4. Semester report

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Title: *Computer simulations of neutrophil swarming and phagocytosis at various stages of inflammatory events*

1. Introduction

Our research aims at modelling and simulating two distinct but biologically crucial processes in innate immunity: neutrophil swarming and phagocytosis.

Regarding neutrophil swarming, video recordings – [1] for instance – show the migration of neutrophils migration towards sites of injury. While previous studies have proposed hypotheses and conjectures to explain the causes of (or the parameters that influence) these observations, there remains a lack of quantitative frameworks to rigorously assess or complement those claims. A key feature of swarming is the relay-like propagation of chemoattractant signals through neighbouring cells, a phenomenon that has been established. However, the emergence mechanisms underlying this collective signalling behaviour remain poorly understood.

With respect to cell shape dynamics during phagocytosis, mechanistic analyses of single-live-cell encounters with microbes are scarce in the biomedical literature. This shortage can be attributed, among others, to methodological limitations of traditional single-cell experiments. Nevertheless, previous studies made it clear that localised forces strongly alter the shape of cell membranes from inside [3]. However, it remains challenging to design a minimal system of those forces which explains the morphological dynamics observed during phagocytosis.

2. Summary of research work carried out in the previous three semesters

2.1.Neutrophil swarming

We started by developing an early 2D model to simulate diffusion equation with sources and sinks on a lattice grid using the finite difference methods to numerically solve the diffusion equation.

Subsequently, we extended the model to include a more biologically realistic source term that captures ligand secretion dynamics by cells, as well as the effect of an intracellular inhibitor that modulates ligand production. This improved model was implemented in 3D using the finite volume method, which ensures mass conservation by design.

Alongside the modelling work, we studied several 2D multi-particles tracking tools to extract quantitative data from experimental videos provided by biologists. Those extractions are crucial for validating our model. Among those tools are CellProfiler, Cell Tracker GNN and TrackMate7 combined with Cellpose.

Cell dynamics was modelled following an agent-based approach where, at each time step, cell positions are updated considering three key factors: (i) the persistency of the motion, (ii) stochastic fluctuations and (iii) a chemotactic force which attracts cells in the direction of increasing gradients. In particular, the amplitude of the latter is considered to be proportional

to the ratio between of the chemoattractant gradient and the local concentration sensed by the cell [4].

In addition to qualitative visualisation of the simulations, we performed quantitative analyses to enable objective comparison between simulation results and experimental data. Metrics include the well-known velocity autocorrelation function; a custom-defined "directionality" index, which measures a cell's ability to keep a directional movement towards the wound; the pairwise velocity correlation; and the time-lagged pairwise velocity correlation.

2.2.Cell shape dynamics during phagocytosis

We have reproduced the engulfment of a microbe by a phagocyte by modelling the energetic aspects of the process using the Cellular Potts Model [6], implemented via CompuCell3D [5]. In the specific case of a small, spherical microbe, we identified and characterised a two-phase parameter space distinguishing between complete and incomplete engulfment. Notably, we observed qualitative similarities between the energy landscape associated with this process and those previously reported in full engulfment scenarios during cell segregation.

3. Description of research work carried out in current semester

During the current semester, our efforts were focused on the neutrophil swarming project. We derived the dimensionless form of the equations governing the dynamics of chemoattractant, the intracellular inhibitor, and the cell themselves. This reduction transformed a system of eight independent parameters into one characterised by four dimensionless parameters, thereby, simplifying the analysis and enabling scaled comparison across regimes.

The resulting dimensionless model depends on the following parameters:

- α_c: the ratio of the timescale for ligand c1 production compared to its decay timescale.
- α_R: the ratio of the timescale for inhibitor R1 production compared to its decay timescale.
- β: the ratio of the timescale for ligand c1 production compared to the timescale for inhibitor R1 production.
- γ: the ratio describing how far the concentration c1 exceeds the threshold c2.

To illustrate the effect of parameter variation, Figure 1 presents snapshots from two simulations at the same final time step. In both cases, N = 300 cells (depicted as green dots) migrate in response to a diffusive chemoattractant. Initially, chemoattractant is concentrated at the centre of the medium, with none elsewhere. The colour intensity represents the level of concentration.

The only difference between the simulations is the value of α_R. On the left, cells produce R1 quickly, leading to accumulation of inhibitor. This suppresses their chemoattractant production, leading to loosely clustered cells. On the right, the inhibitor decays faster than it is produced, preventing its accumulation. As a result, cells maintain chemoattractant secretion, leading to tighter swarm and a slightly larger radius of recruitment.

