NEUTROPHIL CHEMOTAXIS IN MULTIPLE CHEMOATTRACTANT GRADIENTS

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THESIS

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Abstract

Chemotaxis, the phenomenon in which cells move in response to extracellular chemical gradients, plays a prominent role in the mammalian immune response. During this process, a number of chemical signals, called chemoattractants, are produced at or proximal to sites of infection and diffuse into the surrounding tissue. Immune cells sense these chemoattractants and move in the direction where their concentration is greatest, thereby locating the source of attractants and their associated targets. Leading the assault against new infections is a specialized class of leukocytes (white blood cells) known as neutrophils, which normally circulate in the bloodstream. Upon activation, these cells emigrate out of the vasculature and navigate through interstitial tissues toward target sites. There they phagocytose bacteria and release a number of proteases and reactive oxygen intermediates with antimicrobial activity.

Neutrophils recruited by infected tissue in vivo are likely confronted by complex chemical environments consisting of a number of different chemoattractant species. These signals may include end target chemicals produced in the vicinity of the infectious agents, and endogenous chemicals released by local host tissues during the inflammatory response. To successfully locate their pathogenic targets within these chemically diverse and heterogeneous settings, activated neutrophils must be capable of distinguishing between the different signals and employing some sort of logic to prioritize among them. This ability to simultaneously process and interpret multiple signals is thought to be essential for efficient navigation of the cells to target areas. In particular, aberrant cell signaling and defects in this functionality are known to contribute to medical conditions such as chronic inflammation, asthma and rheumatoid arthritis.

To elucidate the biomolecular mechanisms underlying the neutrophil response to different chemoattractants, a number of efforts have been made toward understanding how cells respond to different combinations of chemicals. Most notably, recent investigations have shown that in the presence of both end target and endogenous chemoattractant variants, the cells migrate preferentially toward the former type, even in very low relative concentrations of the latter [1]. Interestingly, however, when the cells are exposed to two different endogenous chemical species, they exhibit a combinatorial response in which distant sources are favored over proximal sources [2]. Some additional results also suggest that cells located between two endogenous chemoattractant sources will respond to the vectorial sum of the combined gradients. In the long run, this peculiar behavior could result in oscillatory cell trajectories between the two sources.

To further explore the significance of these and other observations, particularly in the context of physiological conditions, we introduce in this work a simplified phenomenological model of neutrophil chemotaxis.
In particular, this model incorporates a trait commonly known as directional persistence - the tendency for migrating neutrophils to continue moving in the same direction (much like momentum) - while also accounting for the dose-response characteristics of cells to different chemical species. Simulations based on this model suggest that the efficiency of cell migration in complex chemical environments depends significantly on the degree of directional persistence. In particular, with appropriate values for this parameter, cells can improve their odds of locating end targets by drifting through a network of attractant sources in a loosely-guided fashion. This corroborates the prediction that neutrophils randomly migrate from one chemoattractant source to the next while searching for their end targets [3]. These cells may thus use persistence as a general mechanism to avoid being trapped near sources of endogenous chemoattractants - the mathematical analogue of local maxima in a global optimization problem. Moreover, this general foraging strategy may apply to other biological processes involving multiple signals and long-range navigation.
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List of Abbreviations

fMLP        formyl methionyl leucylphenylalanine
IL8         interleukin 8
LTB4        leukotriene B4
C5a         complement component 5a
GPCR        G-protein coupled receptor
PIP3        phosphatidylinositol (3,4,5)-trisphosphate
PI3K        phosphoinositide 3-kinase
PTEN        phosphatase and tensin homolog
OU          Ornstein-Uhlenbeck
BCRW        biased correlated random walk
MSD         mean square displacement
List of Symbols

\( c \)  cell density
\( s \)  chemoattractant concentration
\( D \)  chemoattractant diffusivity
\( \mu \)  random motility coefficient
\( \chi \)  chemotaxis coefficient
\( \tau \)  persistence time
\( v \)  cell velocity
\( \theta \)  cell orientation
\( \theta_s \)  chemoattractant gradient orientation
\( \sigma \)  noise strength
\( \partial W \)  standard Brownian (Weiner) process
\( \alpha \)  magnitude of concentration at source
\( \beta \)  spread (variance) of gaussian source
Chapter 1

Introduction

1.1 Background

1.1.1 Summary of neutrophil chemotaxis

Neutrophils are a class of polymorphonuclear (PMN) leukocytes that form an essential component of the innate immune system in humans. They are the most abundant type of white blood cell in the body, accounting for over half (50∼70%) of the total leukocyte count in the blood. These specialized immune cells are particularly important during the acute (early) phase of inflammation, where they play a central role in immune surveillance and nonspecific host defense (Figure 1.1). A deficiency in neutrophils (neutropenia) or functional impairment of the cells is often associated with increased susceptibility to bacterial and fungal infections (as well as some cancers), thus attesting to the importance of these cells for basal immunity.

In the inactive state, neutrophils circulate in the bloodstream, continuously monitoring the body for foreign matter and signs of tissue damage. Upon activation by chemicals emanating from the site of an infection, they adhere (margination) and crawl through the vascular endothelium (extravasation), migrate through interstitial tissues toward the signal source (chemotaxis) and congregate around the target area to eliminate the infectious agents and cellular debris (Figure 1.2). Neutrophils employ a number of tactics to fight pathogens, including phagocytosis (ingestion), the release of soluble anti-microbials through degranulation and the generation of neutrophil extracellular traps (NETs). In addition to directly attacking microbes, these cells also recruit and activate other cells of the immune system by expressing and releasing their own cytokines, which in turn help to amplify the inflammatory reaction.

Neutrophils have an average diameter of 10∼15 micrometers in peripheral blood smears. To meet normal physiologic needs, a healthy adult produces roughly $10^{11}$ of these cells daily, which are dispatched into the bloodstream following a period of maturation in the bone marrow. Despite their prodigious rate of production, however, neutrophils actually have a shorter lifespan than other phagocytes in the immune system (e.g. macrophages and monocytes). The average circulating half-life of non-activated neutrophils is approximately twelve hours, while activated cells in extravascular tissues survive for only 1∼2 days. Some hypotheses suggest that the transient activity of these cells may be an evolutionary adaptation to suppress propagation of pathogens that parasitize phagocytes, since their prolonged presence may provide refuge to the viruses from other components of the body’s defenses.
Figure 1.1: Neutrophil granulocytes play a key role in the innate immune response. These white blood cells migrate to sites of bacterial ingress or tissue damage through the process of chemotaxis. Note that neutrophils have a distinctive neutral pink color under Wright’s stain, with a nucleus divided into 2~5 lobes. This cell is surrounded by red blood cells (erythrocytes).

In addition to the risk of harboring parasites, the neutrophil response itself can also be a double-edged sword - the microbicidal or cytotoxic agents produced by these leukocytes are in fact harmful to normal host tissues if produced excessively. An overactive neutrophil response is often the primary cause of chronic inflammation and scarring during wound healing, and functional disorders of neutrophils can sometimes lead to persistent or permanent tissue damage in the presence of inflammation. This is observed in a number of medical conditions including pulmonary emphysema, rheumatoid arthritis, gout and psoriasis. Proteinase activity is significantly elevated in chronic wounds such as these, creating a proteolytic environment that prevents the body’s repair processes. The short lifetime of neutrophils may thus help to limit the amount of collateral damage that may be caused during inflammation.

Although their effect is short-lived and occasionally even unfavorable, neutrophils are typically the first responders to any inflammatory event. These highly motile cells can be recruited to the site of an injury within minutes following trauma. This rapid response is made possible by efficient and accurate chemotaxis of the cells to target areas. Chemotaxis in neutrophils consists of the complex coordination of three processes: gradient sensing, polarization and motility. Unlike most chemotactic bacteria, neutrophils can detect spatiotemporal chemical gradients directly, resulting not only in chemokinesis (non-directional increase in activity due to the presence of chemoeffectors), but also true taxis in response to chemoeffector gradients. This ability is particularly well-suited for precise and adaptive tracking of small targets that may be motile or difficult to detect.

Gradient sensing in neutrophils is mediated by the activation of specific cell surface receptors, primarily heterotrimeric G protein-coupled receptors (GPCRs), which are evenly distributed along the plasma membrane. Chemoattractant binding to these receptors activates a complex network of interacting proteins, lipids, and small molecules. This signaling cascade leads to a symmetry-breaking event, also known as polarization, in which a number of regulatory proteins and lipids (initially distributed uniformly on the membrane or in the cytosol) are recruited to either the front or back of the cell. The differential localization...
Figure 1.2: (Figure from [4]) Inactive neutrophils circulate in the bloodstream, where upon detection of chemokines emanating from activated endothelial cells, they adhere and crawl through the blood vessel walls. The cells then undergo chemotaxis through interstitial tissues toward the site of infection to help eliminate infectious agents and cellular debris.

of these components produce morphologically and functionally distinct leading and lagging edges that both mimic and amplify the extracellular gradient. This internal polarity, characterized by an actin-rich lamella at the anterior end and a tail-like uropod at the rear, serves as a compass to orient the migrating cells.

The chemotactic signaling pathway in neutrophils culminates in activation of the cytoskeletal machinery that drives cell motion. The cytoplasmic events that characterize polarization dictate the alignment and re-organization of cytoskeletal components (specifically F-actin polymerization and actomyosin contraction) to dynamically alter the cell morphology. Neutrophil locomotion is not a process of swimming as with most chemotactic bacteria; these amoeboid cells typically translate through the continuous extension of pseudopods (lamellipodia), which allow transmission of motile force to solid substrates in the environment (e.g. extracellular matrix fibrils). This in turn allows the cells to crawl on two-dimensional surfaces or within three-dimensional structures.

1.1.2 Current limitations in modeling neutrophil chemotaxis

All signal transduction networks serve to relay environmental cues to the cell interior, where they may trigger various cellular responses. In particular, the chemotactic signaling network in neutrophils is very complex, quite likely involving over a hundred components. Like other eukaryotic signaling systems, this network is not arranged in a simple linear configuration, but is believed to involve a number of branched cascades and parallel pathways that lead to multiple functional outputs. Some elements in the network also participate in other regulatory functions in the cell, leading to additional difficulties in delineating the full pathway and identifying its components.

In particular, one of the biggest hurdles in deciphering chemotaxis at the intracellular level has been the lack of a complete description of pathway interactions in their full spatial context (within the cytosol and cell membrane). Spatial heterogeneity is a defining aspect of gradient sensing and polarization, but monitoring
Figure 1.3: In an activator-inhibitor model, polarization is achieved from the interplay between a local activator, which catalyzes its own production, and a global inhibitor. In the presence of a chemoattractant gradient (purple), a membrane-bound activator (red circles) recruits other activator molecules to nearby regions on the membrane via a positive feedback mechanism (blue arrows). Simultaneously, the membrane-bound activator also triggers the release of fast-diffusing inhibitor molecules. The inhibitors act in a long-range fashion to suppress activation on the opposite end of the cell (green lines). This results in the emergence of functional polarity.

these processes in realtime, especially for motile cells within controlled microenvironments, remains a difficult challenge even with current visualization technologies. As such, while many of the key proteins involved in gradient sensing and motility have been separately identified, the cell-scale mechanisms for polarization and directed cell motion remain poorly understood. Most existing models address particular aspects of the system or focus on specific molecular interactions, providing only limited insight into the overall process of chemotaxis.

