A Mathematical Model for Epithelial Cellular Networks
Development, Analysis, and Integration

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A Mathematical Model for Epithelial Cellular Networks
Development, Analysis, and Integration

MASTER OF SCIENCE THESIS

For the degree of Master of Science in Systems and Control at Delft University of Technology

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Abstract

Epithelia are sheets of connected cells that are essential to morphogenesis (the development of organisms and organs) across the animal kingdom. In this MSc thesis a modeling framework is developed to increase understanding of the mechanical properties and the dynamic behavior of epithelia during the early stages of embryo morphogenesis. Experimental observations suggest that the dynamic behavior of many single-layered epithelial tissues during morphogenesis has a strong analogy with the dynamics of a specific type of mechanical systems, namely networks consisting of masses connected through spring-damper elements. A Mass-Spring-Damper (MSD) framework is derived from existing biological knowledge, and employed to build a three-dimensional model, which is tailored to the study of the formation of grooves in the single-layered epithelium of the *Drosophila melanogaster* embryo. The model is adapted to the actual cellular geometry, by incorporating data obtained by 4D confocal microscopy experiments of the embryo. The insights generated by the model help to formulate and test biological hypotheses, and to design new experiments. In doing so, this thesis contributes to the understanding of physical and mechanical properties of the epithelium of the *Drosophila melanogaster* embryo during morphogenesis, and enables the study of how the dynamics of these properties relate to local genomic processes. The derived modeling framework can be tailored to study morphogenesis of the general class of single-layered epithelial cellular networks.
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Chapter 1

Introduction

This MSc thesis reports on a graduation project in Systems Biology. Mathematical modeling and engineering principles are applied in the study of a problem in developmental biology, more specifically in embryo morphogenesis. Before introducing the research project, Section 1-1 introduces the field of developmental biology and embryo morphogenesis. Section 1-2 introduces systems biology, first from a general perspective and subsequently within the context of embryo morphogenesis. Lastly, Section 1-3 introduces the research project and indicates the objective and challenges addressed in this MSc thesis.

1-1 Developmental biology and embryo morphogenesis

The field of developmental biology can be described as the study of the process by which organisms grow and develop. Modern developmental biology investigates the genetic control of cell growth, differentiation and morphogenesis. Morphogenesis is the process that gives rise to tissues, organs and organisms.

The general paradigm in developmental biology research is to alter genes, and observe the effects. Observations of these processes lead to insight into interactions and causalities in signaling pathways and thus implications in development and morphogenesis. Besides the fundamental insight, findings in developmental biology can contribute to an understanding of certain (developmental) human conditions, such as spina bifida [1] and certain types of hereditary cancers.

Morphogenetic processes control the organized spatial distribution of cells during the embryonic development of an organism. Examples of embryo morphogenesis are epithelial morphogenesis, i.e. the formation of epithelia to serve as the outer protective layer of an organ, and gastrulation, a process through which a fertilized egg forms different tissue layers. The phenotypes studied in this project relate to processes in epithelial morphogenesis.

Morphogenic processes are induced by a wide variety of biological events, ranging from hormone activity to direct gene expression, and from radionuclides to mechanical stresses. All
these events lay the basis for the formation of organic structures and their mechanical properties.

1-2 Systems biology

Understanding complex biological systems brings the necessity of modeling. However, the increased complexity in biological studies poses a challenge to model and quantify these systems. Systems biology combines biological and engineering sciences to model and analyze biological problems on a system level, using a reverse engineering approach [40, 13, 23]. The substantial progress in both measurement and computational techniques has allowed engineering science to enter the biological field, by applying and developing computational tools to interpret large amounts of experimental data. This collaborative approach has amounted into a clearer understanding of a range of complicated and relevant problems [8, 27, 2, 3].

In the following, Subsection 1-2-1 introduces the Kitano cycle to give further insight into a systems biology approach. Subsection 1-2-2 places this systems biology approach within the context of embryo morphogenesis.

1-2-1 The Kitano cycle

The Kitano cycle of systems biology research provides a systematic overview to addressing biological problems [23] by using a reverse engineering approach, pictorially presented in Figure 1-1.

The cycle begins with the selection of biological knowledge of a given phenomenon, and the creation of a model representing the phenomenon. The model represents a computable set of assumptions and hypotheses which need to be tested or supported experimentally through
simulations and analyses. Inadequate models will result in inconsistencies with established experimental facts, and will thus need to be rejected or modified. Models that pass this test become subjects of system analysis, which most ideally results in the formulation of a number of predictions. A set of predictions that can discern a correct model among competing models is selected for biological experiments. Successful experiments are those that eliminate inadequate models. Models that survive this cycle are deemed to be consistent with existing experimental evidence.

This project follows the Kitano cycle to systematically identify challenges, and collaborations between different disciplines in the project team. Furthermore, the method motivates the members of the research team to evaluate own contributions, and reflect on how these can enable new activities and insights in the overall project. In Section 1-3 the project will be explained using this method.

1-2-2 Systems biology in epithelial morphogenesis

Relating gene and protein expressions and signaling pathways at a cellular level to the mechanical structure of a epithelial cellular network is an interesting but complex task, which has not been widely addressed.

Three aspects determine the complexity of these studies complex, and call for a system biological approach exploiting mathematical modeling. Firstly, cells and cellular networks are composed of innumerable components, of which many contribute to the mechanical structure. Modeling these structures easily leads to high-dimensional models which are difficult to analyze. Secondly, the influence of genetic processes on these mechanics is currently still investigated. Conducting even the smallest manipulations in the expression of certain genes or proteins can cause a significantly different mechanical structure. Thirdly, the dynamical behavior of epithelial cellular networks is often influenced by many different force mechanisms that act simultaneously. Hence, isolating individual contributions of force mechanisms can be a hard task.

A striking example is the study of planar cell polarity (PCP), which is the spatial organization of planar arrays of cells. PCP is required for normal physiological functions of numerous adult tissues, as well as for the correct execution of developmental events. PCP is regulated by complex signaling mechanisms, which can be found throughout the animal kingdom. In [3] reaction-diffusion Partial Differential Equation (PDE) modeling and simulation was used to study PCP signaling in the development of hairs in the wing epithelium of the Drosophila melanogaster, also referred to as the fruitfly. On a healthy wing all hairs grow towards the distal tip of the wing. Through a poorly understood mechanism, cell clones that have mutations in some of the PCP signaling components cause polarity disruptions, a phenomenon referred to as domineering nonautonomy. The sufficiency of the developed model and the experimental validation of the model predictions reveal how specific protein-protein interactions govern PCP and the observed mutants. This project illustrates the importance of mathematical modeling to understand complex biological systems.
1-3 Project introduction

This section introduces the research project. Subsection 1-3-1 discusses the problem setting, and defines the research objectives and challenges. Subsequently, Subsection 1-3-2 explains the project set up. Finally, Subsection 1-3-3 formulates specific tasks that are executed in this MSc project and lines out the structure of this thesis.

1-3-1 Problem setting and research objectives and challenges

Epithelia are sheets of connected cells that are essential to organ morphogenesis across the animal kingdom. In this project a systems biological approach is applied to develop a novel quantitative dynamical model to investigate epithelial morphogenesis through synthetic experiments. More specifically, it aims at studying the mechanical properties and the dynamic behavior of single-layered epithelia during morphogenesis. This project is first executed in the investigation of Segmental Groove Formation (SGF), a specific phenotype observed during morphogenesis of the epithelium of the Drosophila melanogaster embryo, see Figure 1-2. SGF is a morphogenic event by which grooves form in the epithelium. These grooves divide the embryo into segments. Developmental biologists are interested to learn which force mechanisms and variations of mechanical properties in the epithelium determine SGF. The signaling pathways and cellular processes that are thought to play a role in SGF, are found in many species, including humans. They play a role both in physical functioning and developmental events. However, these processes are currently still to be investigated. Hence, more insight will help to understand epithelial morphogenesis on a larger scale. Table 1-1 presents biological challenges that need to be addressed in the investigation of SGF.

For the overall research project, two important milestones are defined. The first milestone encompasses the first three biological challenges in Table 1-1, i.e. the investigation of the mechanical and dynamical behavior of the epithelium and all the forces and mechanical properties that play a key role in SGF. The second milestone is to unveil the relation between the well studied signaling pathways and the mechanical epithelial structure of SGF. Since the insight in these processes is too poor to do mathematical modeling, challenges d and e in Table 1-1 are left outside the scope of this thesis.

Figure 1-2: Drosophila melanogaster embryo during Segmental Groove Formation.
Table 1-1: Biological challenges. Those addressed in this MSc project are printed in bold.

<table>
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<tr>
<td>b To investigate the origin and contribution of force mechanisms active in the development of the epithelium of the <em>Drosophila melanogaster</em> embryo</td>
</tr>
<tr>
<td>c To investigate the variation in time and space of mechanical properties in the epithelium of the embryo</td>
</tr>
<tr>
<td>d To investigate the signaling pathways involved in SGF, and how these relate to forces and the non-uniformity of mechanical properties in the epithelium of the embryo</td>
</tr>
<tr>
<td>e To investigate the earlier epithelial development of the embryo, such as cell motility and cell division</td>
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This Master thesis addresses the first milestone. The main objective is to develop a quantitative mathematical model for the dynamics of the epithelium to investigate the role played by forces and the non-uniformity of its mechanical properties.

1-3-2 Project set-up

Figure 1-3 presents a structure for the project, that is inspired by the Kitano cycle (see Subsection 1-2-1). It depicts the structure of this project, indicates what efforts need to be done, and clarifies the communication between different disciplines.

This project is set up as a wide multidisciplinary collaboration. Developmental biologists at the Axelrod Laboratory at Stanford University, School of Medicine provide biological knowledge and experimental data. Computers scientist from the Università degli Studi di Padova collaborate to develop computer vision and image processing tools to enable the collection of focused data for model building and simulation. The engineering efforts are done from both the Delft Center for Systems and Control (DCSC) at Delft University of Technology (TU Delft), and the Hybrid Systems Lab at the Electrical Engineering and Computer Sciences (EECS) department at University of California, at Berkeley (UC Berkeley).