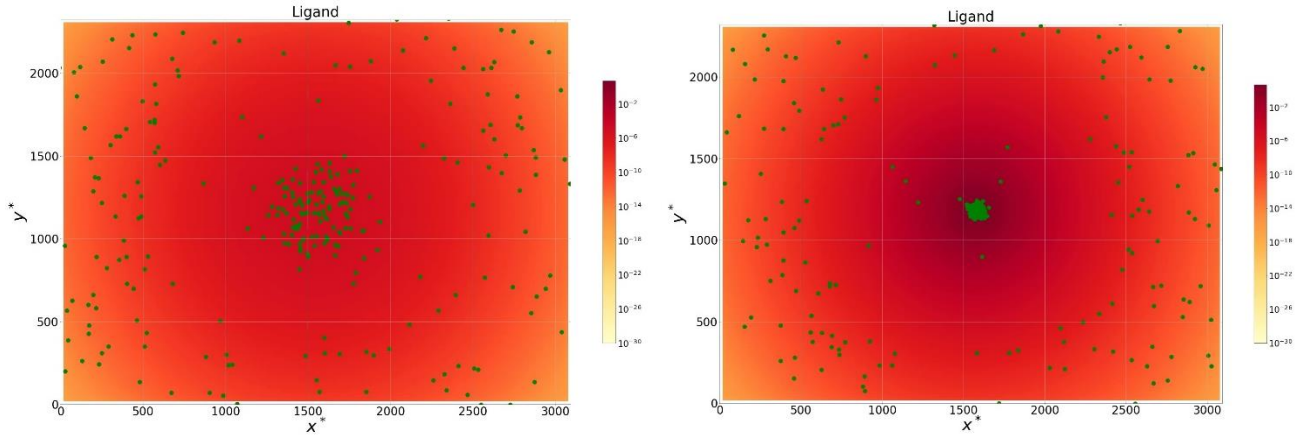

Figure 1: Simulation of neutrophil swarming. Left: α_R = 0.1. Right: α_R = 10.1

We also analysed experimental data using the previously defined metrics. Figure 2 and Figure 3, derived from the same experiment, offer two complementary perspectives of the directionality index.

In this experiment, photoactivation of the chemoattractant was triggered at the 20th minute, simulating a localised injury. To capture spatial heterogeneity in cell behaviour, the directionality index was computed per radial band, grouping cells according to their distance from the site of injury.

Both figures reveal key features of the response: the radius of attraction, and the magnitude of the directional motion exhibited by the cells.

