

# Modelling and Identification of Immune Cell Migration during the Inflammatory Response

PhD Viva

A. Kadochnikova<sup>1</sup>

<sup>1</sup>Department of Automatic Control and Systems Engineering  
The University of Sheffield

June 30, 2019



# Outline

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



## 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



## 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



## Experimental studies

Recruitment  
via chemotaxis

!!!

Environment  
unobserved

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



## Experimental studies

Recruitment  
via chemotaxis

!!!

Environment  
unobserved

Random walk  
or fugetaxis?

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



## Experimental studies

Recruitment  
via chemotaxis

!!!

Environment  
unobserved

Random walk  
or fugetaxis?

Sensing to motion  
via subcellular signals

## Mathematical models

RDS models  
for populations

RWs do not reflect  
global environment

RDS models for  
subcellular species

Morphodynamics as  
intermediate level



## Experimental studies

Recruitment  
via chemotaxis

!!!

Environment  
unobserved

Random walk  
or fugetaxis?

Sensing to motion  
via subcellular signals

## Mathematical models

RDS models  
for populations

RWs do not reflect  
global environment

RDS models for  
populations  
Models not  
informed by  
experimental data  
at intermediate level



Common concept:  
Complicated model → Realistic simulations.



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



## Systematic approach:

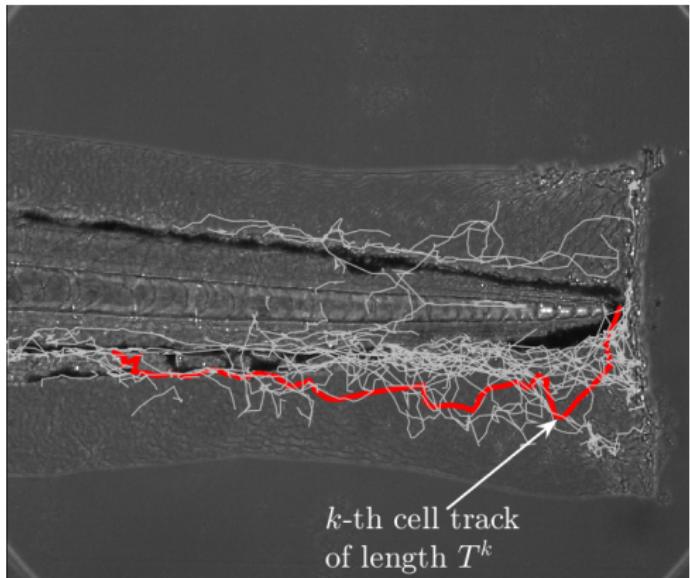
Simplified models → Linking to data → Meaningful inferences.

### 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.



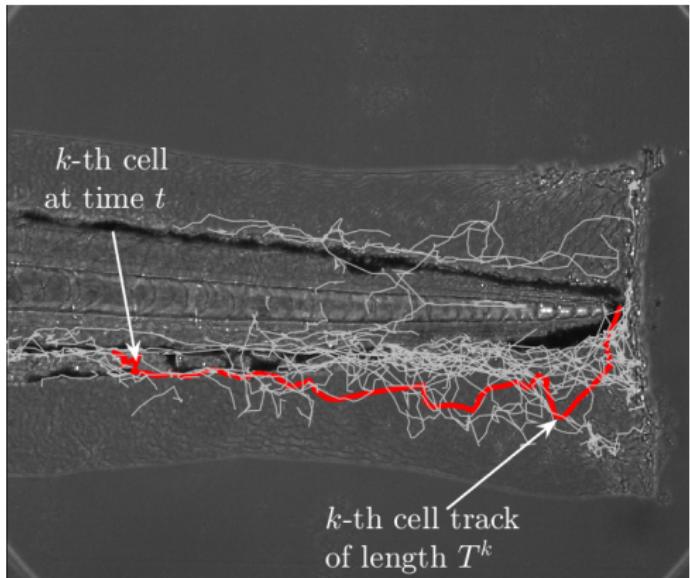
# Hidden chemoattractant



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:  
 $\mathbf{u}_t^k = \mathbf{u}_t^k(s) = \nabla \mathcal{U}(s)$ .

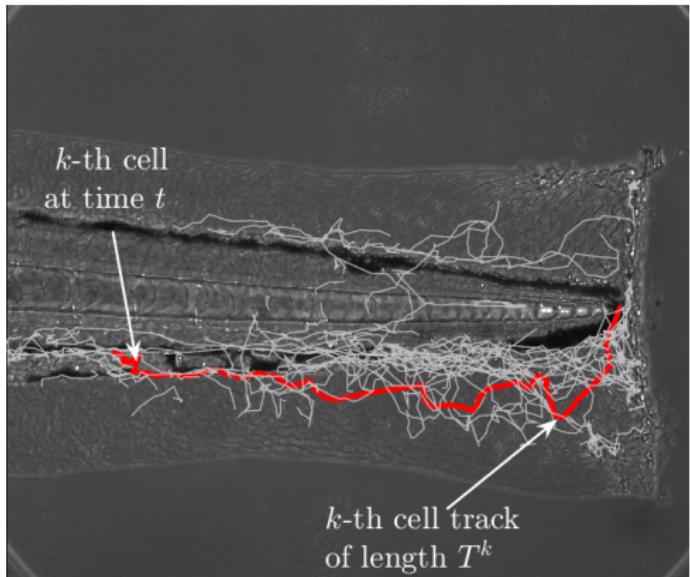
# Hidden chemoattractant



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:**  
 $\mathbf{u}_t^k = \mathbf{u}_t^k(s) = \nabla \mathcal{U}(s)$ .

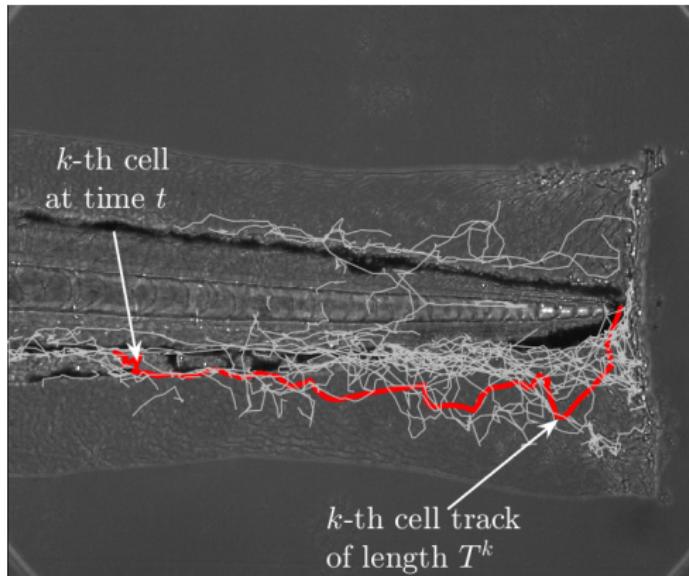
# Hidden chemoattractant



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:**  
 $u_t^k = u_t^k(s) = \nabla \mathcal{U}(s)$ .

