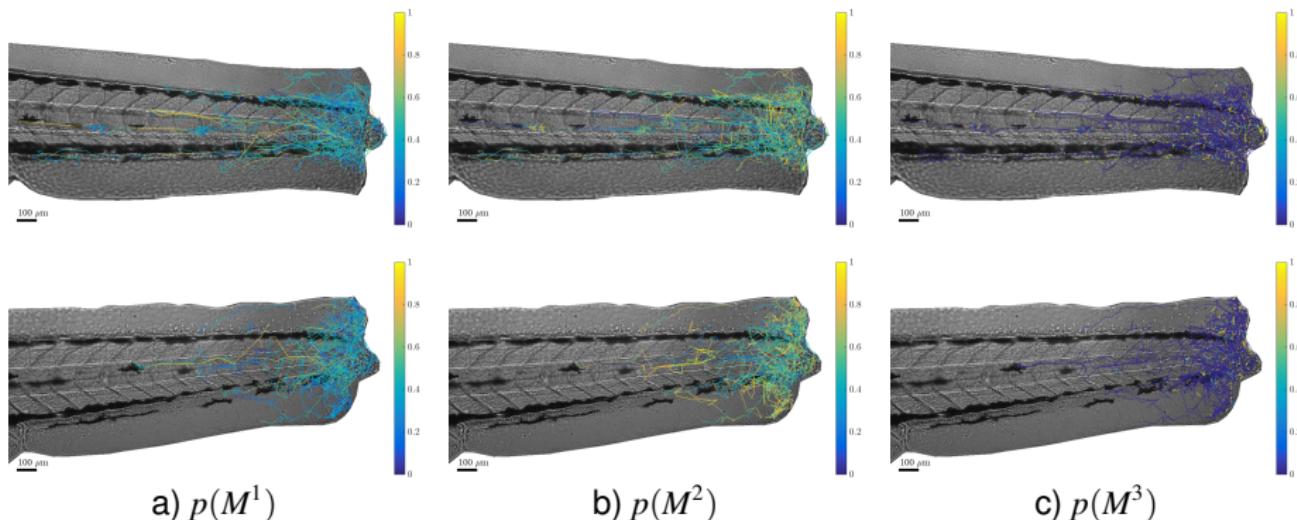
b) M^2



c) M^3



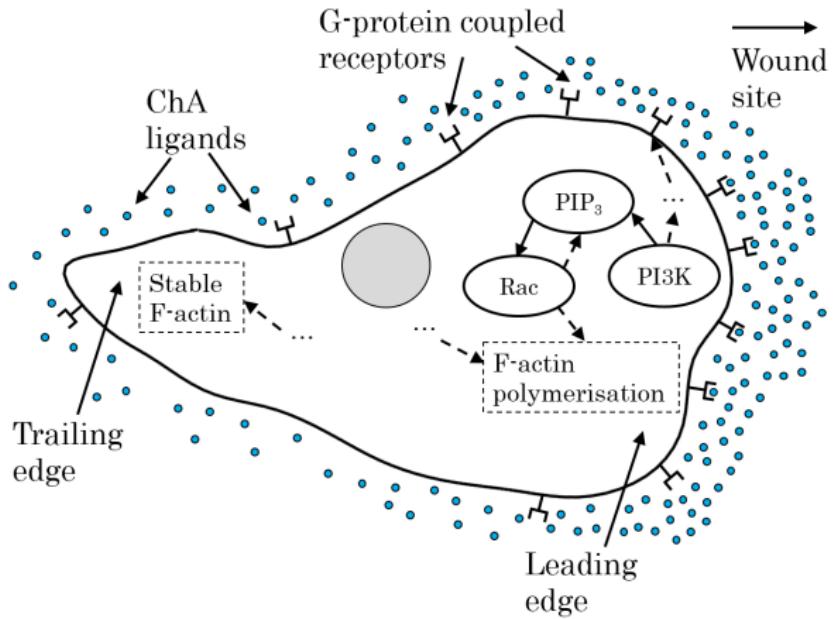
Reverse migration



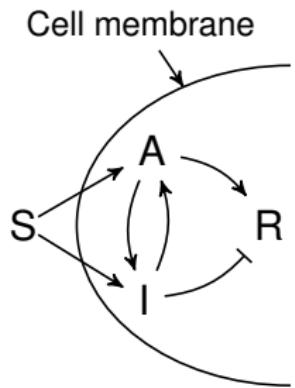