To bridge the gap between these seemingly disparate models, several groups have worked toward formulating coherent functional strategies at the whole cell level (allowing spatial variability in component distributions), based on simplified representations of the pathway and experimental observations. These models attempt to explain how neutrophils generate amplified and sustained responses to external gradients of chemoattractant, in addition to how they adapt to different transient stimuli in uniform environments. For example, a mechanism proposed by Xu et al. implicates the reciprocal inhibition between actin and actomyosin in the maintenance of polarization in activated cells [5]. This model, however, does not provide an adequate explanation for how cell symmetry is initially broken.

A number of other models have also been proposed based on the Turing reaction-diffusion model of morphogenesis. Some of these models are based on a local excitation, global inhibition (LEGI) principle [6], in which receptor occupancy triggers both a fast, local excitatory signal and a slower, global inhibitory signal (Figure 1.3). Qualitatively, this mechanism can account for the observed gradient sensing response of most molecules that have been shown to localize to either the front (e.g. Ras, PI3K, PH domains, actin-binding proteins) or rear (e.g. PTEN, myosin) of a cell within a gradient. It also correctly captures the activation of certain proteins on the cell cortex during uniform stimulation of the cell. However, the LEGI model by itself cannot fully explain the switch-like behavior observed in the spatial distribution of PH (pleckstrin homology) domains, in which the rear of the cell does not show a discernible response.
An alternate model, known as the balanced inactivation model [7] shares some of the features of the LEGI mechanism, including the receptor-mediated production of two opposing signals - a local activator and global inhibitor. The main difference is the addition of a third component - a membrane bound inactivator that is mutually antagonistic to both second messengers - that induces a switch-like response to external gradients. The limitation of this model is the absence of a molecular substrate that fits this description; although a number of locally-generated inhibitors that can diffuse throughout the cytosol have been suggested, no such fast-diffusing molecules have yet been identified in neutrophils. Other recent models that combine LEGI mechanisms with autocatalytic reactions [8] or positive feedback loops [9] have also demonstrated bistable kinetics and symmetry breaking during polarization in the presence of gradients, but these models also suffer from similar issues.

Models based on known molecular interactions [10], such as "first hit" mechanisms [7] or "fast and slow positive feedback loops", have also been shown to correctly predict cell polarization in isotropic chemical fields, but these models are not able to adapt to rapid transient changes in the environment as observed in experiments. A more recent model, known as the adaptive control model, allows cells to detect spatial gradients and remain responsive to changes in the direction of the gradient after initial polarization. In this model, localized temporal sensing through pseudopods is linked to whole-cell integration of temporal information, and allows for successfully predictions of stochastic responses to the initiation of gradients. Again, the shortcoming of this model is that it does not explain the initial intracellular divergence of components.

Neutrophils are also known to exhibit remarkably high chemotactic sensitivities, capable of detecting extremely shallow gradients with as little as 1~2% differences in chemoattractant concentration between their leading and trailing ends. This observation has led to several proposed models in which amplification is achieved by strong positive feedback loops. Many of these models share some basic features, including a response that is locally controlled by receptor occupancy and a locally-generated diffusing inhibitor. Most importantly, this local response triggers a positive feedback loop in which the signaling readout enhances its own production either through autocatalytic effects, substrate delivery or inhibition of its own degradation. These models all achieve greater amplification than the basic LEGI mechanism, and the shape of the response becomes nearly independent of the original signal magnitude.

Rao and Onsum recently proposed a model for gradient sensing and spontaneous polarization that does not require a global inhibitor [11]. In this model, polarization is achieved by the switch-like activation of a coincidence circuit that requires both Ras (a small GTPase) and phosphatidylinositol-3-kinase (PI3K) to transmit a signal. This phase-separating circuit was able to reproduce experimental observations including the effect of F-actin inhibitors, and demonstrated that the known dynamics of Rho GTPase and PI3K activation are sufficient for both gradient sensing and polarization. While this paper provides an interesting re-examination of previous models, further evidence and experiments are needed to validate this claim.
In spite of constructive efforts to resolve the mechanisms governing chemotaxis in neutrophils, it is apparent that no single model yet provides a satisfactory account of the process in its entirety. As models have grown in size and complexity, their utility in forming accurate predictions has become increasingly limited. Moreover, researchers are confronted with the challenge of reconciling polarization mechanisms with cell morphology and motility, since these are also inherently coupled processes - while gradient sensing biases motility, cellular deformations also have an impact on the signaling, either because of temporal changes in chemoattractant concentration or because of changes in the membrane topology. However, at present, our rudimentary understanding of cytoskeletal mechanics precludes the construction of a comprehensive model that can take these factors into consideration.

1.2 Modeling chemotaxis on the macroscale

Due to the inherent complexity of the chemotactic signaling network in neutrophils, the details of the full pathway are purposefully omitted from this work. In lieu of the different microscale models listed above, we instead introduce a macroscale model to investigate neutrophil motion based solely on properties that can be directly observed under a microscope. These include measurable parameters such as cell velocity, orientation, turn angles and mean square displacement. Such a top-down deconstruction permits exploration of cell behavior by abstracting away the intracellular details, allowing us to make inferences about the internal properties without explicit knowledge of the mechanisms involved.

Existing macroscale models for chemotaxis have generally adopted one of two approaches - fine-grained "agent-based" methods, which are devised to capture motion at the level of individual cells, and more coarse-grained continuum models, which are typically used to describe population behavior in the limit of large cell numbers. By far, the most popular of the latter models is the celebrated Keller-Segel equation, a continuum model originally devised by Keller and Segel [12] in modeling the movement of bacterial slime molds. This partial differential equation (PDE) model was applied predominantly in early models of bacterial populations, and has since been used with success in modeling population migration behavior of different cells in a variety of situations.

Much of the success of the Keller-Segel model can be attributed to extensive studies using a number of analytical techniques, including traveling wave analysis, perturbation theory and numerical simulations. The results from such studies have been employed to compare model predictions with experimental findings, allowing for bounds to be set on model parameters. Mathematically, the generalized Keller-Segel model takes the form:

\[
\frac{\partial c}{\partial t} = \nabla \cdot (\mu(s) \nabla c) - \nabla \cdot (\chi(s)c \nabla s) + g(c, s) - h(c, s),
\]

\[
\frac{\partial s}{\partial t} = D \nabla^2 s - f(c, s),
\]
where \( c = c(x, t) \) is the density of the chemotactic cell population, \( s = s(x, t) \) is the chemoattractant concentration at time \( t \) and spatial position \( x \), \( \mu(s) \) is the cell diffusion coefficient, \( \chi(s) \) is the chemotactic coefficient, \( D \) is the diffusivity of the chemoattractant, \( g(c, s) \) and \( h(c, s) \) are functions describing cell growth and death, respectively, and \( f(c, s) \) is a function describing attractant degradation. Variants of the Keller-Segel have diverged primarily in their definitions for \( \mu, \chi, f, g \) and \( h \).

It is important to note that the Keller-Segel model was not originally derived on the basis of a fundamental description of individual cell movement. As a result, it inherently has no relevance to experiments at the single-cell level. Furthermore, independent applications have now revealed critical flaws in the model’s ability to predict certain observed population phenomena. Primary among these issues is the expected response to steep chemoattractant gradients; the model cannot predict that responses must asymptotically approach a limiting cell density flux as gradients become increasingly steep. This asymptotic limit has indeed been observed experimentally for both bacteria and neutrophils due to the finite rate of cell locomotion. Likewise, the omission of finite size effects is also problematic, as multiple cells can occupy the same point in space at any given time. In the asymptotic limit, these features allow cells to converge toward singularities with increasing speed, leading ultimately to finite-time blowup of the model.

Another phenomenon that cannot be captured accurately with the Keller-Segel model is the response of cells to temporal stimulus gradients. Although the effects of diffusion are considered, the equations do not originally support time variations in the concentration of the chemical source. In addition, there is no production by the chemotactic cells themselves (i.e. autocrine signaling), which has been implicated in some chemotactic processes. There is also no explicit mention of the dependence of the response on chemical concentration. In particular, the coefficients \( \mu \) and \( \chi \) need not be constants, and an \textit{a priori} basis for the functional form of their concentration dependence is desirable. And lastly, but perhaps most relevant to the purpose of this work, the model lacks support for multiple coexistent chemical gradients.

To remedy some of the shortcomings mentioned above, the Keller-Segel model has undergone several revisions since its initial formulation. Some of these updates have relied on empirical modifications in which specific cell responses were incorporated into the equations. Other efforts, most notably those developed by Patlak, Segel, Nossalt and Alt [12], have sought to explain the macroscale parameters in the model on the basis of individual cell behaviors, by re-expressing them using variables from single cell models. These and similar attempts have suffered mainly from the inability to accommodate all of the characteristics of the individual cell response. Owing to the many drawbacks associated with the Keller-Segel and similar continuum models for chemotaxis, this work will focus primarily on the characteristics of single cell motion and rely instead on agent-based models of chemotaxis.
Figure 1.4: To combat invading pathogens \textit{in vivo}, neutrophils must efficiently migrate from the vasculature to specific sites within infected tissues. Recruited neutrophils are likely to encounter a combination of multiple chemoattractants, including end target signals produced at or proximal to the pathogenic source (e.g. fMLP and C5a), and endogenous chemokines produced by the host immune response (e.g. IL-8 and LTB4). Successful navigation requires a mechanism for sensing and accurately interpreting this complex chemical environment.

1.3 Neutrophil chemotaxis \textit{in vivo}

Neutrophils perform chemotaxis toward a number of different chemicals including (i) peptides with formylated N-terminal methionine groups secreted by infecting microbes, such as formyl-met-leu-phe (fMLP) (ii) C5a, a glycoprotein fragment produced by the complement system; (iii) leukotriene B4 (LTB4), a product of phospholipid metabolism secreted by sentinel mast cells; and (iv) various chemokines that regulate immune cell trafficking such as interleukin 8 (IL-8 or CXCL8) and macrophage inflammatory protein 2 (MIP-2). Each chemoattractant binds a unique G protein-coupled receptor (GPCR) on the cell surface, which allows for independent detection of different chemicals and their gradients. Using this information, the cells are able to interpret their environment to effect migration in the appropriate direction (Figure 1.4).