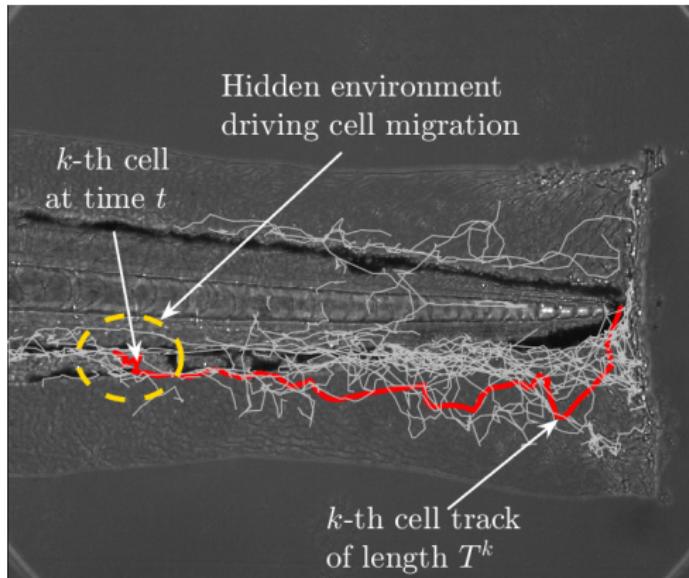
# Hidden chemoattractant



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:  
 $\mathbf{u}_t^k = \mathbf{u}_t^k(\mathbf{s}) = \nabla \mathcal{U}(\mathbf{s}).$

# Hidden chemoattractant



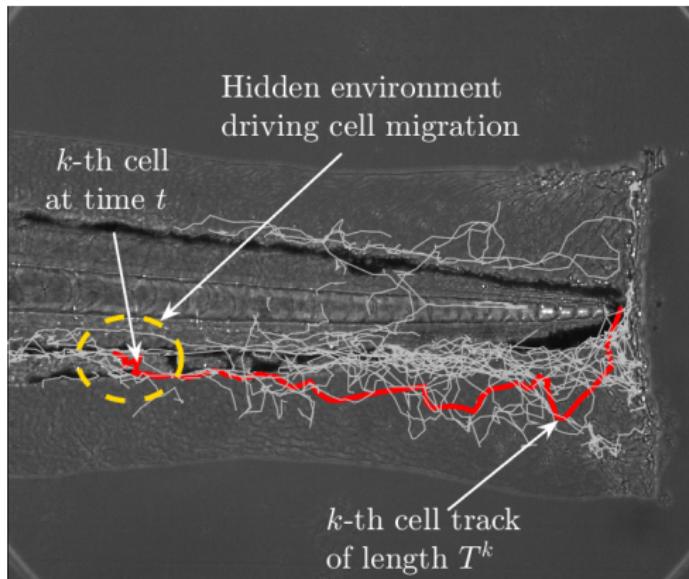
Time series data:

- $K$  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:  
 $\mathbf{u}_t^k = \mathbf{u}_t^k(s) = \nabla \mathcal{U}(s)$ .

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



# 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:  $\mathbf{u}_t^k = \mathbf{u}_t^k(s) = \nabla \mathcal{U}(s)$ .

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:

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



# 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:

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



# 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:

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

- Hidden chemoattractant environment is time-invariant:

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



# 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:

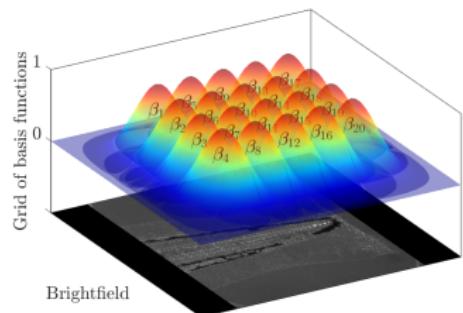
$$\dot{v}(t) = -\rho v(t) + \sqrt{\sigma} \mathbf{W}(t) + \nabla \mathcal{U}(\mathbf{s}(t)).$$

- Hidden chemoattractant environment is time-invariant:

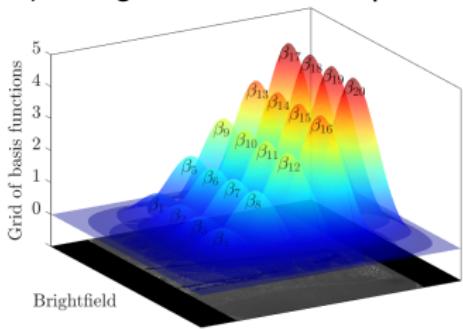
$$\mathcal{U}(\mathbf{s}(t)) = \mathcal{U}(\mathbf{s}).$$



# Decomposition of the environment



a) 5x4 grid of tensor B-splines



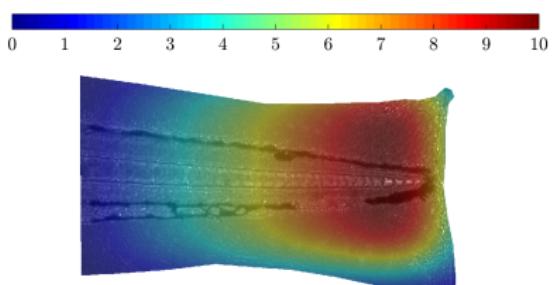
b)  $\theta_h$  defines magnitude of  $\beta_h(s_x, s_y)$

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

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

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

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



Example of the resultant field.



# Model of neutrophil dynamics

Discrete time SSM of the k-th cell :

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

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

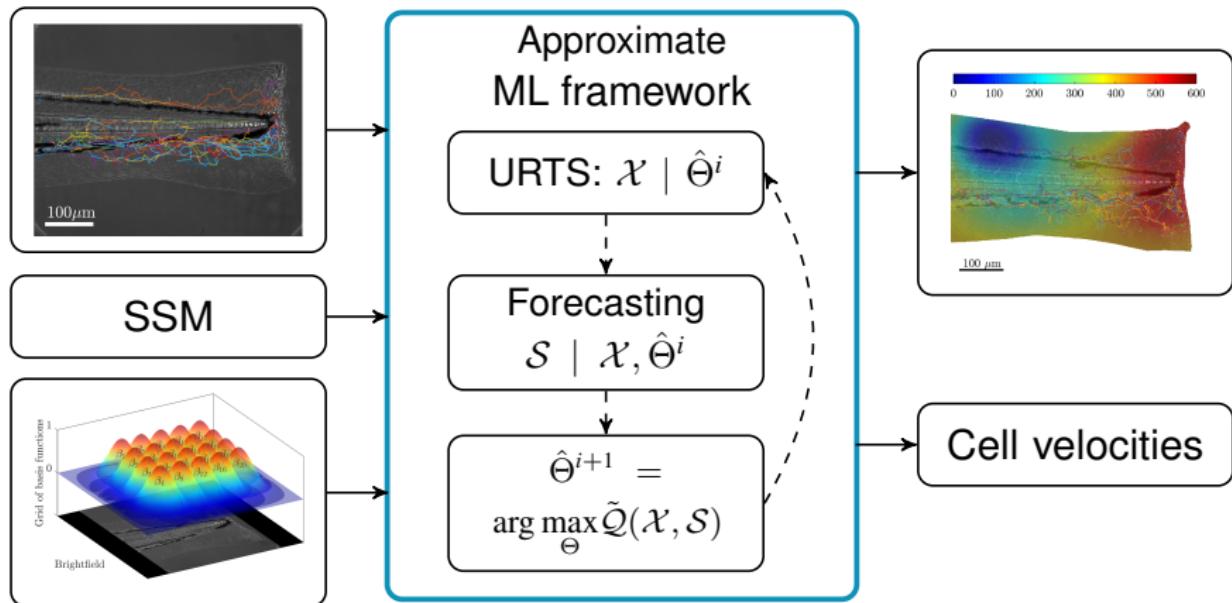
where  $\boldsymbol{x}_t^k = [s_x, s_y, v_x, v_y]^\top$ ,