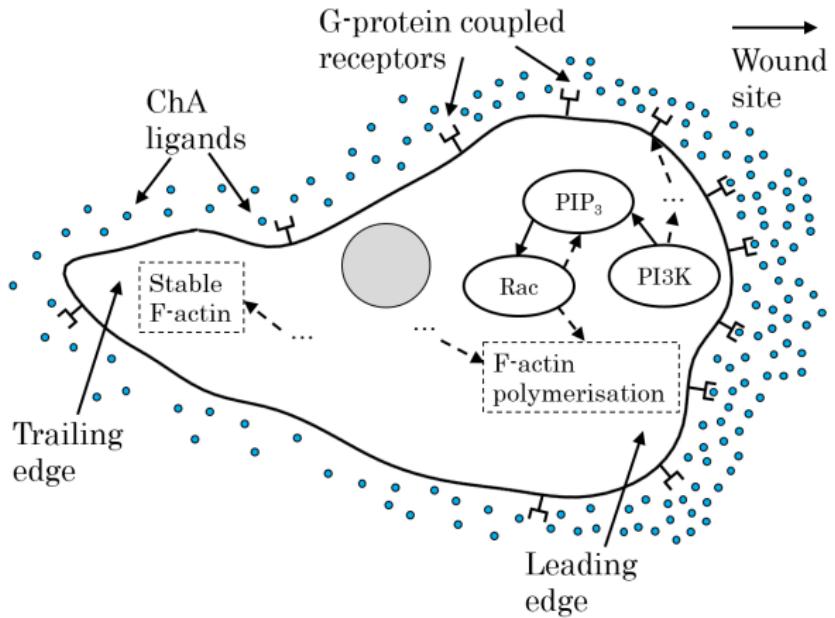
For 4 datasets there is higher probability of neutrophils diffusing away from the wound.



