



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

DOTTORATO DI RICERCA IN
INGEGNERIA BIOMEDICA, ELETTRICA E DEI SISTEMI
Ciclo 38

Settore Concorsuale: 09/G1 - AUTOMATICA

Settore Scientifico Disciplinare: ING-INF/04 - AUTOMATICA

MODELING, PREDICTION AND CONTROL METHODS FOR TUMOR DYNAMICS

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Esame finale anno 2026

Modeling, Prediction and Control Methods for Tumor Dynamics

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2025

Abstract

Cancer cell dynamics can be seen as a complex, multiscale dynamical system whose behavior emerges from interactions among heterogeneous cells, their microenvironment, and external inputs. This thesis proposes a set of systems-theoretic methods for modeling, prediction, and control of tumor dynamics. Thanks to the combination of theoretical control and optimization tools with data and testbeds from experimental oncology, three novel approaches for cancer system scenarios are presented. First, a novel multi-cell tracking algorithm is proposed for time-lapse microscopy data. By modeling cell dynamics as an open multi-agent dynamical system, simultaneous prediction, tracking, and inference of biological cell-to-cell interaction parameters is performed. This method considers cells heterogeneity, migration, and proliferation within a single estimator that connects single-cell dynamics to population observables. Second, a systems-theoretic analysis of Extended Cellular Potts Models (ECPMs) investigates how haptotaxis, extracellular-matrix degradation, and stochasticity drive cancer invasion. Viewing ECPMs through the lens of feedback control, the work derives analytical results for tumor invasion times and related probabilities, energy evolution, and steady-state behaviors in simplified configurations, clarifying how microscopic local interaction rules determine macroscopic invasion speed and morphology. Third, an *in silico* Nonlinear Model Predictive Control (NMPC) approach regulates non-small cell lung cancer growth under multi-drug low-dose protocols. Built upon experimentally identified growth and pharmacodynamics models from *in vitro* imaging, dose-response assays, and viability tests, the NMPC framework explores constrained, multi-drug low-dose combination strategies while accounting for uncertainties. Together, these works contribute toward a unified research direction that integrates data acquisition, modeling, parameter identification, theoretical analysis, and model-based optimal control. By demonstrating how systems and control principles can quantify, predict, and regulate tumor behavior across scales, this work contributes to the emerging field of cybergenetics, thus aiming at bridging control and optimization methods with experimental oncology.

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CHAPTER 1

Introduction

This doctoral research project was carried out as part of a recently established collaboration between the University of Bologna—specifically the Center for Research on Complex Automated Systems (CASY)—and the IRCCS Rizzoli Orthopedic Institute (IOR). The initiative brings together distinct scientific domains—systems and control engineering on one side, and biology and cancer research on the other—to enable the study and regulation of complex biological phenomena. The research activities presented in this thesis are conducted in close interaction with both institutions, integrating experimental investigations with computational modeling. In addition, part of the work was performed at the Department of Engineering Mathematics at the University of Bristol, where I spent my research period abroad, further extending the interdisciplinary scope of the project and promoting international cooperation within the systems biology field.

1.1 Motivation and Challenges

The modeling, analysis and control of tumor dynamics represent a critical challenge in modern oncology. Tumor progression involves complex interactions across multiple scales, where genetic and molecular alterations drive cellular behaviors, and collective cell dynamics influence tissue-level phenomena such as invasion, metastasis, and therapeutic resistance. Despite significant advances in experimental and imaging technologies, our ability to predict and regulate tumor growth and its response to treatment remains limited. Bridging this gap necessitates the integration of mathematical modeling, data-driven inference, and control tools tailored to the biological and clinical complexities related to cancer systems.

A growing body of research has demonstrated how principles from systems and control theory can be applied to biological processes. The field of *Cybergenetics* [1, 2], for instance, has recently emerged as a framework for the feedback control of cellular dynamics, combining theoretical control design with real-time biological experiments. Related developments in synthetic biology and control engineering [3, 4] have provided systematic tools for the modeling, analysis, and regulation of genetic and metabolic systems, enabling the rational design of feedback loops that maintain stability, achieve adaptation, or drive specific dynamic behaviors in living cells. These approaches exemplify how classical control concepts can be extended to biological systems to achieve desired functions at the cellular or molecular scale.

However, the extension of these ideas to multicellular and heterogeneous systems remains an

open and rapidly evolving frontier. In such contexts, complexity arises not only from intracellular regulatory mechanisms but also from spatial organization, stochasticity, and dynamic cell–cell and cell–medium interactions. A prominent example of this complexity is found in cancer systems, where tumor progression and therapeutic response emerge from the interplay between individual cellular behaviors and the surrounding microenvironment. As highlighted in the reviews [5, 6], cell-based and multiscale computational models have become indispensable for bridging the gap between microscopic cellular behaviors and macroscopic tumor dynamics. These frameworks enable the exploration of how local rules of proliferation, migration, and extracellular matrix remodeling give rise to specific invasion patterns and morphological diversity.

At the same time, the regulation of tumor growth and treatment response naturally calls for a control-theoretic perspective. The formulation of optimal and feedback-based strategies for cancer therapy has been explored through the lens of mathematical control theory [7], offering a rigorous framework to design adaptive, low-dose, or combination treatments grounded in predictive modeling. These efforts illustrate how control principles can be translated from engineering to oncology, aiming not only to understand but also to actively steer biological dynamics toward desired therapeutic outcomes.

Within this broad interdisciplinary landscape, the present thesis aims to contribute to the modeling, analysis, and control of multicellular biological systems, with a particular focus on cancer invasion and therapeutic response. Across diverse scenarios and experimental contexts, the common thread lies in developing frameworks that couple experimental observations with mathematical representations. Specifically, this thesis addresses the following challenges:

- modeling and tracking heterogeneous cell populations in time-lapse microscopy experiments, focusing on cell division, migration, and interactions among different cell types;
- analyzing stochastic, spatially structured models of tumor invasion, linking cellular dynamics with extracellular matrix degradation to derive theoretical results and insights on tumor invasion times and steady-state behaviors;
- designing feedback control strategies for cancer growth inhibition, aiming to minimize drug resistance and toxicity, accounting for pharmacodynamics modeling and experimental constraints.

These challenges are unified by a shared vision: to connect experimental investigation, data acquisition, modeling, and control into a cohesive framework for studying cancer systems. By combining concepts from control theory, systems biology, and computational modeling, this work helps to advance model-based approaches for regulating multicellular tumor dynamics.

1.2 Summary of Main Contributions

This thesis presents three main contributions that address the above challenges through distinct yet complementary approaches.

The first contribution is the design of a novel cell tracking algorithm for *in vitro* experiments under optical microscopy. The algorithm leverages multi-agent systems concepts for modeling cell population dynamics. Unlike classical approaches, the proposed method integrates biophysical modeling and filtering to capture the dynamics of interacting cell types. Specifically, cell migration, proliferation, and interactions between different cell types are described through a multi-agent representation, formulated as an open dynamical system. In particular, model parameters are identified

from time-lapse co-culture data capturing tumor–stromal interactions. The calibrated model is then embedded within an Extended Kalman Filter to predict and reconstruct cell migration, mitosis events, and cell lineage across video frames. This framework enables robust tracking in complex biological scenarios while providing interpretable, model-based insights into local cell–cell interactions and global population dynamics.

The second contribution is a theoretical analysis of haptotaxis-driven tumor invasion within Extended Cellular Potts Models (ECPMs). By reformulating the model as a closed-loop stochastic dynamical system coupling tumor cell configuration with ExtraCellular Matrix (ECM) dynamics, the study bridges the gap between computational simulations and system theory tools. Analytical results are derived for the energy evolution of the system, for the asymptotic behavior of the ECM field, and for the transition probabilities of tumor configurations. In simplified settings, closed-form expressions for invasion time probabilities reveal how invasion dynamics depend on haptotactic strength, ECM degradation rates, and target cell volumes. These findings provide a promising foundation for the study of tumor–matrix interactions in Potts-based models.

The third contribution addresses the modeling and control of Non-Small Cell Lung Cancer (NSCLC) growth under multi-drug low-dose treatments. Using EGFR-mutant PC9 cells as a case study, an *in silico* experimental framework is developed that integrates growth modeling, drug response identification, and Nonlinear Model Predictive Control (NMPC). The proposed cancer growth under drug perturbations model is calibrated via time-lapse microscopy, dose–response, and cell viability assays data, capturing both untreated growth dynamics and inhibition effects under single-drug exposures. The model is then used within a NMPC scheme that optimizes multi-drug, low-dose regimens under uncertainty, accounting for time delays, drug resistance, and other experimentally-inspired constraints. The regulation scheme reproduces observed experimental trajectories in open-loop experiments and enables exploration of multi-drug, low-dose therapy design.

Overall, these three research directions advance both the methodological and application-oriented aspects of modeling and control in biological systems. The thesis contributes to the design of model-based approaches—ranging from stochastic cell-level dynamics to predictive control of population growth—to study, analyze and regulate complex biological phenomena.

1.3 Thesis Organization

The thesis is organized as follows.

Chapter 2 provides a foundational overview of cancer biology, experimental platforms, and therapeutic strategies. It reviews cellular mechanisms underlying tumor progression, cell migration, extracellular matrix remodeling, and drug resistance, alongside experimental techniques—including time-lapse microscopy, fluorescence imaging, and microfluidic platforms—that enable data acquisition and analysis of tumor dynamics. These foundations establish the biological and experimental context for the modeling and control approaches developed in subsequent chapters.

Chapter 3 first presents the multi-cell tracking framework. Then, It details the mathematical formulation of the proposed open multi-agent interpretation of cell culture systems, parameter identification from microscopy data, and the embedding of this model within a tailored filtering technique for predicting cell states. Finally, the overall novel algorithm is described, along with its application to *in vitro* time-lapse microscopy data of tumor–stromal co-cultures. Validation results and comparisons with existing tracking methodologies are also discussed.

Chapter 4 develops the theoretical analysis of ECPMs. It introduces a novel closed-loop system formulation highlighting the sparse structure of energy terms that determine tumor migration.

Subsequently, the analysis of steady-state behaviors, invasion times and related probabilities are presented in a simplified scenario. Finally, theoretical findings are compared with numerical simulations.

Chapter 5 focuses on the control-oriented modeling and NMPC-based therapy design for NSCLC growth control. It discusses the modeling choices and assumptions, the identification of growth and pharmacodynamic parameters based on *in vitro* data, and the design of the predictive control problem. Results regarding different drug-combination strategies are presented *in silico*, showing the potential of the proposed framework for optimizing multi-drug low-dose therapies.

Chapter 6 concludes the dissertation by summarizing the main findings and discussing their implications within the broader framework of cybergenetic control of multicellular systems. Future research directions are also outlined.

CHAPTER 2

Biological Systems and Experimental Platforms

This chapter introduces the biological framework and experimental methodologies that underpin the data acquisition, modeling, and control strategies explored in subsequent chapters. We begin with an overview of cellular biology, cancer mechanisms, and therapeutic strategies, followed by a discussion of standard and modern experimental techniques for investigating tumor dynamics.

2.1 Foundations of Cancer Biology and Therapeutic Strategies

In this section, we provide a concise overview of fundamental biological concepts pertinent to cancer biology and therapeutic strategies. We begin with the cellular basis of life, encompassing genetic information flow and protein function. We then explore the molecular mechanisms underlying cancer, including mutations, apoptosis, and drug resistance. Finally, we discuss therapeutic approaches, contrasting traditional chemotherapy with targeted small-molecule inhibitors.

These concepts constitute the foundational basis for systems and synthetic biology approaches to modeling, analyzing, and controlling complex biological systems, such as multi-cell tumor dynamics, as elaborated in later chapters.

2.1.1 The Central Dogma of Molecular Biology

The cell constitutes the fundamental unit of living organisms, comprising key components such as the plasma membrane—which delineates the intracellular environment from the extracellular space—the cytoplasm, organelles (e.g., mitochondria and endoplasmic reticulum), and the nucleus, the repository of genetic material in the form of deoxyribonucleic acid (DNA) [8]. The structural organization of a typical eukaryotic cell is illustrated in Figure 2.1.

Genes encoded within DNA are *transcribed* into messenger ribonucleic acid (mRNA), which is subsequently *translated* into proteins that fulfill structural, enzymatic, regulatory, and signaling roles. This unidirectional flow of genetic information, the so-called *Central Dogma of Molecular Biology* (see Figure 2.2), governs cellular function [9].

Proteins exhibit conformational states (active or inactive) that are dynamically regulated through post-translational modifications, such as phosphorylation, or by subcellular localization; for in-

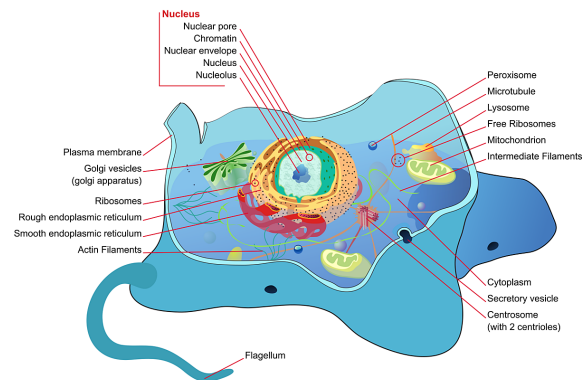


Figure 2.1: Schematic of a typical eukaryotic cell, illustrating the plasma membrane, nucleus, cytoplasm, and major organelles.

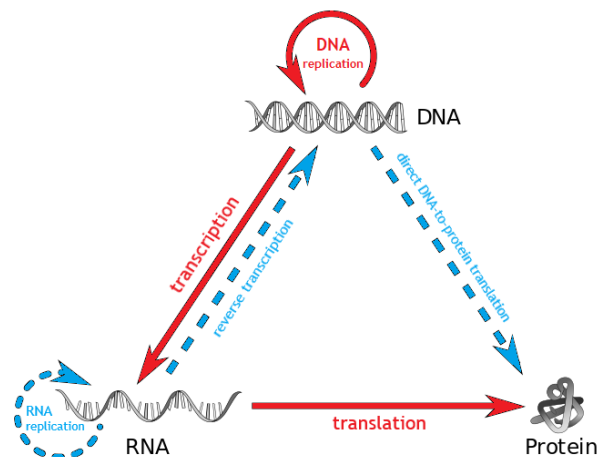


Figure 2.2: The central dogma of molecular biology describes the flow of genetic information from DNA to RNA to protein. DNA is transcribed into messenger RNA (mRNA), which is then translated into proteins that perform various cellular functions.

stance, certain regulatory proteins remain inactive in the cytoplasm until translocated to the nucleus [10]. Mutations in DNA can perturb protein structure, regulation, or expression levels, thereby disrupting these processes and contributing to pathological states [11].

2.1.2 Cancer, Genetic Mutations and Cell Cycle

Mutations are changes in the DNA sequence that can disrupt the normal function of genes, potentially leading to uncontrolled cell growth and cancer. These genetic alterations may occur spontaneously during DNA replication, be inherited from parents, or arise from environmental factors such as ultraviolet radiation, carcinogenic chemicals, or oncogenic viruses. Although the cell cycle includes redundant control mechanisms to ensure proper replication and division, occasional replication errors (mutations) can persist and be passed to daughter cells [11]. If such changes occur within coding regions of genes that regulate cell-cycle progression, they may result in the production of faulty proteins that compromise the fidelity of cell-cycle checkpoints. Small disruptions, such as changes in how cyclin-dependent kinases bind to cyclins¹, can speed up the cell cycle by weakening the cell's ability to control and repair itself. Over time, these small errors build up, allowing mutated cells to grow and divide faster than normal cells, which can lead to the formation of a tumor. Therefore, cancer arises as a result of accumulating genetic errors that upset the balance between cell growth, specialization, and death [12].

Key categories of genes implicated in this process include *oncogenes*, *tumor suppressor genes*, and *DNA repair genes*. Oncogenes are mutated or overexpressed versions of normal *proto-oncogenes*, which normally encode positive regulators of the cell cycle. When proto-oncogenes acquire activating mutations, they can drive cell-cycle progression independently of regulatory cues—for example, mutations in the *RAS* gene family² result in constitutive activation of signaling pathways that promote proliferation [11]. Conversely, tumor suppressor genes encode negative regulators that restrain cell division or initiate apoptosis, a programmed cell death mechanism that eliminates damaged or unnecessary cells, in response to DNA damage. When their function is lost the cell fails to halt division despite genomic instability [13]. A dysfunctional *p53* protein³, for instance, may not detect or repair DNA lesions, nor trigger apoptosis, allowing cells carrying deleterious mutations to survive and divide unchecked. This interplay between proto-oncogenes and tumor suppressor genes is illustrated in Figure 2.3. The interplay between these genetic alterations and the tumor microenvironment further accelerates cancer progression. Mutations enabling evasion of apoptosis, resistance to immune surveillance, or sustained proliferative signaling promote malignant transformation and metastasis.

While cancer biology is not the focus of this thesis, these brief biological foundations provide a conceptual model for understanding how system-level perturbations can lead to emergent, uncontrolled behavior in complex biological systems.

2.1.3 Directed Cell Migration and the Role of Extracellular Matrix

The *extracellular matrix* (ECM) is a complex network of proteins and polysaccharides that provides structural and biochemical support to surrounding cells. It maintains tissue architecture and reg-

¹Cyclins are proteins that control the progression of a cell through the cell cycle by activating cyclin-dependent kinases.

²RAS, from “Rat sarcoma virus”, is a family of related proteins that are expressed in all animal cell lineages and organs. All RAS protein family members belong to a class of protein called small GTPase, and are involved in transmitting signals within cells.

³p53, also known as tumor protein p53 (TP53), is a regulatory transcription factor protein that is often mutated in human cancers.

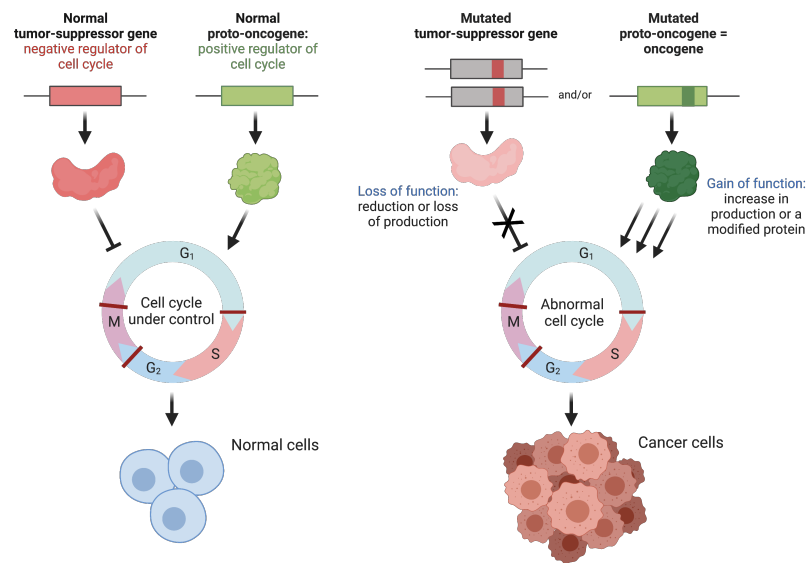


Figure 2.3: Tumor suppressor genes and proto-oncogenes produce proteins that regulate the cell cycle. Tumor suppressor genes are negative regulators that, when inactivated, fail to prevent uncontrolled growth. Proto-oncogenes are positive regulators that, when mutated or overexpressed, promote excessive cell division. Together, imbalances in these pathways underlie the genetic framework of cancer development.

ulates key cellular processes such as adhesion, migration, and signaling. Major ECM components include collagen, elastin, fibronectin, laminin, and proteoglycans, which together create a dynamic and spatially heterogeneous environment influencing cell behavior [14].

In cancer, the ECM is not a static scaffold but an active participant in tumor progression. Cancer cells and stromal cells remodel the ECM by secreting enzymes that degrade or reorganize its components, thereby generating a microenvironment that promotes invasion and metastasis. Such remodeling can alter ECM stiffness, density, and composition, in turn modulating cellular signaling and enhancing proliferative or survival pathways [15]. Within this evolving microenvironment, tumor cells migrate directionally in response to both biochemical and biomechanical cues. Two principal mechanisms underpin this directed migration: *chemotaxis* and *haptotaxis*. In Figure 2.4, we schematically compare these two migration modalities.

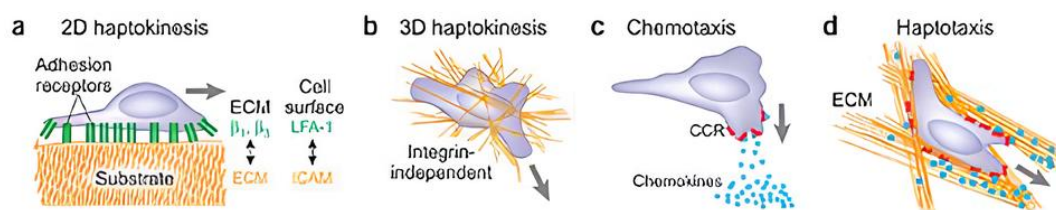


Figure 2.4: Schematic comparison of directed migration mechanisms: (a) 2D haptokinesis on a substrate with immobilized ECM cues and adhesion receptors; (b) 3D haptokinesis in a three-dimensional matrix; (c) chemotaxis toward a soluble chemo-attractant; (d) haptotaxis along gradients of ECM-bound cues in the extracellular matrix.

Chemotaxis refers to movement along gradients of soluble signaling molecules, such as chemokines

or growth factors, which are often secreted by tumor, stromal, or immune cells. These gradients guide cancer cells toward vasculature or distant niches [16].

Haptotaxis, by contrast, is migration guided by gradients of immobilized or substrate-bound signals within the ECM. Spatial variations in ECM composition, ligand density, or stiffness create adhesion landscapes sensed through integrins⁴, enabling tumor cells to move along favorable matrix paths [17]. This process links biochemical signaling with mechanical contact-based interaction, as cells dynamically probe and remodel their surroundings while responding to matrix-bound cues.

The interplay between ECM remodeling, chemotactic gradients, and haptotactic guidance defines the complex migration dynamics underlying cancer invasion and metastasis. From a systems biology perspective, these processes can be viewed as coupled feedback mechanisms, where local environmental cues regulate cell behavior, and migrating cells, in turn, reshape their microenvironment. Understanding and modeling these interactions provide a conceptual basis for quantitative descriptions of tumor spread and its control.

2.1.4 Drug Resistance and Toxicity

Drug resistance remains a major obstacle to effective cancer therapy, often leading to relapse or treatment failure. It arises from a combination of genetic, epigenetic, and microenvironmental factors that enable tumor cells to survive therapeutic pressure [18]. Numerous studies classify resistance determinants into categories including tumor burden, physical barriers, microenvironmental influences, and evolutionary selection. Collectively, these factors contribute to the emergence of resistant subclones and therapeutic insensitivity. In Figure 2.5, we illustrate the diverse nature of drug resistance in cancer.

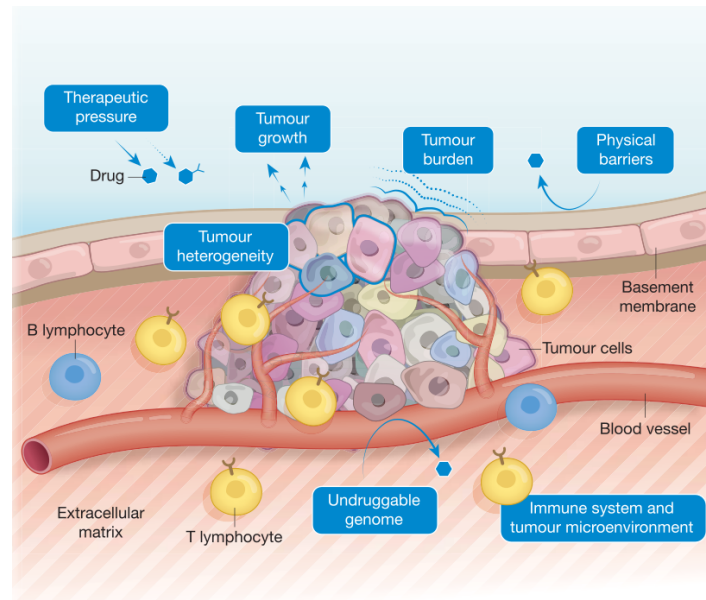


Figure 2.5: Biological determinants of drug resistance. Tumors are heterogeneous and exist within a complex microenvironment comprising vasculature, immune cells, and extracellular matrix components. Genetic, epigenetic, and physical factors collectively contribute to therapeutic resistance [18].

At the cellular level, resistance mechanisms involve mutations in drug targets, activation of sig-

⁴Integrins are transmembrane receptors that mediate cell-cell and cell-ECM adhesion.

naling pathways, epigenetic reprogramming, and phenotypic plasticity. For instance, secondary mutations can prevent drug binding or enhance drug efflux, while redundant pathways sustain proliferative signaling despite inhibition of primary oncogenic drivers. The tumor microenvironment further promotes resistance by restricting drug penetration and providing survival cues through stromal and immune interactions.

Limiting drug-induced toxicity is an equally significant challenge in cancer therapy. Both cytotoxic chemotherapies and targeted agents often affect healthy proliferating tissues, resulting in dose-limiting side effects that constrain the therapeutic window. Escalating doses to counteract resistance may worsen systemic toxicity, while insufficient dosing can accelerate the selection of resistant cell populations. Recognizing and managing treatment-related toxicity is essential not only for patient quality of life but also for sustaining therapeutic adherence and achieving optimal clinical outcomes [19].

Recent approaches aim to delay or mitigate resistance by combining agents with complementary mechanisms, implementing adaptive or intermittent dosing schedules, and integrating molecular monitoring to detect resistance early [18]. These strategies align with control-theoretic principles, emphasizing adaptive feedback and multi-drug dosing optimization to balance efficacy and safety.

2.1.5 Therapies: Chemo-Drugs VS Targeted Small-Molecule Inhibitors

Cancer treatment strategies have evolved considerably, from broad-spectrum approaches that target all proliferating cells to precision therapies designed to exploit cancer-specific molecular vulnerabilities. Understanding these therapeutic modalities is essential for developing rational dosing strategies and control-theoretic frameworks aimed at optimizing treatment efficacy while minimizing toxicity.

Traditional chemotherapy acts as a broad-spectrum control input that suppresses proliferative activity irrespective of molecular context. Cytotoxic compounds disrupt critical processes such as DNA replication and mitosis, thereby eliminating rapidly dividing cells. However, this lack of selectivity results in substantial off-target toxicity to healthy proliferating tissues, including the bone marrow, gastrointestinal epithelium, and hair follicles.

Conversely, *targeted small-molecule inhibitors* represent precision therapeutic inputs that modulate specific signaling nodes within dysregulated oncogenic networks. These agents bind selectively to oncogenic proteins—such as mutated kinases or overexpressed receptors—thereby exploiting cancer cell dependencies for therapeutic advantage [20]. Compared to conventional chemotherapy, targeted inhibitors typically exhibit improved selectivity and can be deployed as monotherapies or in rational combinations. Clinically successful examples include Imatinib for chronic myeloid leukemia and Erlotinib for non-small cell lung cancer (NSCLC) [20, 21]. A canonical example of targeted pathway modulation is inhibition of the epidermal growth factor receptor (EGFR) signaling cascade, a well-characterized driver of epithelial cancers. Figure 2.6 illustrates therapeutic strategies that target distinct levels of EGFR signaling. Small-molecule EGFR inhibitors—including first-, second-, and third-generation tyrosine kinase inhibitors—selectively block EGFR kinase activity by competing with ATP binding at the catalytic site. These agents have demonstrated strong clinical efficacy in tumors harboring activating EGFR mutations or receptor overexpression, though resistance frequently emerges through secondary mutations or activation of alternative signaling routes.

Key challenges in therapeutic design include optimizing dosing regimens (continuous versus intermittent), mitigating mechanisms of acquired resistance—such as target-site mutations, activation of compensatory pathways, or overexpression of efflux pumps [23]—and designing multi-target combinatorial strategies that delay resistance and extend progression-free survival [24]. From a system and control standpoint, chemotherapy and targeted inhibitors differ fundamentally in both speci-

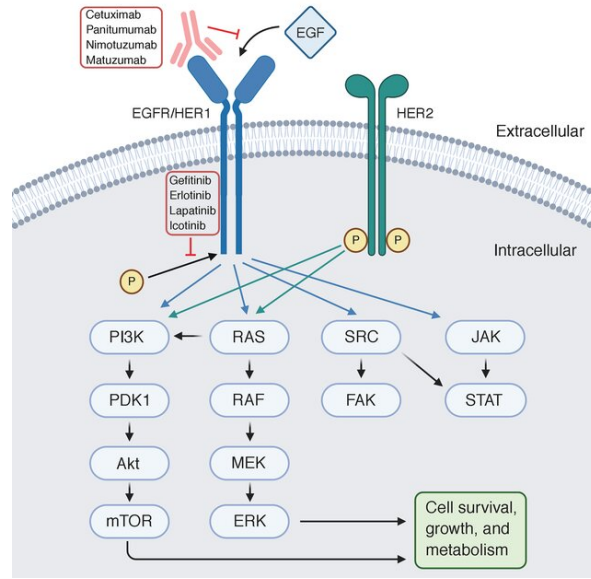


Figure 2.6: Schematic of EGFR and its downstream pathways, showing monoclonal antibodies exerting their effects at the extracellular domain and small-molecule inhibitors blocking phosphorylation intracellularly [22].

ficity and feedback potential. Chemotherapy represents a coarse, largely open-loop perturbation to cellular proliferation, whereas targeted inhibitors enable refined, pathway-specific interventions that lend themselves to model-based prediction, feedback adaptation, and optimal control of therapeutic response dynamics.

2.2 Experimental Platforms for Studying Tumor Dynamics

This section outlines the principal experimental methodologies and platforms used to study tumor dynamics at both population and single-cell levels. We focus on *in vitro* cell culture systems, advanced microscopy techniques, and microfluidic platforms that enable precise microenvironmental control and high-resolution imaging. These experimental frameworks provide the data acquisition and analytical foundation for the tumor dynamics modeling, prediction, and control methods discussed in subsequent chapters.

2.2.1 Cell Culture Systems

Cell culture constitutes the foundational experimental framework for studying tumor cell behavior and therapeutic response *in vitro* [25]. In a typical laboratory setting, cells are maintained under controlled environmental conditions—temperature, humidity, and CO₂ concentration—to reproduce physiological conditions as closely as possible. The most common configuration is the *two-dimensional monolayer culture*, in which adherent cells grow as a single layer attached to the surface of a treated plastic or glass substrate.

2D monolayer systems offer a well-defined and reproducible setting for investigating cell proliferation, migration, and drug response. The experimental workflow begins with the expansion of cells in culture dishes, during which the growth medium is periodically renewed to sustain nutrient availability and remove metabolic waste. When cells reach an appropriate confluence, they are detached

and seeded into multi-well plates for subsequent experiments. Each well constitutes an independent experimental unit, allowing the systematic definition of conditions, such as control environments (i.e. standard growth medium with no additional treatments), nutrient deprivation (starvation), or drug exposure. Control wells typically represent cells cultured under standard growth conditions, serving as a reference against which perturbations or treatments are compared. The use of biological replicates within identical conditions ensures statistical reliability and robustness of observed responses. Moreover, this setup can be extended to multiple cell types cultured together in the same well, enabling the study of cell-to-cell interactions between different populations, such as cancer cells and stromal cells.

Although 2D cultures are widely employed due to their simplicity, scalability, and compatibility with automated imaging and quantitative assays, they lack the structural and biochemical complexity of native tissues. In contrast, *three-dimensional (3D) culture systems* [26]—including spheroids and organoids—better reproduce aspects of the *in vivo* microenvironment by enabling multicellular organization and diffusion gradients. While these models provide enhanced physiological relevance, all experimental data analyzed in this thesis derive from conventional 2D monolayer cultures, which offer a controlled and analytically tractable platform for quantitative modeling and control. For completeness, in Figure 2.7, we illustrate the differences between 2D monolayer cultures and more complex 3D systems.

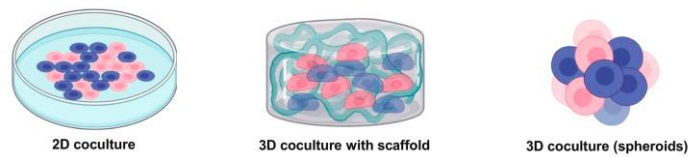


Figure 2.7: Representation of three co-culture setups: standard 2D monolayer (left), 3D with scaffold (middle), and 3D spheroids (right).

2.2.2 Microscopy: Time-Lapse Imaging and Fluorescence

Time-lapse microscopy enables the observation of dynamic cellular events—such as cell division, death, motility, and morphological changes—through periodic imaging over extended time periods. Key technical challenges include maintaining stable focus throughout the acquisition, minimizing phototoxicity (excessive light exposure can damage cells and induce death), and reliably detecting and tracking individual cells across time points. Automated high-throughput systems with image-processing and tracking software try to address these demands [27]. A typical experimental setup is illustrated in Figure 2.8.

Genetically encoded fluorescent proteins and dyes enable visualization of protein localization and dynamic translocation events, such as nucleo-cytoplasmic shuttling, thereby revealing activation patterns of signaling pathways [28].

Fluorescence microscopy techniques—including confocal and two-photon microscopy—provide high-resolution imaging of cellular structures, protein-protein interactions, and signaling dynamics in live cells. Fluorescent biosensors can report intracellular conditions such as pH, calcium concentration, and redox state, offering quantitative insights into cellular responses to therapeutic perturbations [29].

As a representative example, Figure 2.9 presents time-lapse images of PC9 cells, a NSCLC line

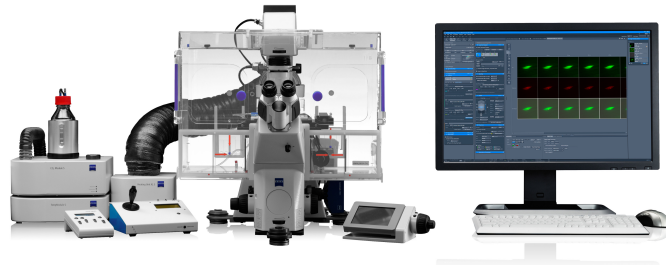


Figure 2.8: Time-lapse experimental setup for live-cell imaging.

harboring an activating EGFR mutation. These cells express fluorescent biosensors that report ERK and AKT signaling activity, enabling real-time monitoring of pathway dynamics in response to targeted therapies. The figure displays image frames captured under a confocal microscope across multiple modalities, including phase contrast and fluorescence channels for three distinct markers.

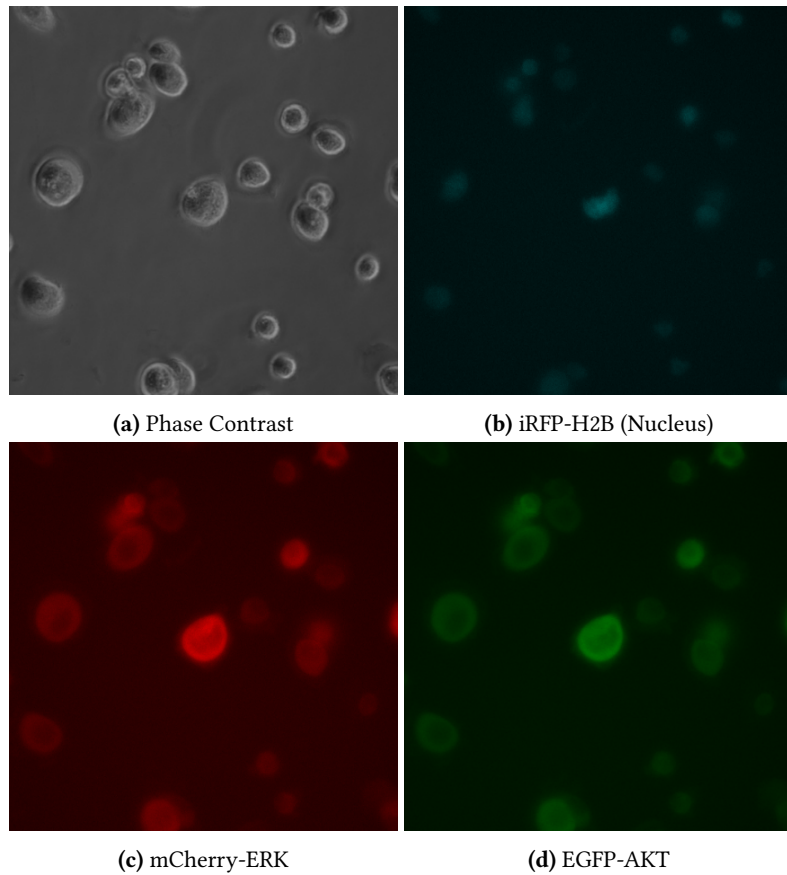


Figure 2.9: Representative time-lapse images of fluorescently tagged PC9 cells in different imaging modalities. The nucleus is labeled with iRFP-H2B (b), while the tags mCherry-ERK (c) and EGFP-AKT (d) report the activity of ERK and AKT signaling, respectively. Phase contrast (a) provides structural context.