Naturally, given the numerous chemoeffectors that have thus far been identified for neutrophils in physiological conditions, several groups have focused their attention on how cells respond to different combinations of chemicals. In particular, a number of studies have shown that neutrophils selectively migrate toward fLMP and C5a, even when opposing gradients of IL-8 and/or LTB4 are present in very high relative concentrations [3] (Figure 1.5). These results suggest that neutrophils are not only able to distinguish between chemoattractants, but also migrate preferentially toward those produced most proximal to infected sites. This apparent intracellular signaling hierarchy classifies chemoattractants as either endogenous (intermediary) species (such as IL-8 and LTB4) produced by affected host tissues or end target species (fMLP and C5a) that represent pathogenic sources. By favoring end target over intermediary chemicals, the cells can ensure efficient convergence on phagocytic targets in the midst of other distractions.
Figure 1.5: (Figure from Foxman et al. [2]) An under-agarose assay demonstrating the preferential migration of neutrophils toward end target chemoattractants. Cells originating in a well containing IL-8 or LTB4 (10 pmol) exhibit consistent migration toward fMLP. However, cells placed with fMLP (10 pmol) do not migrate toward the intermediary chemoattractants IL-8 and LTB4.

Figure 1.6: (Figure from Foxman et al. [2]) [Top images] In the presence of two inverse gradients of LTB4 and IL-8, neutrophils migrate away from one chemokine source toward the other [from the left, images 2 and 5] (almost as well as the control cells [images 1 and 4]). However, in the presence of opposing gradients of the same chemokine, the cells do not migrate well toward the distant source. [Lower image] Similar behavior is observed for two competing chemokine gradients without using under-agarose assays.

A number of theories have been proposed to outline the intracellular mechanisms that govern signal prioritization. One plausible explanation is that fMLP and C5a trigger cross-phosphorylation and desensitization of chemokine receptors [13], thus suppressing the response to intermediary chemoattractants. Several experiments indicate, however, that chemotaxis is not strongly affected by receptor phosphorylation. Other experiments have shown that receptors for fMLP are upregulated in response to IL-8, which causes downregulation of its own receptors [1], or that fMLP may cause downregulation of its own receptors in certain situations [1]. Moreover, some studies have demonstrated that neutrophils actually undergo fugetaxis, or negative chemotaxis, in response to IL-8 [14].

More recently, Heit et al. demonstrated that the different chemoattractant classes may in fact operate along two entirely distinct signal transduction pathways [1]. In particular, chemotaxis to the end target chemoattractants fMLP and C5a is thought to involve the p38 mitogen-activated protein kinase
Figure 1.7: (Figure from Foxman et al. [3]) The preferential motion of cells toward distant sources is observed for any permutation of the chemokines IL-8 and LTB4 placed in the two wells. Thus the observed cell response is statistically significant.

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(MAPK) pathway, whereas chemotaxis towards IL-8, LTB4 and MIP2 involves the phosphatidylinositol-3-OH (PI3K)/Akt/phosphatase and tensin homolog (PTEN) pathway. The study demonstrated that chemotaxis to fMLP and C5a is sensitive to inhibitors of MAPK, while chemotaxis to chemokines is sensitive to inhibitors of PI3K, thus indicating that MAPK signaling attenuates PI3K signaling. The crosstalk between these parallel pathways may involve PTEN; activation of the p38 MAPK pathway appears to direct PTEN to the entire circumference of the cell, where it is believed to antagonize the PI3K/Akt/PTEN pathway [15]. Some results have suggested, however, that chemotaxis in response to fMLP is equally abrogated by PI3K inhibitors, implying that PI3K may also have a more general role in the stability of the chemotactic response [16].

Of greater relevance to the focus of this thesis is the response of neutrophils to combinations of intermediary chemoattractants (chemokines). This subject was first explored by Foxman et al. through a series of controlled experiments using specialized under-agarose migration assays [2]. Neutrophils were initially placed in one of two chemokine-containing wells (Figure 1.6). The wells were filled with either the same chemokine (i.e. both IL-8 or both LTB-4) or different chemokines (i.e. one IL-8 and the other LTB4) to assess how the cells would migrate in response to the resulting dual gradients. The researchers observed that when both the distant and proximal wells contained the same type of chemokine, the net flux was directed toward the more proximal well, as one would predict given the higher concentration and steeper gradient. Interestingly, however, when the two wells contained different chemokines (i.e. in opposing gradients of IL-8 and LTB4), the cells were observed to migrate consistently toward the distant well, independent of the chemokine species.
Figure 1.8: (Figure from Foxman et al. [3]) Two chemoattractant source wells containing buffer, IL-8 or LTB4 are arranged in an equilateral triangle with a third neutrophil-containing well. Given a single chemoattractant source, the cells migrate toward that source only. Given two identical sources, the cells exhibit two fronts that each extend in the direction of the nearest source. However, in the presence of two different chemoattractant gradients, the cells migrate in a broad central front directed between the two sources, suggesting that the cells can vectorially integrate the gradient information.

This peculiar bias toward remote sources was also verified over a broad range of concentrations using other methods (Figure 1.6); in all cases, the cells were found to migrate down the local gradient in response to another chemokine gradient from a distant source (Figure 1.7). In separate analogous experiments, cells were initially placed in a separate well located at an offset from the chemical sources, such that the three wells were arranged in an equilateral triangle (Figure 1.8). In these settings as well, the neutrophils were observed to exhibit similar behavior; in particular, when two different chemokines were used, the cells appeared to respond to the vectorial sum of the combined gradients (toward the midpoint of the sources). Collectively, these results demonstrate that neutrophils are indeed capable of differentiating between different chemoattractant types. Moreover, when multiple chemokine species are present, the resulting chemotactic response appears to be combinatorial in nature; over longer times, this could imply that cells caught in the middle of two opposing gradients would migrate back and forth between the sources in an oscillatory manner.

Neutrophils navigating to a distant target in the body may often face the dilemma of having to migrate away from a local source in order to reach their destinations. From empirical observations, it is now apparent that the cells are indeed equipped to overcome such difficulties - however, the mechanistic basis of this response remains unclear. Foxman et al. proposed that the behavior could be attributed to a form of sensory adaptation, or that the receptor affinities were being actively modulated to become less sensitive to the proximal chemoattractant over time and, as a result, more sensitive to the distant one [3]. Several studies have provided evidence of this type of adaptation mechanism in neutrophils [18][19], which have already been established in many bacterial systems. Quite recently, Oelz et al. also re-examined the phenomenon using a mathematical model. They suggest that the behavior could be the result of a lag in the gradient sensing response - that is, the cells are incapable of rapidly adjusting their sensitivities to new signals, and are thus biased toward other sources in the vicinity [20].
Neutrophils exhibit biphasic dose response characteristics in which there exists an optimal concentration range for accurate gradient sensing. The vertical axis represents the percentage of cells that are oriented in the same half plane as the gradient direction, and may be interpreted as the accuracy of orientation (orientation bias). For this particular formyl peptide variant (FMMM), the optimum lies around $10^{-5}$ M, and is independent of the gradient steepness. The orientation bias is also a function of the steepness of the gradient - note that accuracy increases monotonically with increasing gradient.

Notably, the current hypotheses rely on purely temporal mechanisms to explain the observed response of cells; in particular, neither model gives significant consideration to the spatial effects that may influence how neutrophils respond to their environment. In this work, we present an alternative model in which cells exhibit a bias toward distant sources simply by virtue of their position in space. This spatial mechanism may help to explain how cells can robustly initiate the same response in different environments, regardless of their past trajectory or the amount of time they spend around a particular site. While it may not refute the possibility of the aforementioned mechanisms, this new model can provide deeper insight into all of the factors involved during this experimental scenario - additional work will certainly be needed to decouple and fully characterize both the spatial and temporal aspects of this process.

When a neutrophil translocates between two chemoattractant wells, the local chemical environment is changing constantly as a function of its position. Early experiments by Zigmond et al. have shown that the chemotactic bias of a cell toward a particular chemoattractant depends on the local concentration and the steepness of the gradient [17]. Specifically, neutrophils exhibit biphasic dose response characteristics (Figure 1.9); their sensitivity is optimal only within a certain concentration range. Thus, if the local concentration is either too high or too low, they are unable to accurately detect the gradient direction. Specifically, if the concentration is excessively high, the membrane receptors become saturated with the ligand, leading to a loss in sensitivity to additional receptor binding. This may therefore contribute to a spatial mechanism in which the cells become less sensitive to sources as they drift closer - if the concentration at the source is too great, the local value may actually exceed the threshold for optimal sensitivity. Given the right conditions, the cell is then more sensitive to the other chemoattractants at significantly lower concentrations.
Figure 1.10: (Figure from Foxman et al. [3]) Migrating neutrophils exhibit directional persistence, in which perceptible changes in direction only take place on the order of minutes. At any instant in time, migration is biased toward the forward direction, as indicated by the forward migration index (FMI). The FMI is defined as the ratio of net forward progress to the total path length of cell’s trajectory.

Another key factor in this proposed mechanism is a distinctive feature of neutrophil motility known as directional persistence. In the context of chemotaxis, persistence is defined as the propensity of migrating cells to continue to move forward in the same direction, much like the momentum of a massive moving object [21]. The exact cause of this phenomenon is unclear, though it may be due to an inherent latency in the communication between the gradient sensing and polarization mechanisms of the cell, that in turn introduces a lag in the readjustment of the cell compass. This form of directional memory has been documented extensively in the literature, particularly for cells migrating in uniform concentration fields (Figure 1.10). More recently, other experiments using microfluidic platforms have also demonstrated that persistence may play a role in chemoattractant gradients as well. By leveraging the ability to precisely control cellular microenvironments, they were able to show that neutrophils moving in the presence of a bell-shaped concentration profile will migrate up the gradient, but overshoot the peak by some distance [22] before they turn back around (Figure 1.11).

For primary human neutrophils, the time scale of persistence (the time between significant changes in direction) for cells in an isotropic concentration of fMLP is measured to be on the order of $3\sim5$ minutes. When measured on this time scale, the ratio of net displacement to total linear distance travelled (chemotactic index) is approximately $0.65 \,[\text{r}]$, indicating that the motion of the cell is correlated between consecutive time intervals. This confirms that cells maintain persistence of locomotion even in the absence of a chemoattractant gradient. When measured over longer times, however, the chemotactic index decays toward zero, proving that the overall motion is indeed random in uniform environments and no chemotaxis is observed.
Figure 1.11: (Figure from Jeon et al. [22]) Neutrophils migrating in the presence of a bell-shaped static gradient of IL-8 also exhibit persistence. As the cells migrate up the local gradient, they appear to overshoot the area of peak concentration before turning around.

From the experiments, it has also been shown that activated cells migrate with a mean speed of approximately 10 microns per minute, while the orientation angle is independent of both speed and persistence time.
Chapter 2
Model

2.1 Model description

Traditional macroscale models of neutrophil chemotaxis are incapable of describing the behavior of cells in the presence of multiple chemoattractant gradients. In this chapter we present an updated mathematical model for neutrophil migration to overcome the limitations imposed by these approaches. In particular, this model incorporates some of the features discussed in the previous chapter, such as: (1) the dose response characteristics of cells to varying chemoattractant concentrations; (2) the vectorial integration of different intermediary chemoattractant gradients; and (3) the effects of directional persistence in the presence and/or absence of a gradient. Previous models, such as that proposed by Oelz et al., do not account for these spatial aspects of chemotactic regulation. On the other hand, continuum models like the Keller-Segel equations would have difficulty capturing some of these characteristics; for instance, the inclusion of persistence necessitates an additional variable to provide memory of the internal state of a cell. An agent-based (single-cell) model would be a more sensible approach in this regard. Finally, no models to date have considered the coupled effect of these behaviors in different settings.