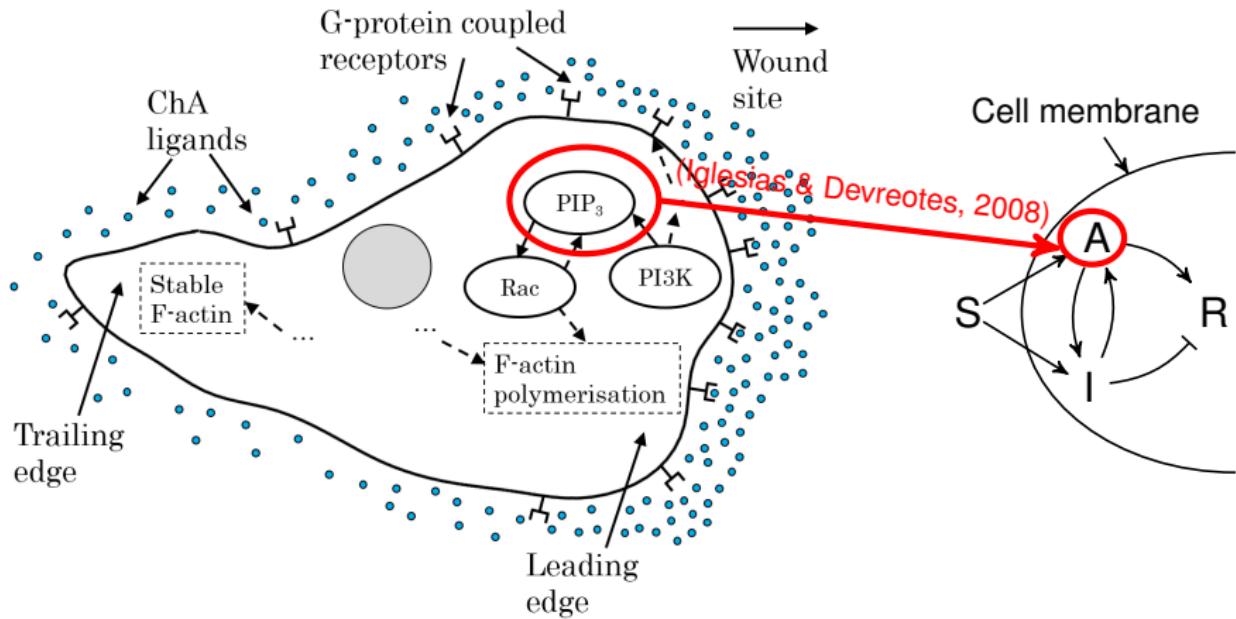
Signal to migration



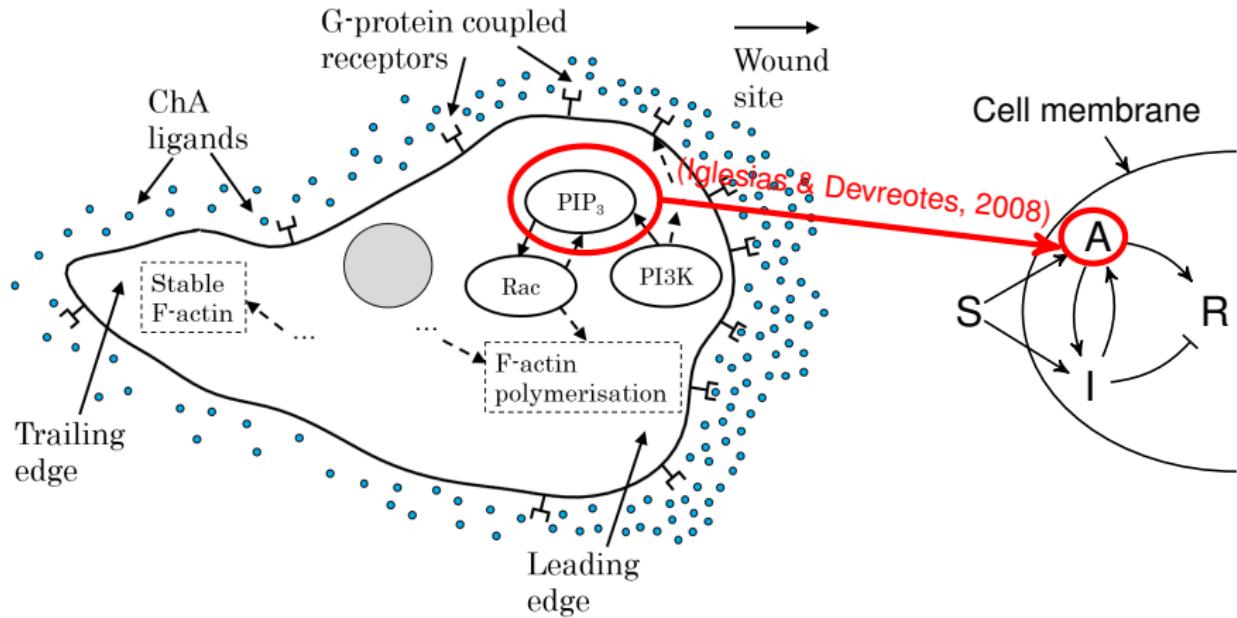
Signal to migration



Signal to migration



Signal to migration



Does PIP₃ activate pseudopod growth in migrating neutrophils?



Defining assumptions

- PIP₃ is the only activator regulating cell membrane protrusions.
- The integrated fluorescence intensity obtained from the imaging data is proportional to the local PIP₃ concentration.
- Local shape change is fully described by the evolution of local normal velocity.

$$\mathbf{v}_{t+1}^k = \mathbf{v}_t^k + \frac{1}{m} \mathcal{F} + \mathbf{w}_t.$$

- The cell is a 2-D curve Γ_t . 3-D effects are accounted for in the random acceleration \mathbf{w}_t .



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** caused by acting regulators along the membrane:

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

- **Surface tension** prevents cell membrane from stretching:

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

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

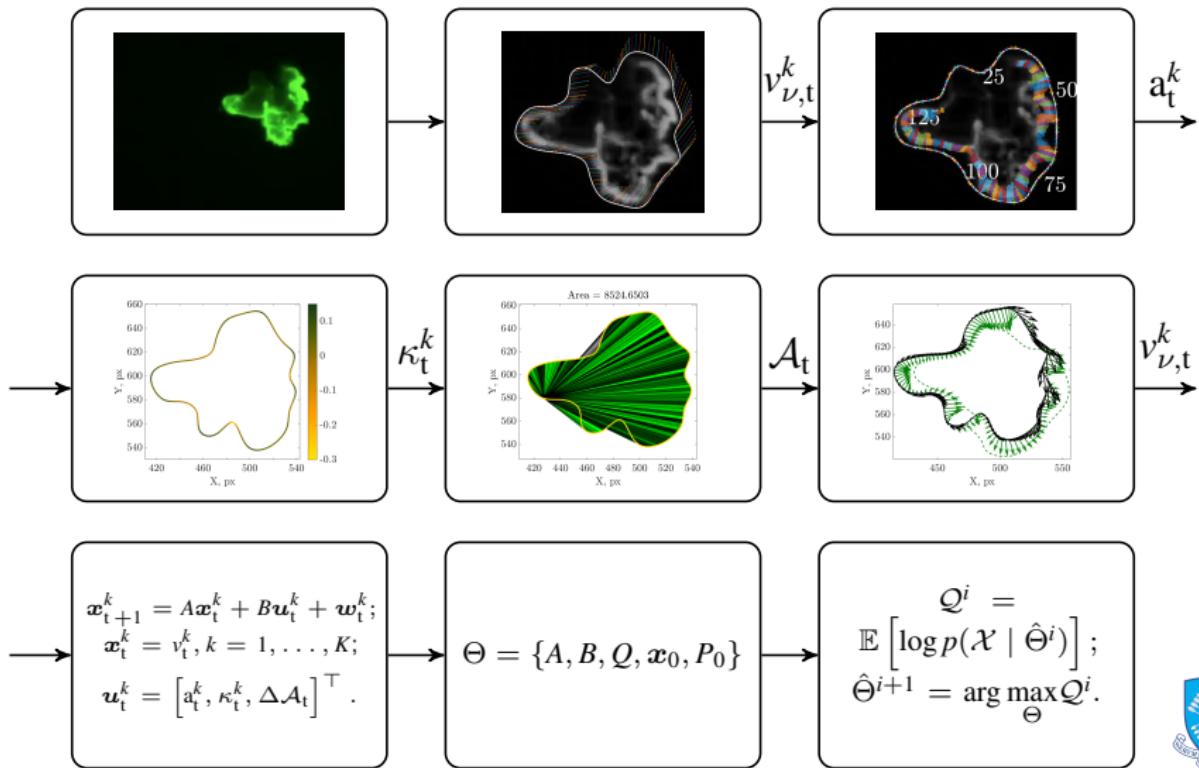
$$\mathcal{F}_{\text{vol}} = \alpha_{\text{vol}} \Delta A_t.$$

- **Viscous force** opposes cell motion:

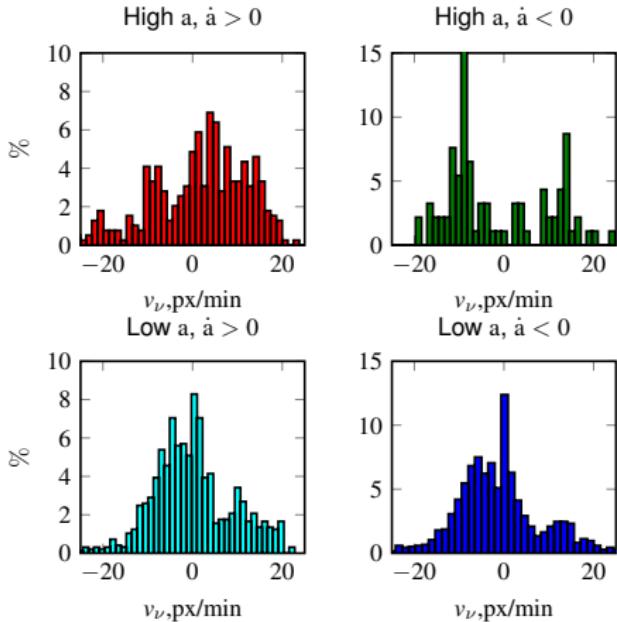
$$\mathcal{F}_{\text{visc}} = -\alpha_{\text{vv}} v_t^k.$$



Image processing framework



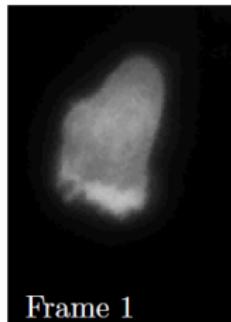
Motile cells observed in vivo



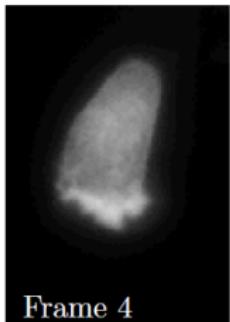
- Very weak correlation between a_{t-1}^k and v_t^k for all cells;
- Mann-Whitney test results: higher concentrations of PIP₃ correspond to accelerated protrusion growth.
- Results in agreement with recent knock-out studies.