2.2.3 Microfluidics and Imaging on-Chip

Microfluidic platforms provide precise control over microenvironmental parameters—including nutrient and oxygen gradients, drug concentrations, flow rates, and shear stress—while enabling prolonged imaging and co-culture experiments [30]. These devices are particularly effective for modeling physiological contexts relevant to cancer invasion, migration, and drug responsiveness [31].

Microfluidic systems typically consist of microchannels and chambers fabricated from biocompatible materials, such as polydimethylsiloxane (PDMS), which allow precise manipulation of fluids at the microscale. These platforms can recreate tumor microenvironments by incorporating extracellular matrix (ECM) components, stromal cells, and immune cells, facilitating the study of cell-cell and cell-matrix interactions [32].

One prominent application of microfluidics is the generation of chemical gradients to study cancer cell migration and invasion. By establishing stable gradients of chemo-attractants or inhibitors, researchers can investigate the mechanisms underlying directed cell movement, such as chemotaxis and haptotaxis [33]. Additionally, microfluidic devices enable high-throughput drug screening by exposing cells to varying concentrations of therapeutic agents in a controlled manner, providing insights into dose-response relationships and resistance mechanisms.

Microfluidics also supports single-cell analysis [34], allowing the isolation and tracking of individual cells over time. This capability is particularly valuable for studying cellular heterogeneity within tumors, as well as for monitoring dynamic responses to treatments at the single-cell level. Furthermore, integration with advanced imaging techniques, such as fluorescence microscopy and live-cell imaging, enhances the resolution and depth of analysis, enabling real-time visualization of cellular processes [29].

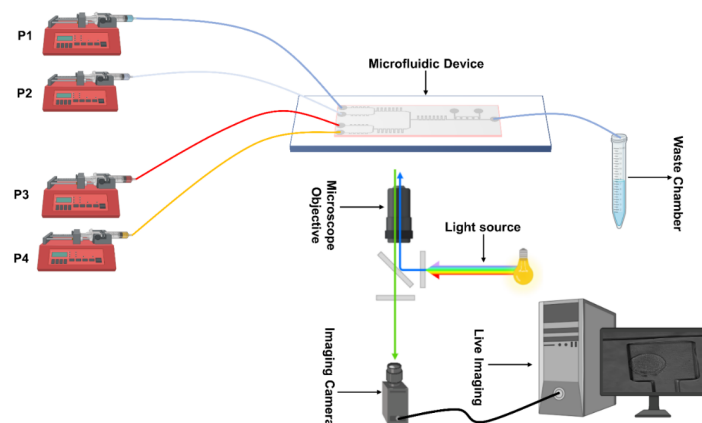


Figure 2.10: A schematic of the experimental setup for cell culturing and imaging on-chip.

Figure 2.10 illustrates a typical experimental setup for cell imaging and control on a microfluidic chip. In particular, it can be seen how the microfluidic device is placed on the stage of an inverted microscope inside a controlled environmental chamber. The setup includes a syringe pumps to regulate fluid flow through the microchannels, enabling precise delivery of nutrients, drugs, or other factors to the cultured cells. This configuration allows for real-time imaging and analysis of cellular responses under tightly controlled conditions, facilitating studies of tumor dynamics and related therapy design.

Modeling and Tracking of Heterogeneous Cell Populations in Time-Lapse Microscopy

This chapter builds upon the findings reported in our journal pre-print [35], which extends our earlier conference paper [36].

3.1 Introduction

The ability to analyze and model cellular behaviors at the single-cell level is essential for understanding tissue-scale biological processes such as cell-to-cell interactions, migration, and responses to microenvironmental cues or external inputs. In this context, cell tracking—the automated reconstruction of individual cell trajectories over time—plays a fundamental role in connecting experimental observations, data analysis, and mathematical modeling.

Building on these motivations, and those introduced in Chapter 1, this chapter presents a novel algorithm for modeling, predicting, and tracking multicellular dynamics in time-lapse optical microscopy videos. As a matter of fact, optical microscopy (see Section 2.2.2) remains a popular technique for observing live-cell behaviors *in vitro*, providing the quantitative information required to investigate multicellular systems and build mathematical models capable of reproducing or predicting biological phenomena.

Particularly informative are co-culture experiments, where distinct cell types—such as tumor and stromal cells—coexist and interact. These systems reveal how local cell-to-cell mechanisms drive collective behaviors, including migration, increased proliferation, and the emergence of drug resistance [37]. Therefore, extracting accurate single-cell trajectories from such experiments is a critical step toward understanding the underlying dynamics of heterogeneous cell populations and their interactions.

In recent years, the systems and control community has shown a growing interest in applying system-theoretic methods to biological populations [38–40]. These developments fall within the emerging field of Cybergenetics [1], which integrates feedback control principles with biological experimentation, and within broader efforts in multicellular systems modeling [41]. Representing individual cells as interacting agents provides a natural framework to capture emergent multicellular behaviors, thereby linking microscopic cell-level dynamics to macroscopic system evolution.

Beyond their role in cell data acquisition and analysis, cell-tracking algorithms also serve as key components in feedback control schemes for biological systems. In cybergenetic applications, a multi-cell tracker can be interpreted as an observer in the systems and control sense—providing real-time state estimates of cell populations to enable state feedback-based interventions, such as the optimal regulation of growth or gene expression in microfluidic environments, like to ones described in Section 2.2.3.

3.1.1 Related Works

A large body of research has addressed the problem of Multi-Cell Tracking (MCT) in biological microscopy data, as reviewed in [42, 43]. This problem has relevance across numerous applications, including cell biology, cybergenetics, and drug discovery. The increasing interest in this field has also led to the creation of benchmark datasets, such as the Cell Tracking Challenge [44], which primarily focus on *in vitro* monoculture experiments. In contrast, the present work investigates a more complex and biologically realistic co-culture scenario involving interacting cell types.

A wide range of algorithms have been proposed for MCT. Deep learning approaches often combine convolutional and recurrent neural architectures to perform segmentation and motion inference simultaneously [45–50]. Other methodologies rely on probabilistic and optimization-based frameworks, such as Markov random fields [51] or variational optical-flow formulations [52]. While these techniques have shown excellent performance on standard benchmarks, they typically treat cell trajectories as independent, neglecting the underlying dynamical and interaction structure of the population. Consequently, they provide limited insight into the mechanisms governing collective behaviors or intercellular coordination. Furthermore, most available tracking algorithms are developed for monoculture data and do not explicitly address heterogeneity across cell types.

Parallel to these algorithmic efforts, multi-agent modeling has emerged as a conceptual bridge between biological and engineered systems [53–56]. Such frameworks represent cells as autonomous agents interacting through local communication, coordination, or signaling processes, and can describe open systems in which the number of agents varies over time. This paradigm offers an appealing foundation for modeling living systems with time-varying populations, where phenomena such as mitosis or cell death introduce non-constant system dimensions. Similar principles have been exploited in the study of synchronization and entrainment in bacterial populations [54] and in the design of bio-inspired coordination strategies for robotics [55].

In addition, several works in systems biology have adopted control-theoretic perspectives to model and regulate biological processes [38–40, 57–61]. These studies have addressed a variety of objectives, including gene regulation, adaptive population control, and therapeutic optimization. However, their focus is often on feedback design rather than on the reconstruction of individual cell behaviors from data. In contrast, this chapter aims to develop a model-driven framework that integrates identification, prediction, and tracking within a single coherent methodology.

3.1.2 Contributions

This chapter introduces a novel framework for tracking heterogeneous cell populations in time-lapse *in vitro* multi-culture experiments, with a specific focus on co-culture settings. The proposed algorithm, termed Heterogeneous Open Multi-Cell EKF-based Tracking (HEOM-EKF), addresses the following key limitations of existing MCT approaches:

- **Modeling of heterogeneous cell populations.** Most tracking algorithms are designed for monoculture settings and do not explicitly model interactions among different cell types.

Contribution of this work: heterogeneous cell populations are modeled as an open multi-agent system [62], in which each cell is represented as an agent governed by parametric interaction terms. These rules explicitly capture both intra-type and inter-type dynamics, enabling the representation of collective behaviors such as chemotaxis-like motion [63]. The open-system formulation naturally accommodates switching population dynamics over time, including cell division and cells entering or leaving the microscope field of view. As a case study, we focus on co-cultures of mesenchymal stromal cells (MSC) and osteosarcoma cells, whose interactions are known to play a key role in tumor aggressiveness and therapeutic resistance [64–67].

- **Interpretability of motion prediction models.** Many state-of-the-art MCT pipelines rely on deep learning-based predictors, which often provide limited interpretability with respect to the underlying biological mechanisms.

Contribution of this work: a structured open multi-agent dynamical model is proposed, whose parameters are identified directly from real experimental data via dedicated system identification procedures. This results in interpretable models that characterize migration, interaction, and proliferation behaviors, allowing biologically meaningful insights to be extracted from the estimated model parameters.

- **Handling of population change events and lineage reconstruction.** Distinguishing mitosis events from cells entering or exiting the field of view remains a challenging problem for many tracking methods.

Contribution of this work: the identified multi-agent model is embedded within an Extended Kalman Filter tailored to open multi-agent systems. The resulting estimator incorporates switching label sets and adaptive state dimensionality, enabling explicit handling of mitosis, cell entry, and cell exit events. This formulation supports recursive state estimation and prediction while allowing reconstruction of the cell lineage tree over time.

To support the proposed methodology, a new annotated dataset has been constructed from raw time-lapse microscopy videos of *in vitro* co-culture experiments involving MSC and osteosarcoma cells. In contrast to most publicly available benchmarks, which are limited to monoculture systems, this dataset provides a challenging and clinically relevant testbed for studying heterogeneous interacting cell populations and validating tracking approaches. Numerical experiments conducted on the newly developed dataset demonstrate the effectiveness of the proposed HEOM-EKF algorithm in terms of tracking accuracy, robustness to population changes, and interpretability of the inferred multi-cell interaction dynamics.

A preliminary version of this work appeared in [36], where a monoculture tracking algorithm based on a standard multi-agent formulation was presented under the assumption of a constant population size. The present study extends that work by introducing an open multi-agent framework capable of handling heterogeneous populations and time-varying numbers of cells, explicitly modeling mitosis events and enabling lineage reconstruction.

3.1.3 Chapter Outline

The remainder of this chapter is organized as follows. Section 3.2 formulates the cell-tracking problem and outlines the main components of multi-object tracking algorithms. Section 3.3 introduces the open multi-agent modeling approach and describes the parameter identification strategy. Section 3.4 presents the proposed HEOM-EKF algorithm. Finally, Section 3.5 describes the dataset and

its use for training and testing, analyzes the identified multi-cell model parameters, and presents numerical experiments that assess the performance of the proposed algorithm.

3.2 Multi-Cell Tracking Problem Formulation

In this work, we consider a cell-tracking problem in a biological scenario in which the goal is to track multiple cells of different types in time-lapse sequences. Throughout this study, we consider video frames in which the number of cells changes over time. These variations may result from biological processes such as mitosis, and/or from cells entering or exiting the microscope camera field of view (FOV).

In this section, we formalize the fundamental concepts behind cell-tracking algorithms. We will delve into more details in Section 3.3, in which we model the cell population as an open multi-agent system and propose suitable formulations for their dynamics. These dynamical models will be a key component of the proposed cell-tracking strategy in Section 3.4.

The proposed framework follows the widely adopted Track-by-Detection paradigm, in which object detections are first obtained independently on each frame and then linked over time by a dedicated tracking module. In this setting, the main building blocks are: *i*) object detection, *ii*) motion prediction, and *iii*) data association. A schematic of this procedure is depicted in Figure 3.1. Object

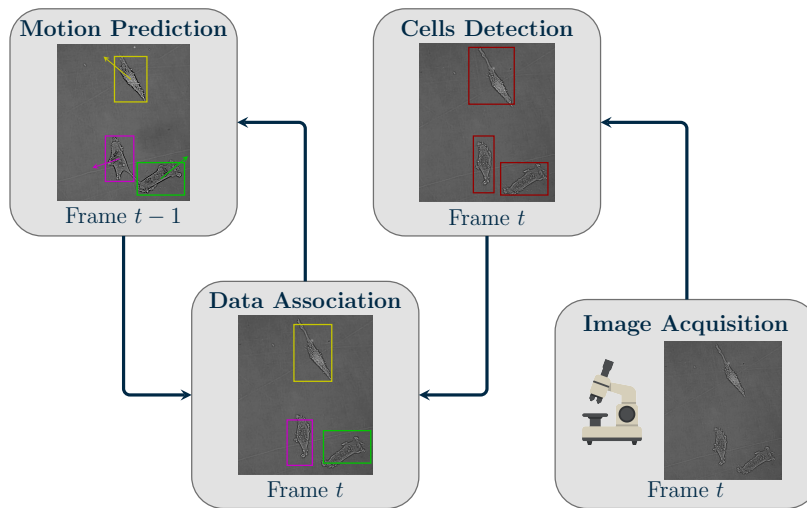


Figure 3.1: Scheme of a generic multi-cell tracking algorithm

detection refers to the identification and localization of cells within images or video frames. Modern object detection methods often employ neural networks trained specifically for this task [68].

Motion prediction aims to estimate future positions of detected cells by combining their past trajectories with current detection measurements. This step is essential for ensuring track continuity and handling gaps or occlusions in the detections. Common techniques include Kalman filters, particle filters, and, most commonly, deep learning-based predictors. However, neural network predictors function as black boxes, limiting interpretability, and require large datasets, which are limited in this field. In contrast, our approach explicitly models multi-cell dynamics and uses a closed-form parameter identification method to ensure reliability and data efficiency.

Data association involves linking detected cells across consecutive frames to preserve their identities over time. This process requires matching detections from different frames and assigning them

to the correct cell track. Motion models and similarity metrics are frequently used to assess the likelihood of accurate associations.

3.3 Heterogeneous Cell Populations as Open Multi-Agent Systems

In this section, we first formalize cell dynamics as an open multi-agent system. Then, we introduce a tailored class of parametric multi-agent terms to describe the interactions between heterogeneous cell populations within a general multi-culture scenario. These dynamical terms constitute the multi-agent model that will be then adopted in the proposed cell-tracking algorithm. The model design draws inspiration from a well-established line of research on bio-inspired multi-agent systems [63]. In particular, we extend interaction terms from swarm-like approaches [69, 70] to an open multi-agent framework. These terms describe both positional and morphological aspects of cell states. The latter terms are weighted by a set of parameters to be identified through data collected from time-lapse experiments.

3.3.1 Open Multi-Cell System Formalization

We model mitosis as an event where a parent cell splits into two new daughter cells. In this process, the parent cell is removed from the system and two new cells appear in its place, retaining the parent's type. To maintain distinct cell identities over time, we assign each cell a unique label i . We consider a discrete-time framework, where $t \in \mathcal{T} \subseteq \mathbb{N}$ denotes the time index over an arbitrary horizon $\mathcal{T}$ (infinite or finite). In the context of a multi-culture experiment, we restrict attention to a finite time horizon, namely $\mathcal{T} = \{1, \dots, T\}$. Here, t will be the discrete-time variable representing the frame at which the system is observed, and with $T \in \mathbb{N} \setminus \{\infty\}$ denoting the final time instant at which the experiment concludes. At any time $t \in \mathcal{T}$, we denote by $N^t \in \mathbb{N}$ the number of cells present within the microscope FOV. Additionally, we define the subset of time instants $\mathcal{T}_s \subset \mathcal{T}$ corresponding to switching times at which events related to cell population changes occur, i.e. when the number of cells within the observed frame changes, indicating a switch in the system dynamics. Moreover, we denote the number of cells entering and exiting the heterogeneous cell population within the microscope FOV at time t by $C_{\text{IN}}^t \in \mathbb{N}$ and $C_{\text{OUT}}^t \in \mathbb{N}$, respectively.

Let $L^t \subset \mathbb{N}$ be the set of labels identifying all the cells present in the scene at time t . Consequently, we introduce its subsets $L_p^t \subset L^t$ and $L_d^t \subset L^t \times L^t$ containing the labels of parent and daughter cells, respectively. Based on that, mitoses occurrences can be described through time-dependent sets of label pairs, identifying parent cells splitting into daughter cells at the current time. Formally, mitoses occurring at frame t are represented by

$$\mathcal{M}^t := \{(i_p, j_d) : i_p \in L_p^t \subset \mathbb{N}, j_d \in L_d^t \subset \mathbb{N}^2\}. \quad (3.1)$$

Instead, new cell entries into the camera field, at time t , are captured by the set $L_{\text{IN}}^t \subset \mathbb{N}$, such that

$$|\mathcal{M}^t| + |L_{\text{IN}}^t| = C_{\text{IN}}^t. \quad (3.2)$$

Similarly, cell exiting from the camera FOV at time t are described by the label set $L_{\text{OUT}}^t \subset \mathbb{N}$, verifying

$$|L_{\text{OUT}}^t| = C_{\text{OUT}}^t. \quad (3.3)$$

Note that $L_p^t \cap L_d^t \cap L_{\text{OUT}}^t \cap L_{\text{IN}}^t = \emptyset$, but for different time instants these sets may have non-null intersections. Moreover, at least one of these sets is non-empty at times $t \in \mathcal{T}_s$. The total number of

cells at any time t , previously introduced as N^t , evolves according to

$$\begin{aligned} N^{t+1} &= N^t + C_{\text{IN}}^t - C_{\text{OUT}}^t \quad \forall t \in \mathcal{T}_s \\ N^{t+1} &= N^t \quad \forall t \notin \mathcal{T}_s. \end{aligned}$$

Moreover, the dynamics of the label set L^t can then be expressed as

$$L^{t+1} = (L^t \cup \bar{L}_d^t \cup L_{\text{IN}}^t) \setminus (L_p^t \cup L_{\text{OUT}}^t),$$

where $\bar{L}_d^t := \bigcup_{(a,b) \in L_d^t} \{a, b\}$ is the set of the individual elements from daughter cells label pairs.

Building upon the concepts introduced above, the following subsection presents the proposed open multi-agent parametric model.

3.3.2 Open Multi-Agent Parametric Model

We now introduce the open multi-cell system that models the cell dynamics for the tracking purpose. In this framework, each generic agent i represents a cell. The state of agent i at time t and its neighbor set are denoted by $x_i^t \in \mathbb{R}^n$ and $\mathcal{N}_i^t \subset L^t$, respectively. To support a general multi-culture scenario in which different cell populations are interacting, we consider $\Gamma \in \mathbb{N}$ distinct agent types, each representing a different cell type. Thus, to each cell type will be associated a parameter vector $\theta_\gamma \in \mathbb{R}^{n_\theta}$, $\gamma \in \{1, \dots, \Gamma\}$. The entries of θ_γ correspond to weights that modulate the strength of different interaction terms in the multi-agent dynamics. We denote

$$\theta := [\theta_1^\top, \dots, \theta_\Gamma^\top]^\top \in \mathbb{R}^{n_\theta \Gamma}, \quad (3.4)$$

the stack of the parameter vectors for each cell type. The constant parameters θ will be learned from experimental data.

Let $L := \bigcup_{t=1}^T L^t$ denote the set of all agent labels across time. Then, each agent $i \in L$ is associated with a parameter vector $\theta_{\gamma_i} \in \mathbb{R}^{n_\theta}$, determined by its type $\gamma_i \in \{1, \dots, \Gamma\}$. Based on that, agent dynamics are modeled according to the discrete-time formulation

$$x_i^{t+1} = x_i^t + f_i \left(x_i^t, \{x_j^t\}_{j \in \mathcal{N}_i^t}, \theta_{\gamma_i}, t \right), \quad \forall i \in L^{t+1} \cap L^t, \quad (3.5)$$

where the function f_i captures the dynamics of agent i based on its current state, the states of its neighboring agents, and its type-based parameters θ_{γ_i} . This formulation captures interactions based on cell states and types, enabling the representation of heterogeneous behaviors in multi-culture settings.

We propose coordinates of a Minimum Enclosing Rectangle (MER) as agent state within the proposed Multi-Cell Tracking (MCT) algorithm. This representation of cell morphology is defined as

$$x_{\text{MER},i} := [p_i^\top, s_i, r_i, \phi_i, v_i^\top, v_{s,i}, v_{r,i}, \omega_i]^\top, \quad (3.6)$$

where $p_i, v_i \in \mathbb{R}^2$ denote the center position and velocity of the cell, $s_i, r_i \in \mathbb{R}$ its area and aspect ratio, and $v_{s,i}, v_{r,i} \in \mathbb{R}$ their respective rates of change. The orientation angle $\phi_i \in [0, 2\pi]$ specifies the rotation of the MER relative to a fixed reference axis, and $\omega_i \in \mathbb{R}$ is the angular velocity. This state representation is especially suitable for tracking cells with fusiform or anisotropic morphologies, as it captures orientation and aspect more faithfully than the standard bounding box format. Moreover, it leads to more informative outputs at the end of the tracking pipeline, supporting better discrimination between different types based on shape dynamics. However, standard performance

metrics for tracking algorithms are typically designed for axis-aligned bounding boxes. As a result, evaluating tracking accuracy on the state format in (3.6) requires custom metrics that are not always readily available or comparable with prior work. To facilitate benchmarking and compatibility with existing evaluation frameworks, our algorithm also supports a simplified state representation based on axis-aligned bounding boxes. Formally,

$$\mathbf{x}_{BB,i} := [p_i^\top, s_i, r_i, v_i^\top, v_{s,i}, v_{r,i}]^\top, \quad (3.7)$$

where the state variables retain the same semantics as in (3.6), but omit orientation and angular velocity. This compact version is especially useful for performance evaluation and comparison with other methods, while still capturing essential information about cell shape and motion.

Based on these two representations, the neighbors of cell i at time t in (3.5) can be defined as

$$\mathcal{N}_i^t := \left\{ j \neq i : \|p_i^t - p_j^t\| \leq R_s, \{i, j\} \subseteq L^t \right\},$$

where R_s is a sensing radius that defines the region of space in which other cells are perceived. Here, we consider R_s as a priori fixed quantity (not learnable).

Building on the generic formulation of the open multi-agent model in (3.5), we now specify the individual terms governing the dynamics of each state component. For better readability, we first define subgroups of the whole state

$$\begin{aligned} \pi_i &:= [p_i^t, v_i^t]^\top \in \mathbb{R}^4 \\ \sigma_i &:= [s_i^t, v_{s,i}^t]^\top \in \mathbb{R}^2 \\ \rho_i &:= [r_i^t, v_{r,i}^t]^\top \in \mathbb{R}^2 \\ \eta_i &:= [\phi_i^t, \omega_i^t]^\top \in \mathbb{R}^2. \end{aligned}$$

Then, for the MER state format, the dynamics of cell i of type γ_i are given by

$$p_i^{t+1} = p_i^t + v_i^t \quad (3.8a)$$

$$s_i^{t+1} = s_i^t + v_{s,i}^t \quad (3.8b)$$

$$r_i^{t+1} = r_i^t + v_{r,i}^t \quad (3.8c)$$

$$\phi_i^{t+1} = \phi_i^t + \omega_i^t \quad (3.8d)$$

$$v_i^{t+1} = v_i^t + f_{i,1} \left(\pi_i, \{ \pi_j \}_{j \in \mathcal{N}_i^t}, \theta_{\gamma_i} \right) \quad (3.8e)$$

$$v_{s,i}^{t+1} = v_{s,i}^t + f_{i,2} \left(\sigma_i, \{ \sigma_j \}_{j \in \mathcal{N}_i^{t-1}}, \theta_{\gamma_i} \right) \quad (3.8f)$$

$$v_{r,i}^{t+1} = v_{r,i}^t + f_{i,3} \left(\rho_i, \{ \rho_j \}_{j \in \mathcal{N}_i^t}, \theta_{\gamma_i} \right) \quad (3.8g)$$

$$\omega_i^{t+1} = \omega_i^t + f_{i,1} \left(\eta_i, \{ \eta_j \}_{j \in \mathcal{N}_i^t}, \theta_{\gamma_i} \right), \quad (3.8h)$$

where

$$f_{i,1} := \theta_{\gamma_i,1} \left(\frac{1}{|\mathcal{N}_i^t|} \sum_{j \in \mathcal{N}_i^t} p_j^t - p_i^t \right) + \theta_{\gamma_i,2} \left(\frac{1}{|\mathcal{N}_i^t|} \sum_{j \in \mathcal{N}_i^t} v_j^t - v_i^t \right) + \theta_{\gamma_i,3} \sum_{j \in \mathcal{N}_i^t} \frac{p_i^t - p_j^t}{\|p_i^t - p_j^t\|^2}, \quad (3.9)$$

captures the tendency to cohesion, alignment, and repulsion behaviors, while the shape-related terms

$$\begin{aligned} f_{i,2} &:= \theta_{\gamma_i,4}^* (S_{\gamma_i} - s_i^t) \\ f_{i,3} &:= \theta_{\gamma_i,5}^* (R_{\gamma_i} - r_i), \end{aligned} \quad (3.10)$$

model the tendency to maintain a certain target area $S_{\gamma_i} \in \mathbb{R}$ and target aspect ratio $R_{\gamma_i} \in \mathbb{R}$, considering cells of type γ_i . Here, $\theta_{\gamma_i,j} \in \mathbb{R}$ with $j = 1, \dots, 5$ represent the learnable parameters associated with cells of type γ_i .

These multi-cell dynamics are inspired by engineering multi-agent models. Specifically, the terms in (3.9) follow swarm-based approaches and incorporate chemotaxis-inspired principles [69, 70]. In contrast, the morphological terms in (3.10), which govern area and aspect ratio dynamics are novel. These terms are designed based on our dataset analysis, where S_γ and R_γ are derived from morphological statistics (see Section V). Together, these components capture a biologically motivated tendency for each cell type to maintain its characteristic shape over time.

Finally, we specify that for the standard bounding box state defined in (3.7), the dynamics can be fully described using only equations (3.8a)-(3.8c) and (3.8e)-(3.8g).

3.3.3 Identification of Model Parameters

We now present the proposed approach for identifying parameters within the multi-agent dynamics discussed in Section 3.3.2. Here, we consider a generic formulation with Γ agent populations captured by parameters $\theta \in \mathbb{R}^{n_\theta \Gamma}$, thus providing a flexible tool scalable to different experimental frameworks. Therefore, we propose an identification method tailored to open multi-cell systems, like those described in Section 3.3.1.

The goal is to identify the parameters and obtain estimates that fit a training set of real cell trajectories extracted from *in vitro* co-culture experiments. Consider a dataset consisting of T image frames with the relative cell segmentations and tracks. From the latter, for each frame t we have access to p_i^t , s_i^t , r_i^t and ϕ_i^t for all cells i . From these, we compute the relative velocities v_i^t , $v_{s,i}^t$, $v_{r,i}^t$ and ω_i^t using differentiation methods. We tested various differentiation methods commonly used in system identification from time series data, such as finite differences, smoothed finite differences, Savitzky-Golay filtering, spline interpolation, and Kalman-based filtering, which are also discussed in the context of data-driven model identification (e.g., in [71]). In the experimental tests, we adopted a Kalman-based differentiation approach, which provided smoother estimates and led to smaller residuals in the subsequent model identification phase. This approach allows us to approximate the necessary time derivatives despite the intrinsic stochasticity and noise in biological cell dynamics and aligns with established practices in the literature.

Since we defined the dynamics in equation (3.5) as a weighted sum of multi-agent state terms, and imposed sensing radius R_s to be not learnable, we end up having a system with linear dependence in θ . By rearranging the dynamics to be linear in θ , we formulate a standard linear least squares (LLS) problem. The solution to the LLS problem is obtained by solving

$$\theta^* = \underset{\theta}{\operatorname{argmin}} \|Y - D\theta\|^2, \quad (3.11)$$

where observation matrices are

$$Y_i := [y_i^1, \dots, y_i^T]^\top \in \mathbb{R}^{n_T}, \quad (3.12a)$$

$$Y := [Y_1^\top, \dots, Y_N^\top]^\top \in \mathbb{R}^{n_T N}. \quad (3.12b)$$

and design matrix

$$D := [D_1, \dots, D_\Gamma] \in \mathbb{R}^{(n_T N) \times \Gamma n_\theta},$$

in which each $D_\gamma \in \mathbb{R}^{(n_T N) \times n_\theta}$ represents the block corresponding to type γ agents. The latter are defined as

$$D_\gamma := [D_{\gamma,1}^\top, \dots, D_{\gamma,N}^\top]^\top \quad \forall \gamma \in \{1, \dots, \Gamma\},$$

where $N := |L|$ is the total number of cells in the dataset, considered here as a single time-lapse experiment.

For all the cells $i \in L$, we specify the entries of the observation matrices (3.12a) as

$$y_i^t = \begin{cases} [x_i^t - x_i^{t-1}]^\top & \text{if } i \in L^t \cap L^{t-1} \\ \mathbf{0}_n^\top & \text{otherwise} \end{cases}.$$

Instead, the single-cell design matrices are constructed as

$$D_{\gamma,i} = \begin{bmatrix} d_{i,1}^1 & d_{i,2}^1 & \dots & d_{i,n_\theta}^1 \\ \vdots & \vdots & & \vdots \\ d_{i,1}^T & d_{i,2}^T & \dots & d_{i,n_\theta}^T \end{bmatrix} = \begin{bmatrix} d_i^1 \\ \vdots \\ d_i^T \end{bmatrix} \in \mathbb{R}^{nT \times n_\theta},$$

verifying that for each t

$$d_i^t = \mathbf{0}_{n \times n_\theta} \quad \forall \gamma \neq \gamma_i \text{ or } i \notin L^t.$$

This condition, adding sparsity to the design matrix, ensures feasibility by limiting the data to cells present at the current frame t and by selecting different weights based on type γ_i . Otherwise, the entries of cell $i \in L^t$ are functions of the considered cell and its neighbor states

$$\begin{aligned} d_{i,1}^t &= f_{i,1} \left(x_i^t, \{x_j^t\}_{j \in \mathcal{N}_i^t} \right) \\ &\vdots \\ d_{i,n_\theta}^t &= f_{i,n_\theta} \left(x_i^t, \{x_j^t\}_{j \in \mathcal{N}_i^t} \right). \end{aligned} \tag{3.13}$$

Note that, since $nTN \gg \Gamma n_\theta$ then $(D^\top D)^{-1}$ exists if and only if $\text{rank}(D) = \Gamma n_\theta$. The only case where this condition is not met is when, for an instant of time $t \in \{1, \dots, T\}$, there are no agents present in the scene, meaning that $L^t = \emptyset$. However, in our framework, we can assume that $L^t \neq \emptyset \forall t$ since we work with videos in which at least one cell is always present throughout the entire duration of the experiment. The solution of (3.11) that best fits cell trajectories extracted from a single experiment is

$$\theta^* = (D^\top D)^{-1} D^\top Y. \tag{3.14}$$

Note that while $nTN \gg \Gamma n_\theta$ is necessary for the LS problem to be well-posed, invertibility of $D^\top D$ additionally requires full column rank. This depends on the informativeness of the collected cell trajectories. For instance, if cells exhibit minimal motion, certain dynamical terms may remain insufficiently excited, potentially causing rank deficiency despite abundant data. To verify these conditions, we analyzed the design matrix constructed from our dataset. We confirmed that $\text{rank}(D) = \Gamma n_\theta$, ensuring full column rank. Specifically, we obtained a condition number of $\approx 4.76 \cdot 10^3$, a value that is generally regarded as numerically safe for least-squares computations in double-precision arithmetic. To further improve numerical stability and reliability, we applied Cook's distance metric [72] to identify and remove influential outliers, removing observations exceeding a threshold of $4/(nTN - \Gamma n_\theta)$. The latter is a common heuristic for outlier detection in regression analysis. This outlier removal reduced the sum of squared residuals (SSR) from approximately 20.63 to 6.79, significantly enhancing both the fit quality and the robustness of the parameter estimates. Figure 3.2 illustrates the Cook's distance values across samples, highlighting the detected outliers (3.6% of total data). Since at least one cell is present in every frame ($L^t \neq \emptyset \forall t$), all rows of the design matrix correspond to valid observations, thus ensuring consistency of the identification procedure.

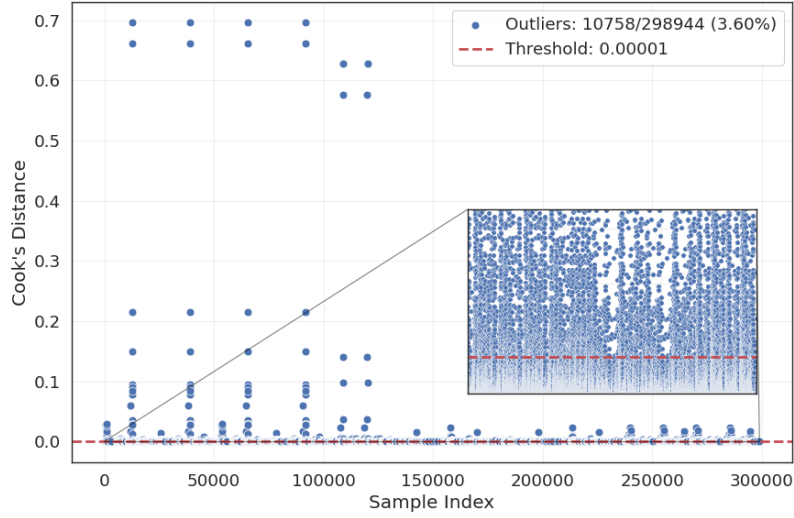


Figure 3.2: Cook's distance values across samples, highlighting detected outliers (blue dots) based on an empirical threshold (dashed red).

In the case of multi-experiment data, such as in our scenario, the identification process involves stacking observation vectors and design matrices for each data collection. Moreover, considering the scarcity of data in biological cell experiments, transformed versions of the same data sequences can be included to enhance robustness with respect to invariant transformations, such as rotations, and horizontal and vertical flips. Details on the augmentation of the dataset are given in Section 3.5 on numerical experiments.

3.4 Multi Cell Tracking Algorithm

In this section, we detail our solution to the cell-tracking problem outlined in Section 3.2. Our Heterogeneous Open Multi-Cell EKF-based Tracking (HEOM-EKF) algorithm introduces a novel motion prediction step employing a tailored Extended Kalman Filter for Open Multi-Agent systems that leverages the models described in Section 3.3. Moreover, our algorithm includes a procedure to estimate cell population changes and reconstruct the cell lineage tree. The main steps of the algorithm are detailed in Algorithm 1.

Here, the set estimations $\hat{L}_p^t$, $\hat{L}_d^t$, $\hat{L}_{OUT}^t$, $\hat{L}_{IN}^t$ and $\hat{\mathcal{M}}^t$ refer to the previous definitions of true quantities introduced in Section 3.3 defined in (3.1)-(3.3). We also provide a graphical illustration (see Figure 3.3) of how HEOM-EKF behaves in the presence of a mitosis event and how these label set estimates change accordingly across frames.