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

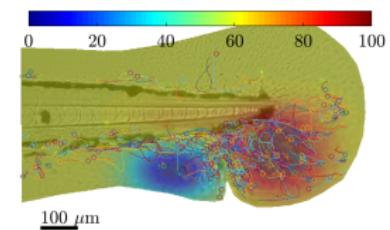
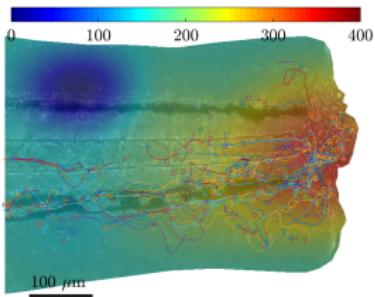
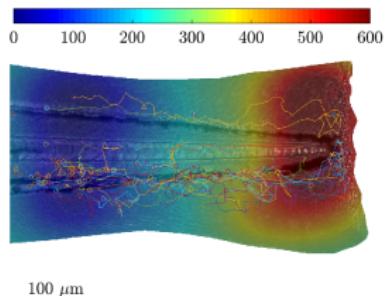
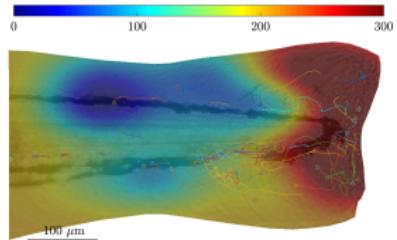
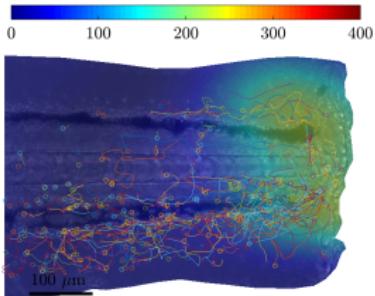
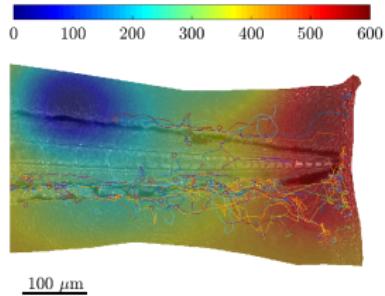
$$A = \begin{bmatrix} \mathbb{I} & T\mathbb{I} \\ \mathbb{O} & (1 - T\rho)\mathbb{I} \end{bmatrix}; B = \begin{bmatrix} \mathbb{O} \\ T\mathbb{I} \end{bmatrix}; G = \begin{bmatrix} \mathbb{O} \\ T\mathbb{I} \end{bmatrix}; C = [\mathbb{I} \quad \mathbb{O}].$$



# ML estimation with missing data



# Inferred chemoattractant concentration



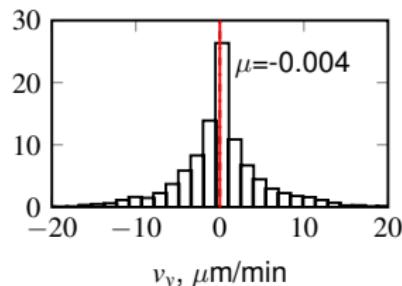
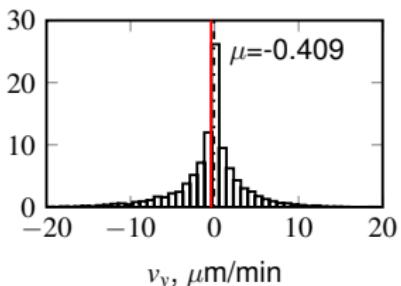
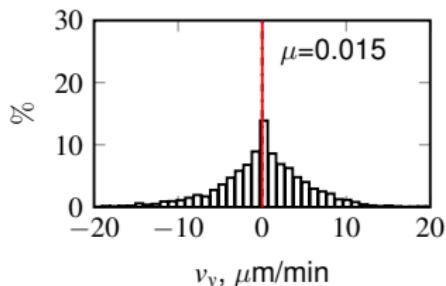
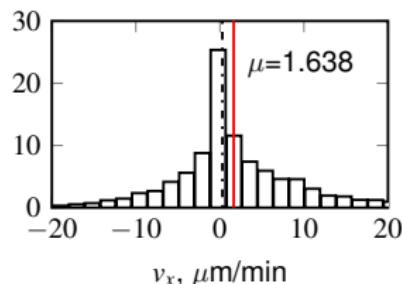
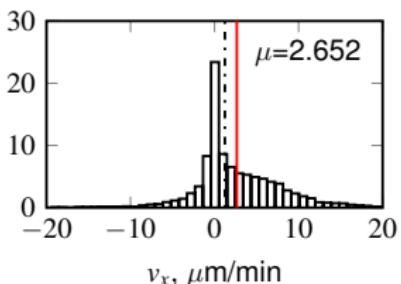
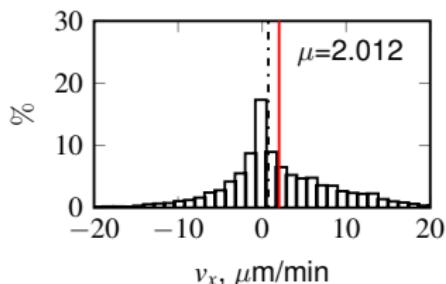
a) normal injury  
(6 datasets)

b) severe injury  
(2 datasets)

c) mild injury  
(6 datasets)



# Estimated cell velocities



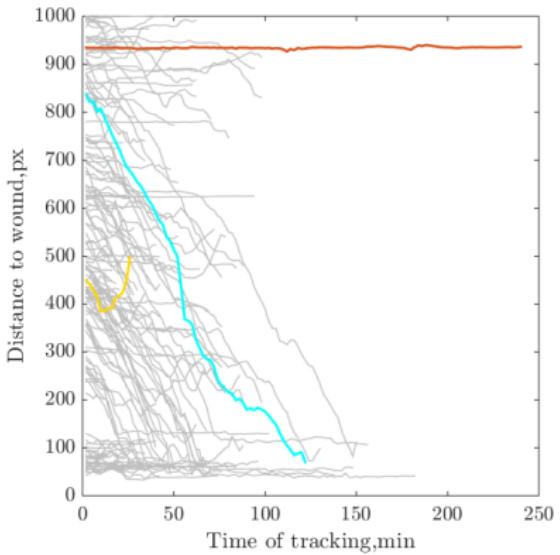
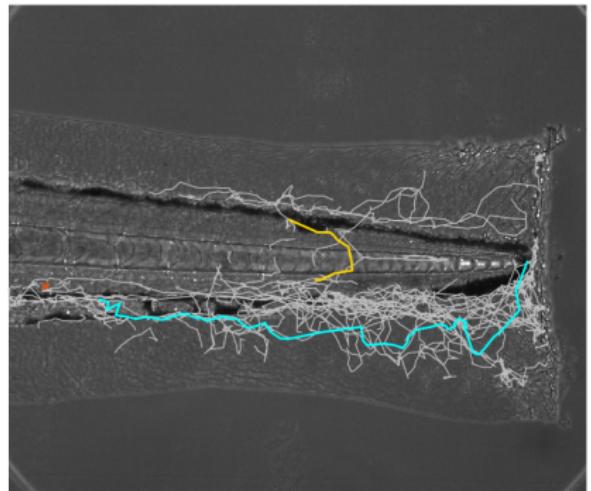
a) normal injury