To begin, the problem is formulated in two dimensions, where cell migration occurs on a surface. Since the majority of experimental data on neutrophil chemotaxis are in this format, this will allow for future evaluation of the model against actual observations. Neutrophil motion is idealized as the continuous limit of a biased and correlated (persistent) random walk (BCRW). The model ignores the underlying signal transduction pathways and tracks only the position \((x(t), y(t))\) and the idealized orientation \(\theta(t)\) of an individual cell (which we can interpret to be the location of intracellular gradient sensing markers that define the cell compass or the directional bias in which new pseudopods are generated) as function of time \(t\). In mathematical form, the position of a neutrophil is given by the following ordinary differential equations:

\[
\begin{align*}
    dx &= v \cos \theta \, dt \\
    dy &= v \sin \theta \, dt,
\end{align*}
\]

where the constant \(v\) represents the linear velocity of the cell and the combination of \(v\) and \(\theta\) form a vector describing the cell trajectory.
The model describes cell motion as a biased correlated random walk (BCRW), where the internal state of the cell, or the orientation of the cell compass, is given by the variable $\theta$. The local chemoattractant environment of the cell determines the perceived gradient toward each chemoattractant source, denoted here by $\theta_{IL-8}$ and $\theta_{LTB4}$. The cell then integrates these signals by taking the vectorial sum, denoted by $\theta_s$, and attempts to reorient itself in this direction. In the presence of a single chemokine gradient, a low $\tau$ results in a rapid response toward the source, while a higher $\tau$ leads to a pronounced lag. In the presence of two competing chemokine gradients, a low $\tau$ value results in cells that are caught between two sources, while a higher $\tau$ leads to oscillatory behavior.

The direction of cell migration $\theta(t)$ is given by the phenomenological Langevin equation:

$$d\theta = \tau^{-1} \xi_i(s_i, \nabla s_i) f(\theta_s - \theta) \, dt + \sigma(\tau) \, dW_{\theta}(t) \quad (2.3)$$

where $\theta_s$ represents the cell’s interpreted target direction relative to the combined chemoattractant gradients, the difference $(\theta_s - \theta)$ is taken to be the minimal distance on the periodic domain $\theta \in (-\pi, \pi)$, and the expression $W_{\theta}(t)$ represents a standard bivariate Brownian process with zero mean and unit variance, multiplied by the noise strength $\sigma$. The function $\xi$ specifies the strength of the chemotactic response to the attractants in relation to their concentrations ($s_i$) and gradients ($\nabla s_i$), while $f$ defines the rate of reorientation of the cell as a function of the cell’s current orientation and the target direction. Possible definitions of $f$ are provided later in this chapter. Finally, the parameter $\tau$ provides a measure of the persistence time, which serves to dampen the reorientation rate imposed by $f$. Note that $\tau$ is defined to be independent of cell speed, which is consistent with the assumption that turning behavior is uncoupled from translocation.

To define the functional form of $\theta_s$ and $\xi$, we allow for the existence of multiple superimposed chemoattractant gradients. Let $k_i$ represent the receptor binding affinity for the $i^{th}$ ligand, where $s_i = s_i(x, y)$ represents the ligand concentration field for the $i^{th}$ ligand. By basic receptor-ligand kinetics, we then have that the approximate concentration of bound cell surface receptors for a particular ligand at position $(x, y)$
Figure 2.2: A simulated neutrophil undergoing a biased correlated random walk as described by the model. Note that the true random walk is approximated by discretizing the time steps. The orientation at each step is correlated with that of the previous time step.

on the cell surface is

\[ C_i(x, y) = \frac{\chi_0 N_T s_i(x, y)}{k_i + s_i(x, y)}, \]  

(2.4)

where \( \chi_0 \) represents the chemotactic sensitivity of each individual receptor and \( N_T \) is the total number of receptors per cell for the \( i^{th} \) ligand. Note that in this equation we assume a Hill coefficient of 1, based on experimental evidence of either non-cooperativity or even slightly negative cooperativity in the receptor binding of some chemoattractants [19]. The perceived gradient of the cell can then be obtained by taking the gradient of these bound receptor distributions over space in two dimensions. This yields the effective signal in both the \( x \) and \( y \)-directions, which we can denote as a vectorial sensitivity \( \omega_i \).

\[ \overrightarrow{\omega_i}(s_i) = \left( \frac{\partial C_i}{\partial x} \right) \hat{x} + \left( \frac{\partial C_i}{\partial y} \right) \hat{y} = \frac{\chi_0 N_T k_i \nabla s_i}{(k_i + s_i)^2}. \]

(2.5)

The vectorial summation of \( n \) different chemokine signals is then represented by the following:

\[ \overrightarrow{\omega} = \sum_{i=1}^{n} \overrightarrow{\omega}_i, \quad \theta_s = \angle(\overrightarrow{\omega}), \quad \xi = |\overrightarrow{\omega}|, \]

(2.6)

By this definition, the cell interprets the target direction as a linear combination of the perceived chemokine gradients in the environment. In addition, we note that the receptor occupancy exhibits biphasic dose response characteristics, where \( C_i(x, y) \) has a maximum at approximately \( k_i \), the equilibrium dissociation constant of ligand \( i \). We will discuss later how this translates into a similar optimal concentration range for the orientation bias in the context of the overall cell behavior. Finally, the steepness of each gradient \( |\nabla s_i| \) also influences the turning rate through \( \xi \), where stronger gradients result in sharper responses. In particular, the orientation bias increases asymptotically toward perfect directional bias as a function of gradient steepness, as expected. These combined features differentiate our approach from a previous stochastic
model by Tranquillo et al. [23], which also captures the phenomenon of persistence but does not explicitly accommodate multiple chemical species.

The implementation of this new model for neutrophil chemotaxis is based on a few important assumptions. First, we assume that the signaling dynamics are fast relative to the time scale of cell movement, and that the cell has perfect knowledge of its environment. Thus, gradient sensing is modeled as an exact and instantaneous process. We also assume that the noise term $\sigma$ is a function of the persistence time - in the absence of any gradients, the first term in the expression for $\theta$ evaluates to zero, but we know that persistence is also observed in isotropic environments, so the second term must necessarily be a function of $\tau$ as well. And lastly, the velocity of the cell is assumed to be constant. This approximation is likely inaccurate, as experimental evidence suggests that cell speed may be a function of a number of factors, including intracellular calcium levels [24], surface properties and concentration [25] [26]. Nevertheless, as the exact dependence remains unknown, and for simplicity in analysis, a constant (mean) value is employed for the purposes of this investigation.

The completed model thus describes the motion of a single neutrophil cell in the presence of multiple chemokine gradients. Note that the variables $x(t)$, $y(t)$ and $\theta(t)$ are inherently coupled in this framework, since the orientation of the moving cell affects its trajectory in space, while the change in position modifies the local environment to which the cell reacts. Unlike a typical random walk model, the directional persistence produces a BCRW with a spatially-dependent directional bias and whose direction of motion is inherently correlated over short times. This implies that the location at each step of the random walk is non-Markovian (as it depends on the sequence of previous locations), and the trajectory itself is non-holonomic - at any given time, the state of the system is path-dependent. The usual framework for describing such correlated random walks is a velocity jump process, first described by Othmer, in which the variable following a Markov process is the cell’s velocity rather than the position [27]. In one dimension, the solution to this problem is described a hyperbolic governing function known as the telegrapher’s equation, which can be solved for the probability distribution of the position provided the initial distribution is given. Obtaining the solution for higher dimensions, however, is non-trivial since no closed form analytical solution has been found to exist for BCRWs in two or more dimensions [28].

Since an exact solution is difficult, we instead employ a Monte Carlo realization of this problem in two dimensions; computationally, we can discretize the random walk in both space and time, where the random component in $\theta$ can be accounted for through the repeated sampling of computer-generated (pseudo) random numbers. This yields the idealized trajectories of cells as a function of time given a prescribed set of initial conditions and concentration fields, where each instance represents a possible outcome of the system. Over many repetitions, we can then analyze the collection of individual cell paths for their dependence [29] on factors such as different parameter values, or alternate initial conditions. We can also calculate various macroscopic parameters such as the cell migration direction and the mean square displacement of populations.
of hypothetical cells. The advantage of this approach over forming the Fokker-Planck expansion

\[
\frac{\partial c}{\partial t} + \frac{\partial}{\partial x} (cv \cos \theta) + \frac{\partial}{\partial y} (cv \sin \theta) + \frac{\partial}{\partial \theta} (c\tau^{-1}\xi_i(s_i, \nabla s_i)(\theta_s - \theta)) = D_\theta \frac{\partial^2 c}{\partial \theta^2},
\]

(2.7)
is that we can look at the behavior of the model at the single-cell resolution. While this deterministic PDE model can be solved numerically to show how the probability density function of \(x, y\) and \(t\) evolve in time, we are also interested in visualizing the paths taken by individual cells undergoing this BCRW.

### 2.1.1 Models for Reorientation

In this section, we consider two possible ways in which the rate of reorientation \(f(\theta_s, \theta)\) can be defined in the model. In both schemes, \(f\) is a function of \((\theta_s - \theta)\), which implies that at every time step the difference between the current orientation \((\theta)\) and the target direction \((\theta_s)\) is diminished. This allows the cell to align its movement with its intended target direction over time. Interestingly, both models result in similar steady state distributions in \(\theta\) given a fixed gradient direction \(\theta_s\). More careful investigation, however, reveals that they diverge significantly in their transient effect on the overall cell trajectory within a spatial context. In particular, with a moving or spatially-dependent gradient direction, the models produce very different outcomes for the cell trajectories. Ultimately, upon thorough analysis of both schemes, the linear orientation model is chosen for both its biophysical justification and its desirable behavioral characteristics.

#### Sinusoidal Reorientation

By the definition of \(\theta\), the functional form of \(f(\theta_s, \theta)\) must exhibit the following symmetry properties: (1) \(2\pi\) periodicity, or \(f(\theta_s, \theta + 2\pi n) = f(\theta_s, \theta)\) for any integer \(n\); and (2) polar symmetry, \(f(\theta_s, \theta + \pi) = -f(\theta_s, \theta)\), since the polarity of the system should flip whenever the gradient direction is reversed. We should also consider the mechanism for how a migrating cell regulates its turning behavior in order to align with the gradient direction. If we assume that the rate of reorientation is dictated by comparisons between binding events on either side of its polarity axis (as in the model by Tranquillo \textit{et al.} [23]), we can include an additional symmetry condition; namely, (3) reflection symmetry, or \(f(\theta_s, -\theta) = -f(\theta_s, \theta)\). The simplest form of \(f\) fulfilling these requirements is then:

\[
f(\theta_s, \theta) = \sin(\theta_s - \theta); \quad -\pi \leq (\theta, \theta_s) < \pi,
\]

(2.8)

where again \(\theta_s\) corresponds to the preferred direction of motion.