In particular, the algorithm starts by initializing the state guess with detections at the first frame and setting up the EKF matrices. Then, throughout each frame, the algorithm iterates through three main steps. First, get the cell detections related to the current image. Second, it predicts the next state via the EKF prediction step leveraging the interaction dynamics learned from real data, whose identification of parameters is described in Section 3.3.3. Third, it associates detections to predictions and updates the state estimate through the EKF update step. Unlike standard filtering schemes, this step also takes into account changes in the number of cells. Specifically, when a cell population change is predicted at current frame, through MITOSIS-DETECT and IN-OUT-DETECT procedures (see

Algorithm 1 HEOM-EKF**Initialization**Obtain Z^0 from detectorInitialize EKF: $\hat{X}^{0|0} = Z^0$, $P^{0|0}$, Q^0 , R^0 and H^0 Initialize mitosis set: $\hat{\mathcal{M}}^t = \emptyset \forall t \in \mathcal{T}$ **for** each frame $t \in \mathcal{T}$ **do****Object Detection:**Obtain Z^t from detector**EKF Prediction Step:**

$$\hat{X}^{t|t-1} = \hat{X}^{t-1|t-1} + f(\hat{X}^{t-1|t-1}, \theta^*) \quad (3.15)$$

$$F^{t-1} = \frac{\partial f}{\partial X} \Big|_{\hat{X}^{t-1|t-1}} \quad (3.16)$$

$$P^{t|t-1} = F^{t-1} P^{t-1|t-1} (F^{t-1})^T + Q^t \quad (3.17)$$

Associate Detections to Predictions:Get S^t via (3.25) and A^t solving (3.26)Reorder detections IDs in Z^t based on A^t **EKF Update Step:**

$$K^t = P^{t|t-1} H^{t\top} (H^t P^{t|t-1} H^{t\top} + R^t)^{-1} \quad (3.18)$$

$$\hat{X}^{t|t} = \hat{X}^{t|t-1} + K^t (Z^t - H^t \hat{X}^{t|t-1}) \quad (3.19)$$

$$P^{t|t} = (I - K^t H^t) P^{t|t-1} \quad (3.20)$$

Search for Population Change Event:

$$\hat{\mathcal{M}}^t, \hat{L}_p^t, \hat{L}_d^t = \text{MITOSIS-DETECT}(Z^t, \hat{X}^{t|t}, S^t, d_{\max}, IoU_{\min})$$

$$\hat{L}_{\text{IN}}^t, \hat{L}_{\text{OUT}}^t = \text{IN-OUT-DETECT}(Z^t, \hat{X}^{t|t}, S^t, t_{\max}, d_{\max}, IoU_{\min}, \epsilon)$$

if $\hat{L}_p^t \cup \hat{L}_d^t \cup \hat{L}_{\text{OUT}}^t \cup \hat{L}_{\text{IN}}^t \neq \emptyset$ **then**

$$\hat{\mathcal{T}}_s \cup \{t\}$$

Update $\hat{X}^{t|t}$, $P^{t|t}$, Q^t , R^t and H^t via Algorithm 4**end if****end for****return** Cell tracks $\hat{X}^{t|t} \forall t$ and cell lineage tree $\hat{\mathcal{M}}^t \forall t$

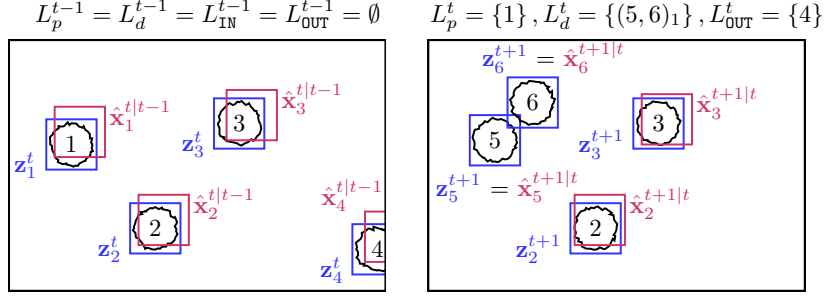


Figure 3.3: An illustrative example of label sets at different frames in a tracking scenario.

Algorithms 2-3), the EKF update variables are adapted to account for dimensionality variations (see Algorithm 4). We now detail all the steps in the algorithm.

3.4.1 Object Detection

The object detection phase serves as the initial step in our algorithm, with the primary goal of localizing cells in consecutive frames of the input video. All quantities and computations in our algorithm are directly based on the pixel coordinates of the detected cells. This ensures consistency with the spatial information provided during the object detection step. In particular, the measurement of cell i obtained via object detection at frame t is an oriented bounding box. Formally

$$z_i := [p_i^\top, s_i, r_i, \phi_i]^\top. \quad (3.21)$$

Here, p_i denotes the center coordinates of the bounding box for the i^{th} cell. The terms $s_i, r_i \in \mathbb{R}$ represent the area and aspect ratio of the detected region, respectively, while $\phi_i \in [0, 2\pi]$ indicates the orientation angle.

It is worth noting that the object detection is performed independently on each frame, producing frame-specific cell detections and measurements, denoted as z_i^t . The identity labels i are assigned arbitrarily by the detector, regardless of the method used. To ensure consistent tracking across frames, prediction and data association steps (see Sections 3.4.3 and 3.4.2) are needed to reorder and match cell identities over time.

3.4.2 Motion Prediction

At each time t , the object detection phase yields a collection of measurement vectors $z_i^t \in \mathbb{R}^{n_z}$ (cf. Section 3.4.1). Based on that, we can now detail the motion prediction step.

Let $x_i^t \in \mathbb{R}^n$ denote the true, unknown state of cell i at time t , represented in the format of equation (3.6). Accordingly, the detection process at each frame t can be modeled by the following measurement equation

$$z_i^t = I_{n_z \times n} x_i^t + w_z, \quad \forall i \in L^t, \quad (3.22)$$

in which $I_{n_z \times n}$ is the observation matrix, which is assumed to be constant and identical across all measurements. The term $w_z \in \mathbb{R}^{n_z}$ denotes zero-mean Gaussian noise with covariance R^t .

Let us introduce $\hat{x}_i^{t|t-1} \in \mathbb{R}^n$ as the predicted state of i^{th} cell at time t , obtained after a prediction step in which the measurement z^{t-1} is known. Instead, $\hat{x}_i^{t|t} \in \mathbb{R}^n$ represents the updated estimate

through the measurement z_i^t obtained after an estimation update step. To retrieve the aforementioned predictions, our tracking algorithm adopts an Extended Kalman Filter [73] incorporating as nonlinear dynamics the open multi-agent model introduced in Subsection 3.3.2.

We first define

$$\begin{aligned} X^t &:= [x_1^{t\top}, \dots, x_{N^t}^{t\top}]^\top \in \mathbb{R}^{N^t n} \\ Z^t &:= [z_1^{t\top}, \dots, z_{N^t}^{t\top}]^\top \in \mathbb{R}^{N^t n_z}, \end{aligned}$$

as the stack of cell population states and measurements. Similarly, we denote by $\hat{X}^{t|t-1}$ and $\hat{X}^{t|t}$ the stacks of predicted and updated state estimates, respectively. According to expression (3.22), the stack measurement vector is given by

$$Z^t = H^t X^t + W_z^t, \quad (3.23)$$

where $H^t \in \mathbb{R}^{(N^t n_z) \times (N^t n)}$ and $W_z^t \in \mathbb{R}^{N^t n_z}$ are the stacks $\forall i \in L^t$ of observation matrices and measurement noise vectors, respectively.

The multi-agent dynamics used to predict the i^{th} cell state belong to the class of models introduced in Section 3.3.2, i.e.

$$\hat{X}^{t|t-1} = \hat{X}^{t-1|t-1} + f(\hat{X}^{t-1|t-1}, \theta^*, t) + W_x^t,$$

which is the predicted version of equation (3.5) written in compact form. In particular, $f : \mathbb{R}^{nN^t} \times \mathbb{R}^{n_\theta \Gamma} \times \mathbb{R} \mapsto \mathbb{R}^{nN^t}$ suitably stacks the dynamics in (3.8) for all the agents present at current time, and $W_x^t \in \mathbb{R}^{nN^t}$ stacks the noise vector w_x for all the cells. The noise $w_x \in \mathbb{R}^n$ is a Gaussian random variable with zero mean and covariance Q . The optimal parameters in (3.15) are learned by solving (3.14) with properly constructed D and Y (see Section 3.3.3) on real data. The step in (3.17) is the update of the so-called estimation error covariance matrix $P^t \in \mathbb{R}^{nN^t \times nN^t}$. The steps in (3.18)–(3.20) instead perform the update steps of the EKF. Here, $K^t \in \mathbb{R}^{nN^t \times n_z N^t}$ is the Kalman gain matrix.

Thanks to this motion prediction design, our tracker can operate on a unified state representation for the heterogeneous cell populations taking advantage of the sparse structure of the dynamics. Indeed, the motion prediction of each cell is performed considering interactions between neighboring cells rather than independent dynamical entities.

3.4.3 Data Association

The data association step bridges the gap between detected cells across consecutive frames, preserving their identities over time. Indeed, given two consecutive time frames $t-1$ and t , the i^{th} cell may be detected and associated with different labels. At the generic time t , the EKF predictions in (3.15) provide the estimate of a novel bounding box $\hat{B}_i$ for cell i . This predicted box is centered in $\hat{p}_i^{t|t-1}$ and has area and aspect ratio $\hat{s}_i^{t|t-1}$ and $\hat{r}_i^{t|t-1}$, respectively. Considering the cell state as in (3.6), also the predicted box orientation $\hat{\phi}_i^{t|t-1}$ is present.

Similarly, let B_j denote the j^{th} bounding box found by the detection step. Let us introduce the similarity matrix $S^t \in \mathbb{R}^{N^t \times N^t}$ at image frame t , with single entry

$$[S^t]_{ij} = S(\hat{B}_i, B_j), \quad (3.24)$$

where S is a similarity function computed between the predicted bounding box of i^{th} cell and the detected one of j^{th} cell at time t . In this work, we use the so-called Intersection Over Union (IoU)

metric [74], so that

$$[S^t]_{ij} = \frac{|\hat{B}_i \cap B_j|}{|\hat{B}_i \cup B_j|}. \quad (3.25)$$

Given S^t , the data association problem regards finding an optimal assignment matrix A^t at each frame of the video that maximizes the total similarity across all associations. This means solving at each time t the following linear-assignment problem

$$\begin{aligned} & \max \sum_{i,j} [S^t]_{ij} a_{ij} \\ \text{s.t. } & \sum_j a_{ij} = 1 \quad \forall i, \quad \sum_i a_{ij} = 1 \quad \forall j, \quad a_{ij} \in \{0, 1\}, \end{aligned} \quad (3.26)$$

where each entry $a_{ij} = 1$ in A^t indicates that j^{th} detection is assigned to i^{th} prediction, and $a_{ij} = 0$ otherwise. This problem can be solved in polynomial time leveraging, e.g., the Hungarian algorithm [75]. To reject associations with a poor similarity index, we enforced a threshold for the IoU function in (3.25), denoted as $IoU_{\min}$.

We now define the set $\mathcal{U}$ containing unmatched elements at time t , which can be further divided into unmatched predictions $\mathcal{U}_x^t$ and unmatched detections $\mathcal{U}_z^t$. Specifically, the set of unmatched is defined as

$$\mathcal{U}^t := \left\{ (i, j) : [S^t]_{ij} \leq IoU_{\min} \right\}.$$

Then, we derive the subsets for unmatched predictions and unmatched detections

$$\mathcal{U}_x^t := \bigcup_{(i,j) \in \mathcal{U}^t} \{i\}, \quad \mathcal{U}_z^t := \bigcup_{(i,j) \in \mathcal{U}^t} \{j\}. \quad (3.27)$$

To provide better context, unmatched predictions and detections arise within a tracking algorithm where associations between predicted states and detections may not always be successful. In such cases, elements that fail to match are categorized into these sets to facilitate further analysis or handling in the tracking process.

3.4.4 Cell Population Changes and Cell Lineage Estimation

In this subsection, we define three routines used in our cell tracking strategy HEOM-EKF. We first introduce a method for estimating cell mitosis events (Algorithm 2). Then, we describe how to detect the entry and exit of cells within the microscope FOV (Algorithm 3). Lastly, we present the procedure to update the dimensions of the EKF matrices in response to the changes in the cell population (Algorithm 4).

We begin by introducing some quantities used within Algorithm 2 and 3. Let $N_z^t \in \mathbb{N}$ be the number of detected cells and $N_x^t \in \mathbb{N}$ the number of predicted cell states. Also, let $\bar{\mathcal{U}}^t \subset \mathcal{U}^t$ be the subset of unmatched predicted and detected cells whose bounding boxes have no intersection and whose distance exceeds the mitosis threshold $d_{\max}$. This distance is determined based on real observed behaviors within the co-culture experiments, and it represents the maximum center distance

at which a mitosis event is usually detected. Formally,

$$N_z^t := \frac{\dim(Z^t)}{n_z}, \quad N_{\hat{x}}^t := \frac{\dim(\hat{X}^{t|t-1})}{n} \quad (3.28)$$

$$\bar{\mathcal{U}}^t := \left\{ (i, j) \in \mathcal{U}^t : [S^t]_{ij} = 0, \|\hat{p}_i^t - p_j^t\| \geq d_{\max} \right\} \quad (3.29)$$

$$\bar{\mathcal{U}}_{\hat{x}}^t := \bigcup_{(i,j) \in \bar{\mathcal{U}}_{\hat{x}}^t} \{i\}, \quad \bar{\mathcal{U}}_z^t := \bigcup_{(i,j) \in \bar{\mathcal{U}}_z^t} \{j\}. \quad (3.30)$$

The ability to accurately determine mitosis events is important for extracting relevant information about the evolution of heterogeneous cell populations over time. A possible approach consists of constructing the cell lineage tree for each cell. To this end, in the following, we present Algorithm 2.

Algorithm 2 MITOSIS-DETECT : Get set estimate $\hat{\mathcal{M}}^t$

Require: $Z^t, \hat{X}^{t|t}, S^t, d_{\max}, IoU_{\min}$

if $\mathcal{U}_z^t \neq \emptyset$ **and** $N_z^t > (N_{\hat{x}}^t - |\mathcal{U}_{\hat{x}}^t| + |\bar{\mathcal{U}}_{\hat{x}}^t|)$ **then**

for $i \in \mathcal{U}_z^t \setminus \bar{\mathcal{U}}_z^t$ **do**

 Find $j = \underset{j}{\operatorname{argmin}} \|z_i^t - z_j^t\|$

 New prediction labels: from (i, j) to (j_{d_1}, j_{d_2})

 Update daughters set $\hat{L}_d^t = \hat{L}_d^{t-1} \cup \{j_{d_1}, j_{d_2}\}$

 Update parent set $\hat{L}_p^t = \hat{L}_p^{t-1} \cup \{j\}$

end for

end if

Update $\hat{\mathcal{M}}^t$ based on definition (3.1)

return $\hat{\mathcal{M}}^t, \hat{L}_p^t, \hat{L}_d^t$

The proposed method estimates mitosis occurrences at a given frame t by computing the set estimates $\hat{L}_p^t, \hat{L}_d^t$, and thus $\hat{\mathcal{M}}^t$. In particular, the algorithm leverages the sets in (3.27) and (3.30), together with the quantities in (3.28), to distinguish new detections due to mitosis, cells entering the camera FOV or unmatched detections. After tracking is completed across the entire video sequence, the lineage of each cell is constructed using the parent-child relationships stored in the sets $\hat{\mathcal{M}}^t$ for all $t \in \mathcal{T}$. Note that $\hat{\mathcal{M}}^t = \emptyset$ at frames where no mitosis events are detected.

We now present Algorithm 3 to estimate the occurrence of cells entering or exiting from the camera FOV.

Analogously to Algorithm 2, this method begins by checking for unmatched detections at the current frame t , using the sets defined in (3.27) and (3.30), along with the values in (3.28). It then examines the set $\bar{\mathcal{U}}_z^t$ to identify detected cells that are ϵ -close to the image boundaries $(\bar{x}, \bar{y})$, i.e., cells that have recently entered the field of view. To detect cells that are leaving the microscope FOV, the algorithm instead looks for predictions that remained unmatched over the past $t_{\max}$ time steps. If these predictions are also ϵ -close to the image boundaries, they are considered as outcoming cells.

Algorithm 4 takes care of adapting the dimensions of EKF matrices. Specifically, it reorganizes the terms $\hat{X}^{t|t-1}, \hat{P}^{t|t-1}, H^t, R^t$, and Q^t to ensure feasible tracking. Especially, let us define the

Algorithm 3 IN-OUT-DETECT : Get set estimates $\hat{L}_{\text{IN}}^t, \hat{L}_{\text{OUT}}^t$

Require: $Z^t, \hat{X}^{t|t}, S^t, t_{\max}, d_{\max}, IoU_{\min}, \epsilon$

if $\mathcal{U}_z^t \neq \emptyset$ **and** $N_z^t > (N_{\hat{x}}^t - |\mathcal{U}_{\hat{x}}^t| + |\bar{\mathcal{V}}_{\hat{x}}^t|)$ **then**

for $i \in \bar{\mathcal{U}}_z^t : \min(p_{x,i}^t, \bar{x} - p_{x,i}^t, p_{y,i}^t, \bar{y} - p_{y,i}^t) \leq \epsilon$ **do**

New prediction label $i \rightarrow j_{\text{IN}}$

Update entry set $\hat{L}_{\text{IN}}^t = \hat{L}_{\text{IN}}^{t-1} \cup \{j_{\text{IN}}\}$

end for

end if

for $i \in \{\mathcal{U}_{\hat{x}}^{t-t_{\max}} \cap \dots \cap \mathcal{U}_{\hat{x}}^t\}$ **do**

if $\min(p_{x,i}^t, \bar{x} - p_{x,i}^t, p_{y,i}^t, \bar{y} - p_{y,i}^t) \leq \epsilon$ **then**

Remove prediction label i

Update exit set $\hat{L}_{\text{OUT}}^t = \hat{L}_{\text{OUT}}^{t-1} \cup \{i\}$

end if

end for

return $\hat{L}_{\text{IN}}^t, \hat{L}_{\text{OUT}}^t$

functions used for including new daughter cells into the tracking procedure as

$$g_d(a, b) := \begin{bmatrix} a \\ b \end{bmatrix} \in \mathbb{R}^{(N+1)n},$$

in which $a \in \mathbb{R}^{Nn}, b \in \mathbb{R}^n$. Also, let

$$G_d(A, b) := \begin{bmatrix} A & b_{1:n-1} \\ b_{1:n-1}^\top & b_{:-1} \end{bmatrix} \in \mathbb{R}^{(N+1)n \times (N+1)n},$$

in which $A \in \mathbb{R}^{Nn \times Nn}, b \in \mathbb{R}^n, b_{1:n-1} \in \mathbb{R}^{n-1}$ and $b_{:-1} \in \mathbb{R}$. Instead, the functions used to remove a parent cell within HEOM-EKF are

$$g_p : \mathbb{R}^{Nn} \times \mathbb{R}^n \rightarrow \mathbb{R}^{(N-1)n}$$

$$(a, a_i) \mapsto [a_1^\top, \dots, a_{i-1}^\top, a_{i+1}^\top, \dots, a_N^\top]^\top,$$

in which a_i belongs to $a = [a_1^\top, \dots, a_N^\top]^\top$, and

$$G_p : \mathbb{R}^{Nn \times Nn} \times \{1, \dots, N\} \rightarrow \mathbb{R}^{(N-1)n \times (N-1)n}$$

$$(A, i) \mapsto A_{\setminus i},$$

with $A_{\setminus i}$ being the matrix A with the i^{th} row and column removed.

3.5 Numerical Experiments

In this section, we first introduce the dataset used in our simulations. Then, we present the results of the proposed cell-tracking algorithm, analyze the optimal multi-agent model parameters that confirm cell type discrimination, and discuss the extracted cell lineage tree.

Algorithm 4 Update EKF dimensionality**Require:** $\hat{L}_p^t, \hat{L}_d^t, \hat{L}_{OUT}^t, \hat{L}_{IN}^t, \hat{X}^{t|t}, P^{t|t}, Q^t, R^t$ and H^t

```

for  $i_p \in \hat{L}_p^t$  and  $j_d \in \hat{L}_d^t$  do
     $\hat{X}^{t|t} = g_p(\hat{X}^{t|t}, \hat{x}_{i_p}^{t|t})$ 
     $\hat{X}^{t|t} = g_d(\hat{X}^{t|t}, z_j^t) \quad \forall j \in j_d$ 
     $P^{t|t} = G_p(P^{t|t}, i_p)$ 
     $P^{t|t} = G_d(P^{t|t}, [\mathbf{0}_{n-1}^\top, 1]^\top) \quad \forall j \in j_d$ 
end for
for  $i_{OUT} \in \hat{L}_{OUT}^t$  do
     $\hat{X}^{t|t} = g_p(\hat{X}^{t|t}, \hat{x}_{i_{OUT}}^{t|t})$ 
     $P^{t|t} = G_p(P^{t|t}, i_{OUT})$ 
end for
for  $i_{IN} \in \hat{L}_{IN}^t$  do
     $\hat{X}^{t|t} = g_d(\hat{X}^{t|t}, z_{i_{IN}}^t)$ 
     $P^{t|t} = G_d(P^{t|t}, [\mathbf{0}_{n-1}^\top, 1]^\top)$ 
end for
Redefine  $Q^t, R^t$  and  $H^t$  according to  $N_z^t$  and  $N_{\hat{x}}^t$ 

```

3.5.1 Dataset Description

The dataset used in this study was derived from raw time-lapse image sequences captured using a confocal microscope (A1R MP microscope, Nikon) at IRCCS Istituto Ortopedico Rizzoli. The human 143b line were obtained from the American Type Cell Culture Collection (ATCC) and were cultured in Minimum Essential Medium Eagle Alpha modification (Sigma) with 10% fetal bovine serum (FBS, Sigma) and 1% penicillin/streptomycin (P/S) (Euroclone) and used for co-culture experiments after trypsinization and semi-confluence. Human adipose-derived MSC (ATCC) were maintained in complete alpha-MEM with 10% fetal bovine serum (FBS, Sigma) and 1% P/S and used for co-culture experiments after trypsinization and semi-confluence. Cells were incubated at 37 °C in 5% CO₂. GFP-transfected MSC cells, obtained as previously described [66], were used in co-culture experiments. For co-cultures, 143b cells and GFP+ MSC were mixed 1:3 and seeded in at low density in complete alpha-MEM in petri dishes for time-lapse imaging. The transfection with GFP allowed us to clearly distinguish MSC from 143b cells and, consequently, accurately assign the measured features between the two cell types. The set of experiments carried out consists of 4 videos with 172 frames each with images captured every 4 minutes (25x immersion objective, NA 1.1, RI 1.333).

Initial processing of the raw images was performed by using NIS Elements AR 5.40.01 software (Nikon), employed on all the images to perform semantic segmentation, labeling, and classification by cell type. We performed these tasks by combining manual adjustments with automated image processing tools. In particular, we first manually segmented representative cell regions and then used the software to automatically propagate and refine the contours across subsequent frames by tuning intensity thresholds and edge-detection parameters. When cells overlapped or formed dense clusters, segmentation was instead carried out entirely by manual annotation. The primary objective of this step was to extract accurate data for training and testing the proposed tracking algorithm. Since the process could be subject to human and/or software automation errors, we refer to these annotations as the gold standard, rather than the actual ground truths. Following segmentation, the

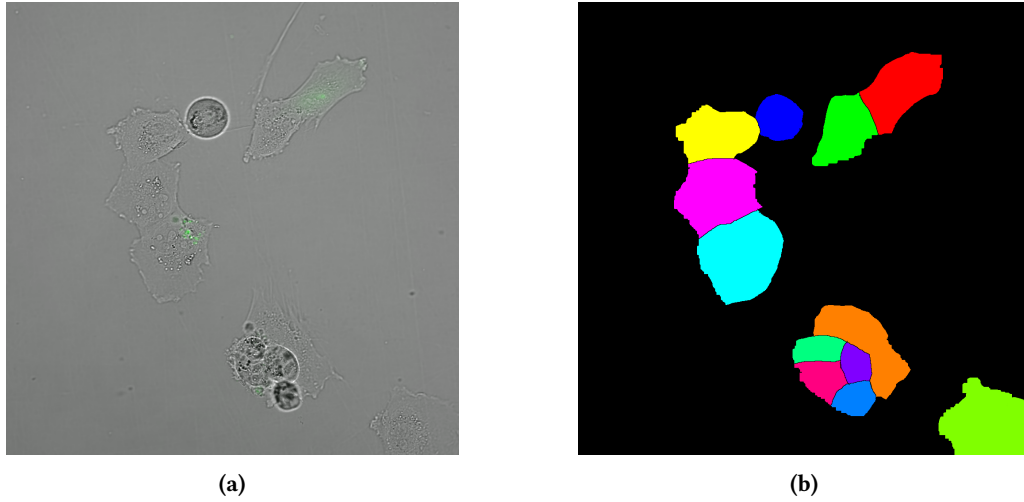


Figure 3.4: Comparison between the original microscope image (a) and its final segmented version (b).

software extracted around 70 features per segmented object, including morphological and positional properties, allowing for detailed temporal analysis of each cell. These features, after filtering only the ones relevant to our purposes, provided a comprehensive characterization of the cells' dynamics and their interactions over time. To refine the dataset further, additional image processing techniques were applied, leveraging the Python package OpenCV [76], focusing on denoising and fixing any labeling or segmentation errors. The latter post-processing corrections ensured enhanced data quality. An example of a segmentation result is shown in Figure 3.4.

Before presenting the final results, we report summary statistics of key cell features extracted from the dataset (Figure 3.5). These metrics reveal clear morphological differences between MSC and 143b cells and motivate the design of the proposed open multi-agent dynamics. Although perimeter, circularity, and elongation are not explicitly modeled as state variables, their morphological information is implicitly encoded in the bounding box representation. In particular, the aspect ratio r_i provides a proxy for circularity and elongation, capturing type-specific shape characteristics. The target aspect ratio R_{y_i} for each cell type is derived from the mean circularity observed in the training data, thereby embedding morphological priors into the tracking framework. As expected, MSC display a more elongated, fibroblast-like morphology, whereas osteosarcoma (143b) cells tend to be more rounded. On average, MSC are larger than 143b cells in terms of both area and perimeter.

Finally, we want to highlight that this dataset is a unique resource to study cellular behavior in co-culture frameworks, setting it apart from most publicly available datasets, such as the ones from the Cell Tracking Challenge, which only focus on monoculture experiments.

3.5.2 Cell Detection in Video Frames

In our experiments, we adopt a semi-automatic approach using the proprietary image-analysis software included with our microscope. This choice offers several advantages: (i) it simplifies the detection pipeline, allowing us to focus on assessing the performance of our novel motion prediction module; (ii) it avoids the need for large, annotated datasets typically required for supervised training; and (iii) it ensures compatibility and consistency with the data acquisition process, as the detection tool is tailored to the imaging system. For each video frame, the software provides region proposals (cell segmentations), from which axis-aligned or oriented bounding boxes can be extracted using

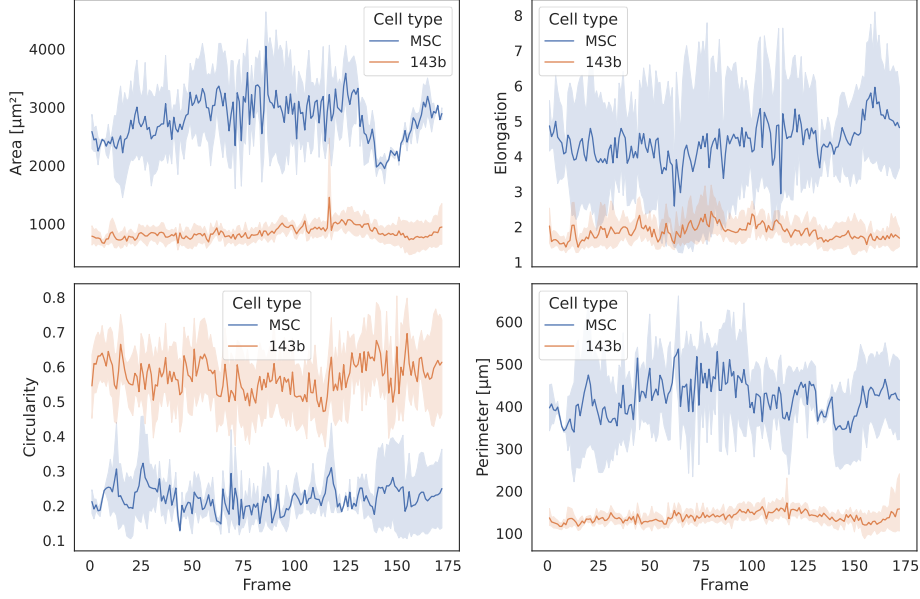


Figure 3.5: Mean and variance over time for the area, outer perimeter, circularity, and elongation, categorized by cell type.

basic image processing techniques. State-of-the-art cell segmentation tools rely on training and deploying a dedicated deep learning model for cell detection, as in [77, 78]. However, these approaches typically require a large amount of data and do not discriminate between different cell populations. Our multi-agent approach to cell tracking aims to introduce dynamical system knowledge into the process. This improves tracking performance even when the object detection component cannot exploit a large amount of data.

3.5.3 Experimental Results

We implemented the proposed methodology in PYTHON programming language, testing our MCT algorithm on co-culture videos from *in vitro* experiments involving MSC and osteosarcoma cells.

Given the limited dataset of only four videos, we employed a cross-validation approach [79]. Specifically, we trained the models described in Section 3.3, by computing the optimal solution θ^* as in (3.11) considering three videos regarding a co-culture scenario ($\Gamma = 2$). Then we evaluated the tracking performances on the remaining one. We repeated this procedure for all possible combinations of training and test videos.

To improve the robustness of our estimation and mitigate overfitting due to the small dataset, we adopted data augmentation techniques [80]. Specifically, we applied rotations of angles $\alpha \in 90^\circ, 180^\circ, 270^\circ$ and orthogonal flips to each segmented image to then extract the corresponding rotated versions of p_i^t , s_i^t , r_i^t , and ϕ_i^t for all cells i at each frame t . After computing the required time derivatives, these transformed values were used to enlarge Y and D on the LLS problem. Moreover, we worked on normalized data sequences to ensure better-conditioned matrix inversion, prevent feature predominance, and enhance the interpretability of parameters. The data augmentation and the subsequent identification process were performed offline.

The model constants in equations (3.10), specifically the target area S_{y_i} and aspect ratio R_{y_i} with $y_i \in \{\text{MSC}, 143b\}$, were derived directly from dataset statistics regarding mean area and elongation

observed across cell trajectories over time (refer to Figure 3.5). We further analyzed the learned model parameters by examining their distributions across different training sets. This allowed us to evaluate if our open multi-agent model could distinguish between the two cell types (MSC and 143b cells) by capturing their distinct behaviors. Automatically distinguishing cells in co-culture models, such as MSC and osteosarcoma cells, is crucial. Both originate from the same mesenchymal progenitor line and appear morphologically similar under direct microscope observation. Consequently, identifying and analyzing cellular behaviors and interactions between these two cell types to gain new insights into cancer progression and aggressiveness is currently limited to systems that are expensive, complex, and often impractical to use.

Specifically, we examined the parameter sets obtained from training on all possible combinations of the four videos, i.e., the power set of the dataset, which consists of 2^4 different training sets. For each training set, we computed the identified parameters and analyzed their statistical distribution using kernel density estimation (KDE) to obtain a smooth approximation of the underlying data distribution. Figure 3.6 illustrates the KDE-based distribution of the identified parameters for the two cell types across different training sets. The analysis reveals distinct parameter distributions, supporting the hypothesis that the proposed model effectively differentiates MSC from 143b cells. It is worth noting that the displayed parameter values are numerically small because the models were identified on normalized data. This reflects the chosen scaling of the variables rather than weak underlying behavioral tendencies.

From a biological perspective, the identified parameter weights can be interpreted as follows. The cohesion parameter $\theta_{y_i,1}$ quantifies the tendency of cells to remain in proximity to their neighbors. It reflects the propensity of cells to lose cohesion, detach from the tissue, and migrate to distant sites, a phenotype commonly associated with tumor cells and increased aggressiveness and metastatic potential. In line with this interpretation, our results indicate that mesenchymal stromal cells (MSCs) exhibit a lower tendency to lose cohesion than tumor cells. The alignment parameter $\theta_{y_i,2}$ measures the tendency of cells to migrate in the same direction as their neighbors. The lower values with smaller variance observed in tumor cells suggest a more heterogeneous and less coordinated migratory behavior compared with MSCs, consistent with the disorganized migration typical of cancer cells that have lost responsiveness to local cues required for coordinated tissue dynamics and structural integrity. Further support for these findings is provided by the repulsion parameter $\theta_{y_i,3}$. Tumor cells display a greater tendency to distance themselves from neighboring cells than normal cells, indicating a disruption of the interaction mechanisms characteristic of organized tissues. Finally, the morphological parameters $\theta_{y_i,4}$ and $\theta_{y_i,5}$, which regulate the tendency to maintain the cell-type-specific target area S_{y_i} and aspect ratio R_{y_i} , show similar distributions between the two cell types. MSCs maintain elongated and relatively stable morphologies around their characteristic shape, whereas 143b cells exhibit increased variability around their own, more rounded morphology, consistent with their enhanced motility and well-documented morphological plasticity. Overall, these results demonstrate that the identified parameters not only distinguish MSCs from 143b cells statistically, but also capture impaired regulation of cell-cell interactions. As such, they represent informative indicators of phenotypic differences between normal and tumor cells and may serve as indirect measures of tumor aggressiveness in biological assays, even in the presence of normal cells capable of modulating tumor behavior.

To further detail the tracking algorithm, we now describe the initialization and tuning of the EKF terms used within Algorithm 1. In our implementation of the Extended Kalman Filter, the error covariance matrix $P^{0|0}$ is initialized by assigning higher variance values to state components that are initially unobserved, reflecting our uncertainty in these estimates. Instead, the measurement covariance matrix R and the process covariance matrix Q are set based on baseline estimates obtained

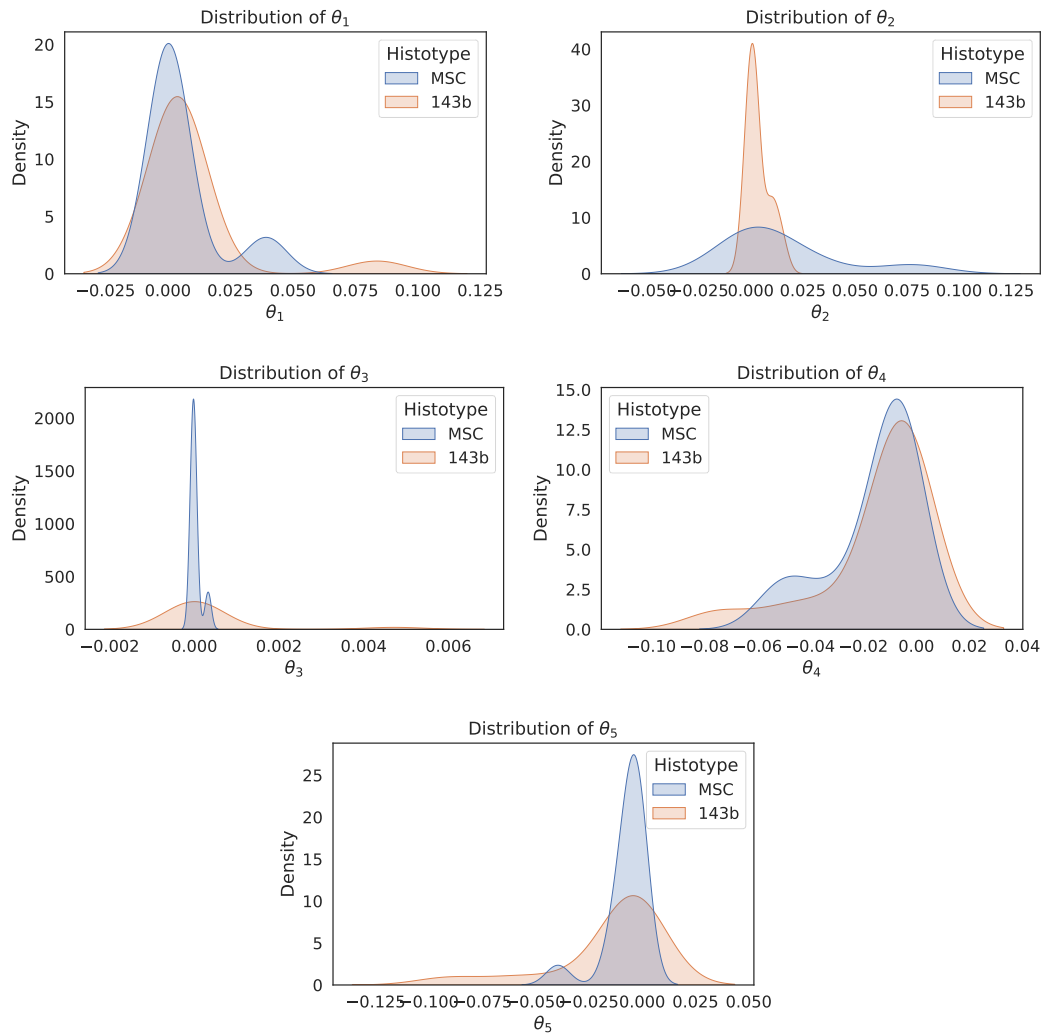


Figure 3.6: KDE distributions of the identified model parameters for MSC and 143b cells across different training sets.

from preliminary noise analyzes of the dataset. To account for different noise conditions, we further explored scaled versions of these baselines using factors of 0.5, 1.0, and 2.0. This systematic exploration enabled us to quantify the impact of these initialization choices on tracking performance and so identify the optimal settings for our application.

We want to underline that, since our focus is on evaluating the performance metrics of our tracker rather than the detector, we conducted experiments simulating the detection process via gold standard annotations with injected Gaussian disturbances. Specifically, the measurement errors were simulated considering random noise with zero mean and defined covariance for each annotation. In the following, we present some results considering the state format (3.7) since, being the standard detection format, it enabled us to perform a comparison with other methodologies. Figure 3.7 shows a video snapshot regarding the numerical experiments on one of the co-culture videos. Here, we display both the bounding boxes predicted by our algorithm and the original gold standard annotations.