b) severe injury

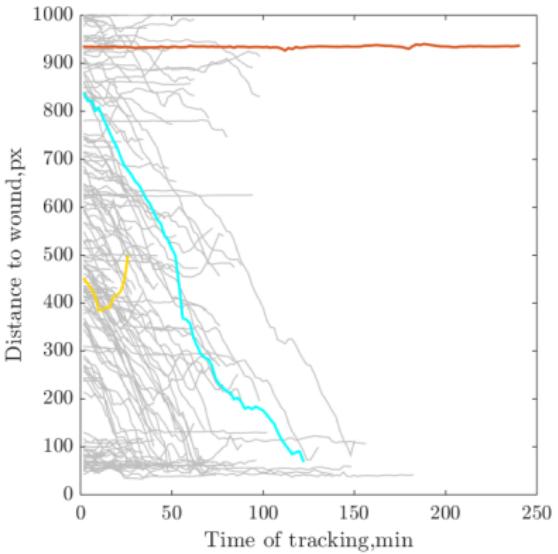
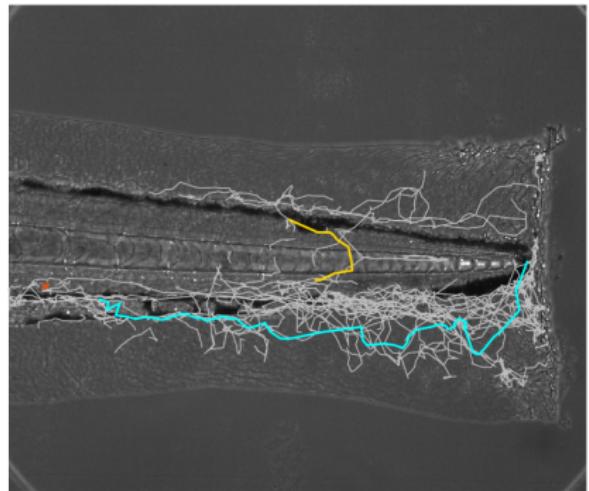
c) mild injury



# Heterogeneous cell behaviour



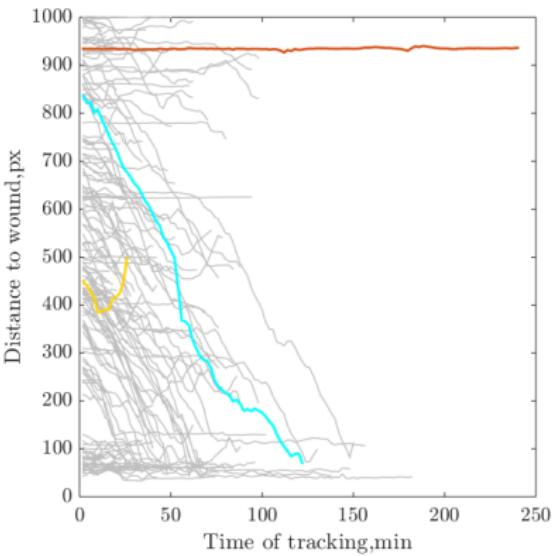
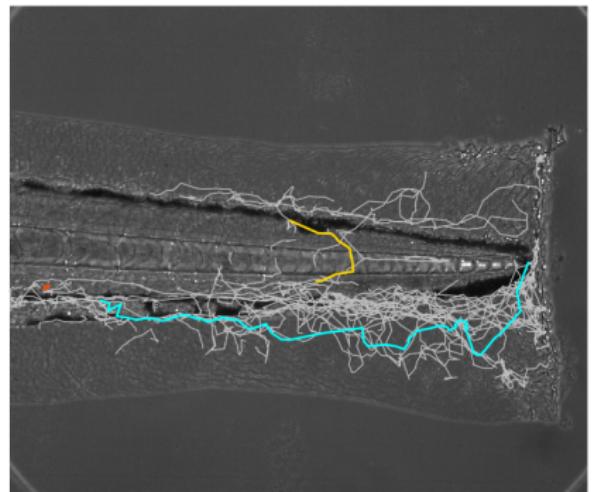
# Heterogeneous cell behaviour



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.



# 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.



# Jump Markov system

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

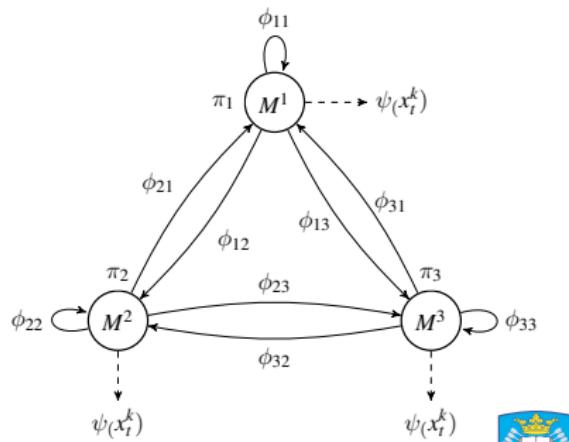
Cell modes:

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

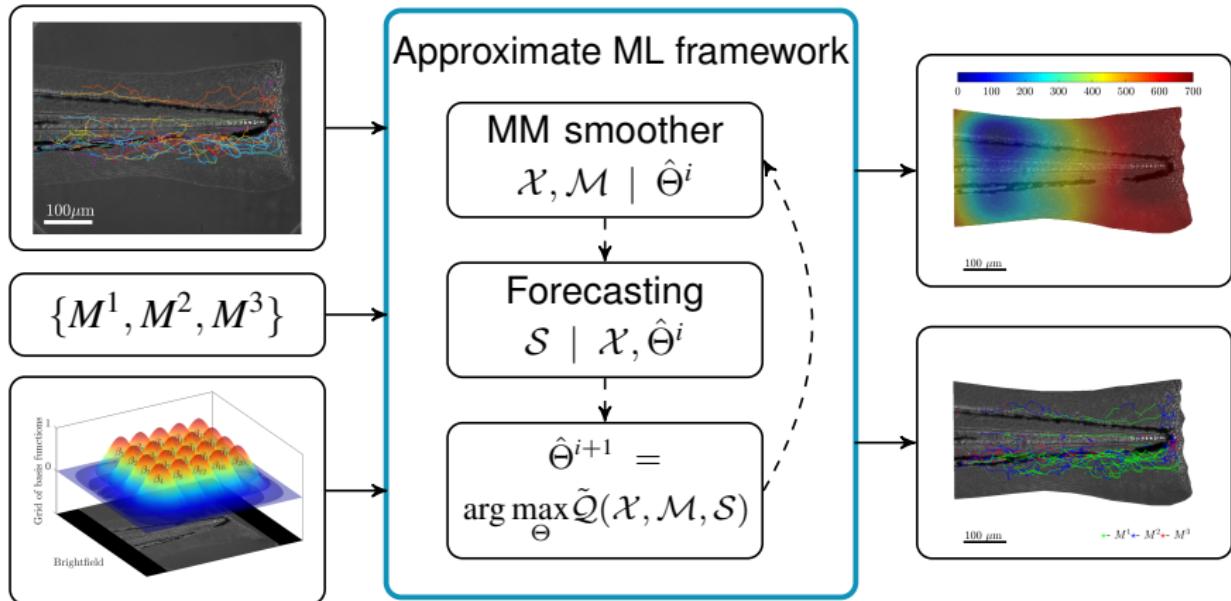
$$M^2 : A = \begin{bmatrix} \mathbb{I} & T\mathbb{I} \\ \mathbb{O} & (1 - T\rho(M^2))\mathbb{I} \end{bmatrix} B = \begin{bmatrix} \mathbb{O} \\ \mathbb{O} \end{bmatrix}$$