The stochastic differential equation in \(\theta\) is then characterized by the drift term \(\tau^{-1}\xi_i(s_i, \nabla s_i)\sin(\theta_s - \theta)dt\) and noise term \(\sigma dW_\theta(t)\). In particular, if we consider the problem of a single static linear gradient such that \(\theta_s\) and \(\sigma\) are constant in time (with the coordinate system defined such that \(\theta_s = 0\)), then from extensive
Figure 2.3: In the presence of two different chemokine sources and no random noise component, the sinusoidal reorientation model captures the expected behavior of biased motion toward the distant source. However, we also find that the trajectory of the cell is irregular in comparison to the linear model.

work on circular statistics by Mardia [30] and Batschelet [31], the normalized steady state solution of the Fokker-Planck equation in $\theta$ can be solved explicitly to yield the Von Mises distribution:

$$f(\theta) = M(\theta; \theta_s, \kappa) = \frac{1}{2\pi I_0(\kappa)} e^{\kappa \cos(\theta - \theta_s)}, \quad (2.9)$$

where $I_0$ denotes the modified Bessel function of the first kind order and order zero, $I_0(\kappa) = \frac{1}{2\pi} \int_{-\pi}^{\pi} e^{\kappa \cos \theta}$, and $\kappa = \frac{2\xi_s(s_i, \nabla s_i)}{\sigma^2}$ in this case. The mean resultant, or the first moment for this distribution is then $\overline{R} = \frac{I_1(\kappa)}{I_0(\kappa)}$, where the circular variance is given by $1 - \overline{R}$.

This sinusoidal reorientation model can correctly reproduce some of the desired characteristics for our model; for instance, in simple linear gradients, the simulated cells are observed align correctly with the gradient vector, and in the presence of two opposing chemokine gradients, the model exhibits preferential migration toward the distant source (Figure 2.3), as desired. Interestingly, however, we also observe that the resulting cell trajectory is rather unstable, such that small perturbations in the initial condition may have a significant effect on the long term trajectory. Visually, we see that the cell may respond very sharply to one source, sometimes resulting in long unfavorable excursions away from the nearby optimum. This phenomenon is likely due to the assumption of reflection symmetry - by this definition of $f$, a cell with its axis in line with the gradient but moving in the opposite direction has very little motivation to turn around, since it cannot distinguish between left and right with respect to its environment. This issue is addressed in the following section by the linear reorientation model.
Figure 2.4: The linear reorientation model also captures preferential migration toward the distant source in the presence of two different chemokine sources and no random noise component. However, unlike the sinusoidal reorientation model, the trajectory appears to form a stable steady state orbit with respect to the two sources.

**Linear Reorientation**

Unlike the sinusoidal model, the linear reorientation model is based on the assumption that neutrophils migrating 180 degrees away from the target direction exhibit the highest turning rate, since the cells are capable of detecting concentration differences along their own axes as well. By eliminating the assumption of reflection symmetry, the functional form of $f$ is thus given simply by the linear difference between the angles:

$$f(\theta_s, \theta) = \theta_s - \theta; \quad -\pi \leq (\theta, \theta_s) < \pi,$$

(2.10)

From a biological perspective, this model may be more sensible than the sinusoidal alternative, since the membrane receptors of a cell are known to be uniformly distributed along its perimeter, and mechanistically, the cells do not appear to have functionally distinct left and right halves. In addition, a cell’s morphology constantly evolves as it changes direction, so gradient sensing itself is likely a spatially homogeneous process in order for the cell to respond impartially to different gradient directions.

For this reorientation function, the Fokker-Planck expansion in $\theta$ can be described as a Hull-White model, a term often used in financial mathematics to describe a dynamic mean-reverting process with an evolving mean. If we also assume that the gradient is linear and remains fixed in time (with $\theta_s = 0$), and the coefficient of the noise term $\sigma$ is also a positive constant, then the system is in the standard form of an Ornstein-Uhlenbeck (OU) process, whose normalized steady state solution (given the appropriate boundary conditions) is readily shown to be the wrapped normal distribution [32]:

$$f(\theta) = B(\lambda) \exp(-\lambda \theta^2), \lambda = \frac{\xi_i(s_i, \nabla s_i)}{\tau \sigma^2},$$

(2.11)
where $B(\lambda)$ is the normalization function defined by

$$B(\lambda) = \sqrt{\lambda} (\sqrt{\pi} \text{erf}(\pi \sqrt{\lambda}))^{-1}. \quad (2.12)$$

The first moment of the steady state solution to this OU process is given by $\exp(-\frac{\sigma^2}{2})$, where $\sigma$ represents the circular standard deviation. In directional statistics, the Von Mises distribution described earlier is in fact a very close approximation to this wrapped normal distribution. In fact the Von Mises is generally easier to work with analytically, and is thus often the preferred distribution for many applications. In particular, if the first moments of the Von Mises and wrapped normal distributions are equal in magnitude, then we can relate the reciprocal of the variance ($\kappa$) of the former to the circular standard deviation of the latter ($\sigma$) by the equation

$$\frac{I_1(\kappa)}{I_0(\kappa)} = \exp(-\frac{\sigma^2}{2}).$$

The linear orientation model was shown to yield more favorable results in cell simulations as well, as observed in Figure 2.4. Unlike the sinusoidal model, this description of $f$ produces stable oscillatory trajectories between the two chemokine sources, such that the cells remain closer to the local optima as they migrate back and forth in their orbits. We also see what appears to be convergence toward a stable manifold over longer times (assuming a static gradient with no noise component). This behavior is a more accurate model of what is observed in experiments, since the majority of neutrophils were shown to consistently migrate toward the distant sources in all cases. With these qualifications in mind, the remainder of this work will focus especially on the application of this linear reorientation model to describe neutrophil migration.

### 2.2 Model derivation

A fundamental challenge for any neutrophil migration model is in rationalizing the relationship between persistence time in uniform chemoeffector environments and directional orientation bias in attractant gradients. The model needs to explain why an activated cell moving in a given direction would want to change its direction in the absence of any macroscopic attractant gradient, and it must also address why at any instant a certain fraction of the cells in a gradient are oriented in the wrong direction. These observations cannot be explained by heterogeneity in particular cell properties, since all cells undergo transient directional changes in uniform attractant concentrations. Even in attractant gradients, there are periods during which any given cell moves toward lower as well as higher chemoattractant concentrations. A coherent picture of cell migration in response to chemoattractants thus requires that both the persistence time and the orientation bias be explained within a common unifying framework.

One common approach to this problem is to deconvolve the motion of cells into two distinct processes. In uniform environments, neutrophils undergo chemokinetic behavior, in which the extracellular concentration affects the amplitude or frequency of the motile character of the cell, such as the direction of migration. In
contrast to chemotaxis, chemokinesis has a random, non-vectorial moiety. Some results have indicated that chemokinesis in neutrophils consists of approximately 80% orthokinetic activity (change in rate of locomotion due to a change in concentration) and 20% klinokinetic (non-directional change in rate of turning due to change in concentration) behavior [33]. When a chemoattractant gradient is present, however, the cells clearly show a more directed response. This can be thought of as the contribution from a chemotactic component, in which gradient sensing allows the cells to bias their motion in a particular direction. These two components are thought to act in tandem to regulate the cell response - specifically, random motility is governed only by chemokinesis, while in a gradient, both chemokinesis and chemotaxis contribute to cell motion.

The strength of a BCRW model lies in its ability to describe both the random and directed motility aspects of neutrophil motion, while maintaining an account of directional persistence. To date, there have been a number of documented attempts in the literature to model eukaryotic chemotaxis as a BCRW in two dimensions [34] [32]. The stochastic chemotaxis model by Tranquillo et al. also uses a BCRW to describe cell turning behavior. In our implementation, neutrophil motion is characterized by an omnipresent random component in $\theta$ (the noise term represented by a Weiner process) and a directed component that only surfaces in the presence of a gradient ($\xi$ is zero in the absence of a gradient). The noise term adds a degree of randomness in the behavior of individual cells, while the latter term allows us to prescribe a preferred direction of motion through $\theta_s$.

In this derivation, the strength of the random motility component in relation to the chemotactic term is undoubtedly an important factor in the overall description of the cell response. The scaling of $\sigma(\tau)$ in effect determines how the persistence time in uniform environments relates to that observed in gradient conditions. To determine the functional form of $\sigma$ with respect to $\tau$, we consider the case in which there is no gradient, such that the first term of $\theta$ is zero; the model then describes an unbiased (normal) correlated random walk. From extensive work by Alt and Othmer on random walks of this form, we then have that the directional persistence time is defined mathematically by:

$$\tau = \lim_{t \to \infty} \frac{2t}{\langle \theta^2 \rangle}$$

(2.13)

where $t$ denotes the observation time, and $\theta_t$ represents the angle formed by the cell polarity axis at time $t$ relative to the initial direction. But we can also see that the denominator is simply equivalent to the circular variance $\sigma$, so that $\sigma$ can be expressed in terms of $\tau$ by $\sigma = \sqrt{\frac{2}{\tau}}$. Thus, our completed model can be written as:

$$d\theta = \tau^{-1} \sum_i \xi(s_i, \nabla s_i) f(\theta_s - \theta) \, dt + \sqrt{\frac{2}{\tau}} dW_{\theta}(t)$$

(2.14)

which can be solved for the individual cell trajectories using Monte Carlo random sampling and conventional approaches to solve ODEs, such as implicit Euler or higher order Runge-Kutta methods.
Figure 2.5: The relative error between the MSD of simulated cells and the approximate value. Note that the error remains within ±5% over the duration of the experiment.

To confirm that this scaling factor for random motility is indeed reasonable, we also examine the diffusion limit of this transport equation in the absence of a gradient. In two dimensions, the mean square displacement (MSD), or \( \langle r^2 \rangle \), of a cell undergoing a correlated random walk is approximated by:

\[
\text{MSD} = \langle \|r(t)\|^2 \rangle = 2v^2\lambda(t - \frac{1}{\lambda}(1 - e^{-\lambda t})),
\]

(2.15)

where \( \lambda = \tau^{-1} \), \( r \) represents the displacement from the initial position and \( t \) is the duration of the observation. Using this information, we can then compare the MSD of simulated cells in a uniform environment (as described by Equation 2.14) to the approximate values obtained using this formula. A plot of the resulting relative error between the expected values and those computed from independent Monte Carlo simulations of cells is given in Figure 2.5, where the horizontal axis represents time. This is repeated for various values of \( \tau \), ranging from \( \tau = 2 \) (\( \sigma = 1 \)) to \( \tau = 50 \) (\( \sigma = 0.2 \)). From the plot, it is evident that the relative error remains within ±5%, suggesting that the model is reasonable.