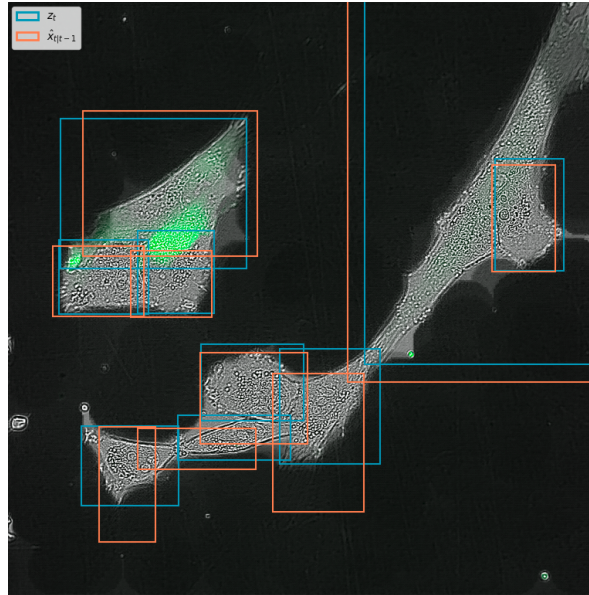


Figure 3.7: Comparison of predicted bounding boxes (orange) and gold standard annotations (blue) in a co-culture video frame.

We compared our scheme against two baseline multi-object tracking methods: SORT [81] and DeepSORT [82]. Both use a Kalman filter for motion prediction and the Hungarian algorithm for data association. SORT performs association using IoU only, whereas DeepSORT employs a neural network trained for re-identification to extract appearance features to improve association. For a fair comparison, the DeepSORT appearance model was fine-tuned on the images from our co-culture dataset. We initialized SORT and DeepSORT with the same matrices as in Algorithm 1 and simulated the detection process for our proposed solution. The chosen metrics are the Multiple Object Tracking Accuracy (MOTA), Multiple Object Tracking Precision (MOTP) [83] and Identity F1 Score (IDF1). We also report the average per-frame processing time, denoted as $T_{\text{avg}} \in \mathbb{R}_{>0}$ for each algorithm. MOTA depends on the number of misses, false positives, and mismatches. MOTP compares the predicted bounding boxes against the real ones. IDF1 measures the accuracy of identity assignment by comparing correctly matched detections to the average number of ground-truth and predicted detections. Higher values of these metrics indicate better tracking performance.

Table 3.1 summarizes the results of performing the cross-validation procedure on different experiments, referred to as “Well”. This comparison highlights the effectiveness of our approach, as evidenced by a good MOTA score, which reflects the algorithm ability to track cells accurately over time. Furthermore, the MOTP metric demonstrates that the proposed tracking model outperforms SORT and DeepSORT, providing more precise predictions. Note that, DeepSORT does not improve over SORT. We attribute this to scarcity of data: the appearance network in DeepSORT is a relatively large model, whereas our total dataset consists of only four videos. Fine-tuning such a network on so few sequences is unlikely to generalize and can even degrade association performance compared to a purely geometry-based method. Moreover, Table 3.1 reports the results of an ablation study that isolates the impact of the motion-prediction component. Indeed, HEOM-EKF, SORT, and DeepSORT share the same Track-by-Detection structure and a KF-based tracking scheme; the key difference lies in the open system model and relative enhanced version of EKF. In HEOM-EKF, this model is identified from real co-culture data and explicitly tailored to heterogeneous, time-varying cell populations, whereas SORT and DeepSORT rely on standard single-object kinematic models. Keeping detection and association fixed isolates the contribution of the proposed multi-agent motion model within the EKF prediction step. In terms of per-frame computation time, our method is slower than SORT but substantially faster than DeepSORT. This outcome is expected since, relative to SORT, HEOM-EKF employs a more sophisticated motion model and an EKF whose state dimension scales with the number of cells (while avoiding the computationally intensive appearance feature extraction required by DeepSORT). Despite this increased complexity, the per-frame runtime remains on the order of tens of milliseconds, which is negligible relative to the minute-scale image acquisition intervals typical of cell culture experiments, thereby ensuring compatibility with real-time microscopy.

Table 3.1: Performance comparison

Metric	Experiment	SORT	DeepSORT	Alg. 1
MOTA	Well 1	74.23 %	55.30 %	83.56 %
	Well 2	40.78 %	52.77 %	67.58 %
	Well 3	76.38 %	54.09 %	87.02 %
	Well 4	79.97 %	55.63 %	96.17 %
MOTP	Well 1	93.68 %	85.33 %	96.72 %
	Well 2	91.45 %	84.04 %	95.51 %
	Well 3	93.34 %	83.56 %	96.03 %
	Well 4	94.33 %	85.38 %	97.18 %
IDF1	Well 1	62.61 %	50.31 %	69.32 %
	Well 2	54.20 %	38.79 %	66.67 %
	Well 3	65.59 %	47.76 %	71.07 %
	Well 4	82.02 %	48.12 %	80.88 %
T_{avg} [ms/frame]	Well 1	2.33	204.98	59.73
	Well 2	0.94	94.07	5.33
	Well 3	3.32	184.42	60.72
	Well 4	1.76	170.57	40.27

Additionally, the proposed algorithm predicts changes in cell population over time and, at the end of the time-lapse sequence, returns the estimated cell lineage tree $\hat{\mathcal{M}}^t$ for the frames $t \in \{1, \dots, 172\}$ of

the current *in vitro* experiment. Figure 3.8 shows a prediction result of the latter. Here, the state format in (3.6) was preferred since it allowed for more precise type discrimination and parent-daughter relation prediction. The depicted example of an estimated cell lineage tree is a reliable result despite the absence of a true reference family tree for direct comparison. In fact, MSC cells rarely divide (considering the duration of the single experiment of about 10 hours), while 143b osteosarcoma cells, due to their oncogenic nature, exhibit a significantly higher mitosis rate. The predicted lineage tree captures these expected proliferation patterns, reinforcing its plausibility.

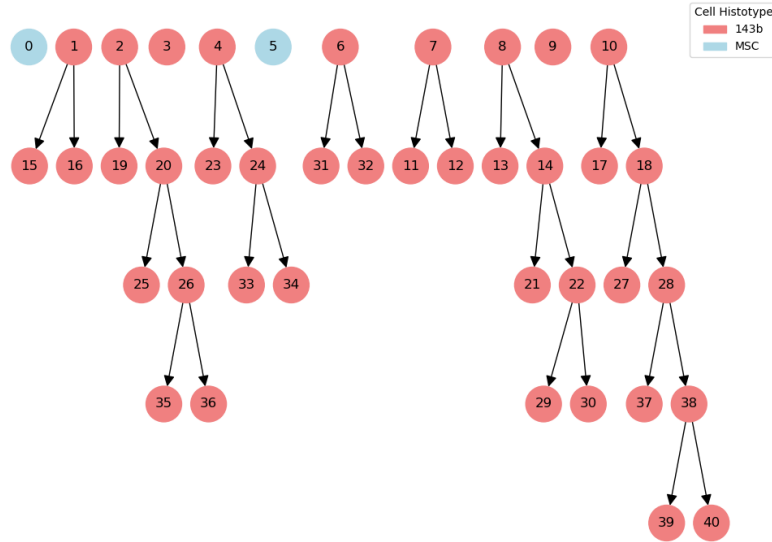


Figure 3.8: Example of a predicted cell lineage tree.

The proposed framework shows promising performance, but the limited dataset size (4 co-culture videos) must be acknowledged. Despite cross-validation and data augmentation, overfitting risks remain. This limitation reflects a broader challenge in cancer-biology: high-quality time-lapse datasets of co-cultures are scarce due to experimental complexity and cost. Thus, the proposed work represents a proof-of-concept in this data-scarce context and highlights that our proposed method can represent a valuable solution as opposed to Machine Learning approaches that typically require large datasets for training. Finally, this study provides a unique dataset for investigating MSC-143b interactions and evaluating tracking in co-culture experiments.

3.6 Summary and Concluding Remarks

This chapter addressed the problem of tracking heterogeneous cell populations in time-lapse optical microscopy, an important task regarding data acquisition and analysis in cancer biology. The inherent complexity of cellular dynamics—driven by migration, mitosis, and cell-cell interactions—poses significant challenges to classical multi-object tracking approaches, particularly in data-scarce experimental contexts and in multi-culture settings where different cell types coexist and interact.

To tackle these challenges, we propose a novel model-based cell-tracking framework leveraging open multi-agent system concepts. In contrast to purely data-driven or detection-based approaches, the presented method explicitly models the dynamics of individual cells and their interactions, allowing the number of agents to vary over time to naturally account for biological events such as

cell division. System identification techniques were employed to learn cell type-specific behavioral parameters directly from experimental data, enabling the framework to capture distinct cell type-specific behaviors and interactions across heterogeneous populations.

The identified multi-agent model was embedded within a generalized Extended Kalman Filter, providing a principled mechanism to fuse prior dynamical knowledge with noisy image-derived observations. This enabled robust motion prediction while accounting for populations change events. The overall tracking algorithm followed a Track-by-Detection paradigm, utilizing standard data association techniques to link predicted cell states with their observations at each time step. A comprehensive validation strategy was adopted, including cross-validation, performance metrics, and comparisons with two baseline multi-object tracking algorithms.

Experimental results, obtained on a novel dataset derived from *in vitro* co-culture experiments involving osteosarcoma cells and mesenchymal stromal cells, demonstrated that the proposed approach consistently outperformed state-of-the-art methods. In particular, the algorithm proved effective in tracking heterogeneous cell types, capturing cell-to-cell interactions, and reconstructing cell lineage trees. Beyond quantitative performance, the use of biologically interpretable models represents a key strength of the approach, as it enables the extraction of meaningful dynamical insights rather than treating cell trajectories as purely geometric entities.

A relevant practical aspect of the proposed methodology concerns the preliminary parameter identification step, which relies on manually annotated data for the specific cell populations under study. While this requirement may initially appear as a limitation, it should be interpreted in the context of the intended application domain. In settings where large-scale repetitions of the same experiment are required, such as systematic perturbation or screening studies, the need for an initial manually annotated dataset can be justified, as the identified parameters can then be reused across multiple experiments involving the same or closely related cell populations.

Moreover, parameter re-identification is not necessarily required for every new experiment. The learned parameters encode fundamental aspects of cell behavior, such as motility patterns and interaction strengths, which are expected to be at least partially transferable across similar cell types, experimental conditions, or culture protocols. This opens the possibility of using “universal” or reference parameter sets. While a systematic study of parameter transferability lies beyond the scope of this thesis, the results presented here suggest that the framework is well suited to incremental refinement and reuse of models across related biological scenarios.

In conclusion, this chapter introduced a novel algorithm for tracking heterogeneous cell populations in time-lapse experiments by modeling them as open multi-agent systems. The framework bridges multi-agent modeling, system identification, and biomedical image analysis, advancing cell tracking under data-limited conditions. By explicitly representing cells as interacting agents with learned, biologically interpretable parameters, the method accommodates time-varying population sizes and heterogeneous cell types. Potential extensions include automated annotation strategies, investigation of parameter transferability across broader cell populations, and embedding this framework into real-time microscopy systems for live-cell analysis and control.

Cellular Potts Models for Tumor Invasion Dynamics: a System Theoretical Viewpoint

This chapter builds upon the findings reported in our pre-print [84], soon available on arXiv.

4.1 Introduction

Building upon the general motivations introduced in Chapter 1, this chapter focuses on understanding the complex mechanisms underlying tumor invasion and progression. Mathematical modeling provides a rigorous means to reproduce the key principles governing tumor dynamics, offering a structured framework to test hypotheses and interpret experimental observations.

Among the various modeling approaches, the Cellular Potts Model (CPM) has emerged as a versatile tool for describing multicellular systems and reproducing cell behaviors and interactions across multiple scales. In its classical formulation, a CPM represents tissues as ensembles of discrete cells governed by energy-based rules accounting for adhesion, surface tension, and volume constraints. This formulation enables the emergence of tissue-level phenomena to be understood as a consequence of local, cell-to-cell interactions. Nevertheless, real tumor microenvironments are considerably more complex, shaped not only by intercellular adhesion but also by interactions between cells and the surrounding extracellular matrix (ECM). These interactions give rise to haptotaxis-driven migration—a key process in tumor invasion in which cells move along gradients of ECM density or adhesion strength (refer to Section 2.1.3 for more details). Classical CPMs, which only account for cell-to-cell interactions, are therefore insufficient to capture these essential mechanisms.

Building on the foundational analysis provided in [85], which discusses the theoretical properties of standard CPMs, this chapter analyses a subclass of Extended Cellular Potts Models (ECPMs) that explicitly incorporate cell-ECM interactions to model haptotaxis. Within this framework, we formalize and analyze a coupled system describing the feedback interconnection between tumor configuration and ECM degradation. Based on this interpretation, our theoretical investigation provides analytical characterizations of the stochastic evolution, invasion time, and asymptotic behavior of the proposed ECPM. By doing so, it bridges the gap between classical CPM theory and its extended formulations for biologically realistic tumor invasion modeling, offering insights into the fundamental mechanisms through which cell-ECM coupling drives invasion dynamics.

Related Works. The literature surrounding CPMs and their extensions provides both a theoretical foundation and a wide range of applications, particularly in tumor invasion dynamics and cell migration modeling.

From a theoretical perspective, early work such as [86] introduced CPMs as a means of simulating cell sorting based on differential adhesion, laying the groundwork for modeling multicellular behaviors through energy minimization principles. Subsequent studies, like [87], explored the mathematical structure of related random-cluster models, revealing critical behaviors and phase transitions that are essential for understanding the long-term properties of CPMs. Further mathematical advancements in [88] examined the continuity of phase transitions in Potts models, contributing to a more rigorous understanding of how these models evolve over time. Additionally, [89] provided a comprehensive examination of Gibbs measures in the context of Potts models, offering valuable insights into their equilibrium properties and biological relevance.

Building on these foundations, [85] offered one of the first systematic theoretical analyses of CPM dynamics, highlighting potential degenerates and structural limitations in the classical formulation. Related developments such as [90] and [91] extended CPM theory by establishing connections between discrete stochastic dynamics and continuum partial differential equation limits. Despite these advances, most theoretical analyses have focused on the statistical mechanics or continuum limits of CPMs without explicitly incorporating ECM dynamics or cell-environment feedback, which are central to haptotaxis-driven invasion.

On the applied side instead, CPMs have proven to be highly adaptable for simulating complex biological processes. In [92], the model was employed to simulate morphogenesis, capturing the transition from individual cells to organized multicellular structures. The study [93] extended the CPM framework to simulate cancer invasion, focusing on the role of intercellular adhesion in tumor progression. Building on this, [94] explored the dynamics of drug treatment failure in cancer, illustrating how modifications to cellular behaviors influence therapeutic outcomes. Computational improvements, such as those presented in [95], introduced parallel implementations of CPMs to enable larger-scale simulations, essential for capturing realistic morphogenetic processes. These contributions established CPMs as powerful computational tools for biological modeling, but they largely remained simulation-oriented, with limited theoretical formalization and analysis of the underlying dynamics.

More recent studies have focused on extending CPMs to incorporate explicit interactions with the extracellular matrix. In [96], the model was adapted to study tumor invasion from cell aggregates, highlighting the interplay between active cell motion and mechanical equilibrium. The work of [97] integrated ECM-guided migration mechanisms into a CPM of angiogenesis, showing how ECM gradients influence collective cell motion. Similarly, [98] reviewed CPM-based approaches to model morphogenesis and tissue organization, emphasizing extensions that include haptotaxis and matrix remodeling. In [99], an extended CPM was developed to describe wound healing, coupling cell migration to a dynamically evolving ECM field. Finally, [100] provided a comprehensive review of ECM representations in cell migration models, discussing how discrete and hybrid frameworks can capture matrix-guided movement.

These studies illustrate how ECM-related phenomena can be incorporated into CPMs. However, most of these models are primarily employed for simulation purposes, aiming to replicate observed behaviors by empirically assigning model parameters, rather than exploiting their underlying theoretical properties. For instance, invasion time analysis is typically performed through extensive simulations rather than leveraging analytical expressions. Therefore, analysis and theoretical results on CPMs extended to model tumor-ECM interactions are still missing.

Overview and Contributions. This chapter introduces a novel system-theoretical framework for analyzing a specific class of ECPMs that explicitly incorporate cell-ECM interactions to model haptotaxis in tumor invasion dynamics. While rigorous theoretical results exist for classical CPMs, such analytical foundations remain absent for ECPMs that capture haptotactic migration driven by ECM gradients and matrix degradation—a gap that this work seeks to address.

We reinterpret the ECPM as a closed-loop dynamical system composed of two interconnected subsystems: (i) a stochastic process governing the tumor cell configuration and (ii) a jump-linear system describing ECM evolution. This formalization captures the feedback between tumor configuration and ECM degradation, enabling a principled study of how local cell-ECM interactions shape emergent invasion patterns.

Building on this representation, we derive analytical results characterizing key properties of the coupled system, including the asymptotic behavior of the ECM field, transition probabilities of tumor configurations, and bounds on the system’s energy evolution. These results provide the first rigorous theoretical description of haptotaxis within the CPM framework. Moreover, the central contribution is the analysis of tumor invasion times in a one-dimensional ECPM, yielding closed-form expressions for the mean invasion time as a function of model parameters and initial state. This reveals how invasion speed and stochastic propagation depend on tumor-ECM coupling strength and degradation mechanisms.

Numerical simulations validate the theoretical predictions, confirming the analytical results and illustrating the parameter-dependent nature of invasion dynamics. This work thus bridges computational and theoretical analyses of ECPMs, advancing the formal understanding of haptotaxis-driven tumor invasion in Potts-based models.

Chapter Outline. The remainder of this chapter is organized as follows. Section 4.2 introduces the standard formalism under ECPM, providing its lattice-based structure, energy formulation, and algorithmic rules. Section 4.3 reformulates the ECPM as a closed-loop dynamical system, emphasizing the feedback interaction between the tumor cell configuration and the ECM field. Section 4.4 presents a theoretical analysis of the model properties, including the asymptotic behavior of the ECM dynamics, the characterization of transition probabilities, and bounds on the stochastic evolution. In Section 4.5, we introduce the concept of invasion time in a one-dimensional ECPM and derive analytical expressions for its probability considering single-cell and multi-cell configurations. Section 4.6 reports simulation results that validate the theoretical findings and illustrate parameter-dependent invasion patterns.

Chapter Notation. Given an arbitrary set S , we denote by $|S|$ its cardinality. Instead, we denote by $\|\cdot\|$ the Euclidean norm. Every function $F : S \rightarrow \mathbb{R}$ can be identified (up to an isomorphism) with a vector in $\mathbb{R}^n$ where $n = |S|$; with abuse of notation we will assume a specific identification and denote such vector also by F . We denote by $\delta(\cdot, \cdot)$ the Kronecker delta function. With $P(\cdot)$ we denote a probability function and $\mathbb{E}[\cdot]$ the expectation operator. We denote by $\mathcal{U}(S)$ the uniform probability distribution over an arbitrary set S . Throughout the chapter we will use the terms “grid” and “lattice” interchangeably, with “lattice” emphasizing the mathematical structure.

4.2 Conventional Algorithmic Formulation of ECPMs

In this section, we present the standard ECPM formalism for modeling tumor invasion within an ECM. This overview provides the foundation for the novel system-theoretical perspective presented in the following sections. In particular, we outline the model key components and governing rules,

including cell-cell and cell-medium interactions, the ECM concentration field, and the total energy of the system.

In ECPMs, the substrate's spatial domain is discretized into a regular grid of sites, each representing a small patch of ECM that may be occupied by cells or remain empty. This grid can be viewed as a finite lattice.

Let us consider a two-dimensional finite lattice

$$G := \mathcal{X} \times \mathcal{Y}, \quad \mathcal{X}, \mathcal{Y} \subset \mathbb{Z},$$

with dimensions $n_x := |\mathcal{X}|$ and $n_y := |\mathcal{Y}|$. On G there exist boundary conditions consisting of zero-flux at the top and bottom boundaries, and periodic conditions along the left and right sides of the grid. Consequently, the lattice is cyclic along the horizontal direction, as if it were wrapped around a cylinder. This could represent a tumor mass invading from an epithelial lining through the basement membrane into the surrounding stroma (which includes the ECM). Zero flux at the top captures the fact that cell masses typically remain in the epithelial boundary layers coating a lumen, thus preventing movement in that direction. Instead, the periodic lateral boundary conditions are designed to simulate a portion of a significantly larger area than what can be represented on the grid.

Now, let us introduce a finite set of labels $L \subset \mathbb{N}$. Then, each grid point $p := (x_p, y_p) \in G$ is associated with a label through the following map

$$\begin{aligned} \sigma : G &\rightarrow L \\ p &\mapsto \sigma_p. \end{aligned} \tag{4.1}$$

Here, each label $\sigma_p \in L$ identifies the entity occupying the grid point p , which can belong to a cell or empty space. Thus, the label map in (4.1) provides a complete representation of the spatial distribution of all entities on the grid. In addition, each label $\ell \in L$ is associated with a type via the following mapping

$$\begin{aligned} \tau : L &\rightarrow \Gamma \\ \ell &\mapsto \tau_\ell, \end{aligned} \tag{4.2}$$

where $\Gamma \subset \mathbb{N}$ is a finite set containing grid point types that represent either cell types (e.g., tumor cells or normal cells) or the ECM.

We now introduce the ECM density scalar field f over the discrete lattice. Formally,

$$\begin{aligned} f : G &\rightarrow \mathbb{R}_{\geq 0}, \\ p &\mapsto f_p, \end{aligned} \tag{4.3}$$

which evolves over time due to degradation by proteolytic enzymes secreted by tumor cells. Before presenting the update rule of (4.3), we introduce some quantities defined on the grid. Consider two arbitrary grid points $p, q \in G$, then we equip the grid with the following metric

$$d_G(p, q) := \max(\Delta_x(p, q), \|y_p - y_q\|),$$

where $\Delta_x(p, q) := \min(\|x_p - x_q\|, n_x - \|x_p - x_q\|)$ accounts for the periodic boundary conditions along the horizontal direction. Then, the closed ball of radius $d \in \mathbb{N}$ centered at grid point $p \in G$ can be expressed as

$$\bar{B}_d(p, d) := \{q \in G : d_G(p, q) \leq d\}$$

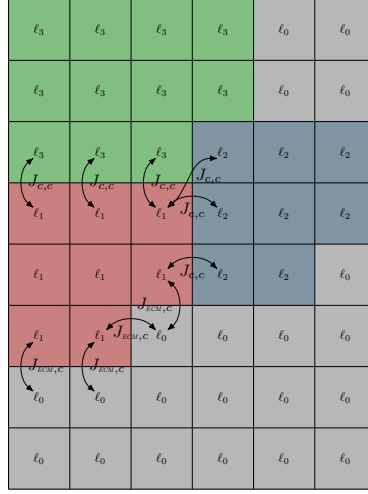


Figure 4.1: Cell-surface interactions in the ECPM are regulated by energy coefficients $J_{c,c}$ (cell-cell) and $J_{ECM,c}$ (cell-ECM) adhesion. A single tumor cell (colored) interacts with neighboring cells and/or ECM (gray).

Accordingly, for $\gamma \in \Gamma$ and integer $d \geq 0$ we define the number of cells of type γ within distance d from p by

$$n_d(p, \gamma, \sigma) := |\{\ell \in \sigma(\bar{B}_d(p, d)) : \tau_\ell = \gamma\}|.$$

Next, we introduce the scalar model parameters that will appear in the f dynamics. For each cell type $\gamma \in \Gamma$, let $c_\gamma^z \geq 0$ be the enzyme secretion rate, $a_\gamma \geq 0$ the spatial decay coefficient, and $D_\gamma \in \mathbb{N}$ the maximal influence radius. Let $c_\gamma^f \geq 0$ denote the ECM degradation rate induced by cells of type γ . With these definitions in place, the proteolytic enzyme produced at p by cells of type γ is given by

$$z(p, \gamma, \sigma) := c_\gamma^z \sum_{d=1}^{D_\gamma} n_d(p, \gamma, \sigma) e^{-a_\gamma d}, \quad (4.4)$$

from which follows the ECM update rule, expressed as

$$f_p^+ = \left(1 - \sum_{\gamma \in \Gamma} c_\gamma^f z(p, \gamma, \sigma) \right) f_p, \quad \forall p \in G, \quad (4.5)$$

where f_p^+ and f_p denote the ECM density at lattice point p at two consecutive instants (i.e. algorithm iterations). To ensure physical consistency, we assume that the parameters are chosen such that

$$\sum_{\gamma \in \Gamma} c_\gamma^f z(p, \gamma, \sigma) \leq 1 \quad \forall p \in G, \quad (4.6)$$

namely, the ECM density cannot become negative after degradation. We now characterize the interaction energy between neighboring grid points by introducing the map

$$J : \Gamma \times \Gamma \rightarrow \mathbb{R}_{\geq 0},$$

which characterizes the interfacial energy between pairs of cell (or medium) types. Biologically, this quantity represents the net energetic cost of maintaining contact between two entities, capturing

coarse-grained effects of cell–cell or cell–matrix adhesion, cortical tension, and other mechanical interactions at the interface. Then, the interaction energy between different labels can be written as

$$\begin{aligned} \tilde{J} : L \times L &\rightarrow \mathbb{R}_{\geq 0} \\ (\sigma_1, \sigma_2) &\mapsto J(\tau_{\sigma_1}, \tau_{\sigma_2})(1 - \delta(\sigma_1, \sigma_2)), \end{aligned} \quad (4.7)$$

where δ is the Kronecker delta function. In Figure 4.1 we provide a schematic representation of cell-surface interactions in the ECPM, regulated by this surface energy function $\tilde{J}$.

Let us define the set of neighbors of lattice point p as

$$\mathcal{N}_p := \{q \in G : d_G(p, q) = 1\}.$$

We can now introduce the core term in CPMs, namely the total energy of the system $H \in \mathbb{R}_{\geq 0}$, given by the following Hamiltonian

$$H := \frac{1}{2} \sum_{p \in G} \sum_{q \in \mathcal{N}_p} \tilde{J}(\sigma_p, \sigma_q) + \sum_{\ell \in L} \lambda \left(\sum_{w \in G} \delta(\sigma_w, \ell) - V_T \right)^2, \quad (4.8)$$

where the first term represents the energy arising from interactions between neighboring sites with different labels (cells), and the second term penalizes deviations of each cell's volume from a predefined target value $V_T \in \mathbb{N}$. The parameter $\lambda \in \mathbb{R}_{>0}$ is a non-negative weight controlling the strength of the volume constraint, thus regulating how strongly cells tend to maintain their target volume.

The ECPM (and CPM) is simulated in practice through a Markov chain Monte Carlo method, the so-called *modified Metropolis algorithm* (MMA) [101, 102]. The latter constitutes an adaptation of the classical *Metropolis algorithm* [103] in the context of the Potts model. The MMA is described step-by-step in Algorithm 5.

Algorithm 5 MMA for simulating an ECPM

```

1: Start from an initial configuration  $(\sigma, f)$ 
2: repeat
3:   Select grid point  $p \sim \mathcal{U}(G)$ 
4:   Pick nearest neighbor  $q \sim \mathcal{U}(\mathcal{N}_p)$ 
5:   if  $\sigma_p = \sigma_q$  then
6:     goto step 3
7:   end if
8:   Define trial configuration  $\sigma'$  such that
      
$$\sigma'_z = \sigma_z + (\sigma_p - \sigma_q)\delta(z, q) \quad \forall z \in G$$

9:   Evaluate energy change  $\Delta H = H(\sigma') - H(\sigma)$ 
10:  Compute total energy
      
$$\Delta E(\sigma, f) = \Delta H + K_{ECM}(f_p - f_q) \tag{4.9}$$

11:  if  $\Delta E(\sigma, f) < 0$  then
12:    Accept the copy and update  $\sigma_q \leftarrow \sigma_p$ 
13:  else
14:    Accept the change  $\sigma_q \leftarrow \sigma_p$  with probability
      
$$e^{-\frac{\Delta E(\sigma, f)}{\beta}} \tag{4.10}$$

15:  end if
16:  Update  $f_z \quad \forall z \in G$  via dynamics (4.5)
17: until stopping criterion

```

In equations (4.9) and (4.10), $K_{ECM} \geq 0$ is a model parameter that weights the influence of ECM gradients on cell migration, while parameter $\beta > 0$ influences the stochastic behavior of the dynamics, in fact it is usually related to environmental noise (such as temperature).

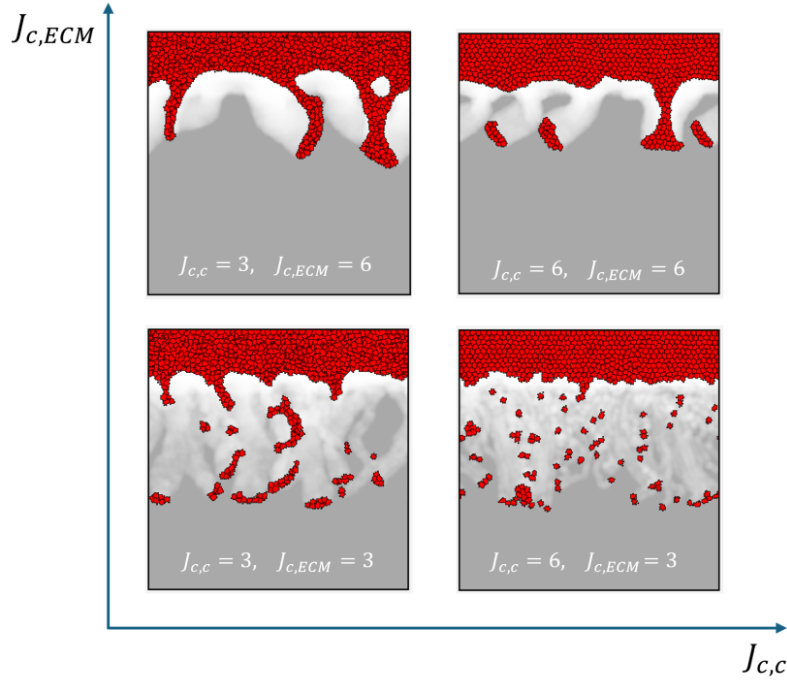


Figure 4.2: ECPM simulation on a 2D lattice showing tumor invasion (red) through ECM (gray) at different adhesion energies $J_{c,c}$ (cell-cell) and $J_{c,ECM}$ (cell-ECM). Higher $J_{c,ECM}$ promotes haptotactic migration along ECM gradients.

This algorithmic description of the ECPM does not provide insights into the dynamical properties of the model. Figure 4.2 illustrates the corresponding procedure through a standard ECPM simulation on a 2D lattice with two types (tumor cells and ECM), where different choices of the adhesion energies $J_{c,c}$ and $J_{c,ECM}$, representing cell-cell and cell-ECM adhesion, lead to different invasion patterns. However, such simulations remain purely descriptive and do not allow for a systematic analysis of the underlying dynamics. To address this limitation, in the next section we cast the ECPM algorithmic procedure into a system-theoretic framework.

4.3 ECPM as a Closed-Loop System with Sparse Energy Terms

In this section, rather than treating the ECPM as a lattice-based energy minimization framework under an algorithmic rule, we regard it as an interconnected dynamical system composed of two distinct yet coupled subsystems: (i) a stochastic process describing the discrete evolution of the cell mass configuration σ , which is updated according to a transition probability based on the MMA, and (ii) a “jump” linear system governing the ECM density field f , whose transition matrix changes due to the current tumor state σ .

We start by identifying the ECM transition matrix underlying dynamics (4.5) as

$$A(\sigma) := \text{diag}_{p \in G} \{ A_p(\sigma) \}, \quad (4.11)$$

with the local terms defined as

$$A_p(\sigma) = 1 - \alpha_p(\sigma) = 1 - \sum_{\gamma \in \Gamma} c_\gamma^f z(p, \gamma, \sigma), \quad (4.12)$$

where $\alpha_p(\sigma)$ represents the total degradation rate at site p induced by all cell types present in the current configuration σ . Here, $A_p(\sigma) \in [0, 1]$ since $0 \leq \alpha_p(\sigma) \leq 1$ due to condition (4.6) and being the parameters within (4.4) non-negative. In view of the definition in (4.5), the ECM field can be identified with a vector $f \in \mathbb{R}^{n_x n_y}$. Accordingly, its dynamics can be rewritten as the following discrete jump-linear system

$$f^+ = A(\sigma)f. \quad (4.13)$$

Moreover, since the acceptance probability in (4.10) depends on the current local ECM values f_p and f_q (see ΔE definition at MMA step 4.9), the σ -dynamics are thus influenced by f . Formally, the stochastic process associated to the evolution of σ can be generally written as

$$\sigma^+ \sim P(\sigma, f) \quad (4.14)$$

where the transition probability is given by

$$P(\sigma, f) := \begin{cases} 1 & \text{if } \Delta E(\sigma, f) < 0 \\ e^{-\frac{\Delta E(\sigma, f)}{\beta}} & \text{otherwise} \end{cases}, \quad (4.15)$$

that is the piecewise Boltzmann-weighted probability derived from the MMA acceptance rule (see Algorithm 5). The resulting feedback interconnection between the subsystems (4.13) and (4.14) is illustrated in Fig. 4.3.

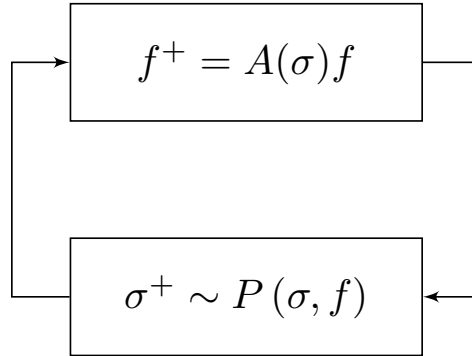


Figure 4.3: System-theoretical view of the ECPM. The discrete stochastic process is coupled to a jump-linear system.

Based on this reformulation, we now proceed by analyzing the computation of the Hamiltonian variation ΔH . Each σ -copy attempt from a selected site p to one of its neighbors $q \in \mathcal{N}_p$ requires computing the energy change ΔH , and a direct computation of H at every step is costly. However, the local nature of cell-cell interactions implies that ΔH is sparse, i.e. only a small subset of terms changes between two successive configurations. To highlight this sparsity, we first split the expression in (4.8) into two terms as follows

$$H_J := \frac{1}{2} \sum_{p \in G} \sum_{q \in \mathcal{N}_p} \tilde{J}(\sigma_p, \sigma_q) \quad (4.16)$$

$$H_V := \lambda \sum_{\ell \in L \setminus \{\ell_0\}} \left(\sum_{w \in G} \delta(\sigma_w, \ell) - V_T \right)^2, \quad (4.17)$$

where H_J collects all local interaction contributions between neighboring sites, while H_V being the volume penalty term that favors label-wise occupancy close to a target value. In (4.17), ℓ_0 represents the label corresponding to empty lattice sites covered by ECM (i.e. any $p \in G : \sigma_p = \ell_0$ with $f_p > 0$). Here, since ℓ_0 does not correspond to an actual cell, it is excluded from the summation in H_V . This ensures that the volume term is applied only to actual cells, as empty or ECM-occupied sites do not have a target volume or contribute to the volume energy term. With these definitions in place, we present the following result.

Lemma 4.3.1. *Consider a current cell mass configuration σ and ECM field f . Consider a trial configuration σ' obtained through a copy attempt from lattice site $p \in G$ to its neighbor $q \in \mathcal{N}_p$ at iteration step k . Then, the total energy change $\Delta E(\sigma, f)$ can be decomposed into sparse energy terms as follows*

$$\Delta E(\sigma, f) = \Delta H_J(\sigma) + \Delta H_V(\sigma) + K_{ECM}(f_p - f_q), \quad (4.18)$$

where the adhesion-energy change is given by

$$\Delta H_J(\sigma) = \sum_{w \in \mathcal{N}_q} \tilde{J}(\sigma_p, \sigma_w) - \tilde{J}(\sigma_q, \sigma_w), \quad (4.19)$$

and the volume-energy change is expressed as

$$\Delta H_V(\sigma) = \lambda \sum_{\ell \in \{\sigma_p, \sigma_q\}} [1 + 2\rho(\sigma_p, \ell)(V_\ell - V_T)] (1 - \delta(\ell_0, \ell)), \quad (4.20)$$

with

$$V_\ell := \sum_{w \in G} \delta(\sigma_w, \ell),$$

and $\rho(\sigma_1, \sigma_2) := 2\delta(\sigma_1, \sigma_2) - 1 \in \{-1, 1\}$.