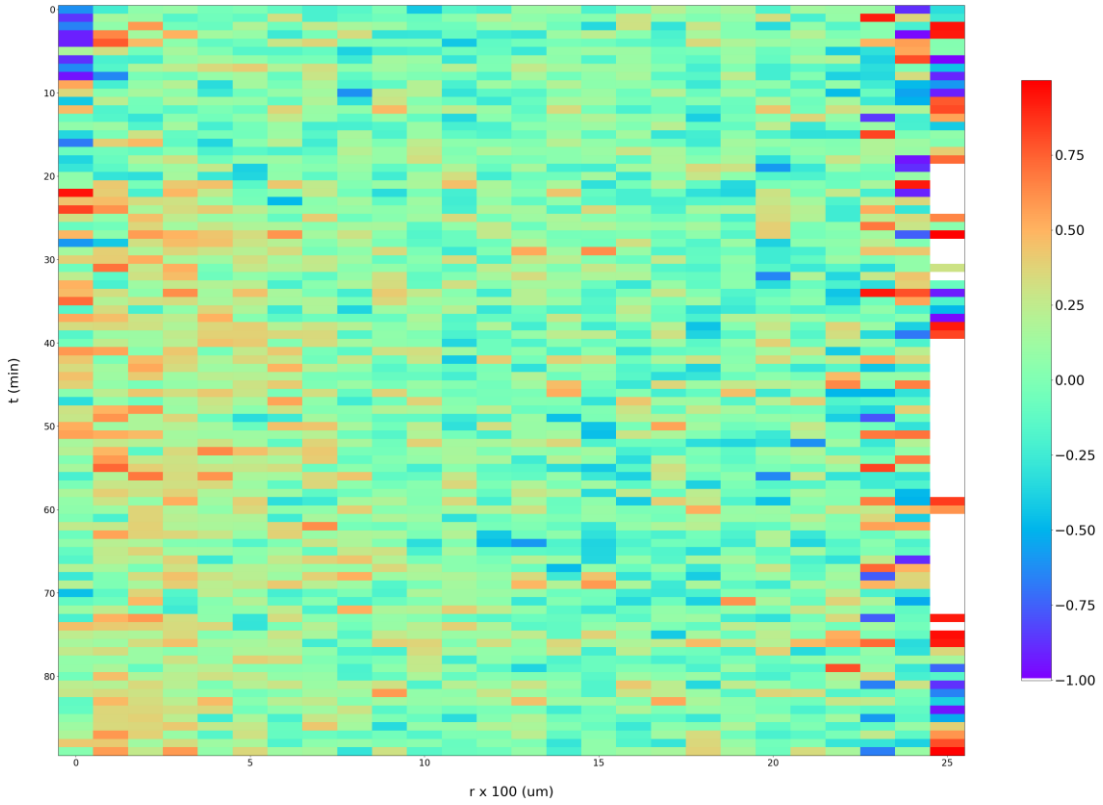

Figure 2: Directionality index from one experiment

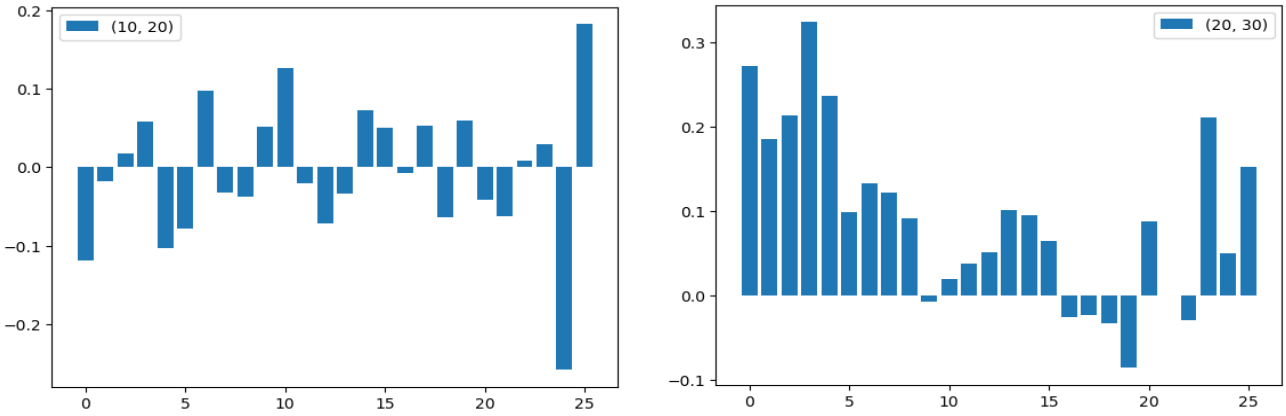

Figure 3: Evolution of the directionality index in time. Left: 10 minutes before photoactivation, Right: 10 minutes after photoactivation

4. Studies in current semester and before

2024/25 Spring semester

- **BIO/07/58** – Data analysis in neurophysiology, *Grade 5*

2024/25 Autumn semester

- **FIZ/3/084** – Data Mining and Machine Learning, *Grade 5*
- **BIO/07/16** – Modelling in Neurobiology, *Grade 5*
- **biophys2f20ex** – Biophysics II, *Grade 5*
- **bioinfub22em** – Bioinformatics L, *Grade 5*
- **bioinfub17gm** – Bioinformatics PR, *Grade 5*

2023/24 Spring semester

- **FIZ/3/071E** – Molecular and biophysical mechanisms of cell motion, *Grade 5*
- **FIZ/3/063E** – Graphs in the bioinformatics, *Grade 5*
- **BIO/06/11E** – Receptors, signalling, cell-cell communication, *Grade 3*
- **biophys1f20ex –** Biophysics I, *Grade 5*

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- **FIZ/3/003** – Statistical physics of biological systems, *Grade 4*
- **FIZ/3/021** – Statistical physics of polymers and membranes, *Grade 5*
- **BIO/3/6 –** Immunology of infections, *Grade 4*

5. Conference participations during the doctoral studies

In November 2023, I attended the conference MIFOBIO (Functional Microscopy for Biology) in Presqu'iles de Giens, France. The conference focused on the acquisition and processing of biological images.

An abstract entitled *"Cell Shape Dynamics of Phagocytes During Phagocytosis"* that I co-authored with Elod Mehes and Tamas Vicsek was accepted for poster presentation at the European Phagocyte Workshop, held in Visegrád, Hungary, from 20–23 March 2024. I attended the workshop and presented the poster during the event.

References

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2. Evelyn Strickland, Deng Pan, Christian Godfrey, Julia S. Kim, Alex Hopke, Wencheng Ji, Maureen Degrange, Bryant Villavicencio, Michael K. Mansour0, Christa S. Zerbe1, Daniel Irimia, Ariel Amir, Orion D. Weiner. Self-extinguishing relay waves enable homeostatic control of human neutrophil swarming. Developmental Cell, Volume 59, Issue 19, (2024)
3. Vutukuri, H.R., Hoore, M., Abaurrea-Velasco, C. et al. Active particles induce large shape deformations in giant lipid vesicles. Nature 586, 52–56 (2020)
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5. M. Swat, Gilberto L. Thomas, Julio M. Belmonte, A. Shirinifard, D.Hmeljak, J. A. Glazier. Multi-Scale Modeling of Tissues Using CompuCell3D. Computational Methods in Cell Biology, Methods in Cell Biology 110: 325-366 (2012).
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