Regarding the complexity of epithelia and their cellular mechanics, this thesis presents a high level abstraction model. A modeling framework, derived from biological knowledge and observations, enables the construction of synthetic models, which resemble the epithelial structures observed in SGF. The constructed models are tractable for simulation and analysis. By reverse engineering, modeling and simulation efforts enable the formulation of predictions for experimental design. New experiments then again serve as new input to improve the accuracy of the models. This way this project iteratively increases understanding of SGF and morphogenesis of epithelial structures in general.

1-3-3 MSc tasks and thesis outline

Enumerated according to degree of relevance, the main tasks performed during this MSc thesis are listed below. Figure 1-3 indicates how these tasks contribute to the main activities of the overall project.
1. Derive a modeling framework to synthesize low-dimensional mechanical models for the cellular mechanics in single-layered planar cellular networks, using existing biological knowledge,

2. Design a data structure which enables tractable representation and simulation of the modeling framework, and which is able to incorporate experimental data from 4D confocal microscopy experiments,

3. Translate the case-based biological knowledge of SGF into hypotheses, which can be tested with simulations,

4. Develop a model building algorithm which employs the modeling framework, and incorporates experimental data to build up models that represent the epithelium of the *Drosophila melanogaster* embryo,

5. Perform simulations and qualitative analyses on the derived models to test biological hypotheses,

6. Enable experimental design with biologists, and data processing with computer scientists to retrieve data to improve model accuracy. Explore the possibilities of novel experimental techniques.

7. Research feasibility of Adjoint-Based Parameter Estimation (ABPE) procedure. It is intended to construct the theoretical framework, which will eventually facilitate practical execution, as soon as focused experimental data comes available.

Chapter 2 elaborates on the motivation for and the derivation of a novel modeling framework, which is used to represent single-layered epithelial cellular networks. Starting from biological knowledge on cellular mechanics, the chapter proposes important modeling criteria. Based on these criteria, two modeling methods are compared. The Discrete Element Method (DEM) is employed to derive a Mass-Spring-Damper (MSD) modeling framework. Finally, the chapter presents the mathematical formulation, and discusses the potential and limitations of the modeling framework.
The plausibility of the modeling framework will be assessed by applying it to the SGF case study. Chapter 3 presents the background knowledge and hypotheses related to SGF. Serving as an introduction, it describes the early embryonic development of *Drosophila melanogaster*. Subsequently, it explains the background of SGF. Lastly, it formulates biological hypotheses, within the frame of this MSc thesis.

Chapter 4 describes the process of transforming experimental data of epithelial cellular networks taken from 4D confocal microscopy experiments into models representing the same cellular network geometry. The chapter starts with explaining the experimental set up, the image processing procedure, and data structure design. Subsequently, it elaborates on the design of a model-building algorithm. Finally, it shows how simulation results can be reproduced and analyzed.

Chapter 5 reports on simulation results, gathered during the testing of biological hypotheses. It first explains the analysis methods used to understand the model, select model parameters, and test hypotheses. Thereupon it presents the simulation results and assesses them based on observed qualitative characteristics. Throughout the discussion suggestions for future research are mentioned.

Chapter 6 presents conclusions and recommendations. It starts with a discussion on the key biological implications relating to the focused challenges. Secondly, recommendations for modeling efforts are listed. Subsequently, recommendations are done for the image processing, and the experimental design. Finally, it gives an overview of the different key contributions made during this MSc project.
This chapter proposes a framework that can model morphogenesis in single-layered epithelial cellular networks. The model derivation is founded on biological knowledge accumulated from literature and performing experiments.

Firstly, Section 2-1 describes cellular mechanics. The epithelial cell is dissected in terms of its structural components and connections to understand its mechanical and dynamical characteristics. Secondly, Section 2-2 gathers all the essential modeling criteria, which should be taken into account during the selection and derivation of a modeling framework. Thirdly, Section 2-3 compares two different modeling methods and utilizes the modeling criteria to identify the superior method. Lastly, Section 2-4 proposes a Mass-Spring-Damper (MSD) modeling framework, exploiting the selected method and set criteria. Moreover, it discusses the potential and limitations of the framework, in order to steer the model building in Chapter 4.

Alongside the derivation of the MSD modeling framework, its stability properties were studied. Appendix D presents a proof of Lyapunov stability for two-dimensional MSD network models.

2-1 Cellular Mechanics

Cells are highly dynamic: they stretch, crawl, change shape and divide. In many critical biological processes, cells both exert and respond to forces in their surroundings [15]. The mechanical properties of the cell are intimately related to this behavior. Cells also continually remodel their internal structure and thereby change their mechanical properties [21]. These properties make the derivation of mechanical models for cellular networks a complex task.

Cellular mechanics are determined by three key aspects, which will all be discussed in the following. Subsection 2-1-1 describes the internal structure of cells, called the cytoskeleton.
Subsection 2-1-2 explains how cell-cell connections work. Subsection 2-1-3 mentions the connection of epithelial cells to other tissue layers, and finally, Subsection 2-1-4 completes the background material with two important mechanical characteristics observed in epithelial morphogenesis.

2-1-1 The cytoskeleton

The cytoskeleton is a biopolymer network consisting of three major components: microfilaments (or actin filaments), intermediate actin filaments and microtubules, all indicated in Figure 2-1. In addition, a myriad of filament cross-linker, motor and regulatory proteins play a critical role in cytoskeletal structure and dynamics and hence in the mechanical properties of the cytoskeleton. A variety of experimental techniques show that cells have both elastic and viscous characteristics, and thus are composed of viscoelastic materials. The cytoskeleton is a complex, heterogeneous and dynamic structure, which makes the study of its properties extremely difficult.

This subsection first discusses the structural organization of the cytoskeleton, and secondly its dynamical characteristics.

![Diagram of cell membrane and circumferential actin belts](image)

![Diagram of intemmediate filaments and microtubules](image)

(a) Indicating the cell membrane (thin, black) and the circumferential actin belts along the cell boundaries (thick, blue)

(b) Indicating the intermediate cytoskeletal components, and cell - cell connections

Figure 2-1: Simplified top-view of a cell in an epithelial cellular network.

Structural components of the cytoskeleton

Tensile forces are sustained by cytoskeletal microfilaments and intermediate actin filaments, which consist of actin [19]. Actin is a protein that is involved in motion and deformation of epithelial cells, and also forms the contractile filaments of muscle cells (together with myosin). For epithelial cells the organization of actin filaments can be divided in two parts.

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First and most significantly, actin accumulates as a circumferential belt along the cell membrane, at a certain apical-basal depth of the cell [5], as depicted in Figure 2-2. Circumferential actin belts of adjacent cells are connected to each other through adherens junctions, see Figure 2-2(b). An adherens junction is defined as a cell junction whose cytoplasmic face is linked to the cytoskeleton, see Figure 2-1(b). The connection of circumferential actin belts of adjacent cells creates a two-dimensional network of actin, within the epithelial cellular network. Actin staining experiments show this actin network characteristic, as depicted in Figure 2-3 [43].

Second, intermediate actin filaments act as ropes between two points on the cell membrane. They are spread around the cell, as can be seen in Figure 2-1(b).

The tension sustaining actin filaments are balanced by interconnected structural elements that sustain compression, called microtubules [37]. Their flexible tube-like protein structure causes microtubules to buckle around significantly. They act as struts pushing the cell membrane outwards, as depicted in Figure 2-1(b). Pushing microtubules and pulling intermediate actin filaments meet at the cell membrane, at adherens junctions. The opposing forces create local equilibrium together with the circumferential actin belts. Adjacent cells are also connected to each other between their cytoskeletal components.

The different components are connected through adherens junction, which among others are represented by integrin and E-cadherin proteins. These proteins are receptors nestled in the cell membrane and are connected to receptors of neighboring cells [47], which is discussed in Subsection 2-1-2. The receptor proteins can freely float through the cell membrane plane. This suggests that the bi-lipid cell membrane itself does not have a significant contribution to the structural balance of the cell, since most of the force is sustained by cytoskeletal components that cross cell membranes.

The discussion so far had a focus on the two-dimensional surface of circumferential actin belts and the intermediate cytoskeleton in between. However, there also exist cytoskeletal

Figure 2-2: Actin accumulation along cell boundaries. (a) depicts apical-basal orientation, the circumferential actin belt at a certain apical-basal level (blue), and cell junctions (magenta). (b) adherens junctions (green) connect different circumferential actin bundles (blue) of the cytoskeletons of adjacent cells along cell boundaries. The black outer lines represent cell membranes.
components connecting the apical and basal cell membrane, i.e. in the third dimension of the cell (the apical-basal axis is the axis from the top of the cell down to the bottom). These filaments govern the stability of the thickness of the cell under stretching loads. Recent studies address the issue of how to model the thickness of epithelial cells, since it is still elusive to retain information on this variable from microscopy data. At the moment a relationship between thickness and cell stress in individual cells can be observed, but not to an extent that enables realistic modeling of thickness and thus volume preservation of cellular networks [11].

Dynamical characteristics of the cytoskeleton

Recent experiments have unveiled the dynamical characteristics of different cytoskeletal components and the cell as a whole [24, 45, 37]. Through severing individual actin filaments and microtubules and analyzing dynamical responses, it was shown that the dynamical characteristics of cytoskeletal components are viscoelastic. The well-damped response can be modeled by a spring and a damper in parallel, also referred to as the Voigt element [18].

2-1-2 Cell-cell connections

In Figure 2-1(b) and Figure 2-2(b) the green double hooks represent cell-cell connections, i.e. adherens junctions. Among others, these junctions are embodied by E-cadherin and integrin proteins. These proteins are nestled in the cell membrane and clamp into each other to form the hook structure. They connect to cytoskeletal components of neighboring cells, creating a cellular aggregate [47].

Adherens junctions can float through the cell membrane. In developing epithelia, cell junctions often rearrange. In many cases rearrangements occur at a high frequency, to facilitate cell motility and cell proliferation. In many other cases with a much lower frequency, to stabilize the epithelium. Because epithelia serve primarily as a structural layer to protect the inner organs and processes, sufficient and stable adherens junctions are needed to seal cells together.
into an aggregate. Hence, depending on the developmental time span, a big difference in the frequency of junction rearrangements can be observed.