Proof. The adhesion-energy change $\Delta H_J = H_J(\sigma') - H_J(\sigma)$ is computed considering all pairwise interactions between neighboring sites (see (4.16)). However, since only the interaction terms involving site q and thus $p \in \mathcal{N}_q$ change, all other terms cancel out, leading to equation (4.19).

For the volume-energy change $\Delta H_V = H_V(\sigma') - H_V(\sigma)$, after the copy attempt from p to q , the affected cell volumes evolve according to

$$V_\ell^+ = V_\ell + \rho(\sigma_p, \ell), \quad \forall \ell \in \{\sigma_p, \sigma_q\},$$

where $\rho(\sigma_1, \sigma_2) = 2\delta(\sigma_1, \sigma_2) - 1$ accounts for the increase or decrease in volume. All the other cell volumes remain unchanged, thus canceling out in the energy difference. Therefore, the Hamiltonian volume energies at σ and σ' can be simplified as

$$\begin{aligned} H_V(\sigma) &= \lambda \sum_{\ell \in \{\sigma_p, \sigma_q\}} (V_\ell - V_T)^2, \\ H_V(\sigma') &= \lambda \sum_{\ell \in \{\sigma_p, \sigma_q\}} (V_\ell + \rho(\sigma_p, \ell) - V_T)^2. \end{aligned}$$

Then, subtracting these two expressions, developing the squares, and simplifying leads to the following volume-energy change

$$\begin{aligned} \Delta H_V(\sigma) &= \lambda \sum_{\ell \in \{\sigma_p, \sigma_q\}} [\rho(\sigma_p, \ell)^2 + 2\rho(\sigma_p, \ell)(V_\ell - V_T)] \\ &= \lambda \sum_{\ell \in \{\sigma_p, \sigma_q\}} [1 + 2\rho(\sigma_p, \ell)(V_\ell - V_T)], \end{aligned}$$

which corresponds to equation (4.20) after excluding the empty label ℓ_0 through the term $(1 - \delta(\ell_0, \ell))$.

The ECM-dependent term $K_{ECM}(f_p - f_q)$ arises directly from definition 4.9 in Algorithm 5, thus completing the decomposition expressed in (4.18). $\square$

This result highlights how the total energy change decomposes into three sparse terms that determine the stochastic evolution in (4.14) following the probability rule in (4.15). Therefore, the state dynamics of the ECPM can be interpreted as a stochastic process driven by a sparse and efficiently computable energy functional, coupled to a jump linear system representing the ECM degradation. This reformulation makes the tumor-ECM interaction dynamics within this class of models more explicit and analytically tractable.

4.4 System Analysis and Properties

In this section we analyze the dynamical properties of the ECPM reformulated in Section 4.3. We begin by presenting a result on the asymptotic behavior of the ECM field f , and then we characterize the transition probabilities related to the σ -dynamics. Furthermore, we study the dynamics of each subsystem, respectively examining the ECM evolution under a fixed cell mass configuration and the cell mass evolution for a fixed ECM concentration, together with the associated transition probabilities. Finally, we define a lower bound on the transition probabilities as a function of model parameters. In the following, we denote with $k \in \mathbb{N}$ the discrete-time index associated with the ECPM state (σ^k, f^k) .

4.4.1 Asymptotic Behavior of the ECM Dynamics

We establish that the ECM concentration f remains bounded and converges to a steady-state value, regardless the evolution of the cell mass configuration σ . Formally, we have the following property.

Lemma 4.4.1. *If $\{(\sigma^k, f^k)\}_{k \in \mathbb{N}}$ is a trajectory for the interconnected system depicted in Figure 4.3, then*

$$\lim_{k \rightarrow \infty} f^k = \bar{f} \in [0, f^0].$$

Proof. Considering the dynamics of f^k , we have for all $p \in G$ that

$$0 \leq f_p^{k+1} = \underbrace{A_p(\sigma^k)}_{\in [0,1]} f_p^k \leq f_p^k \leq f_p^0.$$

Hence, by the monotone convergence theorem the sequence admits a limit in the interval $[0, f_p^0]$. $\square$

4.4.2 Transition Probabilities of Cell Mass Configuration

We first introduce the difference set between two configurations as

$$\Theta(\sigma_1, \sigma_2) := \{p \in G : \sigma_{1,p} \neq \sigma_{2,p}\}.$$

Then, the distance function $d_L : L \times L \rightarrow \mathbb{N}$ counts the number of different points between configurations, i.e.

$$d_L(\sigma_1, \sigma_2) = |\Theta(\sigma_1, \sigma_2)|.$$

Based on that, we state that two configurations σ_1 and σ_2 are adjacent if and only if $d_L(\sigma_1, \sigma_2) = 1$.

Given the current ECPM state (σ, f) , the transition probability $P(\sigma^+ = \sigma' | \sigma, f)$ for the system to evolve to the configuration σ' is zero either if the two configurations are not adjacent, or the two configurations are adjacent with $\Theta(\sigma, \sigma') = \{p\}$, but they differ by an outlier (an isolated label), i.e. $\sigma'_p \neq \sigma'_q, \forall q \in \mathcal{N}_p$. Otherwise, the probability of moving to σ' is always nonzero.

We now characterize the transition probabilities of the cell mass configuration. To this end, we first introduce some notation. Let $\mathcal{E}$ denote the set of all point pairs in G , and define

$$\mathcal{E}_1 := \{(p, q) \in \mathcal{E} : d_G(p, q) = 1\},$$

as the set of point pairs at distance 1 (i.e., nearest neighbors). For a given configuration σ , let $N_U(\sigma) < |\mathcal{E}_1|$ denote the number of edges in $\mathcal{E}_1$ with same label, i.e.,

$$N_U(\sigma) := |\{(p, q) \in \mathcal{E}_1 : \sigma^p = \sigma^q\}|.$$

Next, we partition the set of configurations adjacent to σ based on the sign of the energy variation. Define $L_D^+(\sigma, f)$ as the set of configurations σ' adjacent to σ for which the energy change is non-negative, specifically

$$L_D^+(\sigma, f) := \{\sigma' \in G^L : \Theta(\sigma, \sigma') = \{p\}, \Delta E(\sigma' | \sigma, f) \geq 0\}.$$

Similarly, define $L_D^-(\sigma, f)$ as the set of configurations σ' adjacent to σ for which the energy change is negative, i.e.

$$L_D^-(\sigma, f) := \{\sigma' \in G^L : \Theta(\sigma, \sigma') = \{p\}, \Delta E(\sigma' | \sigma, f) < 0\}.$$

With these definitions in place, we can now express the transition probabilities as follows. For a configuration $\sigma' \in L_D^-(\sigma, f)$ (favorable energy change), the transition probability is

$$P(\sigma^+ = \sigma' | \sigma, f) = \frac{1}{|\mathcal{E}_1| - N_U(\sigma)},$$

where the denominator reflects that only adjacent configurations with differing labels can be reached in one step, excluding the $N_U(\sigma)$ edges with the same label. Instead, for a configuration $\sigma' \in L_D^+(\sigma, f)$ (unfavorable energy change), the transition probability is

$$P(\sigma^+ = \sigma' | \sigma, f) = \frac{e^{-\frac{\Delta E(\sigma' | \sigma, f)}{\beta}}}{|\mathcal{E}_1| - N_U(\sigma)}. \quad (4.21)$$

Finally, the probability of remaining in the same configuration σ is

$$P(\sigma^+ = \sigma | \sigma, f) = \frac{\sum_{\sigma' \in L_D^+(\sigma, f)} \left[1 - e^{-\frac{\Delta E(\sigma' | \sigma, f)}{\beta}} \right]}{|\mathcal{E}_1| - N_U(\sigma)}, \quad (4.22)$$

which accounts for rejected moves to unfavorable configurations.

4.4.3 ECM Evolution under a Fixed Cell Mass Configuration

We now characterize the evolution of the ECM concentration under a fixed cell mass configuration $\bar{\sigma}$. Specifically, we show that f at each lattice point either converges to zero or remains at its initial value.

Proposition 4.4.2. *Let the grid configuration be fixed at $\bar{\sigma}$ and consider the ECM dynamics*

$$f^{k+1} = A(\bar{\sigma})f^k.$$

Then, the asymptotic behavior is given by

$$\lim_{k \rightarrow \infty} f_p^k = \begin{cases} 0 & \text{if } A_p(\bar{\sigma}) \in [0, 1), \\ f_p^0 & \text{if } A_p(\bar{\sigma}) = 1, \end{cases}$$

for all $p \in G$.

Proof. Since $A(\bar{\sigma})$ is diagonal with entries in the interval $[0, 1]$ (see expressions (4.11) and (4.12)), we have

$$f_p^k = [A_p(\bar{\sigma})]^k f_p^0 \quad \forall p \in G.$$

The asymptotic behavior follows directly from the fact that $A_p(\bar{\sigma}) \in [0, 1]$ for any configuration $\bar{\sigma}$, in fact $A_p(\sigma^k) \in [0, 1]$ for any k . $\square$

4.4.4 Cell Mass Evolution under a Fixed ECM Concentration

We proceed to the dual situation in which the ECM concentration is fixed at some value $\bar{f}$ and we want to analyze the evolution of σ . In view of Lemma 4.4.1, this can be interpreted as the asymptotic regime of the interconnected system. In this setting, the grid configuration σ^k at discrete-time step k evolves according to a fixed probability transition function $P(\cdot \mid \sigma^k, \bar{f})$, which depends solely on the current configuration σ^k . As a consequence, the stochastic sequence $\{\sigma^k\}_{k \in \mathbb{N}}$ satisfies the Markov property and is therefore a Markov chain. We formalize this behavior as follows.

Proposition 4.4.3. *Let the ECM concentration be fixed at $\bar{f}$. Then, the process $\{\sigma^k\}_{k \in \mathbb{N}}$ forms a Markov chain with transition probabilities $P(\cdot \mid \sigma^k, \bar{f})$. Moreover, the set Σ_u of uniform cell mass configurations coincides with the set of absorbing states of this Markov chain, and regardless of the initial configuration σ^0 , it follows that*

$$P(\sigma^k \in \Sigma_u, k < \infty) = 1.$$

Proof. Available in [85]. $\square$

4.4.5 Lower Bound on Transition Probability

Provided the formulation of the ECPM as a closed-loop system from Section 4.3, we now proceed by analyzing the bounds on the σ -transition probability defined in (4.14).

Proposition 4.4.4. *For any trial configuration $\sigma' \in L_D^+(\sigma, f)$, the transition probability defined in (4.21) admits the lower bound*

$$P(\sigma^+ = \sigma' \mid \sigma, f) \geq \frac{e^{-(\Delta H_{\max} + K_{\text{ECM}} f^0)/\beta}}{n_x (8n_y - 6) - N_U(\sigma)},$$

where $\Delta H_{\max}$ is an upper bound on the Hamiltonian variation from σ to σ' , determined by the parameters λ and V_T , and by the interaction energy function J in (4.7). The same bound applies to each term of the summation in (4.22), whereas the transition probability defined in (4.4.2) is independent of the ECPM parameters and is therefore unaffected.

Proof. Consider a trial cell mass configuration σ' such that $\sigma'_q = \sigma_p$, with $p \in G$ and $q \in \mathcal{N}_p$. From the proof of Lemma 4.4.1, it follows that the ECM concentration difference satisfies

$$f_p^k - f_q^k \in [-f^0, f^0] \quad \forall k = 1, \dots$$

where f^0 is the initial uniform ECM concentration on the lattice.

Let $\Delta H_{\max}$ and $\Delta H_{\min}$ represent the maximum and minimum variations of the Hamiltonian for the transition from σ to σ' . These depend on the current and trial configurations, model parameters λ and V_T , and the interaction energy function J defined in (4.7). The total energy variation can be bounded as follows

$$\Delta E \in [\Delta H_{\min} - K_{ECM}f^0, \Delta H_{\max} + K_{ECM}f^0].$$

To establish the denominator, we calculate the total number of ordered neighboring pairs in $\mathcal{E}_1$. Each interior point (x, y) , namely $x \in \mathcal{X}$ and $y \in \{2, \dots, n_y - 1\}$, has 8 nearest neighbors. Instead, the top/bottom boundary points at $y = 1$ and $y = n_y$ each have n_x points with 5 nearest neighbors. It follows that

$$|\mathcal{E}_1| = n_x(n_y - 2) \cdot 8 + 2n_x \cdot 5 = n_x(8n_y - 6).$$

Substituting the worst-case energy increase into the expression of the unfavorable transition probability in (4.21) and including the denominator $|\mathcal{E}_1|$, directly yields the stated lower bound. $\square$

4.5 Invasion Time in One-Dimensional ECPM

In this section, we analyze tumor invasion dynamics in a one-dimensional (1D) lattice setting of the ECPM, focusing on the *invasion time*, i.e. the time steps required for the tumor front to reach a specific lattice site given an initial configuration. This quantity provides a direct measure of the tumor invasion speed along the lattice. As highlighted in [104], the 1D setting is relevant in several biological applications since it models migration in narrow ECM fibers, effectively capturing key dynamical features observed in more complex environments. In fact, the 1D-ECPM can be viewed as a reduced representation of tumor invasion, capturing migration dynamics that closely mirror the radial progression observed in three-dimensional settings.

Let us start by considering an ECPM where the 1D lattice consists of n sites, namely G with $n_x = 1$ and $n_y = n$. The initial ECM density is uniform across the lattice, which is a standard initialization for ECPM substrate representation, i.e.

$$f_p^0 = f^0, \quad \forall p \in G, \tag{4.23}$$

where $f^0 \in \mathbb{R}_{>0}$ denotes the initial ECM density at a lattice site. We denote with f^0 the initial ECM vector satisfying (4.23). It follows that $f_p^k \in [0, f^0]^n$ for all $k \in \mathbb{N}$ and $p \in G$ (see Lemma 4.4.1). Now, we can define the invasion time as follows.

Definition 4.5.1 (Invasion time). *Given a grid point $i \in G$, the invasion time T_i^{INV} is defined as the minimum number of ECPM update steps required for the tumor front to reach the lattice site at depth i . Formally,*

$$T_i^{\text{INV}} := \min \{k \geq 0 : \varphi(\sigma^k) \geq i\},$$

where $\varphi(\sigma^k)$ represents the maximum tumor depth at iteration k , defined as

$$\varphi(\sigma^k) := \max \{ p_y \in \mathcal{Y} : \sigma_p^k \neq \ell_0 \}.$$

Here, p_y denotes the vertical coordinate of lattice site $p \in G$.

For clarity, we denote the ECPM state of the interconnected system (in Figure 4.3) at iteration k as follows

$$S^k := (\sigma^k, f^k).$$

We now introduce a compact formulation of the invasion time probability. Let $\mathcal{P}_{i|j}(k | S^0)$ indicate the probability that the tumor reaches lattice site i in exactly k steps, starting from the initial ECPM state $S^0 = (\sigma^0, f^0)$ with maximum tumor depth $j \leq i$. Formally,

$$\mathcal{P}_{i|j}(k | S^0) := P(T_i^{\text{INV}} = k | \varphi(\sigma^0) = j, f^0). \quad (4.24)$$

Building on Definition 4.5.1, the mean invasion time is defined as the conditional expectation of T_i^{INV} given the initial ECPM state. Since T_i^{INV} is a discrete random variable taking values $k \in \mathbb{Z}_{>0}$ with probability mass function (4.24), we obtain

$$\begin{aligned} \bar{T}_{i|j}^{\text{INV}}(S^0) &:= \mathbb{E}[T_i^{\text{INV}} | \varphi(\sigma^0) = j, f^0] \\ &= \sum_{k=1}^{\infty} k \mathcal{P}_{i|j}(k | S^0), \end{aligned} \quad (4.25)$$

provided that the series converges. For numerical evaluation one typically truncates (4.25) at some finite horizon. We now present a result that provides an upper bound on the truncation error under a geometric decay of the invasion time probabilities.

Proposition 4.5.2 (Truncation error bound for mean invasion time). *Consider the mean invasion time defined by the infinite series in (4.25), and fix a truncation horizon $K \in \mathbb{Z}_{>0}$. Assume that the invasion time probabilities satisfy a geometric decay condition, i.e. there exist constants $\eta > 0$ and $q \in (0, 1)$ such that*

$$\mathcal{P}_{i|j}(k | S^0) \leq \eta q^k, \quad \forall k = 1, 2, \dots \quad (4.26)$$

Then, the truncated approximation

$$\bar{T}_{i|j}^{\text{INV}}(S^0) \approx \sum_{k=1}^K k \mathcal{P}_{i|j}(k | S^0), \quad (4.27)$$

admits the truncation error

$$R_K := \sum_{k=K+1}^{\infty} k \mathcal{P}_{i|j}(k | S^0), \quad (4.28)$$

which satisfies the upper bound

$$R_K \leq \eta q^{K+1} \frac{K(1-q) + 1}{(1-q)^2}. \quad (4.29)$$

Proof. Given the assumption in (4.26), we can bound the truncation error in (4.28) as follows

$$\begin{aligned} R_K &\leq \sum_{k=K+1}^{\infty} k \eta q^k = \eta q \sum_{k=K+1}^{\infty} k q^{k-1} = \eta q \sum_{k=K+1}^{\infty} \frac{d}{dq} \{q^k\} \\ &= \eta q \frac{d}{dq} \left\{ \sum_{k=K+1}^{\infty} q^k \right\} = \eta q \frac{d}{dq} \left\{ q^{K+1} \sum_{k=K+1}^{\infty} q^{k-(K+1)} \right\} = \eta q \frac{d}{dq} \left\{ q^{K+1} \sum_{j=0}^{\infty} q^j \right\}, \end{aligned}$$

where we have performed the change of variable $j = k - (K + 1)$ in the last step. Since $|q| < 1$ by assumption, $\sum_{j=0}^{\infty} q^j$ converges to $1/(1 - q)$ being a geometric series, and thus

$$\begin{aligned} R_K &\leq \eta q \frac{d}{dq} \left\{ \frac{q^{K+1}}{1 - q} \right\} = \eta q \frac{(K + 1)q^K(1 - q) + q^{K+1}}{(1 - q)^2} \\ &= \eta q \frac{(K + 1)q^K - Kq^{K+1}}{(1 - q)^2} = \eta q^{K+1} \frac{K(1 - q) + 1}{(1 - q)^2}. \end{aligned}$$

□

The bound in (4.29) allows one to select an appropriate truncation horizon K to ensure that the mean in (4.27) approximates the true expected invasion time within a desired tolerance level.

In the following, we derive analytical expressions for the invasion time probability (4.24) in the 1D-ECPM setting. We consider an initial configuration σ^0 consisting of $N \geq 1$ tumor cells and analyze how the invasion time probability depends on the model parameters. The presentation follows a constructive approach: we first treat the single-cell case in detail, then extend the results to multiple cells.

4.5.1 Invasion Time Probability for a Single Tumor Cell

We start by analyzing the scenario, depicted in Figure 4.4, in which just one tumor cell with label ℓ_1 and initial volume $V_0 := V_{\ell_1}^0$ is considered. Therefore, the corresponding label and type sets are given by

$$L = \{\ell_0, \ell_1\}, \quad \Gamma = \{\gamma_{ECM}, \gamma_c\},$$

with ℓ_0 the label corresponding to an empty grid point, ℓ_1 the label of the cell, γ_{ECM} the type corresponding to an empty grid point covered by ECM, i.e. $\tau_{\ell_0} = \gamma_{ECM}$, and γ_c the tumor cell type satisfying $\tau_{\ell_1} = \gamma_c$.

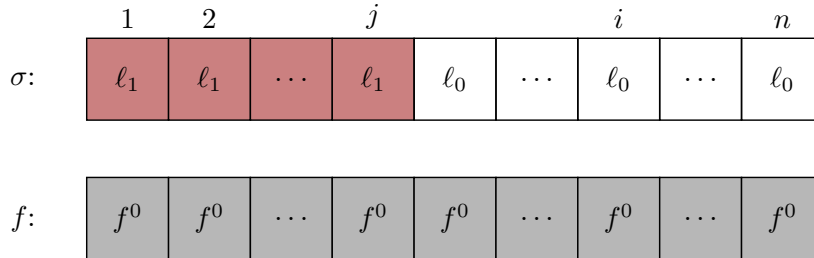


Figure 4.4: Illustration of the starting ECPM state at iteration step $k = 0$ for a 1D grid representing the spatial distribution of a single tumor cell surrounded by extracellular matrix. The top row shows the labeling of grid cells, where ℓ_1 represents the only tumor cell considered, ℓ_0 represents empty lattice sites. The bottom row represents the initial ECM distribution across the grid. Indices are provided above the grid for reference: j represents the deepest lattice point occupied by the cell, $i > j$ is the grid point with respect to which we will perform the invasion time analysis. n denotes the 1D grid length.

In this context, we focus on the pair $(j, j + 1)$ as the only valid grid point selection for the tumor's progression. Specifically, we restrict the admissible changes to the pair (σ_j, σ_{j+1}) , allowing the tumor cell to either advance forward or remain stationary. In contrast, movements where an empty label replaces a tumor label (cell is retreating), such as when the selected neighbor pair is $(p, q) = (j + 1, j)$, are excluded from our analysis. This restriction is biologically and analytically

motivated by the following biological considerations. 1D migration models reflect cell motion along narrow ECM fibers, where cells exhibit stable polarity and primarily advance forward due to localized matrix degradation and traction at the leading edge [104]. Backward motion is rare and transient, as ECM anisotropy and adhesion asymmetry bias movement toward increasing depth. Analytically, excluding reverse copy attempts (ECM-over-tumor) simplifies the stochastic dynamics without affecting the dominant energy-driven invasion mechanism, as such moves would typically increase system energy and occur with negligible probability.

In this framework, the interaction energy function satisfies

$$\tilde{J}(\gamma_{ECM}, \gamma_{ECM}) = 0 \quad (4.30a)$$

$$\tilde{J}(\gamma_c, \gamma_c) = J_{c,c} \quad (4.30b)$$

$$\tilde{J}(\gamma_c, \gamma_{ECM}) = \tilde{J}(\gamma_{ECM}, \gamma_c) = J_{ECM,c}, \quad (4.30c)$$

where $J_{c,c}$ represents cell-cell adhesion energy and $J_{ECM,c}$ represents cell-ECM adhesion energy.

Remark 4.5.3. In the 1D-ECPM single-cell analysis illustrated in Figure 4.4, the invasion dynamics are unaffected by the specific values of $J_{c,c}$ and $J_{ECM,c}$. The adhesion energy contribution ΔH_J remains zero at each iteration, as only one tumor cell exists, which can only occupy empty ECM sites. Thus, no cell-cell interfaces are formed or altered during the invasion. The energy change is primarily influenced by the volume constraint term ΔH_V and the ECM-dependent haptotactic term $K_{ECM}(f_p - f_q)$.

Instead, the interaction energy between ECM sites is zero (see (4.30a)) since the ECM acts as a passive scaffold without self-adhesive properties. This simplification is consistent with the biological behavior of the ECM, which primarily influences tumor dynamics through its interactions with tumor cells.

Based on these considerations, the ECM dynamics in (4.5) simplify into

$$f_p^+ = (1 - c_{\gamma_c}^f z(p, \gamma_c, \sigma)) f_p \quad \forall p \in G, \quad (4.31)$$

in which the enzyme secretion associated to the cancer cell is

$$z(p, \gamma_c, \sigma) = c_{\gamma_c}^z \sum_{d=1}^{D_{\gamma_c}} n_d(p, \gamma_c, \sigma) e^{-a_{\gamma_c} d}.$$

Note that here we consider $c_{\gamma_{ECM}}^f = 0$, i.e. the ECM type does not produce any self-degrading enzyme (only cancer cells can cause a reduction in the ECM density).

First, we present an intermediate result regarding the derivation of $\mathcal{P}_{j+1|j}(k | S^0)$ obtained via a step-by-step analysis of the interconnected system in Figure 4.3. Then, we leverage this result to derive a closed-form expression for the invasion time probability $\mathcal{P}_{i|j}(k | S^0)$ for a generic grid point $i > j$.

Lemma 4.5.4 (Next-site Invasion Time Probability for a Single Tumor Cell). *Consider an initial configuration σ^0 with a tumor cell occupying grid point j in a 1D lattice, with initial volume V_0 , and let either of the following assumptions hold*

(i) *The initial cell volume V_0 and the target volume V_T satisfy*

$$V_T - V_0 < \frac{1}{2}. \quad (4.32)$$

(ii) For all intermediate steps $h \in \{1, \dots, k-1\}$, the parameter λ satisfies the condition

$$\lambda \leq 0 \vee \lambda \geq -\frac{K_{ECM} (f_p^{h-1} - f_q^{h-1})}{1 + 2(V_0 - V_T)}, \quad (4.33)$$

meaning that the total energy variation ΔE^h is non-negative for at least $k-1$ steps. Then, for any $k > 1$, the probability that the tumor cell invades the next lattice site $j+1$ (initially satisfying $\sigma_{j+1} = \ell_0$) exactly at iteration k is given by

$$\mathcal{P}_{j+1|j}(k | S^0) = \prod_{h=1}^{k-1} \left(1 - e^{-\frac{\Delta E^h}{\beta}}\right) P(\Delta E^k), \quad (4.34)$$

in which

$$P(\Delta E^k) = \begin{cases} 1 & \text{if (4.33) holds at } h = k \\ e^{-\frac{\Delta E^k}{\beta}} & \text{otherwise} \end{cases}, \quad (4.35)$$

and ΔE^h is the total energy variation at step h , expressed as

$$\Delta E^h = \Delta H^1 + K_{ECM} (f_p^{h-1} - f_q^{h-1}), \quad (4.36)$$

with the Hamiltonian variation at the first step given by

$$\Delta H^1 := \lambda [1 + 2(V_0 - V_T)]. \quad (4.37)$$

For $k = 1$, the invasion time probability simplifies to

$$\mathcal{P}_{j+1|j}(1 | S^0) = \begin{cases} 1 & \text{if (4.32) and } \lambda > 0 \\ e^{-\frac{\Delta E^1}{\beta}} & \text{otherwise} \end{cases}. \quad (4.38)$$

Proof. As previously stated, consider a cancer cell labeled ℓ_1 occupying the 1D lattice from the starting point of the grid up to position $j < n$. We denote this initial configuration by σ^0 (see Figure 4.4). At the first ECPM step ($k = 1$), the only pair of adjacent lattice points that satisfies the condition $\sigma_p \neq \sigma_q$ is $(p, q) = (j, j+1)$. Based on that, we compute the Hamiltonian variations at the first iteration ($k = 1$) using the sparse decomposition from Lemma 4.3.1. Specifically, applying equations (4.19) and (4.20), we obtain

$$\begin{aligned} \Delta H_j^1 &= 0 \\ \Delta H_{j+1}^1 &= \lambda [1 + 2(V_0 - V_T)], \end{aligned}$$

where $\Delta H_j^1 = 0$ as the copy attempt does not change the tumor-ECM interaction energy, consistent with Remark 4.5.3, and $J_{c,c} = 0$ since there is only one tumor cell in the lattice. Then, the total energy variation at iteration $k = 1$ reads as

$$\Delta E^1 = \lambda [1 + 2(V_0 - V_T)], \quad (4.39)$$

in which the ECM-related term $K_{ECM} (f_p^0 - f_q^0)$ is null due to initial ECM uniform density distribution (refer to (4.23)). Based on that, the acceptance probability at step $k = 1$ reads as

$$P(\Delta E^1) = \begin{cases} 1 & \text{if (4.32) and } \lambda > 0 \\ e^{-\frac{\Delta E^1}{\beta}} & \text{otherwise} \end{cases}. \quad (4.40)$$

Note that λ is generally assumed to be positive in ECPMs, as it weights the volume constraint term in the Hamiltonian. Thus, the condition $\lambda > 0$ in (4.40) is not restrictive.

Let us now consider the next iteration step $k = 2$ at which the ECM term is non-null. The total energy variation can then be expressed as

$$\Delta E^2 = \lambda \left[1 + 2 (V_{\ell_1}^1 - V_T) \right] + \underbrace{K_{ECM} (f_p^1 - f_q^1)}_{<0},$$

in which $V_{\ell_1}^1 \in \{V_0, V_0 + 1\}$ depending on the outcome of (4.40). Here, the ECM term is negative since $K_{ECM} > 0$ and $f_p^1 - f_q^1 < 0$ following from Lemma 4.4.1. As a consequence, the total energy variation is negative if one of the following parameter conditions are satisfied

$$V_T - V_{\ell_1}^1 \geq \frac{1}{2} \quad (4.41a)$$

$$0 < \lambda < -\frac{K_{ECM} (f_p^1 - f_q^1)}{1 + 2 (V_{\ell_1}^1 - V_T)}, \quad (4.41b)$$

in which inequality (4.41a) is the negation of (4.32) if $V_{\ell_1}^1 = V_0$. Otherwise, i.e. $V_{\ell_1}^1 = V_0 + 1$, it becomes $V_T - V_0 \geq \frac{3}{2}$. Therefore, the acceptance probability at second iteration is

$$P(\Delta E^2) = \begin{cases} P(\Delta E^1) & \text{if (4.41)} \\ P(\Delta E^1) e^{-\frac{\Delta E^2}{\beta}} & \text{otherwise} \end{cases},$$

where with condition (4.41) we denote (4.41a) $\vee$ (4.41b). We can now express the probability that a tumor cell invades point $j + 1$ from j at the first iteration as

$$\mathcal{P}_{j+1|j}(1 | S^0) = P(\Delta E^1),$$

which demonstrates (4.38). Moreover, the probability that a tumor cell invades point $j + 2$ from j in exactly two steps is given by

$$\mathcal{P}_{j+2|j}(2 | S^0) = P(\Delta E^1) P(\Delta E^2).$$

We continue the proof by generalizing (4.38) for any arbitrary step $k > 1$. Computing the expression of $\mathcal{P}_{j+1|j}(k | S^0)$ for $k > 1$ means that the tumor remains stationary for $k - 1$ steps, and at the k -th step, it moves to the next grid point. Otherwise, that probability is zero. Having said that, when $\Delta E > 0$ at $k = 1$, then the tumor remains at the same configuration ($\sigma^1 = \sigma^0$) with non-zero probability $1 - e^{-\frac{\Delta E^1}{\beta}}$. Then, at the next iteration ($k = 2$), the ΔH remains equal to the previous iteration and only the ECM-related term changes (becoming negative). Following this reasoning, we express the total energy variation that considers the tumor to remain at the initial configuration σ^0 for $h \geq 1$ steps as

$$\Delta E^h = \Delta H^1 + K_{ECM} (f_p^{h-1} - f_q^{h-1}),$$

being the equivalent of expression (4.36) with Hamiltonian variation computed as in (4.37). Note that, to remain in the same initial grid configuration for $h = k - 1$ steps, we are assuming that the inequality in (4.33) is not met for the same amount of discrete steps, together with condition (4.32). Therefore, we can conclude that the invasion time probability $\mathcal{P}_{j+1|j}(k | S^0)$, which considers the tumor to invade the next grid point $j + 1$ from j in exactly k steps, is given by (4.34), which holds $\forall k > 1$ (in the case of $k = 1$, it simplifies into (4.38)). In the proposed expression, as a result of this step-by-step analysis on the considered 1D-ECPM single-cell framework, the total energy variation terms turn out to be computed as in (4.36) with Hamiltonian variation from (4.37). $\square$

Let us introduce the total amount of ECPM iterations updates up to the s^{th} transition as

$$\kappa_s := \sum_{h=1}^s k_h. \quad (4.42)$$

Then, building on Lemma 4.5.4 and definition (4.42), we present the following result that generalizes the invasion time probability to account for transitions over multiple lattice sites.

Lemma 4.5.5 (Invasion Time Probability for a Single Tumor Cell in a 1D-ECPM). *Let a single tumor cell ($N = 1$) with label ℓ_1 occupy the 1D lattice. Consider to start at configuration σ^0 with $\varphi(\sigma^0) = j$ and reach configuration σ^k such that $\varphi(\sigma^k) = i$ with $0 < i - j \leq k$. The probability that the tumor cell reaches site i from j in exactly k steps, is given by*

$$\mathcal{P}_{i|j}(k | S^0) = \sum_{r=1}^{\binom{k-1}{i-j-1}} \prod_{s=1}^{i-j} \mathcal{P}_{s+j|s+j-1}(k_s^r | S^{s-1}), \quad (4.43)$$

in which $\binom{k-1}{i-j-1}$ indicates the number of $(i-j)$ -partitions of k steps, k_s^r denotes the number of steps associated to the s^{th} transition in the r^{th} partition, and where

$$\mathcal{P}_{s+j|s+j-1}(k_s^r | S^{s-1}) = \prod_{h=\kappa_{s-1}^r}^{\kappa_s^r-1} \left(1 - e^{-\frac{\Delta E^h}{\beta}}\right) P(\Delta E^{\kappa_s^r}), \quad (4.44)$$

with κ_s^r satisfying (4.42), and where the total energy variation for $h \in \{\kappa_{s-1}^r, \dots, \kappa_s^r\}$ is computed as

$$\Delta E^h = \underbrace{\lambda [1 + 2(V_{\ell_1}^{h-1} - V_T)]}_{\Delta H^h} + K_{ECM}(f_p^{h-1} - f_q^{h-1}), \quad (4.45)$$

and the acceptance probability at the s^{th} transition in the r^{th} partition is given by

$$P(\Delta E^{\kappa_s^r}) = \begin{cases} 1 & \text{if (4.33) holds at } h = \kappa_s^r, \\ e^{-\frac{\Delta E^{\kappa_s^r}}{\beta}} & \text{otherwise} \end{cases}.$$

Proof. Consider the tumor's progression from site j to i in k steps. This progression involves $i - j$ transitions, each occurring over a duration $k_1, k_2, \dots, k_{i-j} \geq 1$, such that

$$\sum_{s=1}^{i-j} k_s = k. \quad (4.46)$$

Each transition s corresponds to the tumor advancing by exactly one site, from $s + j - 1$ to $s + j$, requiring k_s steps. This is precisely the single-site invasion scenario addressed by Lemma 4.5.4. Consequently, applying expression (4.34) with initial depth $s + j - 1$ and initial state S^{s-1} , the probability of this one-site transition is given by

$$\mathcal{P}_{s+j|s+j-1}(k_s | S^{s-1}) = \prod_{h=\kappa_{s-1}}^{\kappa_s-1} \left(1 - e^{-\frac{\Delta E^h}{\beta}}\right) P(\Delta E^{\kappa_s}), \quad (4.47)$$

in which ΔE^h is computed as in (4.36) at $h = \kappa_s$, but with the peculiarity that the Hamiltonian variation ΔH^h becomes dependent on the current tumor volume $V_{\ell_1}^{h-1}$, therefore as in (4.45). Here,

$P(\Delta E^{k_s})$ represents the 1-step invasion probability as in (4.34), but with initial configuration σ^0 extended by $s - 1$ positions, thus computed via (4.35) from Lemma 4.5.4.

For a fixed path with pre-defined step durations, namely $(k_1, k_2, \dots, k_{i-j})$, the invasion time probability is given by

$$\mathcal{P}_{i|j}(k | S^0) = \prod_{s=1}^{i-j} \mathcal{P}_{s+j|s+j-1}(k_s | S^{s-1}).$$

To account for all possible paths, we sum over all i -partitions of k . The number of such partitions is given by the binomial coefficient $\binom{k-1}{i-j-1}$. Denoting the r -th partition as $k_1^r, k_2^r, \dots, k_{i-j}^r$, the invasion time probability can be rewritten as in (4.43) substituting the s -th transition in the r -th partition within (4.47) leading to (4.44). $\square$

4.5.2 Invasion Time Probability for Multiple Tumor Cells

In this subsection, we extend the results from Section 4.5.1 to a scenario where the tumor mass consists of an arbitrary number of cells. Specifically, we consider a 1D-ECPM with an initial state as depicted in Figure 4.5, where N tumor cells, labeled $\ell_1, \dots, \ell_N$, occupy the lattice. Each cell has an initial volume V_ℓ^0 for all $\ell \in L \setminus \{\ell_0\}$. The label and type sets are then given by

$$L = \{\ell_0, \ell_1, \dots, \ell_N\}, \quad \Gamma = \{\gamma_{ECM}, \gamma_c\},$$

where ℓ_0 corresponds to empty grid points, $\ell \in L \setminus \{\ell_0\}$ are the tumor cell labels, γ_{ECM} the type corresponding to an empty grid point covered by ECM, i.e. $\tau_{\ell_0} = \gamma_{ECM}$, and γ_c is the tumor cell type such that $\tau_\ell = \gamma_c$ for all $\ell \neq \ell_0$.