### 2.3 Test simulations

#### 2.3.1 Cell migration in a uniform environment

To illustrate the model behavior in a variety of situations, a number of test scenarios were constructed. In the first test, the simulated trajectories of 1000 individual cells in a uniform environment were recorded and analyzed. Figure 2.6 shows the calculated average MSD of these runs using a range of values for the persistence time \( \tau \). Note that the blue and orange dotted lines represent the diffusive and ballistic limits of particle motion. When an object moves ballistically or wavelike, the expectation of the MSD scales with
Figure 2.6: The average MSD of 1000 Monte Carlo simulations of cells in a uniform environment reveal that increased persistence results in more ballistic behavior, as expected. In addition, the length of the ballistic phase is determined by the persistence time. In all cases, the response appears to be super-diffusive.

time quadratically or \( E(\langle r^2 \rangle) \sim t^2 \). This corresponds to the absolute displacement increasing linearly with time, which is a standard property of a wave process. In such cases, the characteristic backtracking and random movement associated with diffusive processes cannot be observed, since each individual effectively moves in a straight line away from the origin for the whole time period. By contrast, in a diffusive process, the expectation of the MSD scales linearly with time or \( E(\langle r^2 \rangle) \sim t \). This is typical of a normal Brownian process.

The simulation results reveal a number of key features of the model. First, in all cases, the initial motion is highly ballistic in nature; however, at larger time scales, we see that the behavior becomes increasingly diffusive. This is a defining property of any correlated random walk. At time scales less than the persistence time, the orientation of the cell is highly correlated between steps, but over longer times, this correlation disappears, allowing cells to turn back and move in the opposite direction. From the plot, we also see that a higher value of \( \tau \) corresponds to more ballistic behavior. In fact, the length of the ballistic phase is determined by the persistence time; in the limit of infinite \( \tau \), we recover the purely ballistic response. Finally, in all cases the response curves lie within the diffusive and ballistic limits. This situation is known as super-diffusion, since the MSD increases at a faster rate than standard diffusion. Super-diffusive processes, often referred to as Levy flights, typically occur when the step lengths are drawn from a distribution with infinite variance.

2.3.2 Chemotaxis in a single unidirectional chemoattractant gradient

In the second test, the correctness of the chemotactic term in the model was assessed by simulating cell motion in a single linear unidirectional attractant gradient. The objective of this experiment was to reproduce the
results obtained by Foxman et al., in which a biphasic dose response curve was observed over different mean concentration values. Thus, in our simulation the orientation bias of the cells were also obtained by counting the fraction of cells within the gradient that were oriented in the correct half-plane (toward increasing concentration) after an arbitrary amount of time. For the unknown parameters, we used realistic values obtained from the literature, including the estimated dissociation constant for fMLP, $k = 2 \times 10^{-8}$ M. Finally, this experiment was repeated for both a 3-fold concentration gradient and a 10-fold gradient, precisely as was performed in Figure 1.9. The resulting sensitivity curves are shown in Figure 2.7.

From the simulation results, we can see that the model captures the macroscopic behavior of cells remarkably well. In particular, the optimal sensitivity range falls in the neighborhood of the dissociation constant for the particular chemoattractant receptor involved. In addition, the accuracy of the chemotactic response diminishes monotonically for concentrations that are higher or lower than this optimal range, much like what was observed in the experimental data, Mathematically, these observations can be attributed to particular aspects of the model’s formulation; at the higher end of the concentration spectrum, the turning rate of the cell is dampened due to the saturation of receptors. This desensitization is reflected in the the turning rate of the cell, $\xi = \frac{\chi_0 N_T k_i \nabla s_i}{(k_i + s_i)^2}$, which was again derived from the kinetics of the receptor binding events. At the lower limit of concentration, the turning rate is also smaller - however, this is due to the fact that the absolute gradient $\nabla s_i$ in the numerator scales with concentration in order to maintain the same relative gradient $\nabla s_i$. This reduction in the response rate also leads to a lower orientation bias. In these regimes, the interplay between random motility and directed motility is particularly significant - if the chemotactic response is attenuated, the random motility component becomes dominant, and vice-versa.
2.3.3 Chemotaxis toward a single chemoattractant point source

The last test of our model involved simulating the migration of a cell toward a single chemoattractant point source. For simplicity, the chemoattractant field in this case was given the form of a static Gaussian function 

\[ s(x, y) \propto \exp \left( -(x^2 + y^2) \right) \] 

which remained fixed in time throughout the simulation. The first objective using this test was to examine the deterministic steady state behavior of the model in the absence of the noise component. The resulting cell trajectory in this simulation is shown in Figure 2.8. Initially, the simulated cell was positioned below the source, oriented away from the gradient direction.

The first thing we may recognize from the cell path is that the cell does not immediately reorient toward the source, but instead undergoes a smooth U-turn to align itself with the gradient. This behavior is caused by the persistence parameter \( \tau \), which restricts the maximum turning rate of the cell. The second key feature of the trajectory is that the cell does not converge to a single fixed point coinciding with the
chemoattractant source in the steady state (as is observed with the Keller-Segel equations). Instead, the system clearly approaches an orbit, or a stable manifold, centered around the source. In particular, the radius of this manifold depends on the magnitude of the persistence parameter - a smaller persistence time leads to a smaller radius, while a large persistence time results in a bigger radius, or even possible divergence away from the source (Figure 2.8). These observations appear to also hold even with the addition of the random motility component (Figure 2.9).
Chapter 3

Results and discussion

3.1 Simulations

3.1.1 Chemotaxis in two opposing attractant gradients

The primary purpose of this investigation was to demonstrate whether our updated model of neutrophil migration could correctly account for the observed behavior of cells in multiple chemoattractant environments. One of the simplest examples of such an environment is the case of two different chemokine sources separated by an arbitrary distance within the plane. Thus, our first experiment would involve simulating the motion of cells within this framework. Note that unlike the previous single chemoattractant examples, this new setting would allow for responses to two different chemokine species simultaneously, through the vectorial integration of the local gradient information ($\sum \omega$). Through these simulations, we wanted to analyze how the adjustment of different parameters in the model would influence the overall cell behavior. To begin, we initially looked at the steady-state behavior of the model in the absence of the noise component - this deterministic version of the model could inform us of both the gradient sensing and purely chemotactic responses of the cells without the contributions from random motility.

The top two images in Figure 3.1 exemplify the trajectories of cells using this deterministic model in the presence of two conflicting chemokine gradients. In the top left pane, for the low persistence case, we immediately see that the cell converges to a steady state attractor centered exactly between the two sources. More interestingly, however, when the persistence value is increased, we see a wildly different response, as shown in the top right. In this case, the cells more closely resemble a satellite in orbit around two gravitational bodies, tracing out sweeping curves as they move back and forth between the gradients. The shape of this stable manifold is not circular or elliptical as in the low persistence case; depending on the conditions, it was shown to adopt a number of different complex looping patterns. In either example, we still observe preferential migration to the distant source, as well as the oscillatory behavior predicted by Oelz et al.. However, we have not relied on any models of temporal processes within the cell to modulate the cells’ sensitivities. The response is purely a function of the position and orientation of the migrating cells. In the lower two images of Figure 3.1, we repeated this example but by also adding the random motility component. Again, we observe that the cells still exhibit similar transient behavior, with lower persistence corresponding to a tighter radius of activity, while higher persistence results in larger oscillations between...
Figure 3.1: [Left] In the presence of two conflicting chemokine gradients and assuming no random motility component, the neutrophils appear to sway back and forth between the two point sources indefinitely. Moreover, the system is shown to gradually converge toward a stable manifold over time. [Right] When the noise term is included, the cells still exhibit oscillatory motion between the two sources, where larger persistence times result in oscillations of greater magnitude.

Figure 3.2: Due to the non-holonomic nature of cell motion as described by this model, the initial conditions can play an important role in the trajectory and/or fate of the cell. Here, we see that subtle differences in the initial orientations lead to vastly different steady state behaviors.
Figure 3.3: Cells are capable of exhibiting oscillatory behavior regardless of the shape of the concentration profiles for the chemoattractants. [Left] A sample cell trajectory in the presence of two gaussian chemokine concentration functions. [Right] Cell behavior for the inversely proportional concentration profile.

Next we investigated how the initial conditions could affect the trajectory of a cell migrating in this environment by selecting different initial positions, as well as adjusting the initial orientation of the cell. From this, we determined another interesting aspect of the model. Due to the path-dependent, or non-holonomic nature of the cell position, the steady state behavior of the cell can also change depending on the course taken by the cell as it approaches the equilibrium. This is shown in Figure 3.2. Notice that in both cases, the cell starts in the same position, but with a slightly different initial orientation. All other parameters in this simulation were kept the same. However, in one case, we see very large oscillations between the two sources - in the other, we see a tighter elliptical manifold centered in the middle. This interesting phenomenon was further explored in the stability analysis section later in the chapter.

We were also interested in determining if the shape of the local concentration profile had any effect on the cell behavior. To this end, we employed an inverse linear chemoattractant distribution centered around the source, proportional to one over the radius, as the the Greens function solution to the problem of diffusion in an infinite volume. The resulting cell trajectories from this example are shown in Figure 3.3. Note that the sharpness of the local gradient around the sources in the inverse linear case causes a different trajectory, as well as a different steady state manifold. However, most parameter choices still resulted in the same general behavior of oscillation between the two sources.

Overall, we were able to demonstrate that our model was capable of correctly interpreting the problem of navigation within two conflicting chemoattractant gradients. In particular, whenever the cells converged into a stable steady state orbit, the cells exhibited preferential migration toward the distant source. The cells were also shown to exhibit a vectorially-directed response to the chemicals, which was expected, given how the gradient sensing process was defined in the model. As in the single chemoattractant case, the persistence time had the effect of changing the radius of the stable manifold - however, in this case, the equilibrium behavior led to a distribution that was elongated in the form of a channel between the two sources.
chemokine sources. A larger persistence time allowed cells to more easily escape the attractive potential of the proximal source, leading to larger oscillations. Finally, we also found that the overall cell behavior can depend on a number of different factors to varying extents, including the initial condition, the shape of the concentration profiles, the separation between the sources, and the magnitude of the concentrations. These factors can influence not only the character of the steady state behavior, but also the question of whether or not the system will ever converge into a stable equilibrium.