Zooming in further, it has been shown that $\alpha$-catenin is the central protein linking the actin cytoskeleton to adherens junction proteins at the cell membrane [42]. An active process of epithelial sheet formation has been discovered that involves the extension, protrusion, embedding and anchoring of filopodia into neighboring cell membranes. This process stabilizes contacts between two membranes and catalyzes adherens junction formation, which in turn promotes the sealing of epithelial cells into sheets. This dynamic process necessitates a major role for actin polymerization and reorganization in cell-to-cell adhesion. It is likely that this mechanism is both operative and important during development, particularly where epithelial sheets must be drawn together. Examples of such situations include dorsal and ventral closure, and wound healing. Actin polymerization has emerged as a force that can push membranes of neighboring cells together, the alignment of which is necessary for intercellular adhesion. This pushing abilities of actin fibers are recently supported by the tensegrity model [26].

2-1-3 The connection of cells to the extracellular matrix

Extracellular Matrix (ECM), and more specifically the basement membrane, is situated underneath the epithelium. The extracellular matrix (ECM) is the extracellular part of animal tissue that provides structural support to any living animal cell in an aggregate. Confluent epithelial cellular networks are loosely connected to the extracellular matrix [33]. The cells are connected to the basement membrane via focal adhesions, which are integrin receptor proteins serving as anchors [47]. Depending on the number of adhesions, the dynamical characteristic of this connection is either viscous and loose, or fixed.

Observing 4D confocal microscopy data of the epithelium of the Drosophila melanogaster suggests that during the patterning developments in the epithelial sheet the epithelium seems to float over the basement matrix, i.e. in a viscous manner [43].

2-1-4 Mechanical characteristics of cells

This section discusses two complex and intensively studied cell-mechanical characteristics. Firstly, Subsection 2-1-4 elaborates on the inherent nonlinear elasticity of all living cells. Subsequently, Subsection 2-1-4 shows the effects of anisotropy in epithelial structures.

Nonlinear elasticity in cellular mechanics

Cellular mechanics is nonlinear. The elastic modulus of a cell depends on the degree of applied or internal stress. Moreover, their elastic behavior depends on the mechanical properties of their environment. For most materials, the elastic constant is independent of the applied stress, i.e. within a certain stress regime. By contrast, networks of semi-flexible polymers often exhibit an unusual property: with increasing applied stress, their elastic modulus increases. This is called stress-stiffening and typically reflects individual filament behavior. A typical nonlinear elasticity characteristic of cells is given in Figure 2-4.
The *overall* elasticity of cells can be attributed to a number of different components, including the cytoskeleton, the cell membrane and cell-cell connections. It can be verified through from experimental results and model simulation that this elasticity is nonlinear [15]. Hence, it can not be modeled with Hookean springs. Experimental results suggest that applied (pre)stress is a major determinant for the nonlinear cellular elasticity of single cells. The relation between applied (pre)stress and the stiffness coefficient has a linear (affine) fashion, both on the cell [45, 21], as well as on the cytoskeletal level [37]. This means that the elasticity characteristic has a quadratic fashion, which will be explained in detail in Subsection 2-4-2.

**Anisotropy and cell shape stability**

The spatial organization of cytoskeletal components clearly creates structures which are inherently anisotropic. For instance, microtubules often determine the direction that a cell elongates [31]. This can be illustrated by considering the epithelial cells as discussed earlier, depicted as a subset of a network in Figure 2-5. The right image shows a stretched version of the left network which is at rest. A phenomenon found in stretching cells is the alignment of the cell boundaries. Hence, the circumferential actin filaments that organize along the cell membrane, tend to sustain and propagate most of the load in the network. This way the cell boundaries contribute to the cell shape stability, taking into account their earlier discussed nonlinear elasticity behavior.

**2-2 Essential modeling criteria**

This section formulates essential modeling criteria, based on the background knowledge, and deduces four important criteria for the derivation of a modeling framework for single-layer epithelial cellular networks. Therefore, these criteria are addressed later in the selection of...
2-2 Essential modeling criteria

Figure 2-5: Anisotropy in cellular networks. left) a network at rest, right) the same network stretched in the direction of the red arrows, and aligned, depicting anisotropy.

a modeling method and the derivation of a modeling framework, respectively dealt with in Sections 2-3 and 2-4.

1. **Cellular architecture is discrete:**
   Epithelial cells behave mechanistically as discrete networks composed of different interconnected cytoskeletal filaments, and are able to sustain both tensional and compressional loads. They do not behave as mechanical (e.g. viscous or viscoelastic) continua. This insight is based on biological experiments [43, 44] and findings in the study of the tensegrity model [19]. On top of that, a large proportion of the actin architecture is organized at a certain depth, creating a two-dimensional surface, governing most of the observed cell dynamics. The discrete nature of this actin network should be incorporated in the modeling framework, to unveil how exactly it affects epithelial morphogenesis.

2. **Cellular geometry determines anisotropy:**
   Cells have highly anisotropic mechanical structures, mainly determined by their geometry. This local anisotropy is often necessary to explain the existence of global morphogenetic events, such as alignment of cell columns or propagation of forces through a cellular network. Taking into account the cellular geometry of the epithelial network, is therefore necessary to study anisotropy and understand its necessity to explain events happening at a network level.

3. **Modeling volume preservation is an elusive task:**
   Volume preservation is an apparent feature of epithelial cells deforming in a network. Modeling this is still elusive, since there is no clear relation between the stress in the surface directions (planar dimension) and the thickness (height dimension) of the cellular network [11]. Enforcing volume preservation in simplistic modeling frameworks can lead to introduction of artificial dynamics, and should therefore only be done under realistic, controlled conditions.

4. **Nonlinear mechanical characteristics are apparent in different structural levels:**
   Both whole cells as well as their components have nonlinear elasticity characteristics.
The cytoskeletal (pre)stress could be a major determinant of this nonlinearity in cell deformability. Relatively simple nonlinear elasticity characteristics relations (quadratic) have been derived on a cellular and tissue level [15, 45, 37], and should therefore be considered when modeling cellular networks.

2-3 Selection of modeling method

The literature survey of this thesis work did a thorough study on earlier work in modeling epithelial structures [16]. It distinguished between two different modeling methods, i.e. the Finite Element Method (FEM) and the Discrete Element Method (DEM).

Firstly, Subsection 2-3-1 briefly introduces these two modeling methods. Secondly, Subsection 2-3-2 assesses both methods, following the criteria derived in Section 2-2. It concludes with a motivation for the selection of the DEM.

2-3-1 Basics of finite and discrete element modeling

Finite and discrete element methods are numerical techniques developed to find approximate solutions of Partial Differential Equations (PDEs), as well as of integral equations. The solution is based either on eliminating the differential equation completely (ending up with steady state problems), or rendering the PDEs into an approximating system of Ordinary Differential Equations (ODEs), which are then numerically integrated using standard techniques such as the Euler method, or Runge-Kutta methods. The common feature of all methods is the application of a mesh discretization of a continuous domain into a set of discrete sub-domains, called elements.

These methods are generally used for solving a set of PDEs over complicated domains, when:

- the domain changes (as the form of a cell)
- the desired precision varies over the entire domain
- the solution lacks smoothness (jumps in stress and strain levels from cell to cell and within cells)
- the mechanistic structure of the system is studied

The highest level distinction between elements is explained in the following, together with important implications in the application to cellular networks:

The FEM considers modeling objects, by meshing them into volumetric elements. For these elements mechanical properties are defined as constant or continuous over the whole element. When volumetric criteria as incompressibility, osmotic pressure or density are important, these elements are indispensable. The FEM assumes that strain and stress vary continuously over the defined elements. This is a sensitive assumption when modeling systems with a discrete mechanical structure.
The DEM considers a nodal mesh in which nodes are considered point masses, which are connected through discrete elements as springs or dampers. Internal forces can be exerted in directions between nodes or combinations thereof. Discrete elements are useful when they can resemble the mechanistic structure of the modeled system. Applying discrete modeling elements to systems with volumetric forces and properties brings implications.

Hybrid combinations with both element types are often applied, e.g. structures consisting of beams (modeled with volumetric finite elements) and rods (modeled with discrete elements) [7].

2-3-2 Comparison and selection of modeling method

The four criteria derived in Section 2-2 are used to assess both modeling methods. This subsection crisply states the result of this assessment, mentioning the different advantages and disadvantages for each criterium in Table 2-1. The extensive elaboration on the assessment can be retrieved from Chapter 4 of the accompanying literature survey [16].

The DEM is selected for the following reasons:

1. The DEM is the only method that is able to represent the discrete tensegrity structure of cellular mechanics, although still to a certain approximation depending on the discretization.

2. The DEM is necessary to study the anisotropy, due to cellular geometry and structural organization.

3. Volume preservation is an elusive task, and therefore not considered in the early stage of modeling framework development. Potentially, new insight can be incorporated in the form of either finite (hybrid models are always possible) or discrete elements representing volume preservation forces/properties.

4. The DEM has shown interesting results in other modeling studies, in which nonlinear mechanical characteristics were incorporated. These studies showed qualitative resemblance of tissue deformation [30, 29, 39] and model stability of high-dimensional models [12], whereas the found literature on the FEM restricts nonlinear analysis to smaller model dimensions.

In the following section the DEM is put into practice to derive a modeling framework, based on the insights gathered in Section 2-1.