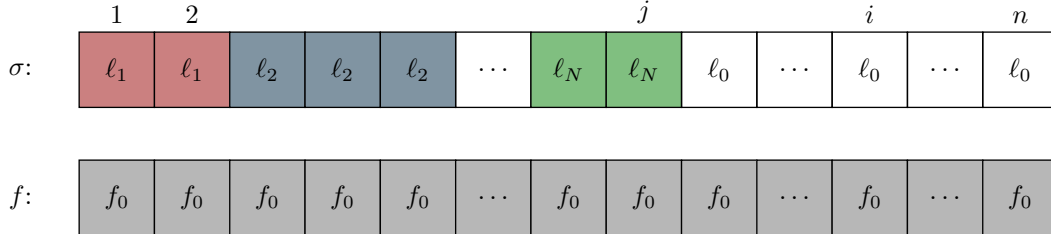


Figure 4.5: Illustration of the initial ECPM state at iteration step $k = 0$ for a 1D grid representing the spatial distribution of multiple tumor cells surrounded by ECM. The top row shows the labeling of grid cells, where $\ell_1, \ell_2, \dots, \ell_N$ represent N tumor cells, and ℓ_0 represents empty lattice sites. The bottom row shows the initial ECM distribution across the grid. Indices are provided above the grid for reference: j represents the deepest lattice point occupied by a cell, $i > j$ is the grid point for which we analyze the invasion time, and n denotes the grid size.

In this multi-cell scenario, the cell with label ℓ_N defines the tumor front position. We now present the main results characterizing the invasion time probability for N tumor cells.

Theorem 4.5.6 (Invasion Time Probability for N Tumor Cells in a 1D-ECPM). *Let $N \geq 1$ tumor cells with labels $\ell_1, \dots, \ell_N$ occupy the 1D lattice. Consider to start at configuration σ^0 with $\varphi(\sigma^0) = j$ and reach configuration σ^k such that $\varphi(\sigma^k) = i$ with $0 < i - j \leq k$. Then, the following results hold:*

(i) **Next-site invasion.** *The probability that the tumor front advances from j to $j + 1$ in exactly $k \geq 1$*

steps is

$$\mathcal{P}_{j+1|j}(k | S^0) = \prod_{h=1}^{k-1} \left(1 - \frac{e^{-\frac{\Delta E^h}{\beta}}}{2N-1} \right) P(\Delta E^k), \quad (4.48)$$

where the total energy variation ΔE^h is computed as

$$\Delta E^h = \underbrace{\lambda [1 + 2(V_{\ell_N}^{h-1} - V_T)]}_{\Delta H^h} + K_{ECM} (f_p^{h-1} - f_q^{h-1}), \quad (4.49)$$

and the acceptance probability is

$$P(\Delta E^k) = \frac{1}{2N-1} \cdot \begin{cases} 1 & \text{if (4.33) holds at } h = k, \\ e^{-\frac{\Delta E^k}{\beta}} & \text{otherwise} \end{cases}. \quad (4.50)$$

In particular, for $k = 1$, the expression simplifies to

$$\mathcal{P}_{j+1|j}(1 | S^0) = \frac{1}{2N-1} P(\Delta E^1), \quad (4.51)$$

where $P(\Delta E^1)$ is defined as in (4.40) with $V_0 = V_{\ell_N}^0$.

(ii) Multi-site invasion. The probability that the tumor front reaches site i from j in exactly k steps is expressed as in (4.43) with each factor is given by

$$\mathcal{P}_{s+j|s+j-1}(k_s | S^{s-1}) = \prod_{h=\kappa_s^r-1}^{\kappa_s^r-1} \left(1 - \frac{e^{-\frac{\Delta E^h}{\beta}}}{2N-1} \right) P(\Delta E^{\kappa_s^r}), \quad (4.52)$$

with κ_s^r satisfying (4.42), and where the total energy variation for $h \in \{\kappa_{s-1}^r, \dots, \kappa_s^r\}$ is

$$\Delta E^h = \underbrace{\lambda [1 + 2(V_{\ell_N}^{h-1} - V_T)]}_{\Delta H^h} + K_{ECM} (f_p^{h-1} - f_q^{h-1}), \quad (4.53)$$

and acceptance probability

$$P(\Delta E^{\kappa_s^r}) = \frac{1}{2N-1} \cdot \begin{cases} 1 & \text{if (4.33) holds at } h = \kappa_s^r, \\ e^{-\frac{\Delta E^{\kappa_s^r}}{\beta}} & \text{otherwise} \end{cases}. \quad (4.54)$$

Proof. The demonstration extends the single-cell framework from Lemmas 4.5.4 and 4.5.5 to incorporate multiple cells, addressing the selection of valid neighbor pairs.

In a 1D lattice with N contiguous tumor cells, the ECPM update requires selecting a pair (p, q) of adjacent sites with distinct labels. Given the restriction to forward copy attempts (tumor-over-ECM), the valid neighbor pairs are: (a) the $N-1$ internal cell-cell interfaces where two distinct tumor labels meet, each contributing two oriented pairs, and (b) the single front-facing pair $(p, q) = (j, j+1)$, where a tumor cell is adjacent to an ECM site. This yields a total of $2(N-1) + 1 = 2N-1$ valid selections, each chosen with equal probability under the uniform sampling $\mathcal{U}(G)$.

Among the $2N-1$ valid pairs, only the pair $(p, q) = (j, j+1)$ can advance the tumor front. Therefore, the probability of selecting this specific pair is $\frac{1}{2N-1}$ at any iteration step. Once this pair

is selected, the acceptance mechanism follows exactly the same energy-based rule as in the single-cell case (refer to Lemma 4.5.4), with the Hamiltonian and ECM contributions computed using the current front cell volume $V_{\ell_N}^{h-1}$.

For $k = 1$, the tumor advances immediately upon selecting the front pair with the corresponding acceptance probability, yielding (4.51). Instead, for $k > 1$, the tumor must remain stationary for $k - 1$ steps before advancing at step k . The tumor front stays at position j at step h either because a non-front pair is selected, which occurs with probability $\frac{2(N-1)}{2N-1}$, or because the front pair $(j, j+1)$ is selected but the move is rejected, which occurs with probability $\frac{1}{2N-1} \left(1 - e^{-\Delta E^h/\beta}\right)$ when $\Delta E^h \geq 0$. Combining these two contributions, the probability of no front advancement at step h is

$$\frac{2(N-1)}{2N-1} + \frac{1}{2N-1} \left(1 - e^{-\frac{\Delta E^h}{\beta}}\right) = 1 - \frac{e^{-\frac{\Delta E^h}{\beta}}}{2N-1}.$$

Multiplying these stationary probabilities over $h = 1, \dots, k-1$ and appending the acceptance probability $P(\Delta E^k)$ (scaled by $\frac{1}{2N-1}$) at step k yields (4.48). Note that this expression reduces to the single-cell result in (4.34) when $N = 1$.

The extension from single-site to multi-site invasion follows the same combinatorial decomposition used in Lemma 4.5.5. The progression from j to i requires $i-j$ successive single-site transitions, each occurring over k_s steps with $\sum_{s=1}^{i-j} k_s = k$. For each fixed partition $(k_1, \dots, k_{i-j})$, the invasion time probability factorizes as a product of single-site probabilities, where each factor is given by the multi-cell single-site expression (4.52). Summing over all $\binom{k-1}{i-j-1}$ ordered partitions of k into $i-j$ positive integers yields (4.43).

The total energy variation ΔE^h in each factor is computed as in (4.45), using the current front cell volume $V_{\ell_N}^{h-1}$, thus leading to (4.53). Moreover, the acceptance probabilities at transition boundaries incorporate the $\frac{1}{2N-1}$ factor consistently with the multi-cell selection mechanism, i.e. the expression in (4.54). $\square$

It is important to note that the number of tumor cells N may vary over time as the tumor evolves. Specifically, as the tumor front expands, it may “overwrite” the label of a neighboring tumor cell, effectively reducing N . However, in practical applications, N is typically large, and its variation over time can often be neglected without significantly affecting the analysis. This assumption simplifies the computation of invasion time probabilities while maintaining the model’s biological relevance.

4.6 Numerical Simulations and Validation

In this section we validate the analytical results derived in Section 4.5 through numerical simulations of the one-dimensional ECPM. The goal is to assess the accuracy of the closed-form expressions for the invasion time probability and the corresponding mean invasion time in both the single-cell and multi-cell scenarios.

4.6.1 Simulation Details

All simulations were implemented in the PYTHON programming language using a custom-developed ECPM simulator to model the coupled tumor-ECM dynamics described in Section 4.3. Each run starts from an initial configuration S^0 , corresponding to the single-cell and multi-cell settings illustrated in Figures 4.4 and 4.5, respectively.

The environmental noise parameter, weighting the probability of energetically unfavorable events, was set to $\beta = 2$. The volume constraint coefficient was fixed at $\lambda = 0.5$, with target volume $V_T = V_0 + 2$. Adhesion energies were chosen as $J_{c,c} = 3$ and $J_{ECM,c} = 6$, reflecting the biological interpretation of tumor-ECM interactions. The enzyme decay rate was defined as $a_{\gamma_c} = \ln(0.1)/D_{\gamma_c}$, the secretion rate as $c_{\gamma_c}^z = 0.4$, and the maximal influence radius as $D_{\gamma_c} = 4$. The ECM degradation rate induced by tumor cells was set to $c_{\gamma_c}^f = 0.02$. The adopted ECPM parameters set is consistent with standard ECPM configurations reported in the literature [86, 93, 94], suitably adapted to the 1D framework for validation purposes.

To estimate the mean invasion time $\bar{T}_{i|j}^{INV}$, we performed 1000 Monte Carlo realizations of Algorithm 5, adopting the stopping criterion $\varphi(\sigma^k) \geq i$. Here, i denotes the target lattice site reached by the tumor front (i.e., $\varphi(\sigma^k) = i$), while j represents its initial position (i.e., $\varphi(\sigma^0) = j$). Each simulation terminates when the front reaches $i > j$, and the corresponding invasion time T_i^{INV} is recorded.

To control computational cost, we imposed a maximum number of iterations $K \in \mathbb{N}$, consistent with the theoretical truncation introduced in equation (4.27). The value of K was selected in light of Proposition 4.5.2, which provides an upper bound on the truncation error. This ensures that the chosen horizon captures most of the probability mass of the invasion-time distribution, yielding accurate estimates of the mean invasion time.

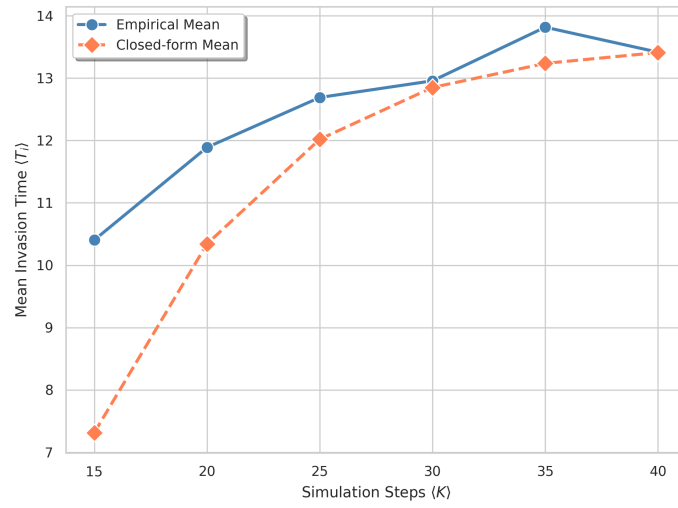
4.6.2 Validation of Mean Invasion Times

We first evaluate the analytical expression of the expected invasion time $\bar{T}_{i|j}^{INV}(S^0)$ against the empirical mean obtained from the Monte Carlo simulations. For a fixed setting of ECPM parameters, we compute the closed-form mean via (4.27) up to truncation horizon K and compare it to the sample average of the recorded invasion times. Figure 4.6 shows the convergence of the theoretical mean to the empirical one as K increases. As it is possible to observe from the numerical simulations, the mean invasion time computed via the closed-form expression approaches from below the empirical mean, thus concluding that the expression in (4.27) constitutes a lower bound for the actual mean invasion time. Convergence in the single-cell scenario (Figure 4.6a) is significantly faster than in the multi-cell case (Figure 4.6b). This difference arises from the analytical form of the invasion time probabilities. In the single-cell scenario, the probability distribution decays exponentially with k , as each step either advances the front with full acceptance probability or maintains the configuration with probability $(1 - e^{-\Delta E^k/\beta})$. In contrast, the multi-cell case introduces an additional factor of $\frac{1}{2N-1}$ in the acceptance probabilities (equations (4.48) and (4.52)), reflecting the reduced likelihood of selecting the front-facing pair $(j, j+1)$ at each iteration. This scaling substantially slows down the probability decay, resulting in longer tails in the invasion time distribution and requiring larger K values to capture the majority of the probability mass.

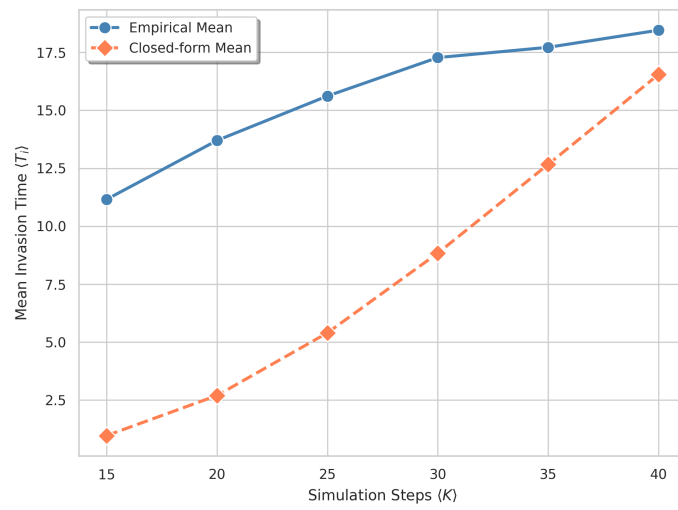
4.6.3 Distribution of Invasion Times

To further assess the model, we analyze the full probability distribution of the invasion time. Figure 4.7 displays the empirical distribution obtained from 1000 realizations of the ECPM in the single-cell scenario, along with the theoretical mean and empirical mean. The two curves show close agreement, confirming that the closed-form probability $\mathcal{P}_{i|j}(k | S^0)$ accurately captures the stochastic variability of the invasion process.

In the multi-cell case, illustrated in Figure 4.8, the invasion time distribution shifts toward larger values as the number of initial tumor cells N increases. This shift is consistent with the reduced



(a) Single-cell scenario



(b) Multi-cell scenario

Figure 4.6: Comparison between theoretical (dashed line) and empirical (solid) mean invasion times as a function of the truncation horizon K for both single-cell and multi-cell scenarios.

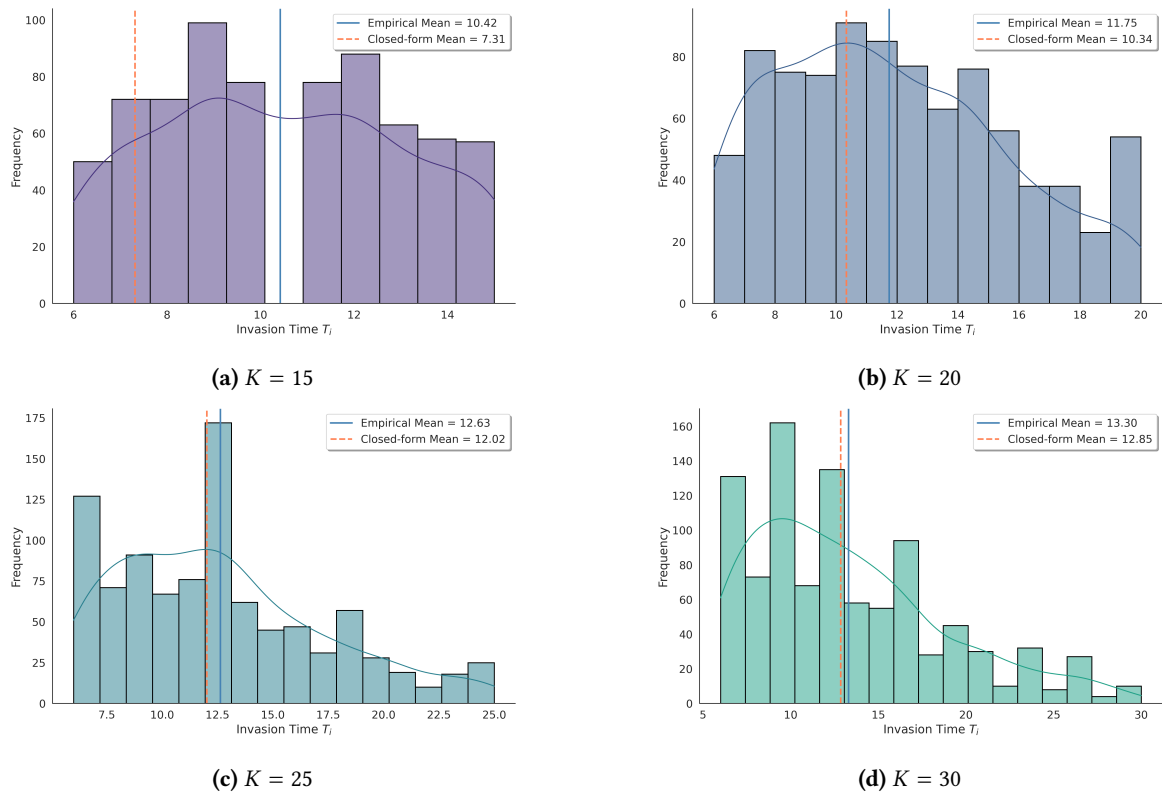


Figure 4.7: Empirical (bars) and theoretical (curve) distributions of invasion times for the single-cell case with varying truncation horizons. Vertical lines indicate empirical (solid) and theoretical (dashed) means.

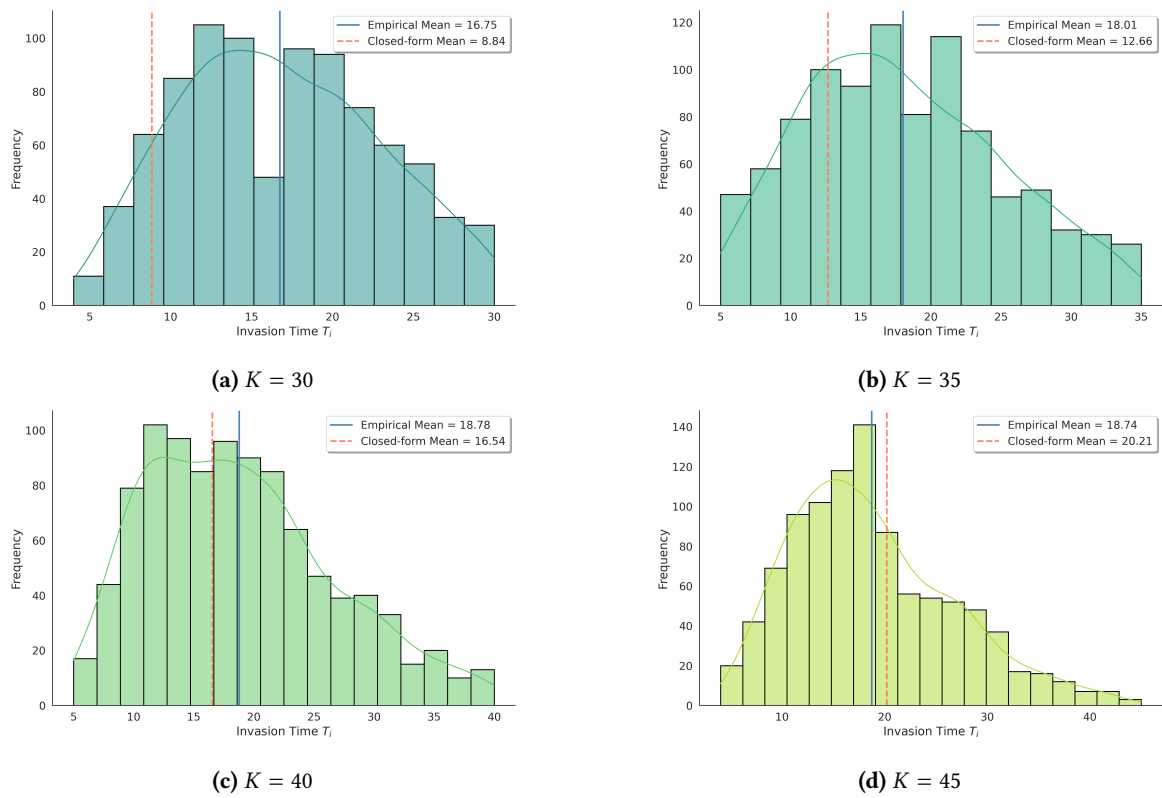


Figure 4.8: Empirical (bars) and theoretical (curve) distributions of invasion times for a multi-cell case with varying truncation horizons. Vertical lines indicate empirical (solid) and theoretical (dashed) means.

probability of selecting the front-facing pair $(p, q) = (j, j+1)$ at each iteration. The analytical results obtained via equation (4.48) successfully capture this behavior. It is worth noting that achieving comparable accuracy between empirical and theoretical invasion times requires significantly higher truncation horizons K in the multi-cell scenario than in the single-cell case. This requirement reflects the slower probability decay discussed earlier, where the additional factor $\frac{1}{2N-1}$ in the acceptance probabilities results in heavier distribution tails. The simulations confirm that the required K scales approximately with N , thus demanding greater computational effort for accurate mean estimation in configurations with multiple tumor cells.

4.7 Summary and Concluding Remarks

This chapter explored tumor invasion from a system-theoretic perspective, specifically focusing on the interaction between cancer cells and the extracellular matrix (ECM). With this purpose, we introduced and analyzed an Extended Cellular Potts Model (ECPM) for modeling tumor invasion in a substrate, incorporating key biological mechanisms such as haptotaxis, ECM degradation, and cell-ECM adhesion.

The main contribution of this work is the reformulation of the ECPM as a closed-loop dynamical system, combining a stochastic process governing tumor mass configuration with a jump-linear system that describes ECM evolution. This reformulation enabled a rigorous analytical approach to haptotaxis-driven invasion, including the characterization of asymptotic behaviors, derivation of transition probabilities between tumor configurations, and the utilization of sparsity in the system's energy variations. In a one-dimensional (1D) context, which captures essential features of radial invasion dynamics in 3D while allowing for analytical tractability, we derived closed-form expressions for invasion time probabilities and established truncation bounds for estimating mean invasion times. Numerical simulations in both single- and multi-cell scenarios validated our theoretical findings.

An important aspect of this work is the biological relevance of the analytical results and their implications for therapy. The reformulated ECPM framework clarifies how model parameters govern macroscopic behaviors. In particular, the closed-loop formulation reveals that invasion speed emerges from the coupled interplay between motility, adhesion, and matrix remodeling, rather than from isolated mechanisms. Consequently, therapeutic strategies targeting single processes—such as protease inhibition—may yield different outcomes depending on how these mechanisms interact, underscoring the importance of accounting for coupled dynamics in tumor invasion. Moreover, the model links Potts parameters to measurable quantities, such as mean invasion time, enabling the estimation of effective motility and degradation rates from experimental invasion assays. This strengthens the potential of ECPMs as a quantitatively interpretable and predictive tool.

In summary, this work demonstrates that stochastic, energy-based Cellular Potts models can be integrated within a rigorous system-theoretic framework without losing biological relevance. By deriving analytical bounds and explicit invasion time distributions, we establish a direct connection between microscopic interaction rules and emergent invasion behaviors, emphasizing how the balance among cell guidance, adhesion, and matrix remodeling fundamentally influences tumor progression. This study bridges tumor-oriented Potts modeling and system theory, providing analytical tools to investigate haptotaxis-driven dynamics and to interpret model parameters in biological contexts. Future developments may extend this framework to higher-dimensional settings and analyzing additional mechanisms such as proliferation, apoptosis, or drug action, ultimately promoting a closer integration between mathematical modeling, systems theory, and experimental oncology.

Model Predictive Control for Cancer Growth Regulation with Multi-Drug Low-Dose Guarantees

The work presented in this chapter was conducted during my research period abroad at the *Department of Engineering Mathematics, University of Bristol, UK*, under the co-supervision of Prof. Lucia Marucci.

5.1 Introduction

Non-small cell lung cancer (NSCLC) remains one of the leading causes of cancer-related mortality worldwide. Despite significant progress in targeted therapies, toxicity and drug resistance (see Section 2.1.4) continue to limit long-term clinical benefits [18, 105]. In particular, EGFR-mutant NSCLC, exemplified by the PC9 cell line, often shows an initial response to tyrosine kinase inhibitors (TKIs) but frequently develops resistance through both genetic and non-genetic mechanisms [106].

Combination and multi-drug low-dose strategies have shown promise in delaying or overcoming such resistance in preclinical studies [107, 108]. However, designing these regimens requires mathematical models and analytical tools that can capture tumor growth dynamics and treatment responses. Control engineering offers a principled framework for addressing these challenges by integrating dynamical modeling, system identification, and optimal control to study treatment scheduling and adaptive therapy [109, 110]. Building on these motivations, together with those introduced in Chapter 2, this research investigates how control-oriented models derived from real experimental data, combined with tailored model predictive control (MPC) schemes [111, 112], can support the design and analysis of novel therapeutic strategies for NSCLC, focusing on the case study of EGFR-mutant PC9 cells. The drug inputs considered in this study belong to the class of small-molecule targeted inhibitors (see Section 2.1.5 for more details), which specifically interfere with key oncogenic pathways including EGFR, RAF, PI3K, and mTOR (similar to the example shown in Figure 2.6).

With an eye toward the microfluidic experimental platforms introduced in Section 2.2.3, we present an *in silico* framework that integrates time-lapse microscopy (see Section 2.2.2) data, dose-response and cell viability assays, and MPC to evaluate diverse multi-drug, low-dose regimens under constraints inspired by *in vitro* experiments in microfluidics (see Figure 5.2).

Related Works. The literature relevant to this work can be grouped into two areas: (i) mathematical modeling of tumor growth and pharmacodynamics, and (ii) control-theoretic approaches to cancer therapy, with particular attention to model predictive control (MPC) in systems biology and tumor dynamics regulation.

Mathematical models of tumor growth range from classical empirical laws—such as exponential, logistic, and Gompertz functions—to more mechanistic or data-driven formulations [113–117]. These models capture key features of uncontrolled proliferation and resource limitation and can be extended to include drug-induced inhibition and resistance mechanisms [106, 118]. Building on these foundations, numerous studies have explored *in silico* optimization of therapy schedules, including adaptive and sequential dosing strategies designed to delay resistance [119]. While these works highlight the potential of mathematical oncology, most remain open-loop and do not exploit feedback control to adapt treatments in real time.

In contrast, control-theoretic approaches provide a systematic means to design and optimize therapeutic strategies. Early studies applied optimal control theory to chemotherapy and targeted therapies, balancing tumor reduction with toxicity constraints [109, 110, 120]. More recent work has adopted feedback and data-driven methods, including iterative optimization of multi-drug and low-dose combinations, to improve efficacy while limiting toxicity [107, 119]. These efforts highlight how combining model-based control with a dynamical understanding of tumor behavior can inform adaptive treatment designs.

Within this context, MPC has emerged as a particularly powerful framework. MPC explicitly handles constraints, manages multiple inputs, and leverages detailed biological models to predict and optimize treatment outcomes [121]. Initial MPC formulations were applied to chemotherapy scheduling and drug-dosing problems using nonlinear models and moving-horizon estimation [122, 123]. More recent studies have extended these ideas to cancer-relevant cellular dynamics, demonstrating how MPC can steer simulated signaling pathways or tumor growth trajectories under treatment while minimizing drug exposure [121, 124, 125]. In parallel, advances in automated microfluidics, real-time imaging, and closed-loop control of living cells [4, 126–128] are creating the experimental infrastructure necessary to translate predictive control concepts from *in silico* simulations to real-time laboratory implementations.

Despite these advances, *in silico* frameworks that combine model identification from time-lapse and dose-response data with Nonlinear MPC (NMPC) are still in their early stages. Existing approaches rarely incorporate realistic experimental constraints, such as actuation or drug-effect delays, nor do they fully address therapy-specific objectives like multi-drug low-dose regimens and resistance mitigation. Moreover, the application of predictive control to EGFR-mutant NSCLC therapy remains largely unexplored. To address these gaps, this work proposes a systematic methodology combining model identification, from experimental data on PC9 cells, with NMPC design under constraints inspired to *in vitro* experiments and with multi-drug low-dose objectives.

Overview and Contributions. This chapter proposes an *in silico* framework for optimal therapy design, tailored to real experimental scenarios. The proposed approach integrates cancer growth modeling, parameter identification from *in vitro* time-lapse cell culture data, and NMPC to test and evaluate multi-drug, low-dose combinations and treatment strategies.

The proposed methodology combines multiple experimental modalities: time-lapse microscopy on PC9 cells provides uncontrolled growth characterization, while dose-response assays quantify drug-induced inhibition through pharmacodynamic models. These complementary datasets enable the identification of control-oriented models that capture both intrinsic tumor dynamics and therapeutic responses. Building on these identified models, we design an NMPC scheme that replicates

observed PC9 growth behaviors under open-loop single-drug treatments while optimizing multi-drug, low-dose regimens. To ensure experimental relevance, the controller explicitly accounts for practical constraints inherent to microfluidic platforms, including discrete drug concentration levels imposed by the actuation systems and measurement uncertainties arising from image acquisition. These design choices enhance the robustness and applicability of the proposed control strategy.

By combining modeling on real data with *in silico* NMPC evaluation, this work contributes to the design and analysis of feedback control strategies in the context of EGFR-mutant NSCLC.

Chapter Outline. The remainder of this chapter is organized as follows. Section 5.2 describes the cybergenetic framework and objectives, and the time-lapse and dose-response *in vitro* experiments performed on PC9 cells, detailing cell-count data acquisition and drug efficacy profiling. Section 5.3 presents the control-oriented modeling framework used to characterize untreated growth dynamics and to incorporate drug inhibition from dose-response data. In Section 5.4, we formulate a generic nonlinear MPC scheme for multi-drug low-dose therapy design, including constraints that reflect experimental conditions. Finally, Section 5.5 presents *in silico* simulation results demonstrating the performance of the proposed NMPC scheme under various experimentally-inspired scenarios.

5.2 Cybergenetics Framework and Experiments

This section presents an integrated framework combining time-lapse microscopy, image analysis, and control-oriented modeling to characterize the growth dynamics of PC9 cells (EGFR-mutant NSCLC) and systematically evaluate multi-drug treatment strategies through *in silico* feedback control experiments.

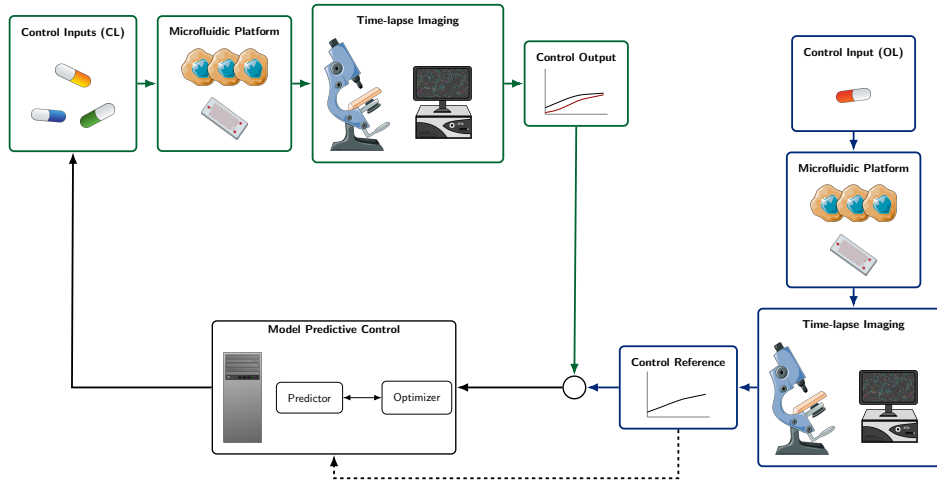


Figure 5.1: *In vitro* feedback control experiment in microfluidics. First, in open loop (OL, blue boxes, right), the growth dynamics of PC9 cells are measured upon treatment with a single drug, to which they are sensitive. Then, these growth dynamics are then used as a control reference in closed-loop experiments (CL, green boxes, left), where the MPC algorithm computes optimal combinations of other drugs for PC9 cells to reproduce the growth behavior observed in OL. The closed-loop experiments are performed in a microfluidic platform that allows real-time imaging and drug administration.

The long-term objective of the research group at the *Department of Engineering Mathematics, University of Bristol* is to establish a fully automated microfluidic feedback control platform. As illustrated in Figure 5.1, this platform would integrate real-time imaging, automated drug delivery,

and closed-loop control algorithms to enable dynamic therapeutic optimization.

In such scenario, PC9 cells are cultured in a microfluidic device that enables real-time imaging and drug delivery. The control loop operates in two phases: (OL) an open-loop characterization phase, where cells are treated with a single drug to which they are highly sensitive (e.g., an EGFR inhibitor), and their growth trajectory is recorded as a reference; (CL) a closed-loop control phase, where the MPC algorithm dynamically computes and administers optimal combinations of alternative drugs to reproduce the reference growth behavior while minimizing drugs exposure.

However, translating this vision into practice requires overcoming substantial experimental challenges. Microfluidic platforms introduce complexities such as device fabrication variability, flow instabilities, and difficulties in maintaining long-term cultures. Moreover, integrating automated microscopy, real-time analysis, and closed-loop control demands considerable technical infrastructure. To avoid resource waste and excessive lab time, a simplified *in silico* scenario provides a pragmatic first step for validating the control framework before proceeding with the full experimental implementation. To address these practical constraints and establish a solid methodological foundation, the present work adopts a stepwise approach that simplifies the experimental setup while preserving the established control-theoretic framework. Specifically, rather than implementing the full microfluidic closed-loop system immediately, we focus on

- **Open-loop growth characterization using *in vitro* monoculture data:** PC9 cells are grown in 2D multi-well plates (see Section 2.2.1) under time-lapse microscopy, enabling data acquisition without the technical overhead of microfluidics. These experiments provide the necessary data for untreated growth dynamics and dose-response characterization.
- ***In silico* closed-loop validation:** Using the identified models, we simulate the NMPC algorithm in a computational environment. This allows systematic evaluation of controller performance, parameter sensitivity, and multi-drug scheduling strategies without the experimental burden of real-time implementation.
- **Reference trajectory generation:** Instead of requiring a preliminary microfluidic open-loop run as in Figure 5.1, we generate reference trajectories by simulating growth under controlled single-drug treatments using the identified models and dose-response data.

Figure 5.2 illustrates this adapted framework.

Our present study focuses on the control of growth dynamics in the EGFR-mutant PC9 cell line, chosen as a representative model system for NSCLC. This corresponds to the first stage of a broader research effort aimed at developing a two-layer cybergenetic platform that will ultimately address

- (i) Growth control using untagged PC9 cells;
- (ii) Gene expression control in tagged PC9 variants for pathway-specific regulation.

While objective (ii) will be addressed in future work, the current contribution concentrates on modeling and *in silico* validation of predictive control strategies for tumor growth regulation, thus on (i).

The overall framework considers three PC9-derived lines differing in their EGFR mutational profile and corresponding drug sensitivities (Fig. 5.3). Specifically, the parental PC9 line (EGFR exon 19 deletion) and two resistant subclones, PC9 SCE4 (C797S exon 20 mutant) and PC9 IRESSA (T790 exon 20 mutant), exhibit progressively reduced response to EGFR inhibitors. In this work, we restrict the analysis to the wild-type PC9 line, which provides a well-characterized and reproducible experimental substrate for developing and validating the control-oriented modeling approach.

The following subsections detail the experimental setup, data acquisition, and analysis methods used to characterize the growth dynamics and drug responses of PC9 cells.