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

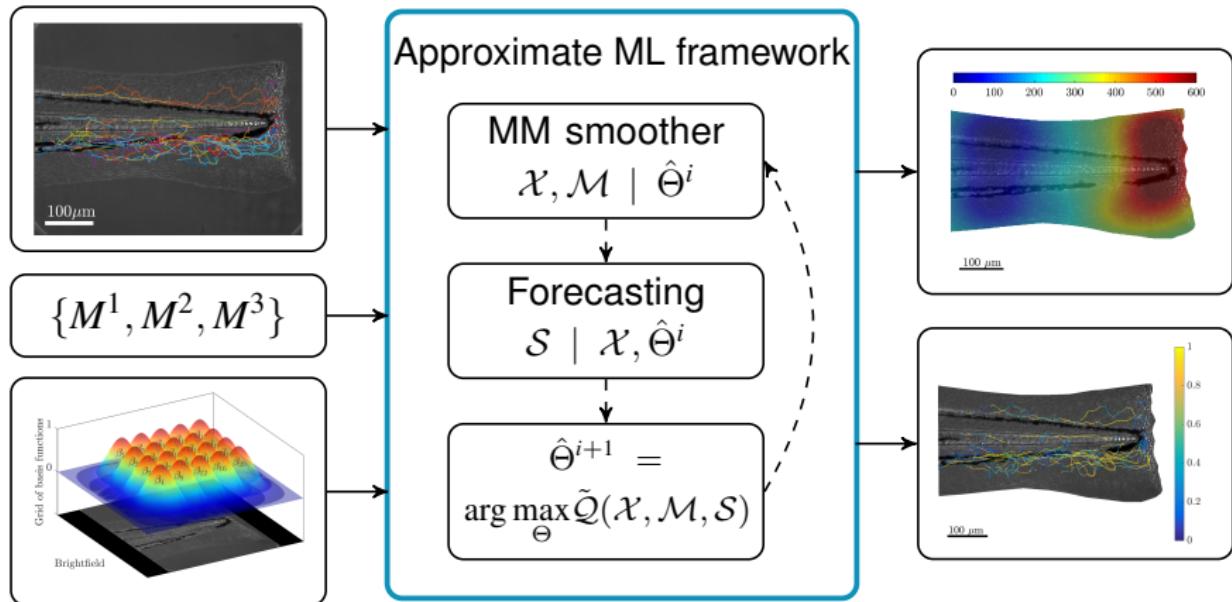
$$Q(M^3) \ll Q(M^1), Q(M^2).$$



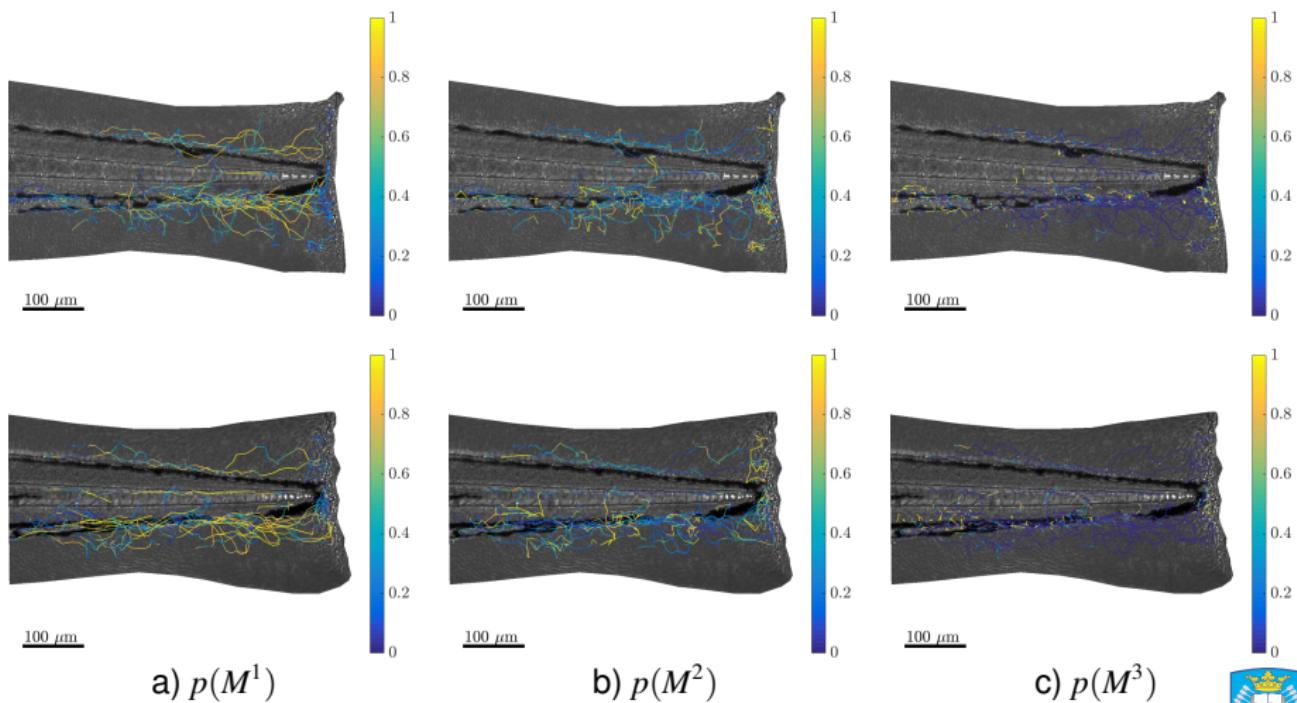
# Inference framework



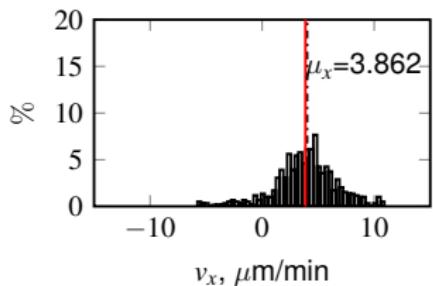
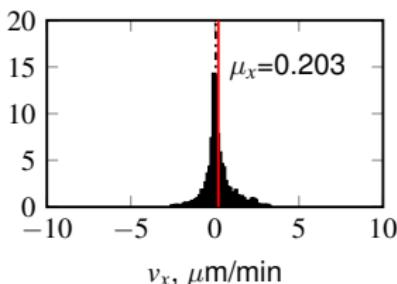
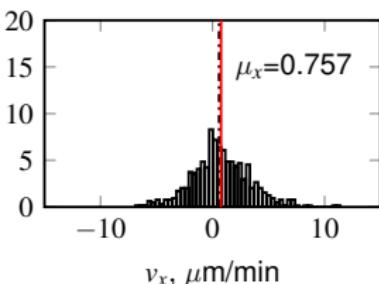
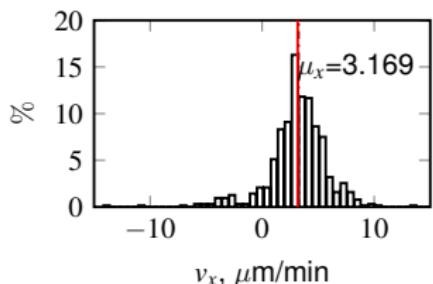
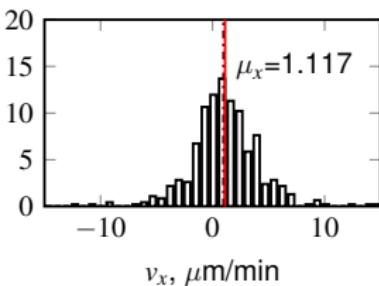
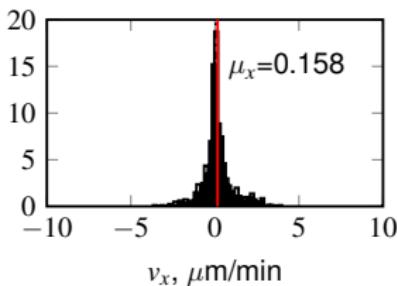
# Inference framework