2-4 The Mass-Spring-Damper modeling framework

This chapter proposes a Mass-Spring-Damper (MSD) framework to model epithelial cellular networks. Using the DEM selected in Section 2-3, it applies a vertex discretization to model a network, which respects the cellular geometry of the epithelium. This way a high abstraction
### Table 2-1: Comparison of modeling methods FEM and DEM

<table>
<thead>
<tr>
<th>Cellular architecture is discrete</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FEM</strong></td>
<td><strong>DEM</strong></td>
</tr>
<tr>
<td>- Can not represent discrete tensegrity structure</td>
<td>+ Can represent discrete tensegrity structure</td>
</tr>
<tr>
<td>+ Can be combined with DEM as hybrid model [7]</td>
<td>- Number of elements per cell is restricted by the discretization to a lumped version of the real cell lay-out, in order to keep computations tractable</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cellular geometry determines anisotropy</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FEM</strong></td>
<td><strong>DEM</strong></td>
</tr>
<tr>
<td>+ Finite elements can be adapted to cellular geometry</td>
<td>+ Discrete elements can be adapted cellular geometry</td>
</tr>
<tr>
<td>- Anisotropy of actin accumulation bundles cannot be incorporated with only finite elements</td>
<td>+ Anisotropy of actin accumulation bundles can easily be incorporated</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Volume preservation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FEM</strong></td>
<td><strong>DEM</strong></td>
</tr>
<tr>
<td>+ Can be enforced through volumetric properties</td>
<td>+ Can only be approximated, not enforced</td>
</tr>
<tr>
<td>- Enforcement results in artifacts and computational (stiffness) problems, due to insufficient knowledge on cell height properties of continuum elements can be use</td>
<td>- Approximation likely means introducing extra undesired mechanical properties, i.e. artifacts</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nonlinear mechanical characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FEM</strong></td>
<td><strong>DEM</strong></td>
</tr>
<tr>
<td>+ Can be incorporated in finite elements</td>
<td>+ Can be incorporated in discrete elements</td>
</tr>
<tr>
<td>- Too much computational burden for average-sized cellular network</td>
<td>+ Nonlinear elasticity resembles tissue deformation qualitatively</td>
</tr>
<tr>
<td></td>
<td>+ Nonlinear elasticity contributes to model stability, i.e. prevents collapsing [12]</td>
</tr>
</tbody>
</table>
model is created that incorporates the mechanistic structure of single-layered epithelial cellular networks.

Subsection 2-4-1 starts with introducing a necessary network notation and introduces different network elements, following graph theoretical concepts. Secondly, Subsection 2-4-2 derives the MSD framework step by step, using the input from Section 2-1. Thirdly, Subsection 2-4-3 collects all mathematical formulation. Finally, Subsection 2-4-4 discusses the potential and limitations of the MSD framework, anticipating its application to a case study in Chapter 4.

2-4-1 Network numbering

Figure 2-6 shows how the network is numbered. A graph theoretical structure is imposed consisting of three different features: vertices, edges, and faces. Consider a set $V$ consisting of $N$ vertices. $V$ stores the vertex index $i \in \{1, 2, \ldots, N\}$ of each vertex $v_i$. In Figure 2-6 vertices are indicated by magenta dots and the indices are depicted with loose numbers (not encircled or boxed). An edge $e_{i,j} = \{v_i, v_j\}$ is defined in between each pair of adjacent vertices $v_i$ and $v_j$, where $j \neq i$ and $j \in \{1, 2, \ldots, N\}$. Edges are stored as rows in a $N_e \times 2$ set $E$. $N_e$ is the total number of edges in set $E$. The edge index is defined by the row number in $E$, and denoted by encircled numbers in Figure 2-6. The graph $\mathcal{G} = (V, E)$ describes the full topology of a network, i.e. all the adjacency information of edges and vertices.

A structured and elegant way of writing the adjacency of the network in a single matrix structure is given by Eq. (2-1) and Eq. (2-2). Consider the $N \times N_e$ incidence matrix $H$ of a

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2-6.png}
\caption{Denotation of the elements in a (cellular) network. Vertices are denoted by purple dots with a straight number, edges connect vertices and are denoted by encircled numbers, faces by squared numbers.}
\end{figure}
graph. Its entries are formulated as

\[ h_{ik} = \begin{cases} 
  +1 & \text{if } v_i \text{ is connected to positive end of } k^{th} \text{ edge} \\
  -1 & \text{if } v_i \text{ is connected to negative end of } k^{th} \text{ edge} \\
  0 & \text{otherwise.} \end{cases} \]  

(2-1)

Now consider a small graph \( G^{\text{small}} \) consisting of three vertices and two edges, as depicted in

![Figure 2-7: A small graph \( G^{\text{small}} \). Loose numbers denote vertices, encircled numbers denote edges.](image)

Figure 2-7. To each edge arbitrarily assign a positive and negative end. For \( G^{\text{small}} \) this yields

\[ H = \begin{bmatrix} 
  1 & 0 \\
  -1 & 1 \\
  0 & -1 
\end{bmatrix}. \]

The \( N \times N \) Laplacian matrix \( L \) describes which vertices are adjacent to each other and is calculated using the incidence matrix [4], as given by Eq. (2-2).

\[ L = HH^\top. \]

(2-2)

For \( G^{\text{small}} \) this yields

\[ L = \begin{bmatrix} 
  1 & 0 & -1 & 0 \\
  -1 & 1 & 0 & 1 \\
  0 & -1 
\end{bmatrix} \begin{bmatrix} 
  1 & -1 & 0 \\
  -1 & 1 & -1 \\
  0 & 1 & -1 
\end{bmatrix} = \begin{bmatrix} 
  1 & -1 & 0 \\
  -1 & 2 & -1 \\
  0 & -1 & 1 
\end{bmatrix}. \]

The Laplacian \( L = L^\top \) tells for each vertex \( v_i \) whether it is connected to another vertex \( v_j \) \((l_{ij} = l_{ji} = -1)\) or not \((l_{ij} = l_{ji} = 0)\). The diagonal represents the degree \( \partial(v_i) = l_{ii} \), which is the number of adjacent edges or vertices of vertex \( v_i \).

The last important features in the network are faces, depicted by boxed numbers in Figure 2-6. In a cellular network a face \( f \) represents a cell and is defined as the set of its vertices. For example, Eq. (2-3) gives the set of vertices of face \( f_1 \), which is depicted in Figure 2-6,

\[ f_1 = \{v_1, v_2, v_3, v_4\}. \]

The degree of a face \( f \) is \( \partial_f(f) \) and denotes the number of vertices/edges it consists of, e.g. \( \partial_f(f_1) = 4 \). In this project the data is delivered as graphs of vertices and edges \((G = (V, E))\). In order to retrieve the face information, a face detection algorithm has been derived, which is presented in Section 4-3.
2-4-2 Derivation

Section 2-2 derived essential criteria for modeling epithelial cellular networks. Following these criteria, this subsection proposes and motivates a MSD modeling framework. Assuming that the cellular geometry (network topology) is known, a mesh is applied to all the cell junctions, i.e., the points where cell boundaries come together, as indicated in Figure 2-8 (2D) and Figure 2-2(a) (3D) by the magenta dots. Models are built up as two-dimensional surfaces, and represented using the graph theoretical network notation given in Subsection 2-4-1. With this data structure, three different modeling elements are assigned to different features of the network, which resemble different structural features in epithelial cellular networks. First,

\[ m_i \ddot{x}_i(t) = \sum \vec{F}_i(x, t), \]

where \( \ddot{x}_i(t) \) is the second time derivative of the position \( x_i \) of point mass \( m_i \). The point masses are placed at cell junctions, where different cell boundaries come together. This modeling choice enables modeling the mechanistic organization of circumferential actin belts in between these cell junctions. Since these belts propagate their elastic energy to other belts through these vertices, a mechanistic equivalent can be resembled and studied in the model. It should be noted, that in reality cell junctions are not stable, i.e., they do rearrange.
with respect to the adjacent cell boundaries, governed by adherens junction rearrangements. At first, this modeling framework neglects these rearrangements. The implications of this assumption are further explained in Section 2-4-4.

The second modeling element models the circumferential actin belts, which organize along cell boundaries at a specific apical-basal depth in the cell. In the modeling process, a distinction is made from the intermediate cytoskeleton (consisting of intermediate actin filaments and microtubules), which are modeled by the third element. As stated above, the circumferential actin belts form along cell boundaries and connect cell junctions. Their mechanical behavior is viscoelastic, and hence modeled as spring-damper elements (Voigt elements) between two adjacent cell junctions, as depicted in Figure 2-8 and Figure 2-9. In the following, these elements are called boundary elements. It is important to notice that one boundary element represents a merger of two adjacent circumferential belts in two adjacent cells, between two shared cell junctions. These two belts are connected through adherens junctions. As explained in the above, the modeling framework assumes that these junctions do not rearrange, which implies that it assumes that adjacent circumferential actin belts do not rearrange either.

Consider a point mass element $m_i$ connected to three other point mass elements $m_j$, where $j \in \mathcal{N}(v_i)$ denotes the set of adjacent vertices of vertex $v_i$. The point masses are connected through spring-damper elements, having stiffness coefficients $k_{i,j}$ and damping coefficient $\nu_{i,j}$, as depicted in Figure 2-10.

The mathematical equation for point mass element $m_i$ connected to the point masses $m_j$
through spring-damper boundary elements, is given by

\[
m_i \ddot{\mathbf{x}}_i(t) = F_{i}^{\text{elastic}}(x) + F_{i}^{\text{damping}}(x) = \sum_{j \in N(v_i)} \left\{ \left( k_{i,j} (l_{i,j} - d_{i,j}) + \nu_{i,j} \frac{dl_{i,j}}{dt} \right) \frac{\mathbf{x}_j - \mathbf{x}_i}{||\mathbf{x}_j - \mathbf{x}_i||} \right\},
\]

where \( l_{i,j} \) is the length of boundary element \( e_{i,j} \) and \( \frac{dl_{i,j}}{dt} \) its time derivative, and \( d_{i,j} \) is the resting length related to the spring at boundary element \( e_{i,j} \). \( \frac{\mathbf{x}_j - \mathbf{x}_i}{||\mathbf{x}_j - \mathbf{x}_i||} \) is the normalized direction vector, resembling the orientation of the element \( e_{i,j} \).