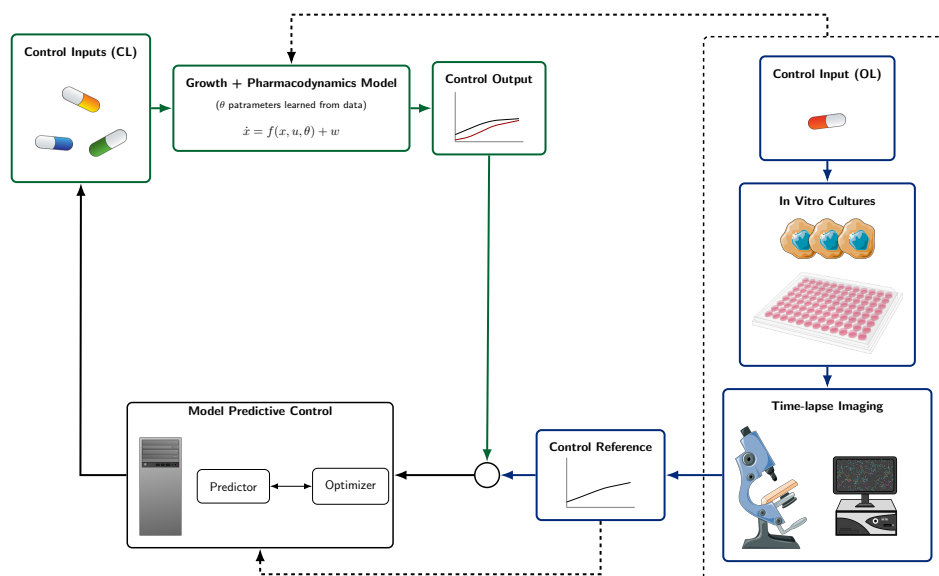


Figure 5.2: *In silico* feedback control experiment. Open-loop experiments (OL, blue boxes, right) are conducted in standard multi-well plates to characterize PC9 growth dynamics and drug-dose responses. Time-lapse microscopy provides cell count trajectories for identifying untreated growth parameters (θ_x), while dose-response assays quantify drug inhibition parameters (θ_u). These identified parameters yield control-oriented models subsequently used in *in silico* NMPC simulations (CL, green boxes, left), where optimal multi-drug schedules are computed and evaluated without requiring microfluidic devices.

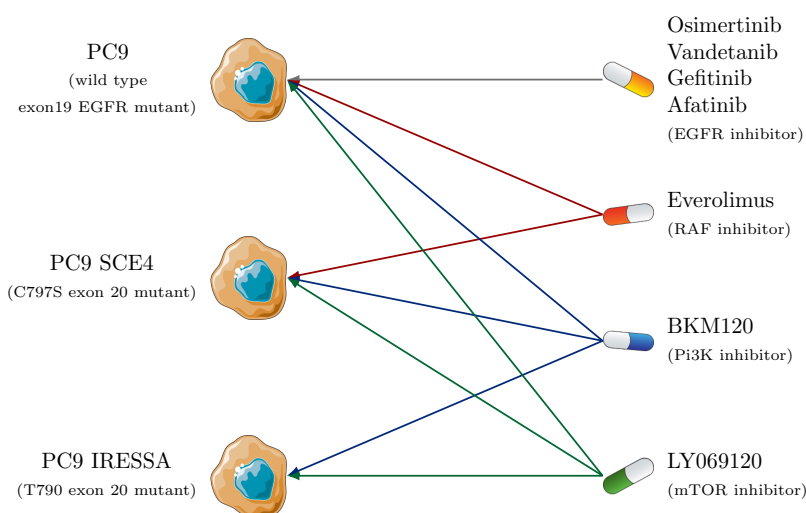


Figure 5.3: Drug sensitivity of PC9 cell line variants to targeted inhibitors. Schematic showing three PC9-derived cell lines: wild-type EGFR exon 19 mutant (PC9), C797S exon 20 mutant (PC9 SCE4), and T790 exon 20 mutant (PC9 IRESSA), and their respective responses to four classes of inhibitors.

5.2.1 Time-Lapse Experiments and Cell Counting.

To characterize the uncontrolled growth behavior of PC9 cells, we performed time-lapse microscopy experiments under standardized *in vitro* conditions. The aim of these experiments is to collect single-population growth trajectories that serve as quantitative data for model identification and validation.

Experimental setup. Cells were cultured in a 24-well plate (4×6 layout), where each well pair represented one biological replicate. Within each row, cells were treated with one of the selected drugs at two concentrations corresponding to IC_{20} and IC_{50} levels, previously determined from dose-response analysis (Section 5.2). The first well pair was reserved for untreated (control) conditions to monitor the intrinsic proliferation dynamics in the absence of pharmacological input. In addition, eight wells (two columns) were dedicated to *starvation* or *normal* culture conditions, serving as additional controls to evaluate metabolic and growth dependencies on nutrient availability. In Figure 5.4, we provide a schematic representation of the well plate layout. Moreover, to minimize measurement errors, the first row and column of the plate were intentionally left empty, as microscope boundary regions typically suffer from focus instabilities and uneven illumination. The experiment was conducted using untagged PC9 cells, since the focus was solely on growth quantification (cell count) rather than protein expression dynamics.

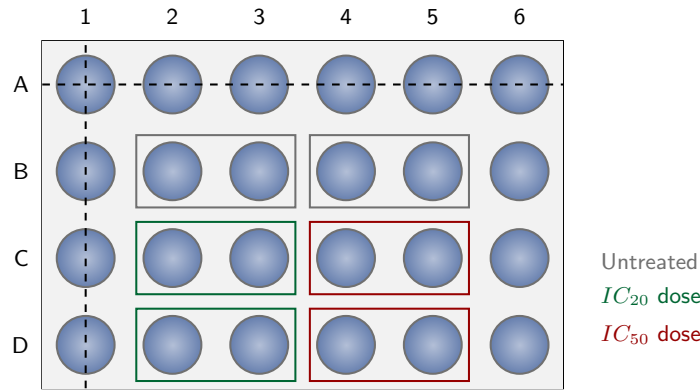


Figure 5.4: Example schematic of a well plate layout for time-lapse microscopy experiments on PC9 cells. Each row represents a different drug, and each well pair corresponds to a biological replicate. The first pair of wells in each row (gray boxes) represents untreated controls cultured under standard conditions, while the remaining pairs represent cells treated with the same drug at low (IC_{20} , green boxes) and high (IC_{50} , red boxes) concentrations. Dashed lines indicate the first row and column, which were left empty due to microscope boundary limitations. The remaining wells (not boxed) can be used for starvation or normal culture conditions as additional controls.

Cell culture and preparation. PC9 cells were maintained in RPMI medium supplemented with 10% fetal bovine serum, which provides the essential growth factors required for cell survival and proliferation. Prior to the experiment, cells were harvested from standard culture dishes and seeded into the wells of the 24-well plate. After seeding, they were allowed to adhere and reach a stable proliferative state over 24-48 h. Drug treatments were then administered in the designated wells, and after a short period (2-3 h), time-lapse acquisition started.

Imaging protocol and analysis. Each well was imaged at 6 non-overlapping fields of view, each of size 512×512 pixels. The total imaged area represents only a fraction of the full well surface, yet

it is sufficient to capture representative population dynamics across spatial replicates. Images were acquired at regular intervals for several days, during which cells remained viable without media replacement, consistent with standard PC9 culture stability over approximately 4–5 days.

The acquired image sequences were analyzed using Cheetah [77], a convolutional neural network (CNN)-based segmentation tool designed to automatically detect and quantify individual cells in each field of view. A key advantage of Cheetah is its ability to perform semantic segmentation, distinguishing between live and dead cells based on morphological features. This capability is essential for reducing measurement errors in live cell count, as it prevents the inclusion of apoptotic or necrotic cells that would otherwise bias growth rate estimates. The resulting cell-count trajectories across acquisition frames for each condition and replicate formed the experimental basis for modeling uncontrolled growth dynamics.

5.2.2 Drug Data Acquisition and Analysis

To characterize the pharmacodynamic response of the EGFR-mutant NSCLC PC9 cell line, we performed dose-response and cell viability experiments under controlled *in vitro* conditions. These datasets provide the basis for developing control-oriented models describing growth inhibition under targeted drug treatments.

Dose-response experiments. Cells were exposed to increasing concentrations of targeted inhibitors representative of different oncogenic signaling pathways. Drug sensitivity assays were performed on parental PC9 cells as well as on two resistant derivatives, PC9 SCE4 and PC9 IRESSA, as schematically summarized in Fig. 5.3. Cell viability was measured after the treatment, and the resulting dose-response curves (see Fig. 5.5) were analyzed using GraphPad Prism software [129]. The data were fitted to a four-parameter logistic (Hill) model [130] to estimate the half-maximal inhibitory concentration (IC_{50}) and IC_{20} values, corresponding respectively to high- and low-dose inhibition levels. These parameters quantify the pharmacological potency of each drug and serve as calibration points for the pharmacodynamics modelling.

It is important also to note that LY306091 was excluded from the simulations due to unreliable dose-response data, as visible in Figure 5.5. The observed variability and lack of a clear sigmoidal profile prevented accurate estimation of the pharmacodynamic parameters (IC_{50} , IC_{20} , and Hill coefficient) required for realistic drug inhibitory effects. Consequently, all multi-drug combinations evaluated in Section 5.5 comprise only those inhibitors depicted in Figure 5.2 aside from LY306091.

Cell viability assays. To assess the impact of different drug concentrations on cell survival, cells were treated with inhibitors at IC_{20} and IC_{50} concentrations previously determined from the dose-response experiments. Cell viability was quantified using the CellTiter-Blue assay (Promega), which measures the metabolic capacity of living cells through resazurin reduction [131, 132]. Data represent the average of six biological replicates, and standard errors are shown (Fig. 5.6). As expected, PC9 wild-type cells exhibited reduced viability under both low- and high-dose conditions, while the resistant variants maintained higher viability across all treatments, confirming their insensitivity to the selected inhibitors.

Collectively, these data-acquisition protocols establish a consistent experimental foundation for modeling the growth dynamics of PC9 cells under drug perturbations.

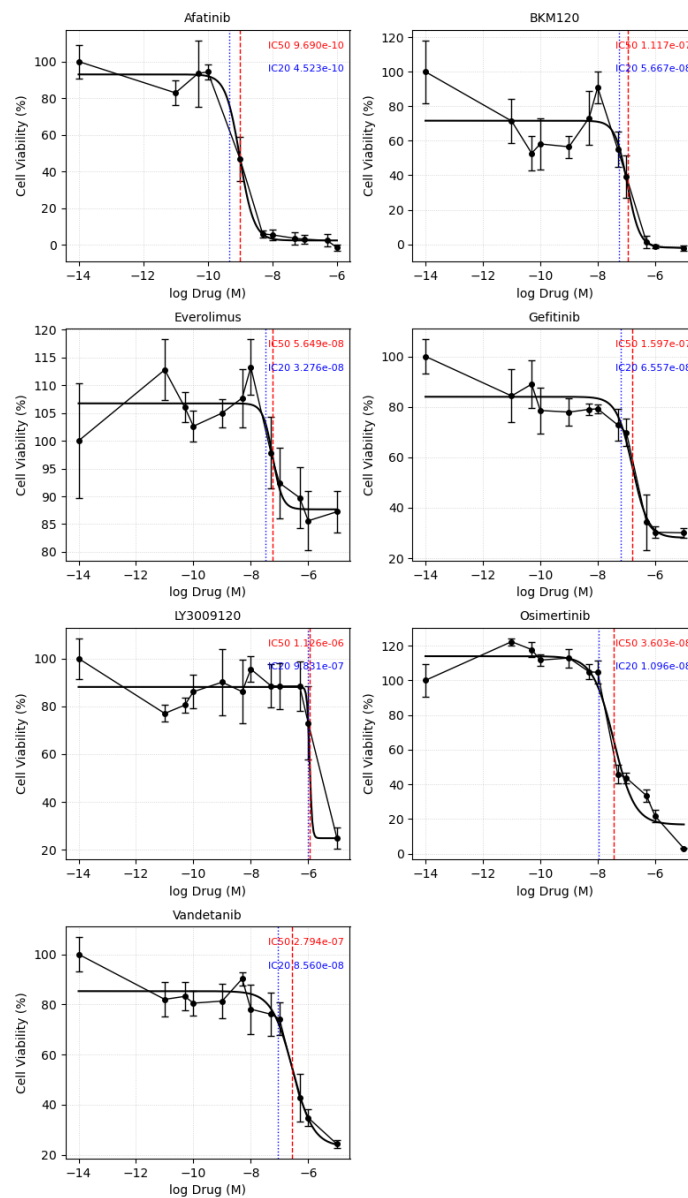


Figure 5.5: Dose-response curves for PC9 cells (wild type) for the inhibitory inputs Afatinib, BKM120, Everolimus, Gefitinib, LY306091, Osimertinib and Vandetanib. Each curve was obtained by nonlinear fitting of experimental data using a four-parameter logistic model. The estimated IC_{50} and IC_{20} values for each drug are indicated by vertical dashed lines, representing the concentrations corresponding to 50% and 20% inhibition of cell viability, respectively.

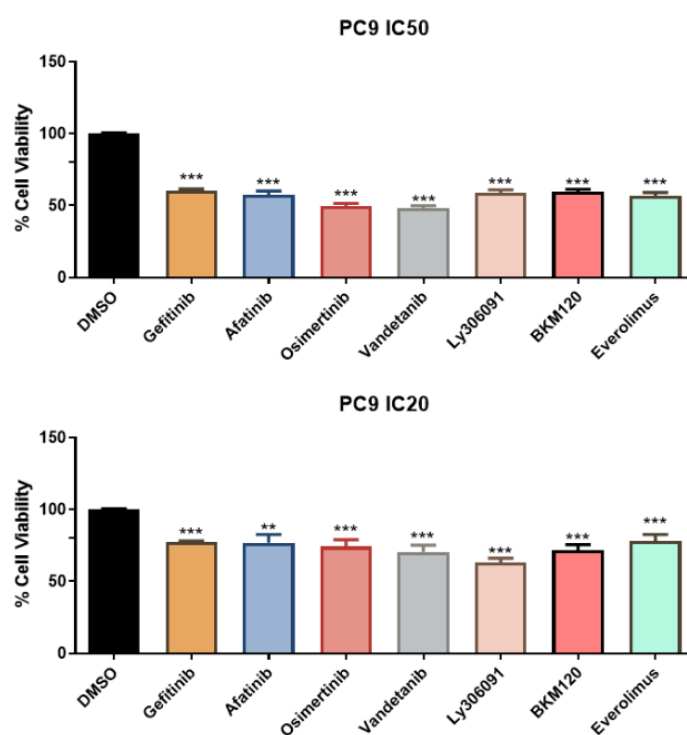


Figure 5.6: Cell viability of PC9 cells after treatment with targeted drugs at concentrations corresponding to IC_{50} (top panel) and IC_{20} (bottom panel), as determined from dose–response curves. Cells were treated with dimethyl sulfoxide (DMSO) as vehicle control, or with Gefitinib, Afatinib, Osimertinib, Vandetanib, LY306091, BKM120, and Everolimus. Viability was measured as the percentage of viable cells compared to DMSO-treated controls. Statistical significance is indicated by asterisks.

5.3 Modeling of Cancer Growth and Pharmacodynamics

In this section, we outline our control-oriented modeling choice for PC9 cells growth and drug inhibition dynamics, computing parameter estimates from cell counts and dose-response data. In particular, we focus exclusively on models that describe the dynamics of tumor cells, excluding those that involve interactions with normal cells. This simplification aligns with our experimental setup, which involves *in vitro* studies conducted solely on NSCLC.

Let $x(t) \in \mathcal{X} \subseteq \mathbb{R}$ the state of the system representing the tumor cell population level (number or concentration of cells) at time $t \in \mathbb{R}_{\geq 0}$. Then, we define the input vector $u(t) \in \mathcal{U} \subseteq \mathbb{R}^p$ as the drug concentrations administered at time t . The parameters governing cancer growth dynamics are denoted by $\theta_x \in \Theta_x \subset \mathbb{R}^{q_x}$, while $\theta_u \in \Theta_u \subseteq \mathbb{R}^{q_u}$ represent the drug inhibition parameters. Based on that, the perturbed growth models considered in our study belong to the following class of nonlinear systems

$$\dot{x} = x(f_x(x, \theta_x) - f_u(u, \theta_u)), \quad (5.1)$$

in which the function $f_x : \mathcal{X} \times \Theta_x \rightarrow \mathbb{R}$ models the tumor growth rate, and $f_u : \mathcal{U} \times \Theta_u \rightarrow \mathbb{R}^p$ models the tumor reduction rate due to the administered drugs. Here, the initial tumor cell population is given by $x_0 := x(0)$.

5.3.1 Untreated Tumor Growth Modeling

Here, we present the characterization of the untreated tumor dynamics, i.e. the growth behavior without applying any drug input ($u(t) = \mathbf{0}_p$, $\forall t \geq 0$). Specifically, inspired by the work in [109], we selected two autonomous nonlinear systems to model the evolution dynamics observed during time-lapse experiments.

Let us first define the growth parameters vector as $\theta_x := [\alpha, K]^\top \in \mathbb{R}_{\geq 0}^2$ with α and K representing the growth rate and the tumor carrying capacity, respectively. The first selected model is the *Gompertz* growth system, given by

$$\dot{x} = x\alpha \ln\left(\frac{K}{x}\right), \quad (5.2)$$

with state domain $\mathcal{X} = \mathbb{R}_{>0}$ and scalar field f_x locally Lipschitz (since $f_x \in C^1$). Moreover, the system in (5.2) is characterized by a single hyperbolic equilibrium $x_{eq} = K$ being a sink. The forward solution of the Gompertz growth system, is given by

$$x(t, x_0, \theta_x) = K \exp\left(\ln\left(\frac{x_0}{K}\right) e^{-\alpha t}\right), \quad (5.3)$$

that exists $\forall t \geq 0$.

In this study we also consider the *Logistic* growth (or Verhulst-Pearl) system, defined as

$$\dot{x} = x\alpha \left(1 - \frac{x}{K}\right), \quad (5.4)$$

with state domain $\mathcal{X} = \mathbb{R}_{\geq 0}$ and scalar field f_x locally Lipschitz (since $f_x \in C^1$). The dynamics in (5.4) are characterized by two equilibria $x_{eq,1} = K$ and $x_{eq,2} = 0$ being a sink and a source, respectively. Its forward solution, that exists $\forall t \geq 0$, is given by

$$x(t, x_0, \theta_x) = \frac{x_0 K e^{\alpha t}}{K + x_0(e^{\alpha t} - 1)}. \quad (5.5)$$

Note that both the Gompertz and logistic growth models can describe sigmoidal tumor growth patterns, but they differ in their intrinsic characteristics. The logistic model exhibits a steeper growth phase around the inflection point compared to the Gompertz model, which displays a more gradual S-shaped curve with earlier deceleration. The choice between these models depends on the characteristics of the experimental data: the Gompertz model often provides better fits for tumor dynamics characterized by early growth saturation, while the logistic model may be more appropriate when proliferation remains near-exponential until closer to the carrying capacity. In practice, both formulations will be evaluated during parameter identification, and the final selection will be guided by goodness-of-fit metrics and the specific growth characteristics observed in the PC9 cell line data.

5.3.2 Pharmacodynamics

We now detail the modeling choice regarding the pharmacodynamics function f_u previously introduced in (5.1). Here, we consider the set of drugs to act as antagonists, thus inhibiting the tumor population growth.

First, we define $u_i(t) \in \mathbb{R}_{\geq 0}$ as the concentration of drug $i \in \{1, \dots, p\}$ given at time t . Then, we call $I_{\max,i} \in [0, 1]$ the maximum efficacy of the administered drug i , instead we define $I_{0,i} \in [0, 1]$ as the baseline value of its effect (namely the natural death rate), and $IC_{50,i} \in \mathbb{R}_{>0}$ being the drug concentration u_i that produces 50% of the maximum inhibitory effect, i.e., $u_i : f_u(u_i, \theta_u) = I_{\max,i}/2$. In our specific scenario, $IC_{50,i}$ can be seen as the drug concentration which halves the PC9 population. Finally, we can introduce the so-called $I_{\max}$ drug model [133, 134], which has been widely used in pharmacodynamics and tumor growth inhibition modeling [135, 136], and it is given by

$$f_u(u, \theta_u) = \sum_{i=1}^p \frac{I_{\max,i} \cdot u_i^{h_i}}{IC_{50,i}^{h_i} + u_i^{h_i}}, \quad (5.6)$$

in which $h_i \in \mathbb{R}_{\geq 0}$ denotes the Hill coefficient of the dose-inhibitor curve relative to the i^{th} drug. Here, the drug effect parameters θ_u are represented by the stack of the single drug parameters $I_{\max,i}$, $IC_{50,i}$ and h_i for all $i \in \{1, \dots, p\}$. The drug-inhibitor parameters for each of the seven available drugs are selected from dose-response curves (see Figure 5.5) which are obtained through the procedures described in Section 5.2. The input domain for equation (5.6) is given by

$$\mathcal{U} := \prod_{i=1}^p [0, IC_{100,i}],$$

i.e. the drug doses are assumed to be always positive and upper-bounded by their maximum inhibitor efficacy concentration IC_{100} . The additive form in (5.6) reflects a non-interacting (additive) model, i.e. each drug contributes independently to growth inhibition and the total effect is the sum of single-drug Hill functions. This choice is consistent with our experimental setup (Figure 5.3), in which the control inputs are designed to allow at most one inhibitor per protein target, for example, one among the four EGFR inhibitors (Osimertinib, Vandetanib, Gefitinib or Afatinib) together with one RAF, one PI3K and one mTOR inhibitor. Under this design, each compound perturbs a distinct signalling node, making additivity the natural baseline assumption [137, 138]. Importantly, synergy seen in 2D *in vitro* PC9 cultures may not translate *in vivo* due to pharmacokinetics and microenvironmental factors [139]. Hence, the additive model is both theoretically grounded and aligned with the structure of our drug-selection strategy.

It is important to note that the present pharmacodynamics model does not explicitly account for acquired drug resistance. Since the purpose of this work is to propose an *in silico* framework

capable of reproducing *in vitro* microfluidic growth control experiments (refer to the cybergenetics framework depicted in Figure 5.1), and given that the typical duration of such experiments is approximately 4–5 days, resistance mechanisms that emerge over weeks or months cannot be directly observed or characterized within this experimental time window. Consequently, the model assumes constant drug sensitivity throughout the simulation period, which is appropriate for the short-term dynamics under consideration.

5.3.3 Identification of Growth Parameters

Now we describe the method used to identify the parameters of the growth models just introduced. Here, the goal is to fit the growth rate and carrying capacity parameters on experimental data derived from microscopy videos, in particular, the *in vitro* experiments regarding the PC9-HIGH cell line. As outlined in Section 5.2, each experiment consists in a video recording of multiple and fixed microscope positions within the same well. After the experiment is concluded, cell counts are extracted at each time frame combining image processing techniques and the Cheetah segmentation tool [77]. Here, we want to highlight that biological replicates refer to experiments performed in different wells under equivalent conditions and protocol. These replicates capture biological variability and help ensure that the estimated growth parameters are robust and representative.

Let $\mathcal{T} \subset \mathbb{R}_{\geq 0}$ denote the set of time points corresponding to the image frames at which cell counts are performed. Then, we define $N_E \in \mathbb{N}$ as the total number of experiments, each associated with a different well, and $M \in \mathbb{N}$ as the number of microscope positions recorded within a single well. For each time point $t \in \mathcal{T}$, microscope position $m \in \{1, \dots, M\}$ and well $e \in \{1, \dots, N_E\}$, let $d_{e,m}(t) \in \mathbb{N}$ represent the number of PC9 cells detected. We also define the total cell count within experiment e at time t as $d_e(t) := \sum_{m=1}^M d_{e,m}(t)$. Then, we formalize the dataset with the following set

$$\mathcal{D} = \bigcup_{e=1}^{N_E} \mathcal{D}_e, \text{ with } \mathcal{D}_e = \{(t, d_e(t)), \forall t \in \mathcal{T}\},$$

in which the subsets $\mathcal{D}_e$ contain the data sequences regarding the e^{th} biological replicate of a single-well experiment. In Figure 5.7 we show these data sequences for a well experiment.

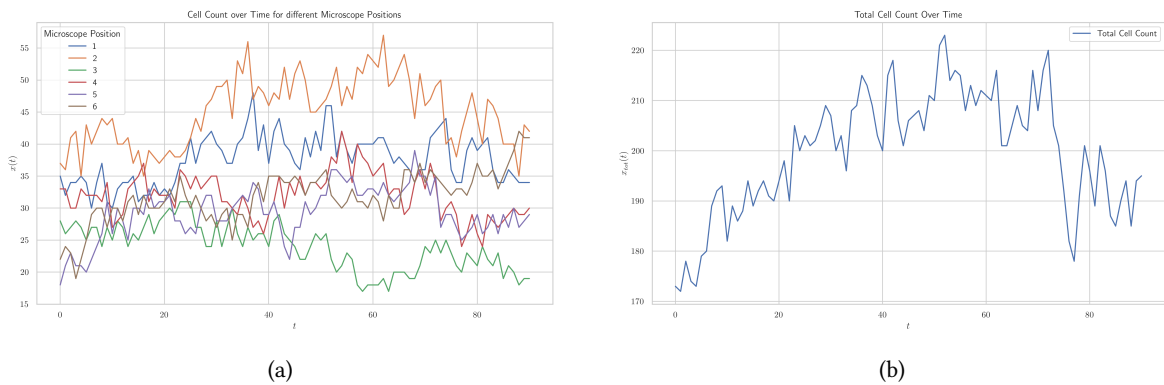


Figure 5.7: An example of single well recorded cell counts of PC9-HIGH cells across time frames. (a) Time measurements at different microscope positions. (b) Total well cell count.

In this single-well experiment, a decline in the cell population is observed starting around time point 70. Such behavior is inconsistent with the expected monotonic increase in cell count under

untreated conditions and is likely the result of experimental artifacts that produce biologically implausible measurements. Time-lapse microscopy experiments are inherently prone to measurement disturbances arising from multiple sources, such as cells moving out of the recorded field of view, transient acquisition defects in the microscope, or segmentation inaccuracies after image processing. These effects can introduce anomalous points or biased counts that do not reflect true biological dynamics. The presence of such disturbances motivates the use of robust parameter identification techniques capable of attenuating the influence of outliers.

We proceed by describing the identification method used for fitting the growth parameters θ_x on the dataset $\mathcal{D}$. Here, we consider the forward solutions in (5.3) and (5.5), with unknown Gaussian noise $w(t) \sim \mathcal{N}(0, \sigma_w^2)$, as our dynamical models. The parameters that we want to fit, i.e. α and K , on biological replicates are obtained by solving the following nonlinear least squares (LS) problem

$$\min_{\theta_x} \frac{1}{2} \sum_{i=1}^{N_E} \sum_{t \in \mathcal{T}} \left(\underbrace{d_e(t) - \psi(t, d_e(0), \theta_x)}_{=: r_e} \right)^2, \quad (5.7)$$

where $\psi(\cdot)$ corresponds to equation (5.3) if considering the Gompertz growth, otherwise (5.5) for the logistic model. Here, $r_e \in \mathbb{R}$ denotes the residuals referred to experiment e . Moreover, to ensure robustness to biased LS solution (caused by data outliers), we introduce a class of sublinear functions $\rho : \mathbb{R} \rightarrow \mathbb{R}$ to be embedded into problem (5.7). Therefore, the standard nonlinear LS problem transforms into

$$\min_{\theta_x} \frac{1}{2} \sum_{e=1}^{N_E} \sum_{t \in \mathcal{T}} \rho(r_e^2). \quad (5.8)$$

This problem can be reduced to standard nonlinear LS by modifying a vector of residuals and Jacobian matrix at each iteration, such that the computed gradient and Hessian approximation match the ones of the objective function. This procedure is detailed in [140]. The choices for the sublinear function are

(i) Huber loss:

$$\rho(z) = \begin{cases} z, & z \leq 1 \\ \sqrt{z} - 1, & z > 1 \end{cases};$$

(ii) Soft ℓ_1 loss: $\rho(z) = 2(\sqrt{z+1} + 1)$;

(iii) Cauchy loss: $\rho(z) = \ln(1+z)$;

(iv) Arctan loss: $\rho(z) = \arctan(z)$.

Here, the options (i) and (ii) are relatively mild and give approximately absolute value loss for large residuals. Instead, the sublinear functions (iii) and (iv) give significant attenuation for outliers due to their strong sublinearity. To generalize, we introduce the scaling parameter C and evaluate the loss as

$$\hat{\rho}(r^2) := C^2 \rho\left(\left(\frac{r}{C}\right)^2\right)$$

Note that if residuals r are small we have that

$$\hat{\rho}(r^2) \approx \rho(r^2) \approx r^2$$

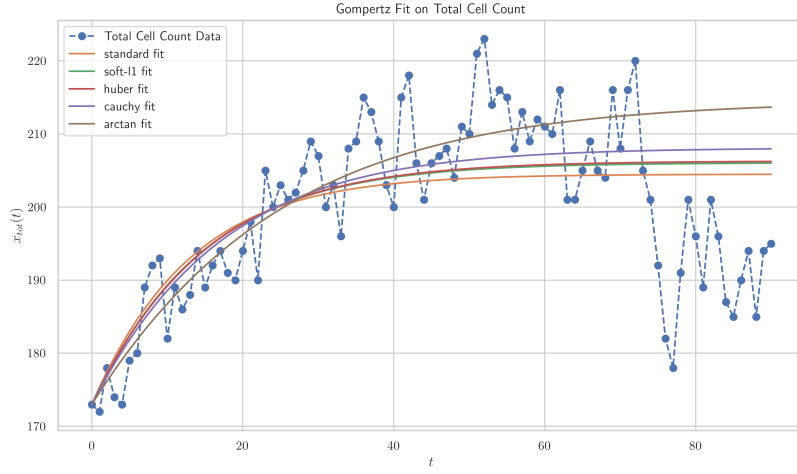


Figure 5.8: Nonlinear regression results with different sublinear functions considering Gompertz model as growth dynamics.

for any $\rho(z)$ defined above.

Figure 5.8 presents the fitting results using the Gompertz growth model. The data used for model fitting come from a single microscope time-lapse experiment on PC9 cells (see Figure 5.7). As previously discussed, the decline in the cell population observed in the last acquisitions is likely due to experimental disturbances rather than true biological dynamics. Under these conditions, strongly sublinear penalties such as (iii) and (iv) are preferable, as they reduce the impact of data outliers and increase the robustness of the model fitting.

It is worth noting that the parameter identification results presented here are based on a limited dataset consisting of a single time-lapse experiment. While this preliminary analysis demonstrates the feasibility of the proposed modeling and identification approach, a more comprehensive characterization of PC9 growth dynamics requires additional experimental replicates under controlled conditions. At present, we are awaiting further laboratory data from the *Department of Biomedical Sciences, University of Bristol*, which will enable robust validation of the identified model parameters across multiple biological replicates and experimental conditions. Once a sufficient dataset becomes available, a systematic model selection procedure will be conducted to determine whether the Gompertz or logistic growth model provides a better description of PC9 population dynamics, and the identified parameters will be refined accordingly.

5.4 Nonlinear MPC Design for Multi-Drug Low-Dose Therapy

In this section, we address the formulation and practical implementation of a nonlinear model predictive control (NMPC) scheme tailored for multi-drug low-dose therapy design on NSCLC. We also discuss how the control algorithm accounts for realistic experimental conditions, including measurement errors and the finite set of available drug dose concentrations. In this context, we want to specify that any practical implementation of NMPC will rely on a sampled-data setting, where state measurements (i.e. microscope acquisitions) are available only at discrete instants and the control input is updated accordingly. We denote by $\delta \in \mathbb{R}_{>0}$ the sampling period of the control loop. At each sampling instant $t = k\delta$, the controller computes a discrete-time input $u^k \in \mathbb{R}^p$, which represents the drug-dose levels applied during the interval $[k\delta, (k+1)\delta)$. Under the standard *zero-order hold*

assumption, this input is kept constant between two consecutive sampling instants, resulting in the continuous-time signal

$$u(t) = u^k, \quad \forall t \in [k\delta, (k+1)\delta).$$

Similarly, since state measurements are only available at discrete sampling instants, we denote the measured state at time step k as $x^k \in \mathbb{R}$, obtained from the continuous-time state trajectory $x(t)$ at sampling time $t = k\delta$. Formally,

$$x^k := x(k\delta).$$

Our control algorithm thus operates on the sampled state sequence $\{x^k\}_{k \in \mathbb{N}}$ rather than the full continuous-time trajectory. Therefore, to obtain an optimization problem suitable for a future real-time *in vitro* implementation in microfluidics, it will be presented a discrete-time formulation of the NMPC problem. Let us start by considering a discretization of the perturbed growth dynamics, given by

$$x^{k+1} = f(x^k, u^k, \theta) + w^k, \quad (5.9)$$

in which the function $f : \mathcal{X} \times \mathcal{U} \times \Theta \rightarrow \mathbb{R}$ is obtained discretizing the system in (5.1) with Runge Kutta methods [141], and the discrete random variable $w \sim \mathcal{N}(0, \sigma_w^2)$ with zero-mean and variance σ_w^2 accounts for measurement errors and/or model uncertainties. Here, we consider all the model parameters stacked into the vector $\theta := [\theta_x^\top, \theta_u^\top]^\top \in \mathbb{R}^q$. Then, given a priori known reference trajectory $x_r \in \mathbb{R}$, we would like to design a controller such that the system state converges to the reference, i.e.

$$\lim_{k \rightarrow \infty} \|x^k - x_r^k\| = 0,$$

and that the state and input constraints, represented by the sets $\mathcal{X}_c$ and $\mathcal{U}_c$, are satisfied for all discrete-time steps k , namely

$$\begin{aligned} x^k &\in \mathcal{X}_c \subseteq \mathcal{X}, \quad \forall k \geq 0 \\ u^k &\in \mathcal{U}_c \subseteq \mathcal{U}, \quad \forall k \geq 0. \end{aligned}$$

Now, by introducing the tracking error

$$e^k := x^k - x_r^k,$$

the reference tracking problem can be reformulated as a set-point stabilization problem of the following error dynamics

$$e^{k+1} = \tilde{f}(k, e^k, u^k, \theta) := f(e^k + x_r^k, u^k, \theta) - x_r^{k+1}.$$

Moreover, let us define $s \geq k$ as the fictitious discrete step and $N \in \mathbb{N}$ as the prediction horizon. We denote the stage and terminal costs with the functions $\ell : \mathbb{N} \times \mathbb{R}^n \times \mathbb{R}^p \rightarrow \mathbb{R}$ and $F : \mathbb{N} \times \mathbb{R}^n \rightarrow \mathbb{R}$, respectively. We also introduce $\mathcal{E}_{k+N} \subset \mathbb{R}$ as the error terminal region, representing a stability constraint within the NMPC. Instead, the variable $\bar{e}^{s|k}$ denotes the fictitious error at predicted instant s , used as a decision variable in the optimization problem at (real) discrete-time k , while $\bar{u}^{s|k}$ represents the corresponding fictitious input. Based on these definitions, the NMPC problem that we want to

solve iteratively is

$$\min_{\bar{e}^{\cdot|k}, \bar{u}^{\cdot|k}} \underbrace{\sum_{s=k}^{k+N-1} \ell(s, \bar{e}^{s|k}, \bar{u}^{s|k}) + F(k+N, \bar{e}^{k+N|k})}_{=: J(e^k, \bar{e}^{\cdot|k}, \bar{u}^{\cdot|k})} \quad (5.10a)$$

$$\text{subj.to:} \quad (5.10b)$$

$$\bar{e}^{s+1|k} = \tilde{f}(k, \bar{e}^{s|k}, \bar{u}^{s|k}, \theta), \quad s = k, \dots, k+N-1 \quad (5.10c)$$

$$\bar{e}^{k|k} = e^k \quad (5.10d)$$

$$\bar{u}^{s|k} \in \mathcal{U}_c, \quad s = k, \dots, k+N-1 \quad (5.10e)$$

$$\bar{e}^{s|k} + x_r^k \in \mathcal{X}_c, \quad s = k, \dots, k+N-1 \quad (5.10f)$$

$$\bar{e}^{k+N|k} \in \mathcal{E}_{k+N}. \quad (5.10g)$$

From this general formulation, various practical designs can be derived and tailored to the multi-drug low-dose therapy framework. The MPC algorithm proceeds iteratively according to the following steps:

- (i) At discrete-time k , measure the current state x^k , solve the open-loop optimal control problem (5.10) and get the optimal input trajectory $\bar{u}^*(s | k) \quad \forall s = k, \dots, k+N$;
- (ii) Apply $u_{MPC}^k := \bar{u}^*(k | k)$;
- (iii) Replace k with $k+1$ and repeat.