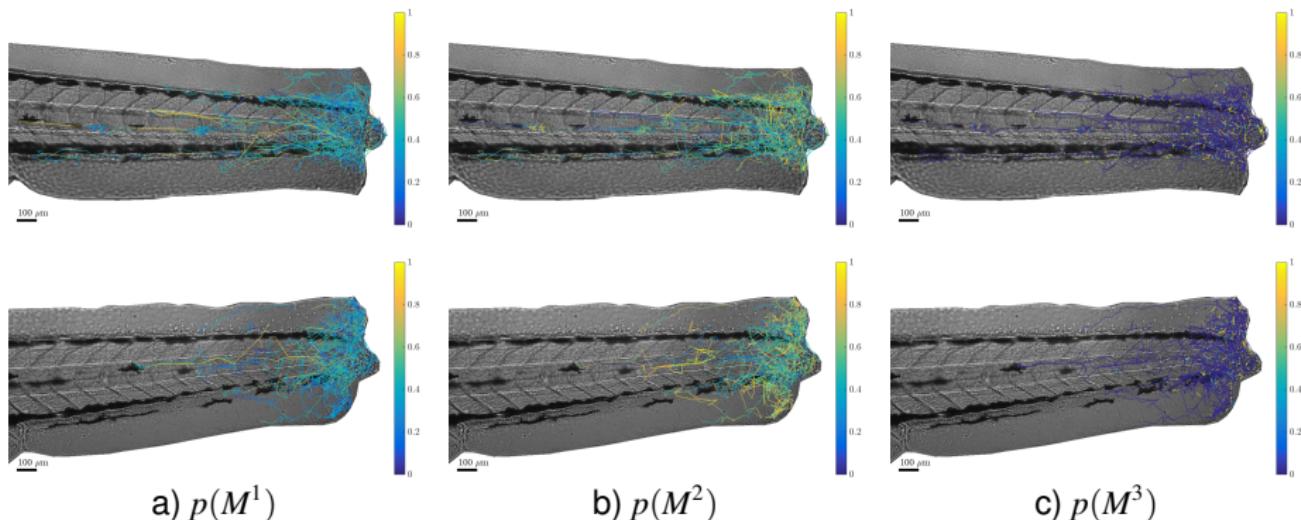
# Migratory modes - normal injury



# Estimated cell velocities

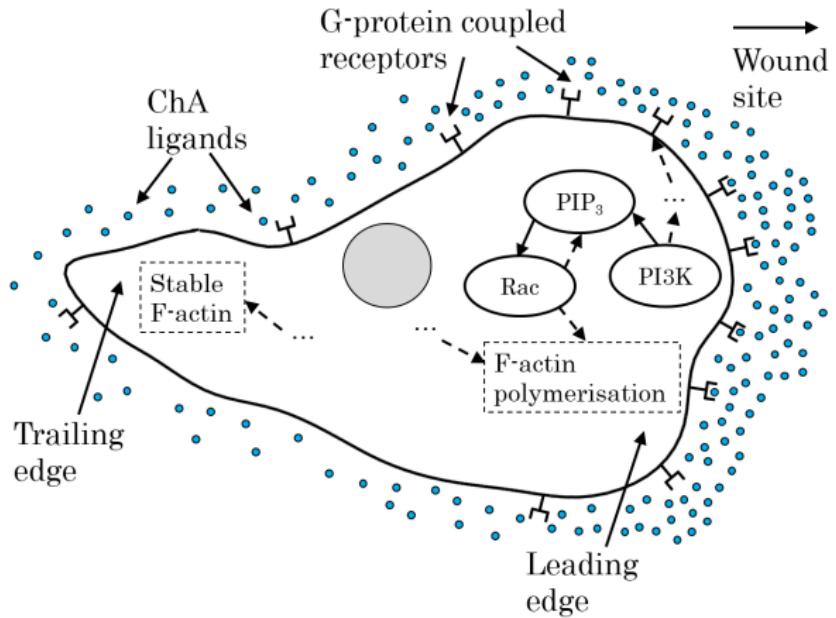
a)  $M^1$ b)  $M^2$ c)  $M^3$ 

# Reverse migration

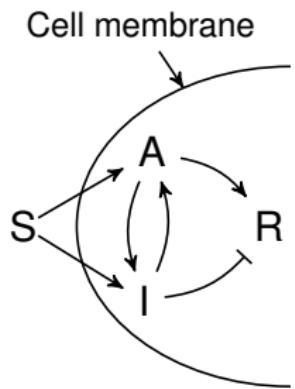
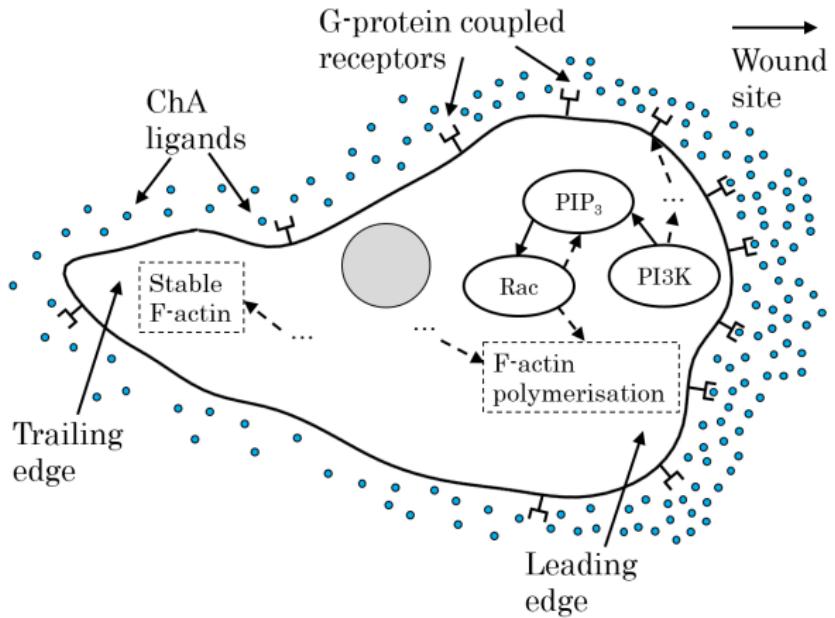


For 4 datasets there is higher probability of neutrophils diffusing away from the wound.