Thirdly, similar to the circumferential actin belts, the intermediate cytoskeleton (consisting of intermediate actin filaments and microtubules, see Figure 2-1) is modeled using spring-damper elements, in the following called \textit{intermediate elements}. Figure 2-11(a) shows a cell with the intermediate cytoskeleton isolated. Following the nodal discretization, intermediate elements are modeled in between all \textit{non-adjacent} mass elements \textit{within a cell}, depicted in Figure 2-8, and isolated in Figure 2-11(b). This way the discrete nature of the intermediate cytoskeleton can be represented, although to a limited extent. Section 2-4-4 explains the limitations of this approach. The mathematical equations for the intermediate elements are identical to those of the external elements, this yields

\[
F_{i}^{\text{int,elastic}}(x) + F_{i}^{\text{int,damping}}(x) = \sum_{j \in N^{\text{int}}(v_i)} \left\{ \left( k_{i,j} (l_{i,j} - d_{i,j}) + \nu_{i,j} \frac{dl_{i,j}}{dt} \right) \frac{\mathbf{x}_j - \mathbf{x}_i}{||\mathbf{x}_j - \mathbf{x}_i||} \right\},
\]

where \( N^{\text{int}}(v_i) \) denotes the set of all vertices which lay in one of the faces adjacent to vertex \( v_i \), but which are \textit{not} directly adjacent to \( v_i \).
The MSD framework assumes that the connections between cells and the ECM are viscous. It implements a friction force $F_{\text{friction}}^i$ that acts on all point mass elements. $F_{\text{friction}}^i$ is inversely proportional to the velocity $\dot{x}_i$ of point mass $m_i$,

$$F_{\text{friction}}^i(x) = -\lambda_i \dot{x}_i ,$$

where $\lambda_i$ is the friction coefficient at point mass $m_i$.

The last feature considers nonlinear elasticity in cellular mechanics. In Subsection 2-1-4 the nonlinear elasticity characteristics in cellular mechanics were discussed extensively. One finding was the relation between applied (pre)stress and stiffness. This relation has a linear (affine) fashion, both on the cell [45], as well as on the cytoskeletal component level [37]. To illustrate this relation, consider a spring, as depicted in Figure 2-12. The classical constitutive equation given by the law of Hooke for the magnitude of the elastic force exerted by a spring is

$$F_{\text{elastic}}(l) = k (l - d) ,$$

where $k$ denotes the stiffness coefficient, $l$ the current length and $d$ the resting length of the spring. Prestress is defined as the fraction of the deviation from the resting length with respect to the resting length, which is $\frac{l - d}{d}$. Now consider an affine relation between stiffness and applied (pre)stress as found in cells, as found in reality [45]. This yields

$$k \propto \frac{l - d}{d} ,$$

$$\Rightarrow k(l) = k^1 \frac{(l - d)}{d} + k^0 ,$$

where $k^0$ is the nominal (linear) stiffness coefficient at resting length and $k^1$ adapts the stiffness due to applied (pre)stress. Plugging Eq. (2-4) into the elastic force, Eq. (2-3), results in a quadratic (and thus nonlinear) elastic force-deformation (stress-strain) relation

$$F_{\text{elastic}} = k(l) (l - d)$$

$$= \frac{k^1}{d} (l - d)^2 + k^0 (l - d) .$$

The stiffness coefficients $k_{i,j}$ of the spring-damper elements in the MSD models are modeled accordingly. This means an extra parameter $(k^0, k^1)$ is introduced for each spring-damper element.

Figure 2-12: One-dimensional spring model to explain applied (pre)stress.
2-4-3 Mathematical formulation and implementation

The former section discussed how and with what kind of modeling elements are needed to represent the essential mechanical and dynamic characteristics of single-layer epithelial structures. All of these components can be mathematically described and gathered to form a system of second-order ODEs, according to the second law of Newton. Consider a point mass \( m_i \) at vertex \( v_i \) representing 6 state variables, i.e. 3 position variables \( \vec{x}_i \) and 3 velocity variables \( \dot{\vec{x}}_i \). The dynamical Ordinary Differential Equation (ODE) for \( m_i \) is

\[
m_i \ddot{\vec{x}}_i(t) = F_i^{\text{elastic}}(x) + F_i^{\text{damping}}(x) + F_i^{\text{friction}}(x) + F_i^{\text{ex}}(x,t)
\]

where \( k_{i,j} \) and \( \nu_{i,j} \) denote the stiffness and damping coefficients of an element between masses \( m_i \) and \( m_j \), \( l_{i,j} \) is the distance between the two masses. \( \mathcal{N}(v_i) \) denotes the adjacent vertices of \( v_i \), and \( \mathcal{N}^{\text{int}}(v_i) \) denotes the non-adjacent vertices within all the adjacent faces of \( v_i \). The stiffness and damping force can only act in the direction of the element between the two masses, which is \( \frac{\vec{x}_j - \vec{x}_i}{||\vec{x}_j - \vec{x}_i||} \). \( \lambda_i \) is the friction coefficient at mass \( m_i \). \( \sum_\mu F_i^{\text{ex},\mu}(x,t) \) denotes the vectorial sum of all \( \mu \) external forces acting on mass \( m_i \). This term depends on the system under study.

The ODEs of Eq. (2-5) are nonlinear, due to the computation of the lengths \( l_{i,j} \) and their time derivatives and the varying stiffness coefficients \( k_{i,j}(l_{i,j}) \). However, a matrix formulation can be derived using the Laplacian matrix \( L \), defined in Eq. (2-2). First define the stiffness/damping matrix \( S \) by considering the structure of Eq. (2-5). Notice that \( ||\vec{x}_j - \vec{x}_i|| = l_{i,j} \) and define the non-diagonal entries \( (i \neq j) \) of \( S \) as

\[
s_{ij} = -\left( k_{i,j}(l_{i,j}) (l_{i,j} - d_{i,j}) + \nu_{i,j} \frac{dl_{i,j}}{dt} \right) \frac{1}{||\vec{x}_j - \vec{x}_i||}
\]

and similarly the diagonal entries as

\[
s_{ii} = \sum_{j \in \mathcal{N}(v_i) \cup \mathcal{N}^{\text{int}}(v_i)} \left\{ k_{i,j}(l_{i,j}) \frac{l_{i,j} - d_{i,j}}{l_{i,j}} + \nu_{i,j} \frac{dl_{i,j}}{dt} \right\}.
\]

Notice that the structure of \( S(k, l, t) \) coincides with the structure of \( L \). Subsequently, \( S \) can be exploited to rewrite the dynamical equations Eq. (2-5) for a whole network of point masses in matrix notation as

\[
\begin{bmatrix}
\dot{x} \\
\dot{\vec{x}}
\end{bmatrix}(t) =
\begin{bmatrix}
\bigoplus_{i=1}^{3N} M^{-1}\mathbb{S}(k, l, t, L) \\
M^{-1}\mathbb{A}
\end{bmatrix}
\begin{bmatrix}
x \\
\dot{x}
\end{bmatrix}(t) +
\begin{bmatrix}
0 \\
M^{-1}\sum_\mu F^{\text{ex},\mu}(x, t)
\end{bmatrix},
\]

with the \( 3N \times 3N \) mass matrix \( M \), the \( 3N \times 3N \) diagonal friction matrix \( \Lambda \). \( S \) is time-dependent and contains all the nonlinearities of the equations. \( k_{i,j}, l_{i,j}, \) and \( \frac{dl_{i,j}}{dt} \) need to be updated continually for all spring-damper elements. \( L \) can be used to assign these updated variables to the right entries in \( S \).

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By using smart vectorial implementations in MATLAB, the simulation algorithm can integrate the dynamics of Eq. (2-6) of a Mass-Spring-Damper network of in the order of hundreds of vertices in a feasible way. The integration method used is Runge-Kutta-4,5. This algorithm continually considers adaptation of the time step in order to produce accurate and stable solutions, based on a comparison of the local truncation error with a set tolerance [17].

2-4-4 Model potential and limitations

The decision to utilize a Mass-Spring-Damper modeling framework is made from a systems biology perspective. It incorporates both engineering and biological principles. Starting from a very simple modeling framework, the equations can easily be extended to include new insight from both experiments and modeling simulations. This way the real potential of this framework can be assessed iteratively, and new experiments can be steered in parallel to the modeling process. Hence, the analysis in Chapter 5 has a focus on what is, and what is not possible to resemble with the given modeling framework.

The list below gives the assumptions made throughout the derivation of the MSD modeling framework, and indicates related limitations in the application to epithelial cellular networks.

1. The internal spring-damper elements represent a lumped characteristic of the cytoskeleton. The intermediate cytoskeleton is scattered around through the cell, but accumulates at a certain height, hereby governing most of the mechanical behavior of cells, as described in Subsection 2-1-1. Besides the intermediate cytoskeleton, there are other factors contributing to the internal mechanics of cells, such as osmotic pressure, and the third dimension of the cell. This modeling framework only represents a lumped two-dimensional model of the internal cellular mechanics. Its main role is to be able to determine the contribution of internal mechanics versus the mechanics in the actin accumulation filaments along cell boundaries. It should be noticed that this representation is not able to represent the highly scattered intermediate cytoskeleton to its full extent, neither can it distinguish between different contributors to internal mechanics.

2. The Mass-Spring-Damper modeling framework assumes a two-dimensional surface structure. It neglects the depth of the cell and only considers the mechanics and dynamics in the surface dimensions. This assumption is necessary, since there is too little insight in the 3D deformation of epithelial structures. Hence, this framework can explain behavior in the surface, but it is not able to give any insight in the volumetric deformations (including volume preservation) of epithelial cells.

3. The mass discretization assumes that cell junctions maintain their configuration. In other words, adherens junction rearrangements are neglected. This means the framework cannot model:
   - cell division
   - cell death
   - cell motility

   Depending on the morphogenetic events considered, these events do or do not play a significant role. It should be stressed that the modeling framework has the potential
to incorporate these characteristics. To do this, however, feasibility studies should be conducted. Therefore these characteristics are neglected within the frame of this thesis research, and recommended as a future topic for studies.