We now explicit our NMPC practical design choices tailored for the multi-drug low-dose therapy design framework introduced in Section 5.2. Specifically, the cost function $J(\cdot)$ within problem (5.10) is defined as

$$J(e^k, \bar{e}^{\cdot|k}, \bar{u}^{\cdot|k}) = \sum_{s=k}^{k+N-1} \left\| \bar{e}^{s|k} \right\|_Q^2 + \left\| \bar{u}^{s|k} \right\|_R^2 + \left\| \bar{e}^{k+N|k} \right\|_P^2,$$

in which $Q \in \mathbb{R}$, $R \in \mathbb{R}^{p \times p}$ and $P \in \mathbb{R}$ represent the tracking error, input drugs and terminal cost weights, respectively. To guarantee feasibility and recursive feasibility of the NMPC problem, we assume that $Q \geq 0$, $R > 0$ and $P > 0$, as standard in the NMPC literature [111]. Moreover, the terminal region $\mathcal{E}_{k+N}$ is typically selected to enforce a level-set constraint on the terminal cost function, i.e.,

$$\left\| \bar{e}^{k+N|k} \right\|_P^2 \leq \alpha, \quad (5.11)$$

where $\alpha \in \mathbb{R}_{>0}$ is a design parameter that determines the size of the terminal set. This constraint restricts the predicted error at the end of the horizon to remain within a neighborhood of the origin, thereby promoting closed-loop stability and recursive feasibility [112]. In the simulation section, we will investigate the NMPC performance both with and without enforcing the terminal constraint (5.10g), comparing the resulting trade-offs between tracking accuracy, drugs usage and optimal control problem feasibility.

Furthermore, to better reflect the constraints of real microfluidic experiments, we constrained the drug inputs to a finite set of admissible concentrations. In fact, in practice only a limited number of dose levels can be delivered at each control instant. Accordingly, in our *in silico* NMPC design we restrict each drug to a small discrete set of possible concentrations. Specifically, for the i^{th} drug we

allow either no administration, a low dose corresponding to $IC_{20,i}$, or a medium dose corresponding to $IC_{50,i}$. Formally, the admissible input set is

$$\mathcal{U}_c := \prod_{i=1}^p \{0, IC_{20,i}, IC_{50,i}\}. \quad (5.12)$$

This formulation captures the finite actuation available in microfluidic devices and allows the NMPC to generate control actions directly compatible with future *in vitro* implementation.

5.5 In Silico Experiments

This section presents *in silico* control experiments assessing the performance and robustness of the proposed NMPC strategy under various design choices and experimental conditions. The feedback control framework emulates *in vitro* scenarios on microfluidic devices (see Figure 5.2), enabling systematic evaluation of the controller's ability to regulate tumor growth while minimizing drug exposure under discrete actuation constraints typical of such platforms.

All simulations were implemented in PYTHON using the CasADi optimization framework [142]. The NMPC problem was formulated as a mixed-integer nonlinear program (MINLP) and solved using the BONMIN solver [143], which efficiently handles the discrete-valued drug inputs arising from the admissible concentration set in (5.12). A sampling period of $\delta = 15$ min and prediction horizon of $N = 30$ were adopted to reflect typical experimental time scales in microfluidic culture systems. Gaussian noise with zero mean and standard deviation σ_w was added to measured states to emulate experimental uncertainty, enabling subsequent robustness analysis across varying noise levels.

Each drug was restricted to three discrete concentration levels—no dose, low dose (IC_{20}), and medium dose (IC_{50})—consistent with practical microfluidic capabilities. Considering the drug sensitivity profile of wild-type PC9 cells (Figure 5.3), we simulated all feasible two- and three-drug combinations, ensuring at most one inhibitor per protein target. This constraint aligns with the additive pharmacodynamic model (5.6) and ensures experimental implementability in microfluidic platforms.

The identified PC9 growth model from Section 5.3 constitutes the predictive model used within the MPC scheme. Reference trajectories x_r were generated by simulating the identified growth model under open-loop conditions with a constant single-drug input at IC_{20} concentration, i.e., by solving (5.1) with $u_i(t) = IC_{20,i}$ for the selected drug i and $u_j(t) = 0$ for all $j \neq i$. This synthetic approach was adopted due to current operational constraints in the biomedical laboratories at the University of Bristol. Specifically, ongoing experimental delays for the open-loop, single-drug experiments prevented the acquisition of real reference trajectories. Therefore, the closed-loop control strategy was evaluated using synthetically generated reference data as an interim solution. Regarding the MPC cost design, the weighting matrices Q , P , and R were tuned to promote low-dose therapies while maintaining tracking performance. A key aspect of the NMPC formulation concerns the inclusion or omission of strong terminal constraints, such as (5.10g), which significantly influences both the control behavior and the resulting trade-off between tracking accuracy and drug usage, as will be demonstrated in the subsequent analysis.

To quantitatively evaluate the performance of the proposed control strategy, we define two indices inspired by the work in [121], namely the normalized *Error Index* (EI) and *Dose Index* (DI),

respectively given by

$$\text{EI} := \frac{1}{x_0} \sum_{k=0}^{T_k} \|e^k\|, \quad (5.13)$$

$$\text{DI} := \sum_{i=0}^p \underbrace{\frac{1}{\text{IC}_{50,i}} \sum_{k=0}^{T_k} u_i^k}_{=: \text{DI}_i}, \quad (5.14)$$

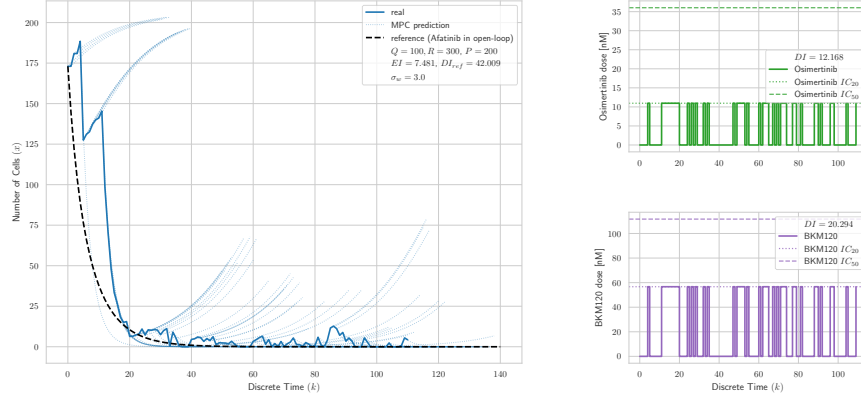
which respectively quantify the tracking accuracy and the overall drug usage over the total discrete-time instants $T_k \in \mathbb{N}$, so that $T_k \delta$ represents the total simulation length. A lower EI indicates tighter regulation of the tumor population around the target reference, while a lower DI reflects a more conservative, low-dose control strategy. These indices are reported for each *in silico* simulation result to facilitate comparison across different NMPC configurations and treatment scenarios.

5.5.1 NMPC without Strong Terminal Ingredients

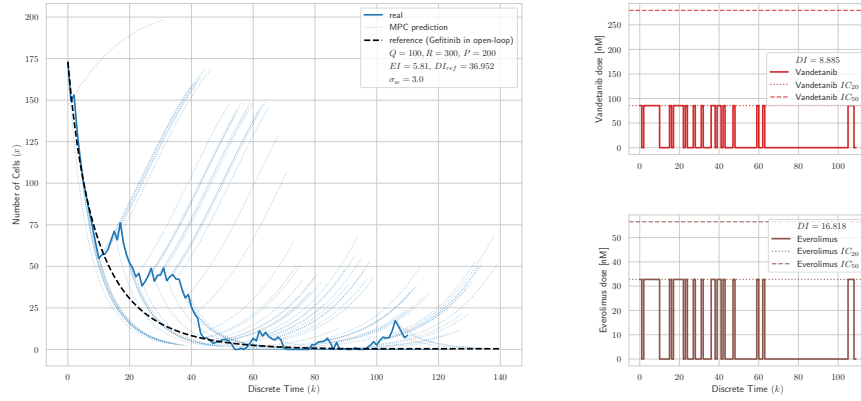
We first consider the NMPC formulation including the terminal cost term (given by the choice of P), but without an explicit terminal constraint (5.10g). Figures 5.9 and 5.10 report representative results for some two- and three-drug combinations. The NMPC successfully drives the tumor cell population toward the open-loop single-drug reference, achieving stable closed-loop regulation while using lower cumulative drug amounts compared to open-loop single-drug treatments. Specifically, the multi-drug low-dose NMPC solution achieves a lower DI value than the corresponding open-loop schedule, confirming its effectiveness in exploiting synergistic drug effects while maintaining a reduced pharmacological burden.

Additionally, in order to present a comprehensive overview across all possible two- and three-drug NMPC configurations, we computed DI and EI values for each feedback-controlled combination and corresponding open-loop single-drug reference. Figure 5.11 summarizes the DI values obtained for all two- and three-drug combinations, providing a systematic comparison of the cumulative drug exposure required by each NMPC configuration to achieve the desired growth regulation. This overview enables identification of which drug combinations achieve effective control at lower total doses, thereby offering a measure of potential toxicity reduction. Notably, certain combinations exhibit substantially lower DI values, suggesting more favorable therapeutic windows where effective tumor control can be maintained with reduced pharmacological burden. The corresponding EI overview in Figure 5.12 illustrates the tracking performance for each drug combination and reference trajectory. This heatmap reveals that while some combinations achieve tighter regulation (lower EI), others require higher drug concentrations to maintain comparable tracking accuracy. Combinations including Everolimus tend to yield higher EIs, reflecting a slightly slower dynamic response associated with mTOR pathway inhibition. Taken together, these results enable a systematic assessment of the efficacy-toxicity trade-off, i.e. drug combinations achieving both low DI and low EI represent optimal candidates for multi-drug low-dose therapy, balancing effective growth control with minimal cumulative exposure.

An interesting property emerging from these results is the so-called *Turnpike phenomenon*, a characteristic behavior of finite-horizon optimal control problems without strong terminal constraints [144, 145]. When the prediction horizon N is sufficiently large, optimal predicted trajectories exhibit a characteristic pattern: they quickly approach and remain near a steady-state manifold (the “turnpike”), deviating only at the initial and terminal portions of the horizon. In our *in silico* experiments, this manifests as follows: during each NMPC iteration, the predicted state trajectory initially

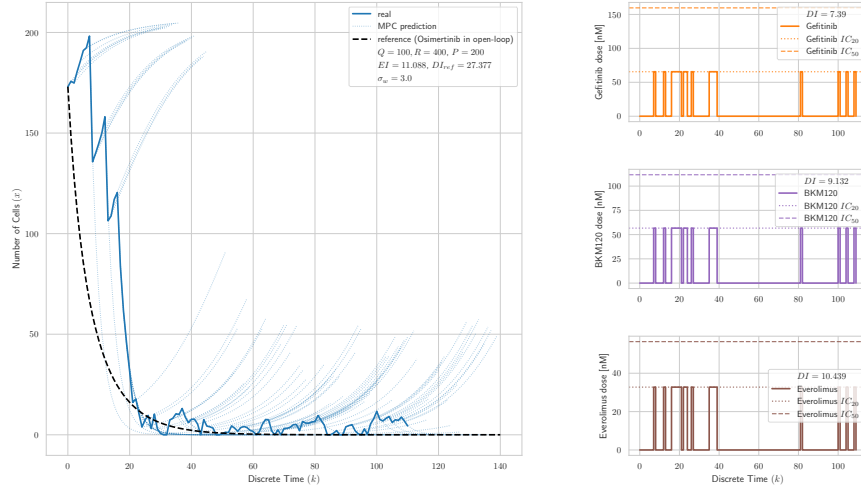


(a) Feedback control using a two-drug combination (Osimertinib and BKM120). The reference trajectory was generated under constant Afatinib treatment at IC_{20} concentration.

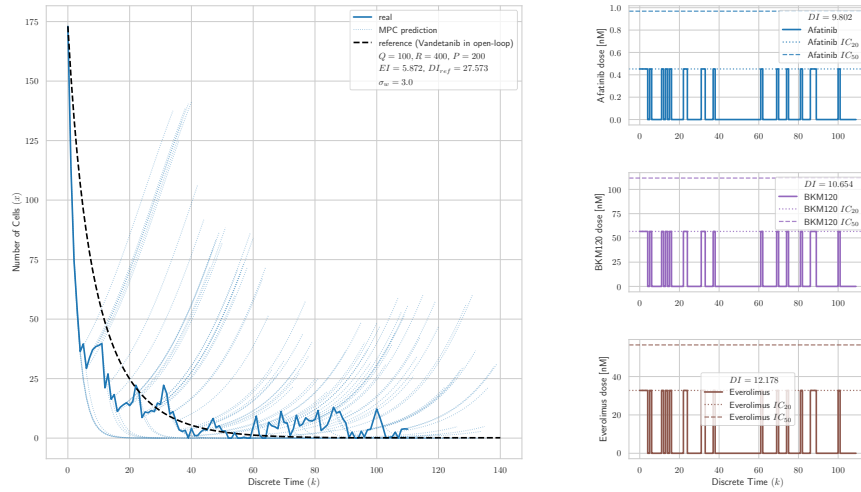


(b) Feedback control using a two-drug combination (Vandetanib and Everolimus). The reference trajectory was generated under constant Gefitinib treatment at IC_{20} concentration.

Figure 5.9: *In silico* NMPC reference tracking for two-drug treatment experiments. The closed-loop MPC achieves a combined dose index (DI) below the open-loop single-drug reference value DI_{ref} , demonstrating reduced cumulative drug exposure through predictive feedback control. Each panel reports the error index (EI) quantifying tracking accuracy, the NMPC cost weights (Q , R , P), and the standard deviation σ_w of measurement noise used in the simulation.

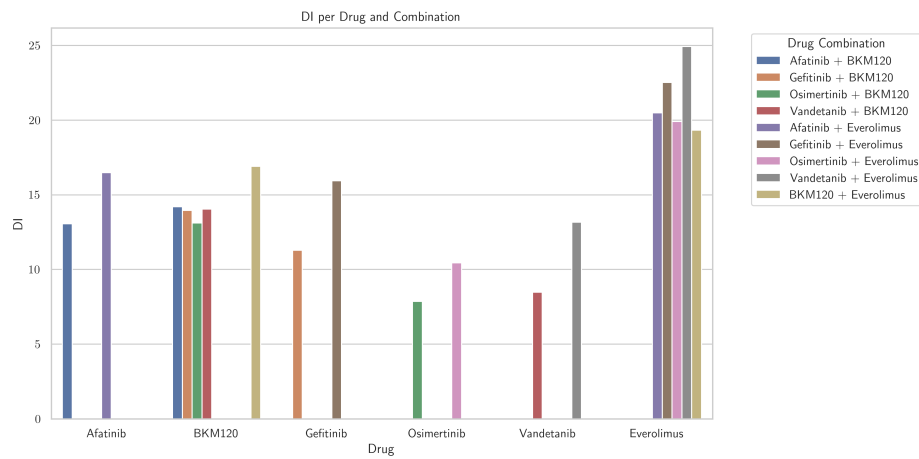


(a) Feedback control using a three-drug combination (Gefitinib, BKM120 and Everolimus). The reference trajectory was generated under constant Osimertinib treatment at IC_{20} concentration.

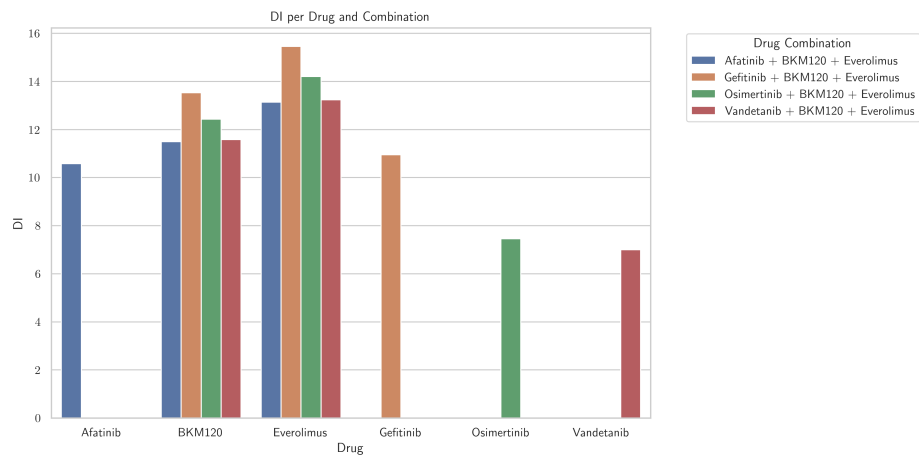


(b) Feedback control using a two-drug combination (Afatinib, BKM120 and Everolimus). The reference trajectory was generated under constant Vandetanib treatment at IC_{20} concentration.

Figure 5.10: *In silico* NMPC reference tracking for three-drug treatment experiments. Since the controller can deliver three drugs simultaneously, the combined dose index (DI) may exceed the open-loop single-drug reference value DI_{ref} (as visible in panel (b)), even though each individual drug index $DI_i < DI_{ref}$. This shows that while multi-drug regimens can reduce the burden of any single inhibitory input, the cumulative pharmacological exposure may increase when multiple therapeutic pathways are targeted simultaneously. Each panel reports the error index (EI) quantifying tracking accuracy, the NMPC cost weights (Q , R , P), and the standard deviation σ_w of measurement noise used in the simulation.

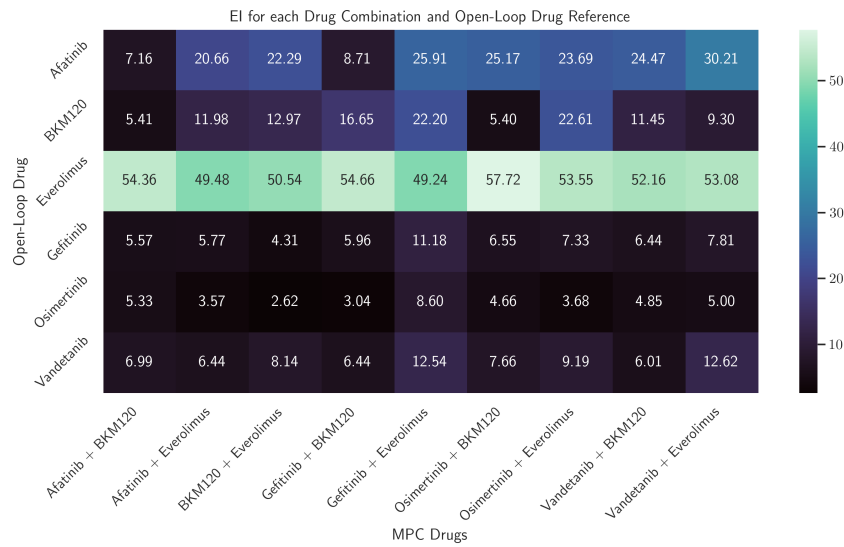


(a) Two-drug combinations

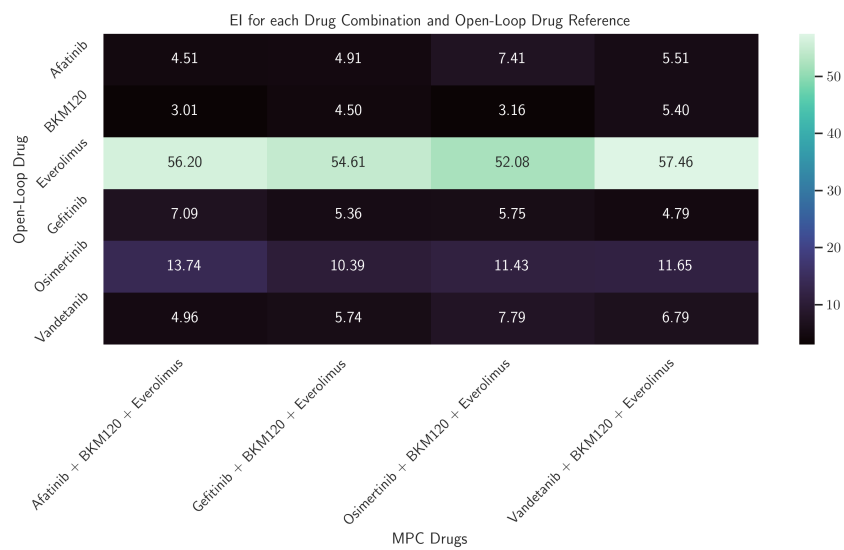


(b) Three-drug combinations

Figure 5.11: DI values for all two-drug (a) and three-drug (b) NMPC combinations and corresponding open-loop single-drug references. Lower DI values indicate reduced cumulative drug exposure under closed-loop control. While the combined DI can exceed the single-drug reference in some three-drug cases—due to the concurrent administration of multiple agents—the per-drug contributions remain below the corresponding open-loop values.



(a) Two-drug combinations



(b) Three-drug combinations

Figure 5.12: EI values quantifying NMPC tracking accuracy for all two-drug (a) and three-drug (b) combinations. Each cell represents the mean EI obtained when using the column combination as the MPC drug set and the row drug as the open-loop reference.

tracks the reference closely, but progressively diverges toward an economically favorable steady state as it approaches the horizon endpoint, prioritizing minimal drug usage over strict terminal tracking accuracy. Crucially, the receding horizon principle ensures that only the first control action is implemented before re-optimization with updated measurements, maintaining effective closed-loop tracking despite apparent long-horizon prediction divergence. When the reference trajectory aligns with the economically optimal (i.e., minimal drug usage) steady state, the predicted trajectory naturally converges toward it, reflecting consistency between tracking and economic objectives.

5.5.2 Effect of Strong Terminal Constraints

We next analyze the case where the NMPC includes the terminal constraint (5.10g), thus enforcing the fictitious state at the end of the prediction horizon, i.e. $\bar{x}^{k+N|k}$, to lie within a neighborhood of the reference, as expressed by condition (5.11). The introduction of this hard constraint mitigates the turnpike-like behavior, as visible in Figure 5.13. While this results in faster convergence and reduced transient oscillations, it also increases the overall drug usage as reflected by a higher DI. It is important to note that excessively tight terminal sets (i.e., small values of α in (5.11)) can render the optimal control problem infeasible, particularly when starting from initial conditions far from the reference or when the available drug concentrations are insufficient to drive the system into the terminal region within the prediction horizon. Therefore, the choice of α must balance the desire for rapid convergence against the need to maintain recursive feasibility throughout the closed-loop operation. These results, illustrated by the example in Figure 5.13, highlight the fundamental trade-

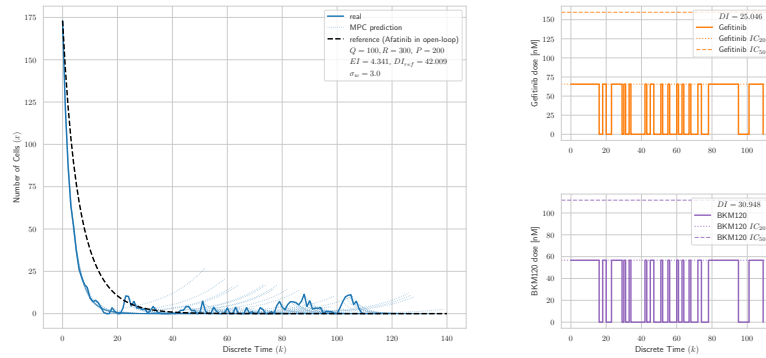


Figure 5.13: Two-drug combination NMPC with strong terminal constraints. Enforcing the terminal region $\mathcal{E}_{k+N}$ improves tracking accuracy (lower EI) compared to Figure 5.9, but requires substantially higher drug exposure: both the total DI and individual drug indices DI_i exceed the open-loop reference DI_{ref} . This illustrates the NMPC trade-off—tighter terminal constraints enhance regulation at the expense of increased pharmacological burden.

off between therapeutic aggressiveness and pharmacological economy. This suggests that the choice of terminal ingredients should depend on the specific clinical context and the desired treatment horizon.

5.5.3 Robustness to Uncertainties

To assess the robustness of the NMPC scheme to measurement noise and/or model uncertainties, we conducted simulations under varying noise levels. In particular, we evaluated the controller perfor-

mance by introducing measurement noise with varying standard deviations σ_w into the discrete-time system dynamics (5.9). In Figure 5.14 we report an example that illustrates the regulator performance when subjected to increasing standard deviations σ_w of the Gaussian measurement noise. As expected, higher noise levels lead to slight increases in both tracking error (EI) and cumulative drug usage (DI), reflecting the controller's compensatory actions in response to uncertain state estimates. Nevertheless, the NMPC maintains stable regulation across a wide range of realistic noise conditions, demonstrating satisfactory robustness to the measurement disturbances typically encountered in time-lapse microscopy experiments.

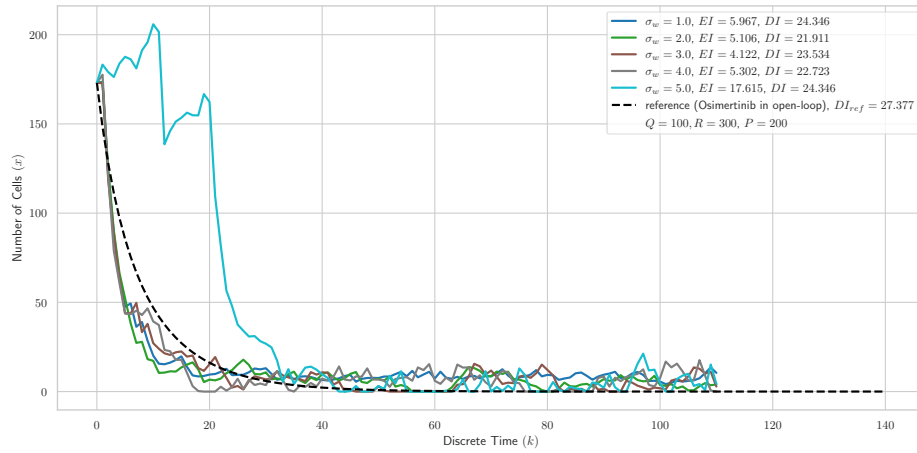


Figure 5.14: NMPC performance under varying measurement noise levels for a representative two-drug combination example (analogous to those presented in Section 5.5.1). The standard deviation σ_w quantifies the magnitude of uncertainty.

5.6 Summary and Concluding Remarks

This chapter presented an *in silico* framework for designing and evaluating multi-drug low-dose therapeutic strategies for EGFR-mutant non-small cell lung cancer (NSCLC), specifically targeting the PC9 cell line. The work integrates control-oriented modeling, experimental data-driven parameter identification, and nonlinear model predictive control (NMPC) to systematically explore feedback-based treatment optimization under constraints reflective of microfluidic experimental platforms.

The proposed methodology combines time-lapse microscopy data for tumor growth characterization with dose-response assays for pharmacodynamic modeling, enabling the identification of control-oriented models that capture both intrinsic tumor dynamics and drug-induced inhibition. Using the Gompertz and logistic growth models as candidate representations, we demonstrated a robust parameter identification procedure capable of handling measurement outliers typical of time-lapse imaging experiments. The identified models were subsequently employed within an NMPC framework designed to regulate tumor growth while minimizing cumulative drug exposure through optimal multi-drug scheduling.

A key contribution of this work lies in the systematic evaluation of NMPC performance across all feasible two- and three-drug combinations, considering realistic experimental constraints such as discrete drug concentration levels and measurement/model uncertainties. The simulation results demonstrate that multi-drug low-dose NMPC can achieve effective tumor growth regulation—matching

or improving upon single-drug open-loop reference trajectories—while significantly reducing total pharmacological burden in many configurations. This finding supports the hypothesis that multi-drug predictive feedback control can exploit synergistic drug interactions to maintain therapeutic efficacy with lower individual drug doses, thus reducing potential toxicity.

The analysis of NMPC formulations with and without strong terminal constraints revealed important trade-offs between tracking accuracy and drug economy. Configurations without hard terminal constraints exhibited the so-called turnpike phenomenon, where predicted trajectories prioritize long-term economic objectives (minimal drug usage) over strict terminal tracking, yet maintain effective closed-loop regulation through the receding horizon mechanism. Conversely, enforcing terminal constraints improved transient tracking performance but at the cost of increased cumulative drug exposure, highlighting the need for context-dependent tuning based on clinical objectives and treatment horizons.

Robustness analysis under varying measurement noise levels confirmed that the proposed NMPC scheme maintains stable regulation across realistic uncertainty ranges, with graceful degradation in tracking accuracy and modest increases in drug usage as noise intensity grows. This robustness property is essential for practical implementation, where microscopy-based state measurements are inherently subject to segmentation errors, focus variations, and cell movement artifacts.

While the present work establishes a solid methodological foundation and demonstrates promising results, several aspects merit further development to strengthen the framework. First, the parameter identification relied on data from a single time-lapse experiment; validation across multiple biological replicates will enhance confidence in the identified model parameters once additional laboratory data become available. Second, the pharmacodynamic model adopts an additive interaction assumption, which provides a tractable and experimentally justified baseline; future extensions incorporating synergistic effects and resistance dynamics could further refine predictive accuracy for longer treatment horizons. Third, reference trajectories were generated synthetically due to operational constraints in the collaborating laboratories; upcoming open-loop microfluidic experiments will provide real reference data to further validate the control strategy. Finally, while the discrete input constraints faithfully represent microfluidic actuation capabilities, incorporating actuation delays and pharmacokinetic effects in future iterations will enhance realism for real-time closed-loop implementations.

In conclusion, this chapter establishes a principled control-theoretic foundation for multi-drug low-dose therapy design in NSCLC, demonstrating through comprehensive *in silico* experiments that predictive feedback control can effectively balance tumor growth regulation with pharmacological economy. The systematic evaluation across drug combinations, terminal constraint configurations, and noise conditions provides valuable insights into the design trade-offs inherent to cancer treatment optimization and lays the groundwork for future experimental validation in microfluidic cybergenetic platforms.

Conclusions and Future Directions

This thesis has addressed the interconnected problems of modeling, prediction, and control of tumor dynamics, with the broader goal of advancing a systems-theoretic understanding of cancer as a complex, multiscale dynamical system. Across the presented case studies—ranging from multi-cell tracking in time-lapse microscopy, to analytical modeling of invasion, and optimal control of tumor growth—the common thread has been the formulation of mathematically grounded yet experimentally validated methods to analyze and regulate tumor behavior.

The first contribution, presented in Chapter 3, introduced the *Heterogeneous Open Multi-Cell EKF-based Tracking* (HEOM-EKF) framework for modeling and tracking heterogeneous cell populations in time-lapse microscopy. By representing cell populations as open multi-agent dynamical systems that capture heterogeneity, division, and migration, and by coupling this model with recursive state estimation via an Extended Kalman Filter, the method enables simultaneous prediction, tracking, and inference of biologically interpretable parameters. This approach advances beyond heuristic or purely data-driven tracking pipelines by providing a biological informed estimator that links single-cell dynamics to population-level observables.

The second contribution, detailed in Chapter 4, presents a system-theoretic framework for analyzing Extended Cellular Potts Models (ECPMs) in the context of haptotaxis-driven tumor invasion. By reformulating the model as a closed-loop system that couples a stochastic process for tumor configurations with a jump-linear system for extracellular matrix (ECM) degradation, we established an analytical foundation for studying cell-ECM feedback in Potts-based models. We characterized the asymptotic behaviors of both subsystems, derived transition probabilities with associated bounds, and leveraged the sparsity of the energy variation terms. Closed-form expressions for invasion time probabilities in one-dimensional settings were derived, emphasizing their dependence on haptotactic coupling, ECM degradation rates, and initial conditions. Furthermore, we established truncation bounds for estimating mean invasion times. Numerical simulations validated the theoretical predictions in both single- and multi-cell scenarios. This work bridges simulation-oriented ECPM studies with a system-theoretical approach to tumor invasion dynamics.

The third contribution, discussed in Chapter 5, proposes an *in silico* framework for optimal therapy design in EGFR-mutant Non-Small Cell Lung Cancer. The approach integrates cancer growth modeling, parameter identification from *in vitro* data, and Nonlinear Model Predictive Control (NMPC) to evaluate multi-drug, low-dose treatment strategies. Specifically, control-oriented models capturing tumor growth and pharmacodynamics are identified by combining time-lapse microscopy

of PC9 cell cultures, dose–response assays, and cell viability tests. Building on these models, the NMPC scheme optimizes multi-drug, low-dose regimens while accounting for microfluidic platform constraints, such as finite input doses and measurement uncertainties. Validations through *in silico* simulations compare various control strategies across two- and three-drug combinations, advancing the understanding of model-based feedback control for cancer growth regulation in realistic experimental settings.

Taken together, these contributions establish a unified framework that integrates data acquisition from time-lapse microscopy, mathematical modeling of multiscale tumor dynamics, parameter identification from experimental assays, theoretical analysis of feedback-driven invasion, and model-based optimal control under realistic constraints. By applying systems and control principles to quantify, predict, and regulate tumor behavior across temporal and spatial scales, this thesis contributes to the emerging field of *Cybergenetics*, bridging control-theoretic methodologies with experimental oncology. While clinical implementation remains a long-term objective, the presented methodologies contribute to the advancement of principled, model-driven approaches in cancer research and optimal therapy design.

Future Directions. The research presented in this thesis opens several avenues for future investigation. Concerning the cell tracking framework in Chapter 3, future work will focus on integrating the HEOM-EKF estimator with deep learning-based segmentation and classification methods to enable fully automated real-time tracking during live microscopy experiments. Additionally, testing the framework on other co-culture datasets and comparing it with existing state-of-the-art tracking algorithms will be important to validate its robustness, scalability, and applicability across different experimental settings.

Regarding the analytical modeling of tumor invasion in Chapter 4, several theoretical and computational extensions are planned. First, extending the invasion time analysis from 1D settings to 2D or even 3D lattices would provide a more realistic representation of *in vivo* tumor progression. Second, incorporating cell proliferation and apoptosis into the ECPM analysis would yield a more complete description of coupled invasion–growth dynamics under ECM interaction. Third, developing systematic parameter estimation methods that map ECPM parameters to quantitative microscopy measurements would bridge the gap between model calibration and experimental data, thereby improving both predictive accuracy and biological interpretability.

For the feedback control study in Chapter 5, several practical extensions are identified. First, incorporating explicit actuation dynamics and delayed drug responses would increase the realism of the *in silico* feedback control scheme. Second, with access to richer open-loop experimental datasets, the model can be validated under diverse dosing schedules and perturbations to assess robustness. In the longer term, implementing closed-loop NMPC schemes directly within microfluidic platforms could enable experimental demonstrations of real-time tumor growth regulation, providing a critical step toward practical *in vitro* growth and gene regulation of NSCLC.

Ringraziamenti/Acknowledgments

Finalmente siamo arrivati alla parte che preferisco: quella dedicata alle persone. Perché, per quanto un dottorato possa sembrare un percorso solitario, è in realtà un viaggio condiviso.

Prima di tutto desidero ringraziare il mio supervisore, Prof. Giuseppe Notarstefano, per avermi dato la possibilità di vivere questa esperienza nel mondo della ricerca, per la pazienza e la disponibilità costanti, per aver creato un gruppo di ricerca straordinario — soprattutto dal punto di vista umano — ma anche per aver creduto nei nostri lavori più di quanto, a volte, ci abbia creduto io stesso.

Un ringraziamento va a tutto il gruppo dell'Istituto Ortopedico Rizzoli, in particolare ai miei co-supervisori, Prof. Nicola Baldini e Sofia Avnet, per avermi dato, insieme a Giuseppe, l'opportunità di fare ricerca in un campo affascinante quanto complesso come quello della biologia.

Grazie al CASY, il miglior laboratorio di ricerca di Bologna, dove tra risate, serpenti volanti (questa è per pochi) e voli in bicicletta, ho vissuto momenti indimenticabili. Un grazie va a tutti i suoi membri: Alice, Riccardo, Alessandro, Guido, Biagio, gli Andrea, i Lorenzi, i Marco, i Davide, Ivano, Carlo e Matteo (sì, in questi anni ne sono passati parecchi!). Un ringraziamento speciale va a Simone, il mio compagno di dottorato e di stanza, con cui ho iniziato e concluso questo percorso. Con lui ho condiviso davvero tanto: posso dire di aver trovato un grande amico, oltre che un grandissimo matematico con cui confrontarmi ogni giorno e con disponibilità.

A big thanks also goes to the people I met in Bristol during my research period abroad. Thanks to Prof. Lucia Marucci for hosting me in her lab and for her support throughout my stay, and to all the members of the Marucci Lab, in particular Antonella, Sara, Vaishnu and Manju. Thank you for your patience and for guiding me through the fascinating world of cybergenetics.

A huge thanks also goes to my Bristol roommates and friends, Iza and Sharik. You have been incredibly kind and welcoming, and I am truly grateful for the time we spent together. I hope we will stay in touch and meet again soon. Thanks as well to my landlord Maciej, a.k.a. "Magic", who was always kind and helpful during my stay — and who even welcomed me into his crew for some unforgettable nights. Knowle West the best!

Un grazie va ovviamente anche alla mia famiglia, che non mi ha mai imposto scelte, ma mi ha sempre fatto sentire libero di seguire le mie passioni, anche quando non erano ancora così chiare.

Infine, il grazie più grande va a Laura: per avermi dato la forza di affrontare questo percorso, per avermi sostenuto nei momenti più difficili, per avermi fatto sorridere quando ne avevo più bisogno e per essere sempre stata al mio fianco in questi anni.

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