3.1.2 Chemotaxis in complex environments

The next study was to extend the predictive power of our cell migration model by introducing a hypothetical end target source into our description. The goal of this heuristic model was to simulate cell behavior in more realistic environments consisting of arbitrarily many chemokine sources randomly distributed throughout the interstitial tissues. This would require implementing the inhibitory effect of fMLP on LTB4 and IL-8 sensitivity; the model was therefore adjusted to account for the reduction in sensitivity toward the endogenous chemokines by increased end target chemoattractant concentration. Since the interaction between the two proposed signaling pathways has not been well-characterized, the sensory inhibition was instead captured at the receptor-level by modeling the chemical kinetics of competitive inhibition. This was achieved by modifying the apparent dissociation coefficient ($k_m$) within our hill equation model:

$$\text{effective } k_m = k_m \left(1 + \frac{[I]}{k_I}\right), \quad (3.1)$$

where $k_I$ represents the inhibitor dissociation constant and $[I]$ denotes the inhibitor concentration. Note that while the maximum rate of reaction is unchanged by this definition, the apparent affinity of the substrate to the binding site ($k_m$) is indirectly decreased. Thus, increasing the substrate concentration (LTB4 or IL-8) can allow the substrate to outcompete the competitive inhibitor (fMLP) in enzyme binding. Thus, for this model system, the sensitivities to each chemoattractant were defined as:

$$\xi_{IL-8} = \frac{\chi_0 R_{IL-8}k_{IL-8}}{k_{IL-8} \left(1 + \frac{[fMLP]}{k_{IL-8}} + [IL8]\right)} \nabla[IL8]$$

$$\xi_{LTB4} = \frac{\chi_0 R_{LTB4}k_{LTB4}}{k_{LTB4} \left(1 + \frac{[fMLP]}{k_{LTB4}} + [LTB4]\right)} \nabla[LTB4]$$

$$\xi_{fMLP} = \frac{\chi_0 R_{fMLP}k_{fMLP}}{(k_{fMLP} + [fMLP])^2} \nabla[fMLP]$$

where fMLP served as a distinct competitive inhibitor to the other chemicals. The parameters in this new model were then chosen to best reproduce the conditions encountered in vivo, which are given in Table 3.1.2.

Using this new definition to incorporate the effect of end target signals, the model was confirmed to exhibit the desired behavior, as shown in Figure 3.4. Initially, an LTB4 and IL-8 source were located in close
Table 3.1: Nominal parameter set for neutrophil model in complex environments

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{IL-8}$</td>
<td>$3 \times 10^{-9} M$</td>
</tr>
<tr>
<td>$k_{LTB4}$</td>
<td>$3 \times 10^{-9} M$</td>
</tr>
<tr>
<td>$k_{fMLP}$</td>
<td>$2 \times 10^{-8} M$</td>
</tr>
<tr>
<td>$k_i$</td>
<td>$2 \times 10^{-11} M$</td>
</tr>
<tr>
<td>$\chi_0$</td>
<td>$1 \times 10^{-4}$ mm/receptor</td>
</tr>
<tr>
<td>$R_i$</td>
<td>$1 \times 10^4$ receptors/cell</td>
</tr>
<tr>
<td>$\tau$</td>
<td>4.0 mins</td>
</tr>
</tbody>
</table>

Figure 3.4: In the presence of IL-8, LTB4 and fMLP, the cell was shown to successfully navigate toward the fMLP source. Toward the top of the image, two similar sources of IL-8 and LTB4 chemokines were placed, while a single distant fMLP source was located toward the bottom.

Figure 3.5: In an alternating array of IL-8 and LTB4 sources, directional persistence plays a key role in regulating the amount of displacement the cell can achieve from its initial position. If the persistence parameter is too low for a particular parameter set, the cell is trapped around one of the sources. Conversely, if the persistence parameter is increased, we observe stepwise migration among the alternating chemokine sources which appears to continue indefinitely.
proximity to the cell’s position. At a slightly distant location, a single distant fMLP was placed. In this scenario, the simulation repeatedly demonstrated that while the cell initially undergoes oscillatory behavior between the chemokines, it quickly senses the presence of the end target chemoattractant. This in turn inhibits the response toward the secondary signals and allows the cell to escape toward the fMLP source.

In realistic environments, however, we are likely to have more than just two endogenous chemoattractant sources at any one time. Thus, for the second step in this investigation we placed an alternating grid of IL-8 and LTB4 sources within the plane, with no end target chemoattractants initially present. The results of these simulations are given in Figure 3.5. Here we see the pivotal role of directional persistence in tuning the response of cells to local concentration maxima. If the persistence parameter is too low for a particular set of conditions, we find that the cell is held up around the nearest source, unable to escape the local attractive potential. By contrast, with higher persistence values we see an extension of the oscillatory behavior observed in the dual gradient scenario - the cell exhibits long excursions from the initial position, where it migrates sequentially between adjacent sources. By restricting the turning rate and setting a fixed velocity, the cells are thus able to avoid getting trapped.

Combining the observations from these separate studies, we were then equipped to paint a more realistic representation of the neutrophils’ native environment. For the final test, a single fMLP source was placed in the middle of the chemokine grid that was described in the previous example, in order to simulate a phagocytic target randomly positioned within activated interstitial tissues. A cell initially located at the edge of this domain was then required to navigate efficiently toward the end target, where it then had to converge to within an arbitrarily small radius. Figure 3.6 shows some of the results obtained from these simulations for different values of the persistence time $\tau$. The first thing we may notice from this plot is that for low persistence values (left image), the behavior is roughly identical to the previous example; again we...
observe that the cell is effectively trapped around the nearest local maximum (left inset). The difference, however, is that in the context of this problem we now see that this response is highly undesirable, as it hinders the cell’s ability to get to its final intended target at the center of the domain. A slightly higher persistence time allows the cell to overcome this issue and move sequentially between nearby sources. This in turn increases the probability that the cell will come within detection range of the end target, allowing it to safely converge on the destination (center inset). We must note, however, that a high directional persistence is not always beneficial. In fact, if the persistence parameter is too high it can be counterproductive for convergence on the target, as the cell is unable to come to rest around the fMLP source (right inset).

### 3.2 Analyzing the effect of directional persistence

#### 3.2.1 The effect of persistence on stability

To characterize the general conditions under which a cell could get trapped in transit by a chemokine source, we decomposed the overall navigation problem back down to the simple case of just two competing chemokine gradients. The model was then reformulated in non-dimensional form to reduce the number independent variables in the parameter space. The dimensionless system is given by:

\[
\begin{align*}
\frac{d\hat{x}}{dt} &= \cos \theta \hat{d}, \\
\frac{d\hat{y}}{dt} &= \sin \theta \hat{d}, \\
\frac{d\theta}{dt} &= T^{-1} \xi (\theta - \theta_s) + \sqrt{2} dW.
\end{align*}
\]

with the unitless variables

\[
\begin{align*}
T &= \left( \frac{v \tau}{b} \right), \\
\hat{x} &= \left( \frac{x}{b} \right), \\
\hat{y} &= \left( \frac{y}{b} \right), \\
\hat{t} &= \left( \frac{t}{\tau} \right), \\
\xi &= \left\| \frac{\nabla \phi_s}{(1+s)^2} \right\|, \\
\hat{s} &= \frac{s}{a}.
\end{align*}
\]

The independent parameters were thus reduced to \( T, a \) and \( b \), where \( T \) denotes the dimensionless persistence measure, \( a \) represents the chemokine concentration at both sources, and \( b \) is a measure of the spread of the gaussian chemokine distributions centered around both sources. The parameter space was then scanned
Figure 3.7: Cross-sectional plot of two different measurements within the parameter space, for the case $a = 1$. In both images, the vertical axis represents the log of the non-dimensional persistence parameter $T$, while the horizontal axis represents the separation between the two sources. [Top left] Colors represent mean L2 norm (euclidean distance) of cell position to steady state orbit center, to show compactness of stable manifold. [Top right] Colors represent mean L2 norm (euclidean distance) from coordinate system origin to steady state orbit center to indicate location of stable manifold. [Bottom] Representative trajectories for the different phases in the two phase diagrams above, with their corresponding locations indicated. (A) Unstable. (B) Elongated stable manifold around origin. (C) More compact stable manifold around origin. (D) Stable manifold not centered around origin.

for two different criteria: (i) the mean euclidean distance of the cell position in the steady state to the center of this orbit and (ii) the mean euclidean distance from the origin of the coordinate system (which was maintained precisely in between the two sources) to the center of the stable manifold. The first criterion was intended to capture the compactness of radius of the steady state orbit, while the second criterion was included to measure the location of the orbit itself. These values were then visualized for every point within the discretized parameter space. Figure 3.7 shows a cross section of this parameter space for the case in which the chemokine sources were kept at a concentration of $a = 1$. In particular, the measurements taken in the phase space exhibited the least amount of change for $a$, so the dependence on $a$ is omitted for this investigation.

The four images (A,B,C,D) in the lower half of Figure 3.7 show some representative cell trajectories for the corresponding regions indicated in two phase diagrams. These trajectories are characterized by the following properties: (A) The cell escapes the attractive potential of both sources and runs off *ad infinitum* - in this instance, no stable equilibrium is observed. (B) In this region, an elongated stable manifold is observed, in which the cell exhibits large oscillatory behavior between the two sources. Notice that the left plot shows a
Figure 3.8: To test the ability of cells to converge on an end target chemoattractant source hidden within a randomly distributed network of chemokines, we initially placed 4000 simulated instances of individual cells randomly within the domain. The persistence parameter was varied with the values (from left to right): $T = 0.125, T = 0.25, T = 0.5, T = 1.0, T = 2.0, T = 4.0, T = 8.0$.

markedly higher value only within this region. (C) This region represents a tighter stable manifold than B, which is also centered around the origin. Note the right plot indicating a value of approximately zero. (D) Finally, this region also corresponds to a stable manifold, but in this case the orbit is not centered around the origin, as seen from the nonzero value in the plot to the right. This suggests that the orbit forms around one of the chemokine sources itself, and not between the sources.

The two phase diagrams tell us a number of interesting things about the stability of this system. First, we see that for any value of the separation $b$, there is an upper limit to the persistence parameter, beyond which the cell escapes the system. This limit falls within the range $T \in (0.1, 1)$. This result is sensible because if we plug in realistic values for the variables composing $T = \frac{v\tau}{\sigma}$, $v \approx 10$ microns/min, $\tau \approx 4$ mins, $\sigma \approx 100$ microns, we recover $T \sim 0.4$. Another important observation to make is that there is only a very narrow region in which we observe the characteristic large oscillatory behavior of cells between the sources. This is a particularly interesting point, because in theory, a cell should want to maximize the area of this domain - if the cells were to actually exploit chemokine sources as guides for incremental migration toward their final targets, an elongated oscillatory trajectory would be more favorable to make transitions between distant sources. In the most ideal case, a cell would exhibit extensive back and forth migration for any separation distance $b$. The actual breadth of the region that we observe may or may not be a fault of the model itself, but this is what was mathematically observed. It would also be interesting to see whether the addition of noise could influence this result, since in this study we have only looked at the behavior of the deterministic model.