2-5 Concluding remarks

This chapter has presented a novel mathematical modeling framework for single-layered epithelial cellular structures. Cellular mechanics has been explained thoroughly. By identifying different structural components it was possible to derive important criteria, which should be respected in the modeling process. Based on these criteria two modeling methods were assessed on their capabilities: the FEM and the DEM. The DEM was selected and used in the derivation of a Mass-Spring-Damper (MSD) modeling framework. The MSD framework was proposed after identifying different analogies between cellular components and mechanical elements. A solution for nonlinear cellular mechanics was implemented in the stiffness characteristic of spring elements. A graph-theoretic notation for the MSD framework was introduced, and the mathematical equations were set up, using a vertex discretization. Finally, it discussed the potential and the limitations of the MSD framework.
The introduction (Chapter 1) presented the investigation of Segmental Groove Formation (SGF) in the ectoderm of the *Drosophila melanogaster* (fruitfly), as a case study to gain more insight into general epithelial morphogenesis. Investigating SGF is a complicated task. This chapter explains the biological background and interprets different biological hypotheses from an engineering perspective. This interpretation leads simulation scenarios which can be tested with a Mass-Spring-Damper (MSD) model, as will be done in Chapter 5.

The chapter is organized in five parts. Section 3-1 explains the embryonic developmental stages preceding SGF. Section 3-2 introduces the phenotype SGF itself. Section 3-3 mentions other biological phenotypes occurring simultaneously with SGF. Section 3-4 presents biological hypotheses from a mechanical engineering perspective, which have been derived in close collaboration with the biologists in the project team. Finally, Section 3-5 summarizes the chapter with concluding remarks.

### 3-1 Embryonic development of *Drosophila melanogaster*

This section gives a brief explanation of the developmental processes which precede the time span and phenotypes considered in the case study.

The embryonic development of *Drosophila melanogaster* has been subdivided into 17 stages, which are defined by prominent features that are easily distinguishable in the living *Drosophila melanogaster* embryo [9]. The timeline starts with a fertilized egg, with an average length of 500µm, and an average diameter of 180µm. The mature egg is enclosed by two envelopes, an inner homogeneous *vitelline membrane* and an outer *chorion*. When the chorion layer is removed one may directly observe certain aspects of the developmental processes through the transparent, structureless vitelline membrane, which is wrapped around protoplasmic contents (colorless plasma). Within just one day the fertilized egg develops from a single cell...
into a complete and autonomous organism consisting of many different organs. At first, a blastoderm is formed from the fertilized egg, forming a superficial layer of cells enclosing the yolk mass. During the blastoderm stages (stage 2 through 5), the approximately 6000 cells, which give rise to all somatic tissues, are located as a single layer epithelium at the surface of the embryo. Subsequently, three different germ layers are created through a process called gastrulation (stages 6 and 7). In inwards order, the three germ layers are the ectoderm, the mesoderm, and the endoderm. Consider Figure 3-1 for a cross-sectional view of the embryo after gastrulation.

In the following stages, the ectoderm differentiates into a number of distinguishable vital organs, such as the epithelium, the Central Nervous System (CNS), and the salivary glands. The mesoderm gives rise to all types of muscles, the fat body, the hemocytes (immune system cells), and the lymph glands. The two endoderm fractions represent the anlagen of the midgut, which gives rise to most of the intestines. The amnioserosa is located on the dorsal side of the embryo and represents an extraembryonic epithelium. It does not contribute to any embryonic tissue and is therefore not assigned to any of the germ layers.

Consider a three-dimensional model of the embryo in a three-dimensional Cartesian space.
(x,y,z). Figure 3-2 indicates the global dimensions and orientation used to point out the position of different features in the embryo, and view angles of image data.

In the next section, the discussion focuses on the development of the epithelium, which is part of the ectoderm. It explains SGF, which occurs during developmental stages 12, 13, and 14.

![Lateral view](image1)

(a) Lateral view

![Lateral view with indication of SGF related features](image2)

(b) Lateral view with indication of SGF related features

**Figure 3-3:** *Drosophila melanogaster* embryo during Segmental Groove Formation. Image taken from the Axelrod Lab.

### 3-2 Segmental Groove Formation in the epithelium of the *Drosophila melanogaster* embryo

While the previous section described the early developmental stages of the *Drosophila melanogaster* embryo, this section zooms in on *Segmental Groove Formation (SGF)*, i.e. the segmentation of the embryo through the formation of grooves in the epithelium.

Subsection 3-2-1 lists the important features in and around the epithelium that are relevant in the explanation of SGF. Subsection 3-2-4 briefly explains the timing of SGF.

#### 3-2-1 Important features in the epithelium

Consider Figure 3-3. Distinguish between the epithelium and the amnioserosa. The epithelium covers most of the embryo and is circumscribed by a yellow line, the amnioserosa covers the dorsal side of the embryo and is circumscribed by a white line. The border between the
epithelium and the amnioserosa is called the leading edge, denoted in magenta. The leading edge is a ring of epithelial cells, which exhibits special properties, which will be explained later on. From the leading edge down, the segmental groove consists of a single column of 16-19 groove cells, indicated by the red lines. A groove cell is defined by the fact that it invaginates, and that it expresses a number of important groove marker proteins. Subsection 3-2-2 describes the groove marker proteins expressed in groove cells.

The column of 16-19 groove cells ends in the groove slit, pictured in green. The groove slit is a pack of a dozen cells that invaginates first, at the start of SGF. Subsection 3-2-3 explains what is known about this phenotype. The ventral epithelium, between the groove slit and the ventral line of the embryo, consists of another dozen rows of cells. These cells are affected by SGF and invaginate depending on their dorsoventral position. By contrast, they are in the groove but not denoted as groove cells, since they do not express groove marker proteins [44].

**Figure 3-4:** Partial cross section of the embryo showing the form of the groove. The coloring matches that of Figure 3-3. The dotted yellow line indicates the outer radius of the embryo, the dashed red line indicates the form of the groove. The groove slit is located where the groove is deepest and indicated in green. Image taken from 3D confocal experiments at Axelrod Lab. The color code is: Green: FasIII, a marker of the lateral membrane of the epithelium at this stage of development. Red: Elav, a neuronal marker to target the CNS. Blue: aPKC an apical marker of epithelial cells. White: Over-expression of the Green Fluorescence Protein in the mesoderm.

### 3-2-2 Groove marker protein expressions

Before and throughout the formation of the segmental grooves, a set of molecules is enriched specifically in the groove cells. These are listed below and pictorially presented in Figure 3-5.

- Odd Skipped (Odd)
- Crumbs (Crb)
- atypical Protein Kinase C (aPKC)
In an early stage (stage 6: 2:50-3 h) the molecule *Odd Skipped (Odd)* is expressed in groove cell precursor.

**Figure 3-5:** Close-up of the groove with indication of groove marker protein expression. The image also resembles the ladder configuration that appears in the segmental grooves. Image gathered through [5].

At embryonic stage 12, the groove cells express the proteins *Crumbs (Crb)* and *atypical Protein Kinase C (aPKC)*. These are enriched at the apical circumference of the groove cells, when the epithelium starts folding. By over-expressing Crb to high levels in a stripe that goes all around the embryo from leading edge to leading edge, just anterior to the groove cells, a manipulated indentation can be made that continues ventrally [43]. This may indicate that Crb has influence on the mechanical properties of groove cells. aPKC collocates with Crb.

When the groove has been formed, at stage 13, the actin-binding molecule *Enabled (Ena)* is enriched at the cell junctions separating groove cells, whereas it is detected only at the vertices in non-groove cells [44].

Moreover, *Armadillo (Arm)* is gradually enriched somewhat later, and specifically in the horizontal cell boundaries between groove cells. Arm is the beta catenin that binds the cell adhesion molecule E-cadherin and anchors the actin cytoskeleton. E-cadherin complexes recruit the circumferential actin belts.

In conclusion, a strong association can be made between the different marker proteins observed in groove cells and the actin organization of groove cells. Especially the sharp variation in comparison with non-groove cells, outside of the grooves, makes these proteins worth studying in more detail. Determining the exact causal relations between the mechanical properties in the groove cells, and the expression of the groove markers, is one of the main challenges addressed in the overall research project.

### 3-2-3 Groove slit invagination

Each groove column consist of approximately 16-19 groove cells, starting at the leading edge and descending ventrally. At the ventral end of the groove column, a special pack of approx-
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imitarily a dozen cells is located, called the groove slit. Figure 3-6 shows a close-up of the groove around the ventral end, in which the groove slit cells are colored blue.

At the start of Segmental Groove Formation, the slits are the first areas of epithelial cells to start invaginate, i.e. to fold inwards. Subsequently, the rest of the groove column invaginates [43]. Figure 3-3(b) and Figure 3-4 depict the slit in green.

Little is known about the exact dynamics of the epithelium around the slit. It is likely that there exists a connection between the ectoderm and the mesoderm, which enables the mesoderm to exert pulling forces on the epithelium. At this point no experimental proof can be given to support this idea. Section 3-4 hypothesizes Mesodermal Forces (MF), which leads to the study of mesodermal interaction in a MSD model.

![Figure 3-6: Lateral close-up of the groove around the slit, showing different cell types. Image taken from the Axelrod lab.](image)

![Figure 3-7: Figure of a cross section around the anterior-posterior axis at a groove site. Showing the different cell types and features.](image)

3-2-4 Timing of Segmental Groove Formation

SGF starts at stage 12 (starting 440 min after egg laying). First the groove slit starts invaginating, which is explained in Subsection 3-2-3. Shortly after, Dorsal Closure (DC) starts, which is explained in Subsection 3-3-1. The groove starts to propagate from both ends of the column of groove cells, until the whole column has invaginated. At the end of stage 13 (starting 560 min after egg laying) the grooves are deepest and define the segments morphologically. Figure 3-4 shows a partial cross section of the embryo, indicating the form of the groove.
3-3 Processes occurring simultaneously with Segmental Groove Formation

In order to understand SGF sufficiently, this section briefly explains phenotypes, which occur simultaneously with SGF. This creates a necessary system level perspective of the whole problem. From this perspective biological hypotheses can be derived in Section 3-4.