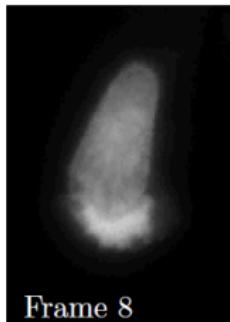
Polarised cell observed in vitro



Frame 1



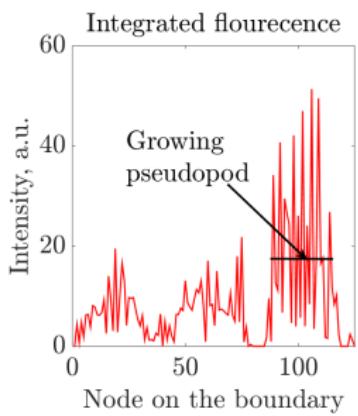
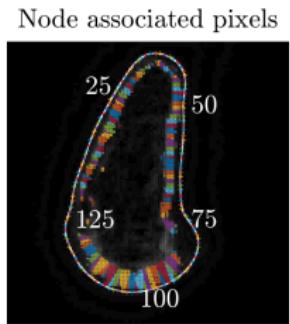
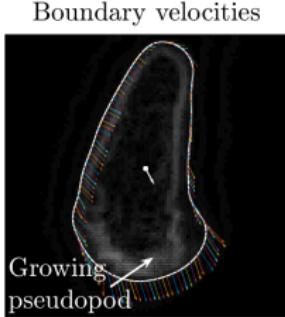
Frame 4



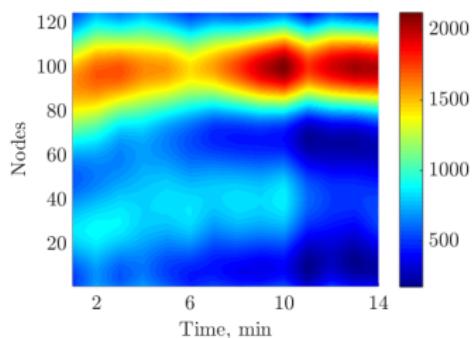
Frame 8



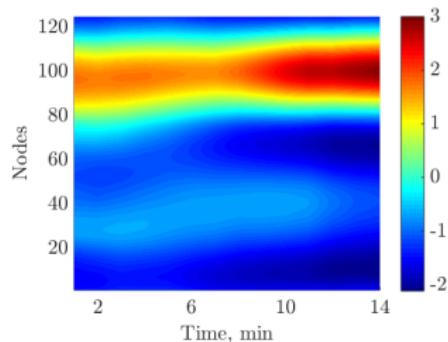
Frame 12



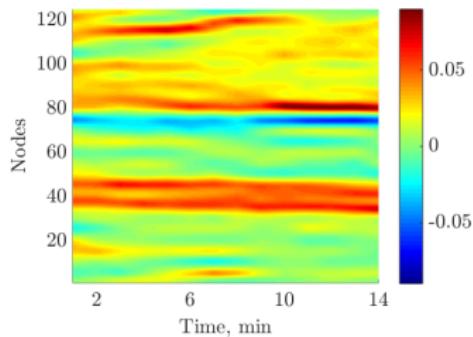
Polarised cell observed in vitro



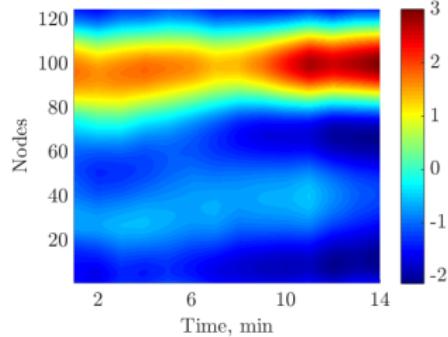
a) Smoothed intensity.



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 environment.
- A statistical framework for simultaneous inference of the global chemoattractant environment and cell behavioural modes.
- An image processing and estimation framework that links local cell boundary evolution to observed subcellular concentrations.



Contributions in field of application

- Investigation of neutrophil-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.



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?