3.2.2 The effect of persistence on convergence

Besides the chemokines, another factor that can influence a cell’s ability to arrive at a phagocytic target is the behavior around the end target chemoattractant source during the final approach. The previous simulation involving a network of chemokine sources and a single fMLP source hidden within it indicated that under some conditions, persistence can in fact be a double-edged sword. In particular, persistence is inherently favorable during migration among the endogenous chemokines, but is not conducive to settling at a particular point in space upon arrival at the site of infection. Thus, to quantify the overall effect of persistence on convergence around the end target chemoattractant source, we repeated the in vivo simulation experiment.
using a Monte Carlo model of 4000 individual non-interacting instances of cells that were both randomly positioned and orientated in space. The simulation domain consisted of a single end target chemoattractant point source in the center, with 10 chemokine point sources distributed pseudo-randomly in the surrounding region. The same parameters were used as listed before in Table 3.1.2; however, the persistence parameter $\tau$ was varied from 0.125 to 8.000. The resulting simulations are shown in Figure 3.8.

From the visualized trajectories of the cells, we immediately notice that for $T = 0.125$, only the cells that are initially located within a certain radius converge toward the end target, while for larger persistence times such as $T = 8.0$, the cells have a difficult time settling on the fMLP source. As a quantifiable measure for the degree of convergence, we then used the mean of the time-averaged root mean square (RMS) euclidean distance between the cell positions and the end target (located at the origin), given by

$$d_{\text{rms}} = \sqrt{\frac{1}{T} \int_0^T (x(t)^2 + y(t)^2) dt},$$  \hspace{1cm} (3.11)

where $t$ was the time of the observation. A plot of these values as a function of persistence $\tau$ is given in Figure 3.9. From this plot we note that the rms norm has the smallest value for intermediate values of persistence (although it may not agree with the experimentally observed value of $3 \sim 4$ minutes). These data corroborate the theory that both low and high persistence can in fact be detrimental to convergence, even in more realistic settings with randomly positioned sources.

### 3.2.3 Role of directional persistence in the biological context

The problem of finding the maximum of a spatially-defined function by moving agents is often referred to as source seeking, or extremum seeking, in the control theory literature. Chemotaxis represents a specific
application of this problem, in which the objective is to find the source of a chemical substance that is produced locally, but which spreads to a greater region over time through a diffusive process. In these cases, the agent is often assumed to be incapable of sensing its own relative positioning or the position of the chemical sources; it is, however, capable of sensing the signals originating from the sources to navigate. For our particular problem, we also had the additional constraint of a constant forward velocity, since the neutrophils were modeled as non-holonomic objects. Mathematically, this assumption led to complications in the behavior of the agent after it converged near the source, since it could not settle at a point and at best could only converge locally to an orbit-like attractor. The richness and complexity of the asymptotic behaviors of our model meant that the problem was not amenable to extensive analysis. However, using studies based on computational simulations, we were still able to illustrate some important features of the model. In particular, we focused on the question of how directional memory affects the chemotactic efficiency of neutrophils migrating toward their targets in vivo.

It had previously been speculated in the work by Foxman et al. that by preferentially seeking distant sources, neutrophils may navigate in a stepwise fashion towards sequentially encountered chemokine gradients [3]. In this framework, the proposed role of the intermediary chemoattractants (e.g. IL8, LTB4) was to facilitate long-range navigation to a target by loosely guiding the cells. This multistep navigational strategy could then serve as an efficient means to increase the probability of cells encountering end target chemoattractant gradients and their associated sources over greater distances. Very recent studies using dynamic in vivo imaging [35] have also corroborated this hypothesis. In this work we were able to demonstrate quantitatively that the phenomenon of directional persistence may in fact play an pivotal mechanistic role in making this behavior possible. In environments where very little spatial information is available, persistent forward motion allows cells to cover more territory in a given number of steps than they otherwise would in a random walk. In this way, time wasted on exhaustive back and forth searching is greatly reduced, thereby enlarging the search area and improving search efficiency.

Our phenomenological model also showed that in realistic environments consisting of multiple chemokine sources randomly distributed in space, increased directional persistence allows the cells to explore a greater area by avoiding getting trapped near the sources, which are in effect the mathematical analogue of local maxima in global optimization problem. This principle is a familiar concept in dual control theory, where a so-called probing or exploratory signal is purposefully injected into the system that may detract from the short term performance of finding an optima (i.e. the local chemokine maximum), but will improve control in the long run (i.e. finding the global optimum or the end target chemoattractant source). We also found in our investigation, however, that an excessively high persistence time has the adverse effect of pushing the cell off track and preventing convergence upon the final target, which can negatively affect the overall efficiency. Thus, directional persistence has both benefits and drawbacks: the cells must strike a fine balance between exploring a wider area and wandering too far from the intended destination. We predict that this
general principle may also apply to other chemotactic processes in the body, particularly in those involving long-range navigation through complex multiple chemoattractant environments.
Chapter 4

Conclusions

4.1 Neutrophil chemotaxis as a foraging problem

In nature, organisms are commonly faced with the challenge of finding sparsely distributed resources occurring randomly over very large areas. These resources, or targets, may also have chaotic dynamics with respect to their location, duration and timing. In such scenarios, the search strategies adopted to minimize the associated energetic costs are referred to as foraging. The act of foraging typically involves two interrelated phases of behavior that serve divergent purposes. In the absence of external cues, most organisms employ a stochastic search pattern in order to maximize the likelihood of random encounters with nearby targets. Conversely, if sensory information is available, a more directed search method is used for faster convergence. Common examples of this optimized behavior include large-scale coordinated searches by insect populations such as ant colonies, swarming dynamics in microorganisms such as bacteria and levy flight-like search patterns that are sometimes observed in bird and fish. Presumably these behaviors have arisen because different environments place different pressures on foraging organisms.

Neutrophils searching for pathogens in the body are in many ways similar to larger organisms foraging for food. These phagocytes must also overcome the problem of locating targets within an unspecified area, which may or may not be distributed randomly in both space and time. Efficiency is also an important factor in this problem, since the cells must work to minimize the extent of damage caused by the infectious agents, while also preventing the systemic spread of the infection. In light of this analogy, some recent studies have attempted to examine the chemotactic behavior of neutrophils and other related eukaryotic cells in the context of foraging theory. In particular, Li et al. [36] studied the migrational patterns of the slime-mold amoeba *Dictyostelium*. They showed that given no exogenous stimuli, a correlated random walk greatly improves the cell’s chances of finding a target relative to performing a normal (uniform) random walk. These findings suggest that persistent motion could be a general foraging strategy employed by other chemotactic eukaryotes, such as neutrophils, to more efficiently locate targets in the absence of chemoattractant signals.

To further expand on the conclusions drawn from this previous work, this thesis presents a new phenomenological model of neutrophil migration designed to better characterize the chemotactic response of cells within their natural environment. This model allows for heterogeneous concentration fields of any number of chemoattractant species within the plane. Such a scenario differs considerably from the typical foraging problem described by Li et al. In particular, this situation involves additional cues, in the form
of endogenous chemoattractants, which serve to facilitate cell navigation toward the end targets. These chemokines can expand the effective detection radius of the cell by communicating the infection event to surrounding tissues, where locally-diffusing end target signals have little effect. By moving among these chemical beacons in stepwise fashion, wandering cells can move more efficiently to within detection range of the end target, where they can then disengage from the chemokine network through suppression of the corresponding signaling pathway. In theory, this chemotactic strategy may significantly improve search efficiency over conventional roaming strategies involving no assisted navigation.

In this updated picture of the immune response, the phenomenon of persistence then serves the auxiliary purpose of preventing excessive detainment of cells around regions of particularly high secondary signal concentration. Since there is no selective advantage to spending large amounts of time at a site that has no targets, this behavior could allow cells to more efficiently partition their migration time (since no persistence could result in little net displacement and therefore no favourable drift toward an end target source). Our mathematical model provides quantitative evidence to corroborate this argument - we’ve shown that varying the persistence time can heavily influence a cell’s ability to navigate successfully to a distant end target. In particular, the phenomenon of directional persistence was shown to have both benefits and drawbacks - in some cases, an excessively high persistence time exacerbated the cells’ ability to use the chemokine sources as effective navigational guides. It was also shown that high persistence times tend to be detrimental to the cells’ ability to converge on their targets. Overall, the effect of persistence may depend on a number of contributing factors, including the concentration of emitted chemokines, the separation between sources and the form of the gradient profiles.

By demonstrating that persistent motion may serve as an effective foraging strategy in both homogeneous and more complicated environments, we have thus introduced a general framework for biological processes involving long-range navigation and homing, particularly in the presence of multiple signals. A challenge for the future is to shed light on the factors that contribute to the efficiency of this foraging strategy in more specific environmental settings. For instance, are strategies that use directional memory efficient in a patchy food environment? If so, how does the optimal persistence time vary with the patch density and size distribution? Are there mechanisms by which the cells are able to manipulate the degree of persistence in their motion? These are some of the questions that we would like to address with additional experimentation and analysis, in addition to confirming the validity of our theoretical model.

4.2 Future work

One of the major challenges to understanding neutrophil chemotaxis is the inherent difficulty of working with primary human neutrophils, which can be very hard to isolate in the resting state, are terminally differentiated, and have short lifespans following purification. Adding to these complications is the fact that
it is almost impossible to monitor these processes \textit{in vivo} as they occur in real-time. Advances in single-molecule technologies such as FRET (fluorescence resonance energy transfer) have only recently made such studies more tractable. Another obstacle is the intrinsic coupling between the spatial and temporal aspects of regulation - since the cells themselves are undergoing constant motion, it is often difficult to decouple the two mechanisms in order to isolate their contributions to the overall cell response. Naturally, these issues will also need to be addressed in future investigations.

The goal of this thesis was to address the fundamental question of how neutrophils behave under realistic conditions, where multiple chemoattractant gradients are likely to influence the cells’ ability to reach their targets. The heuristic model presented here was based on a number of key observations made by previous experiments in the literature. However, it must be pointed out that there are a number of factors that were not considered in this crude model, including the effect of cell morphology, surface properties, physical topography and the effect of fluid dynamics on chemoattractant diffusion and cell behavior. As such, the data presented here is by no means conclusive. In particular, further investigation is necessary into the effect of noise and how directional persistence is mechanically regulated at the intracellular level. Possible follow-up experiments include manipulating the noise parameter within the model to determine if there is an optimal range within which cells can most efficiently find their end targets. Another valuable test would be to determine how the relative concentration of the chemokines emitted by each source influence the cell behavior. This would be a relatively straightforward experiment to test the predictions made by our model. And finally, it is also necessary to empirically confirm whether the persistence time \( \tau \) depends on concentration as speculated.

The motivation for studying neutrophil chemotaxis stems from both the crucial biological and medical importance of the process in homeostasis, inflammation and pathophysiology. A more rigorous understanding of the chemotactic mechanism in these cells has important implications for identifying potential therapeutic targets in modulating chemotactic efficiency or for designing specific pharmacologic interventions to dampen uncontrolled neutrophil responses in conditions such as chronic inflammation. In addition, more accurate computer simulations of other processes involving chemotaxis such as embryo development, bone remodeling, wound healing, tumor growth and infection will improve the predictive power of the model to evaluate untested therapies for related disorders. We hope that this work will contribute to providing deeper insight into the problem of chemotactic regulation in neutrophils beyond what can be achieved by conventional intracellular studies.
References


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