The section discusses three phenotypes. Firstly, Subsection 3-3-1 explains DC. It discusses the mechanical effects on the epithelium and mentions the issue of timing. Secondly, Subsection 3-3-2 mentions germ band retraction, a wide rearrangement of epithelial tissue over the whole epithelium, that occurs just before SGF. Thirdly, Subsection 3-3-3 explains the development of the CNS and how it is located with respect to the epithelium.

3-3-1 Dorsal Closure

Dorsal Closure (DC) is a fusion of the epithelium on the dorsal side, which occurs through the bonding of two opposing epithelial sheets. Consider Figure 3-8. Before closure the dorsal side is covered by the amnioserosa, which is a squamous tissue (thin flattened cells). Through

![Figure 3-8: DC exerts a zipping force on the whole epithelium. The epithelium slides over the amnioserosa. Image taken from 3D confocal experiments at Axelrod Lab.](image)

DC the epithelium elongates in the dorsal direction, led by the most dorsal row of epithelial cells, called the leading edge. This is indicated by the magenta arrows in Figure 3-9 and Figure 3-8. Throughout the leading edge an actin-myosin cable is assembled, which nurtures filopodia and lamellipodia [28]. Filopodia and lamellipodia are machinery at the boundary of the cell which ignite the epithelium to slide over the amnioserosa. The finally exert a zipping procedure to close the two epithelial layers at the dorsal line, starting from the anterior and posterior end of the embryo. The zipping aspect refers to the fact that the fusion of both leading edges starts at the anterior and posterior ends and subsequently moves towards the center. In other words, the whole epithelium is pulled up dorsally by a force exerted at the leading edge.

Controversy exists regarding the timing of DC. Some sources claim DC starts at stage 13 [46]. Experimental observations suggest that DC starts earlier, i.e. in stage 12 [43]. Since the segmental grooves start forming in stage 12 as well, an important mechanical relation between these two phenotypes is hypothesized, as presented in Section 3-4. Figure 3-9 indicates the simultaneous occurrence of DC and SGF.
3-3-2 Germ band retraction

During germ band retraction, the posterior end of the embryo retracts to its final posterior position, causing significant movements of epithelium in the ectoderm. These large-scale movements of epithelial sheet precedes the process of DC (starting at stage 12). The epithelium that moves from the dorsal side to the posterior end (dashed red arrow in Figure 3-10(a)) pushes the epithelium there towards the anterior side (dotted white arrow in Figure 3-10(a)), and all the epithelium upwards towards the dorsal line (solid green arrows in Figure 3-10(a)). In the meantime the squamous amnioserosa adopts a new ellipsoidal configuration, as can be seen from both Figure 3-10(b). The cellular processes associated with germ band retraction have been well-characterized in the Drosophila melanogaster embryo. It is possible to knock down the processes governing germ band retraction. Experimental observations show that SGF still occurs, and hence, is not dependent on germ band retraction.

3-3-3 Development of the Central Nervous System

The Central Nervous System (CNS) is a double-cylindrical organ right under the ventral epithelium stretching longitudinally throughout the ventral line of the embryo, as depicted by the red tissue in Figure 3-4 and in the green cylinders in Figure 3-7.
As mentioned before, the groove does fully encircle the embryo, as there is no groove on the ventral side of the ectoderm. The CNS is thus not indented through Segmental Groove Formation, which is clarified in Figure 3-12. There are indications that the CNS mechanically interacts with the ectoderm, and that the mechanical properties of the ventral groove cells relate to the development of the CNS. Therefore, the role of the CNS is considered during the formulation of the hypotheses in Section 3-4.

3-4 Formulation of biological hypotheses

Based on the existing biological knowledge explained in Section 3-2, this section derives hypotheses on SGF. These hypotheses are interpreted from a mechanical engineering perspective. First, Subsection 3-4-1 derives the main hypothesis. Secondly, Subsection 3-4-2 distinguishes between different sub-hypotheses.

3-4-1 Main hypothesis

The ectoderm of the *Drosophila melanogaster* embryo forms grooves at each segment boundary during a precise developmental phase. While genetic analysis revealed that the patterning of these grooves relies on signaling mechanisms, 3D and time-lapse confocal analysis indicates that the morphogenesis of the grooves depends on mechanical forces applied to the epithelium. Moreover, recent studies on the cellular cytoskeleton conclude that the action of forces on cellular aggregates promotes local and temporal variation in mechanical properties. Preliminary analysis indicates that groove morphogenesis involves the fine cooperation of several mechanisms. From a mechanical perspective, this cooperation can be cut up in three mechanisms, which can explain SGF in one main hypotheses,

*All together it is hypothesized that pulling forces generated during the process of Dorsal Closure, and by the mesoderm through slit invagination, in combination with a non-uniformity of mechanical properties in the groove cells, lay the necessary basis for groove morphogenesis.*

1. **Dorsal Closure (DC):**
   At developmental stage 12, the onset of groove formation coincides with the initiation of the DC, where the epithelial layers situated on both sides of the embryo merge on the dorsal side, as indicated in magenta in Figure 3-12. It is claimed that the leading edge cells are pulled up towards the dorsal line. Filopodia and lamellipodia in the leading edge drive the actin belt upwards dorsally, as depicted in Figure 3-9 and Figure 3-8. This imposes a force on the whole epithelium, stretching it dorsally to establish DC with the epithelium on the other lateral side of the embryo, as shown by the magenta arrows in Figure 3-12 and Figure 3-8.

2. **Mesodermal Forces (MF):**
   Second, mutant analysis reveals that the underlying mesoderm is likely to come into contact with the deeper groove cells at the slit, just when these undergo morphogenesis, as indicated in red in Figure 3-12. Experimental observations show that the invagination of the groove starts specifically at the slit. There is no hard evidence yet to support that the mesoderm is pulling the slit cells.
3. Variation of actin organization in groove cell columns:

Thirdly, actin and myosin accumulations were observed along the grooves. There are two indicators for this claim.

(a) groove marker expression:
In each segmental groove a column of groove cells can be identified. In groove cells a number of protein marker expressions has been observed, which distinguish these cells from the rest of the epithelium, see Figure 3-5. Especially, the markers Ena and Arm are known to have an association with the actin organization in the structure of epithelial cells.

(b) actin staining experiments:
See Figure 3-13. This actin staining image shows an accumulation of actin along the groove cells.

![Figure 3-13](image-url)

Figure 3-13: Actin accumulation in grooves, depicted by green frames. Image taken from 3D confocal experiments at Axelrod Lab.

The actin accumulation along the groove cells can be interpreted as a local variation,
causing an increase of stiffness (elastic modulus) with respect to the cells outside of the grooves. This is indicated in Figure 3-12 by the use of thick red lines (groove cell column) and thin black lines (non-groove epithelium).

The higher stiffness in the cells of the groove column will cause a higher stress throughout the column, when perturbed by the above mentioned force mechanisms. The hypothesis claims that this local increase in stress forces the groove column to indent, this way forming the segmental groove.

Figure 3-11 and Figure 3-12 pictorially show how the groove cells indent, with respect to the outer radius of the embryo, and how the three mechanisms mechanically express themselves. The next section gives three additional hypotheses, which have implications for the modeling of SGF.

3-4-2 Other supporting hypotheses concerning Segmental Groove Formation

Ectodermal cells have a nonlinear and/or time-varying stiffness

Live imaging (and fixed embryos) has shown that at the onset of SGF the indentations are formed on both ends (dorsal most groove cell and ventral slit cells), and then converge on the lateral groove cells, through stiffening. Stiffening of cells can be explained in two ways:

1. The cells have a time-invariant nonlinear elasticity characteristic, which means: The more the cell stretches, the higher the stiffness. Time-invariant means that this characteristic is formed upfront and maintained throughout the biological events. This effect is mainly supported by insight from cellular and intracellular mechanics, in which a quadratic elasticity characteristic was found both on the cellular [45], as well as on the filament level [24].
2. The cells have the ability to respond to the mechanical stimuli, by changing their elasticity characteristic. A causal relation between the presence of mechanical stimuli and protein expressions related to actin organization or contraction (myosin activity) should be identified to prove this effect.

It is possible that both effects contribute to stiffening. In order to separate both, different experiments are formulated to isolate both factors.

From a signaling perspective, a distinction needs to be made regarding whether the expression dynamics of the different groove marker proteins are responsive to forces, or just timed by signaling processes. So do question becomes whether the groove marker proteins express in response to mechanical stimuli, or they are regulated independent of mechanical stimuli?

Germ band retraction is not a necessary phenotype for Segmental Groove Formation

Germ band retraction is widely seen as a necessary phenotype to facilitate Segmental Groove Formation (SGF). However, genetic manipulations have shown that segmental grooves still form when germ band retraction is knocked down [43]. Therefore germ band retraction is not considered a necessary phenotype for SGF in model simulations.
The Central Nervous System acts as a physical constraint in Segmental Groove Formation

The column of groove cells does not extend from the leading edge to the ventral line, but stops at the slit. Between the slit and the ventral line non-groove cells are identified, with corresponding mechanical properties.

The CNS is a double-cylindrical organ right under the ventral epithelium stretching longitudinally throughout the ventral line of the embryo. It exhibits a higher elasticity characteristic with respect to the rest of the inner body content. It is claimed that the CNS plays a key role in the mechanics of the epithelium during SGF, by acting as a physical constraint, causing the ventral epithelium to wrap around it at the groove sites, during the indentation caused by the different force mechanisms. This is pictorially shown in Figure 3-12.

There are two reasons to take the mechanical effects of the CNS into account. Firstly, experimental observations of the precise location of the different components (ectoderm, mesodermal muscles, and CNS) suggest that that the epithelium and the CNS interact. The second reason concerns the form of the groove. Normally the groove stops at the ventral epithelium, respecting the shape of the CNS, as clearly indicated in Figure 3-12. By over-activating the Jun Kinase pathway in the whole groove column, i.e. all the way around the whole embryo, a full groove was created, also indenting the CNS [43]. The Jun Kinase pathway is associated with stress accumulation in cytoskeletal actin bundles [5].

These observations suggest a relation between the mechanical properties of the ventral cells in the groove columns and the CNS. The CNS has to be modeled in order to understand the interaction between CNS and epithelium, and how this might affect Segmental Groove Formation.