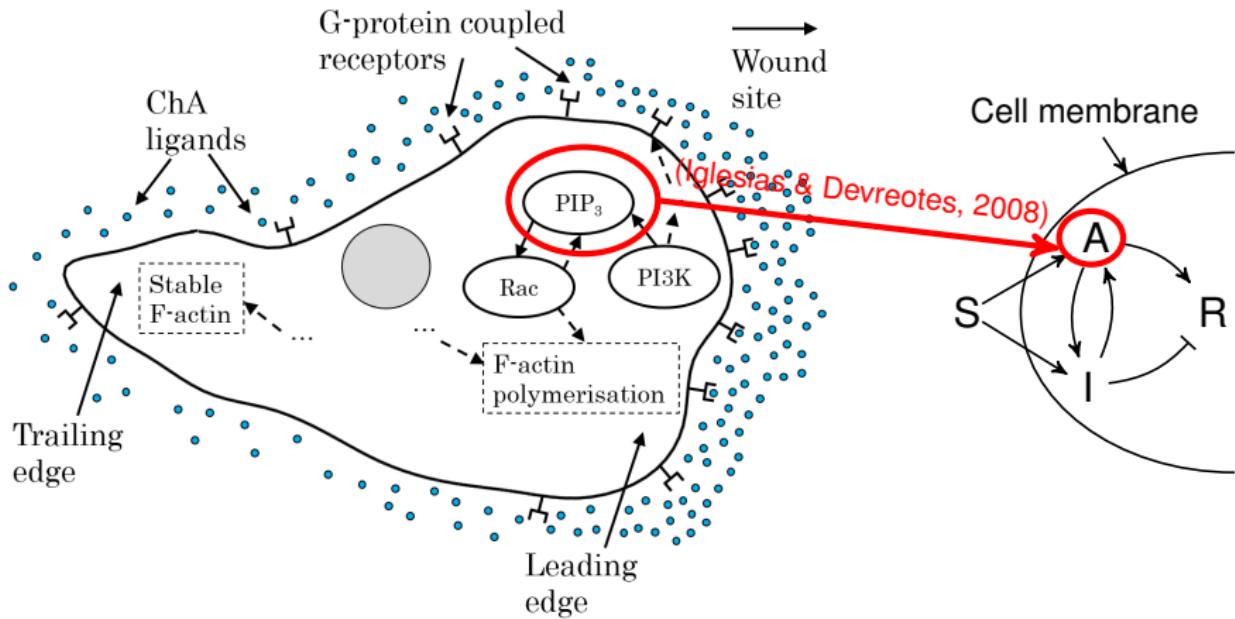
# Signal to migration



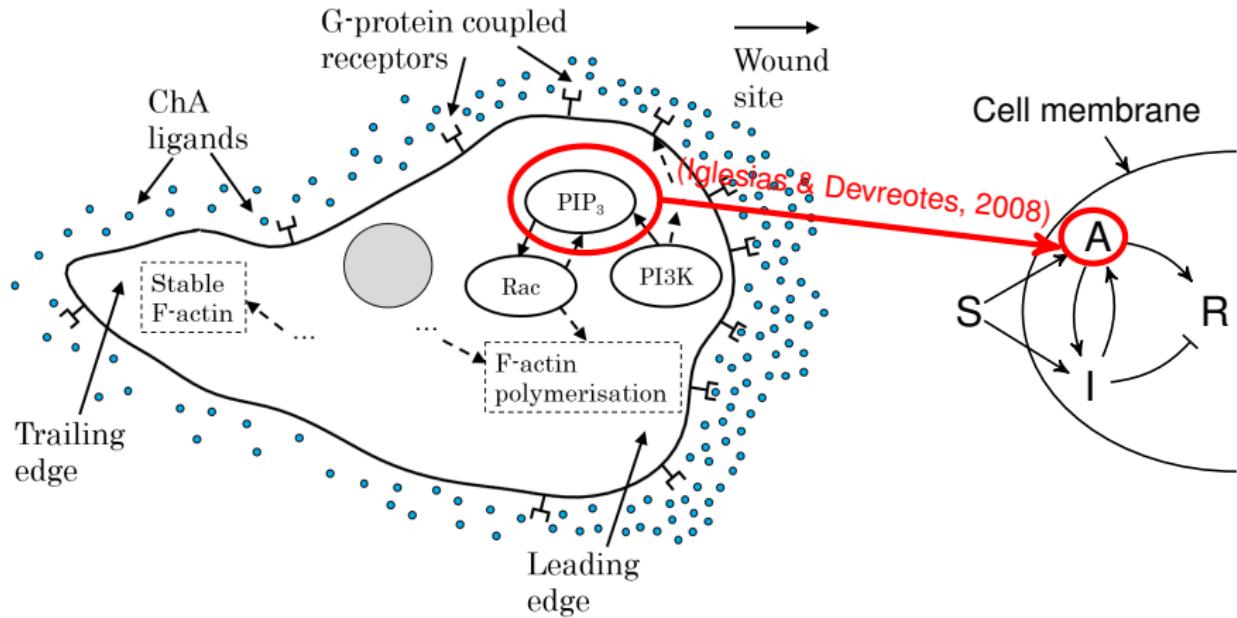
# Signal to migration



# Signal to migration



# Signal to migration



Does PIP<sub>3</sub> activate pseudopod growth in migrating neutrophils?



# Defining assumptions

- PIP<sub>3</sub> is the only activator regulating cell membrane protrusions.
- The integrated fluorescence intensity obtained from the imaging data is proportional to the local PIP<sub>3</sub> 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  $\Gamma_t$ . 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:

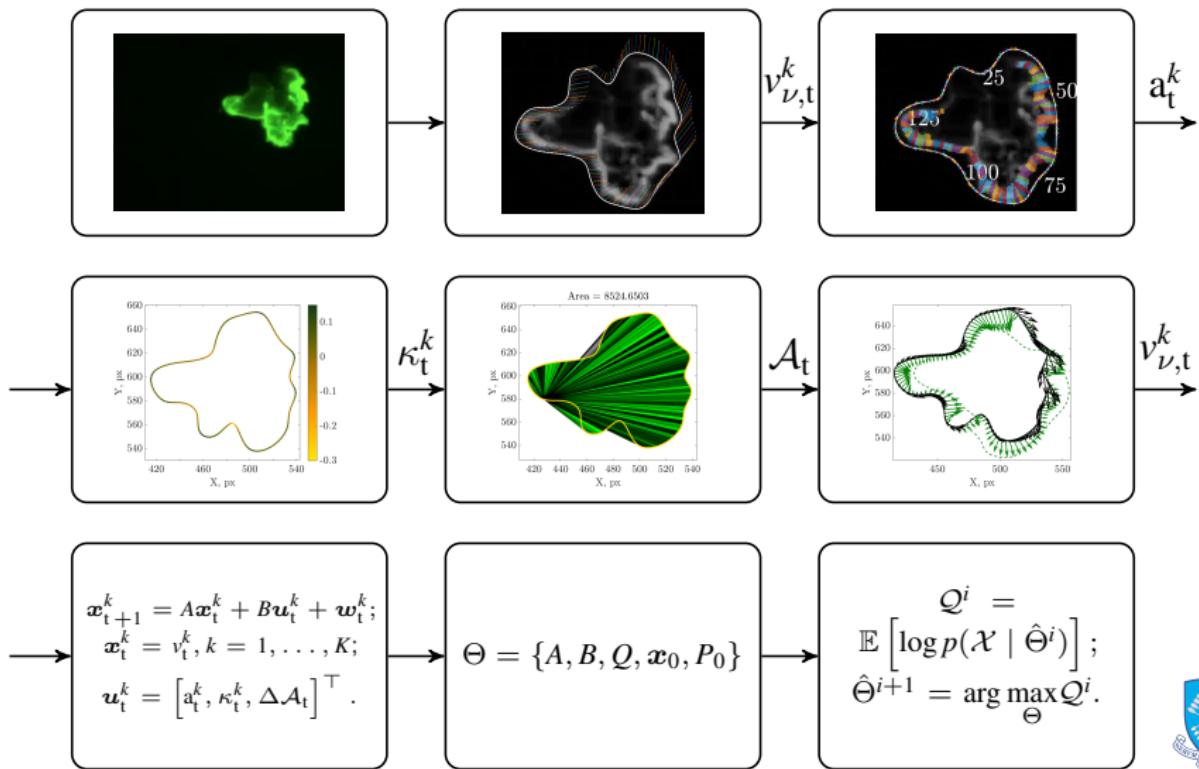
$$\mathcal{F}_{\text{vol}} = \alpha_{\text{vol}} \Delta A_t.$$

- **Viscous force** opposes cell motion:

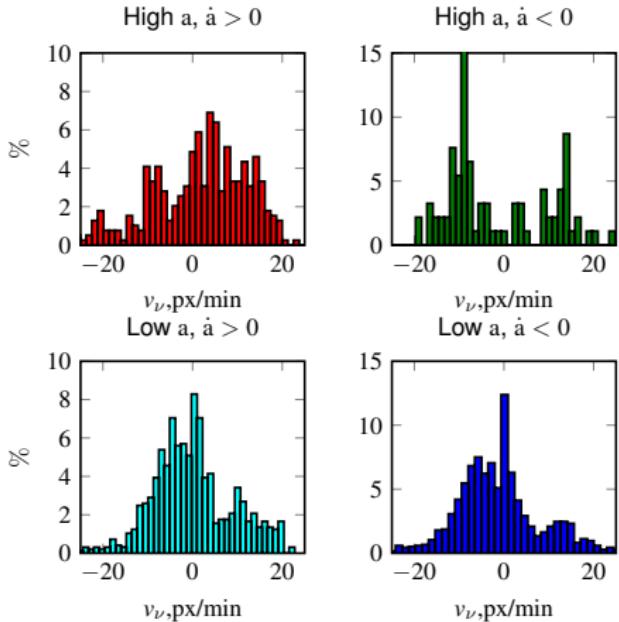
$$\mathcal{F}_{\text{visc}} = -\alpha_{\text{vv}} v_t^k.$$



# Image processing framework



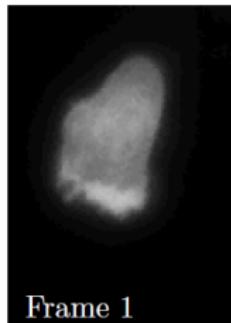
# Motile cells observed in vivo



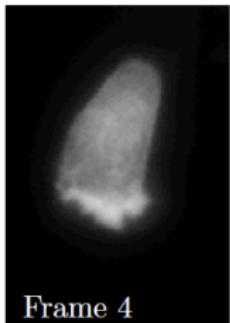
- Very weak correlation between  $a_{t-1}^k$  and  $v_t^k$  for all cells;
- Mann-Whitney test results: higher concentrations of PIP<sub>3</sub> correspond to accelerated protrusion growth.
- Results in agreement with recent knock-out studies.



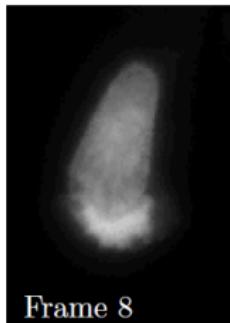
# Polarised cell observed in vitro



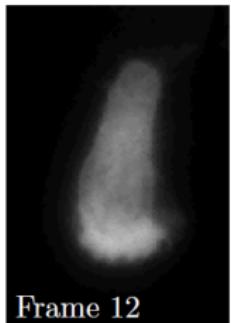
Frame 1



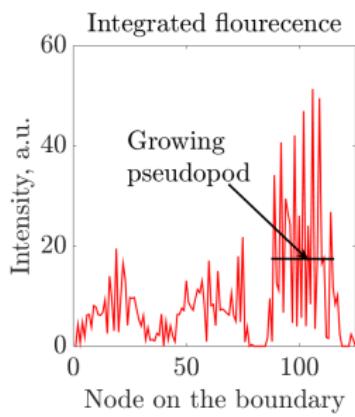
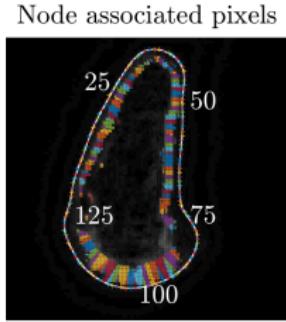
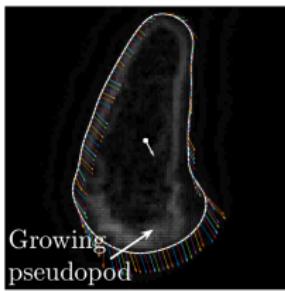
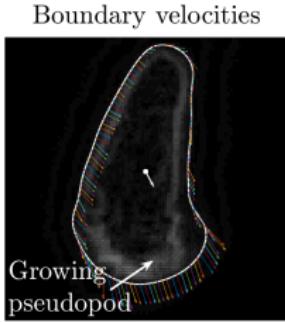
Frame 4



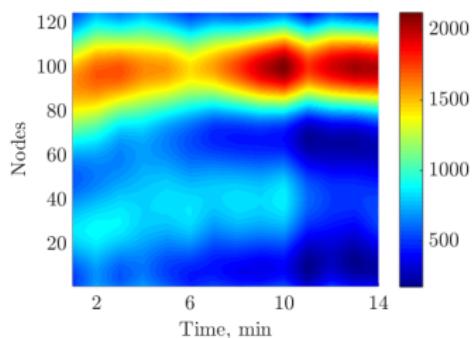
Frame 8



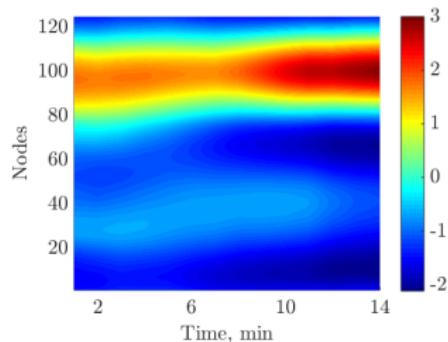
Frame 12



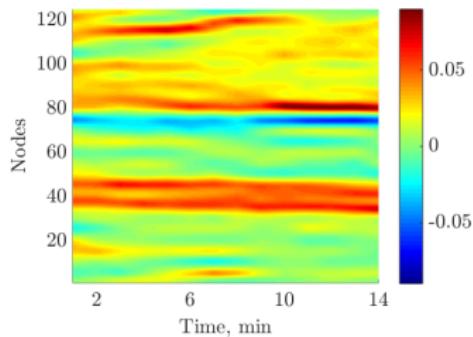
# Polarised cell observed in vitro



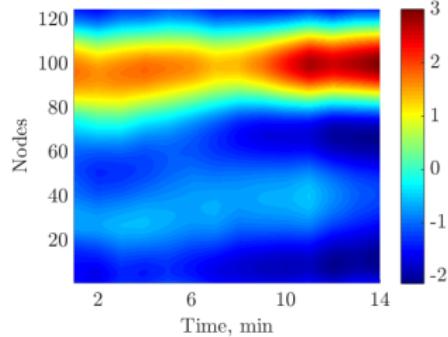
a) Smoothed intensity.



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  $\text{PIP}_3$  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?