3-5 Concluding remarks

This chapter introduced the case study on Segmental Groove Formation (SGF) in the embryonic development of Drosophila melanogaster, and derived accompanying biological hypotheses. The early developmental stages preceding SGF were briefly mentioned. Subsequently, SGF and other important developmental processes were discussed. This lead to the formulation of a main hypothesis and some supporting hypotheses, related to SGF. These hypotheses were derived in close collaboration with biologists, and were interpreted from an engineering point of view, respecting the biological challenges posed in Chapter 1. Section 3-4 presented initial support for the hypotheses and serves as the primary input for the formulation of simulation scenarios later on. In order to test these hypotheses, Chapter 4 first enrolls a procedure to develop three-dimensional synthetic models of the epithelium of the Drosophila melanogaster embryo.
This chapter explains the procedure necessary to advance from experimental 4D microscopy data to a three-dimensional Mass-Spring-Damper (MSD) model that resembles cellular geometry. The earlier MSD modeling efforts presented in Appendix A served as an inspiration for the development of this procedure. These efforts used a MSD modeling framework to build up regularly packed models with hexagonal cells.

To improve the accuracy of synthetic models, this chapter derives a procedure which incorporates experimental data to synthesize the geometry of actual irregularly packed cellular networks. Figure 4-1 indicates the important stages in this process, i.e. biological experiments, image processing, data structure design, and data-driven model building. Section 4-1 describes how the biological experiments work and what type of data these retrieve. Section 4-2 briefly explains the image processing software that is used to extract focused data, which is developed in the project team. Section 4-3 describes the data structure derived in this thesis.
to host the model building and simulation algorithms. Section 4-4 takes the extracted data and shows how a model is built up that resembles the geometry and mechanical structure of embryonic epithelium of the *Drosophila melanogaster* embryo. Finally, a model is ready for simulation to test hypotheses in Chapter 5.

![Diagram of an embryo showing the microscopy range](image)

**Figure 4-2:** Pictorial model of the embryo. The red dashed frame indicates the field covered by the microscope that allows for sufficient resolution of single cells.

### 4-1 Biological experiments

The experimental data that is used to build synthetic models is collected with *4D confocal microscopy*. 4D stands for measuring in three spatial dimensions \((x, y, z)\) and one time dimension \(t\). 4D confocal microscopy detects the fluorescence of targeted genes or proteins. This way expressions can be deduced and analyzed in real time. Green Fluorescence Protein is used to tag actin binding proteins. The fluorescence is measured in consecutive two-dimensional \((x, y)\)-planes, at different lateral depths \(z\), see Figure 4-2. The measurements at different depths \(z\) for one time interval \(\Delta t\) are saved together in a stack file (STK-file).

These stack files contain a great deal of information in space and time. This data is analyzed to filter the information needed for building a model. The STK-file is filtered with fluorescence software. The software goes through all images and collects pixels describing the circumferential actin belts in the network. As discussed in Section 2-1, these belts reflect the dynamics of the epithelium at the depth of the two-dimensional actin surface. After filtering the STK-file, the filtered pixels are gathered in a single two-dimensional projection, and saved as a bitmap file (BMP file). Figure 4-3 shows such a BMP file, composed using this method.

Considering the application in the study of Segmental Groove Formation (SGF), ideally the whole epithelium is captured and eventually modeled, during the whole developmental time span of Segmental Groove Formation. Two issues should be addressed to accomplish this goal. Firstly, the 4D confocal microscope range is limited by the necessary resolution to retain the cellular geometry with image processing algorithms. Secondly, the image processing software can process networks with up to an order of hundreds of vertices.
The covered three-dimensional range can be increased by a *spinning disc* technique, i.e. a mechanism to rotate the microscope around the measured object. See Figure 4-2 for a rough indication of the applied microscopy range. For more detail on the execution of the 4D confocal microscopy experiments, see [44].

Due to the limitations, the microscope focuses on one segmental groove and the surrounding epithelial network, as indicated in Figure 4-3. It tries to capture as many cells between the leading edge and the ventral line. As can be noticed from Figure 4-3, part of the ventral epithelium is still not covered. Hence, related issues to missing ventral epithelium have to be tackled, which is discussed later on in the simulation and analysis phase of Chapter 5.

![Figure 4-3: An image taken by 4D confocal microscopy with Green Fluorescence Protein detection. The red frame indicates the region of the network that is used for image processing to retrieve a data set to build a MSD model. Courtesy of the Axelrod Lab.](image)

### 4-2 Image processing

Earlier efforts in MSD modeling exploited regularly packed networks with hexagonal cells to model epithelial cellular networks. These models thus neglected the irregular packing observed in real cellular geometry. Appendix A briefly summarizes the results and recommendations that followed from the hexagonal MSD modeling efforts. Based on analysis of 4D confocal video data, the biologists suggest that cell shape and patterning play an important role in epithelial morphogenesis, also in SGF [43].

Hence, synthetic mechanistic models should be adapted to the cellular geometry, as contained in the 4D confocal microscopy data. In the project team, computer scientists developed image processing methods to extract this geometry. Subsection 4-2-1 explains the method, and Subsection 4-2-2 describes how the method is used in this MSc project.

#### 4-2-1 Dynamic model generator

Given a specific biological data set, the dynamic model generator method extracts features, and correlates them over time [36], in order to help the researcher to gain a quantitative understanding of the data set. The method consists of three stages, i.e. segmentation,
morphing, and registration. These three stages are briefly explained in the following of this section.

The segmentation stage extracts available information from the data about network elements and relations between elements, and builds a network structure. The morphing stage tries to understand how a given network deforms in time, due to the presence of exogenous forces and interactions between network elements (endogenous forces). The registration stage considers an ensemble of networks for different time steps and investigates the presence of correlations (over time) among their elements.

![Image of isolated cellular network](image)

**Figure 4-4**: The isolated cellular network used for image processing and model building. Courtesy of the Axelrod Lab.

![Straightened Graph vs. Raw Graph](image)

**Figure 4-5**: A processed version of the network depicted in Figure 4-4. The thin red graph is the raw processed version, the thick blue line is the straightened version of the red raw graph.

### 4-2-2 Application of image processing in this project

During the research period the segmentation algorithm has been applied (i.e. the first of three stages). Due to a combination of limited resolution in the 4D confocal microscopy data and the standing challenges to automize the image processing algorithms, the data could not be morphed and registered.

Nevertheless, the image processing software was used to segment individual frames. It was chosen to take a single microscopy image of before SGF takes place, and focus on the cellular network around a single groove, as indicated by the red frame in Figure 4-3. This network is isolated in Figure 4-4.

Applying the segmentation algorithms results in the thin red *graph*, depicted in Figure 4-5. This graph has identified the cell boundaries and cell junctions of the network. The graph structurally consists of straight line elements, called *edges*, connected in between *vertices*. Hence each cell boundary consists of multiple edges, to approximate its *curved* shape, and similarly in between the vertices representing cell junctions, multiple intermediate vertices are defined.
This data is fed to a data structure design, which is explained in the following.

<table>
<thead>
<tr>
<th>Entity</th>
<th>Dimension</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N^0$</td>
<td>$\mathbb{N}_0$</td>
<td>Number of vertices in network (including 4 artificial corner vertices)</td>
</tr>
<tr>
<td>$N^{bv}$</td>
<td>$\mathbb{N}$</td>
<td>Number of vertices at the border of the network</td>
</tr>
<tr>
<td>$N^e$</td>
<td>$\mathbb{N}$</td>
<td>Number of normal edges in network</td>
</tr>
<tr>
<td>$N^{e,int}$</td>
<td>$\mathbb{N}$</td>
<td>Number of intermediate elements in network</td>
</tr>
<tr>
<td>$N^{be}$</td>
<td>$\mathbb{N}$</td>
<td>Number of artificial border edges in network</td>
</tr>
<tr>
<td>$N^f$</td>
<td>$\mathbb{N}$</td>
<td>Number of faces in network</td>
</tr>
<tr>
<td>ID</td>
<td>$N_0^{N\times 1}$</td>
<td>Vector with vertex indices</td>
</tr>
<tr>
<td>$X,Y,Z$</td>
<td>$N\times 1$</td>
<td>Vectors with $(x,y,z)$ position information</td>
</tr>
<tr>
<td>EDGES</td>
<td>$N^e \times 2$</td>
<td>Matrix with pairs of vertex indices for all edges</td>
</tr>
<tr>
<td>EDGESint</td>
<td>$N^{e,int} \times 2$</td>
<td>Matrix with pairs of vertex indices for all intermediate elements</td>
</tr>
<tr>
<td>DEGREE</td>
<td>$N \times 1$</td>
<td>$\mathcal{D}(V)$, i.e. vector with the degrees of all vertices</td>
</tr>
<tr>
<td>pEDGE</td>
<td>$N \times \mathcal{D}(V)$</td>
<td>Matrix with pointers for each vertex to its adjacent edges (edge index is row number in EDGES)</td>
</tr>
<tr>
<td>BORDER</td>
<td>$N \times 1$</td>
<td>Vector with 1 for border vertices and 0 for other vertices</td>
</tr>
<tr>
<td>BORDERdir</td>
<td>$N^{bv} \times 2$</td>
<td>Matrix with left column containing indices of border vertices and right column containing orientation of the border vertex</td>
</tr>
<tr>
<td>FACES</td>
<td>$N^f \times \mathcal{D}(FACES)$</td>
<td>Matrix in which the $i$th row contains the vertex indices of face $f_i$</td>
</tr>
<tr>
<td>fDEGREE</td>
<td>$N^f \times 1$</td>
<td>$\mathcal{D}(FACES)$, i.e. the vector with the face degrees of all faces in FACES</td>
</tr>
<tr>
<td>FACEstr</td>
<td>$\sum_{f=1}^{N^f} {fDEGREE} \times 3$</td>
<td>Matrix with triangle elements, first two columns give pairs corresponding to the edge of a triangle, third column gives the face index</td>
</tr>
<tr>
<td>pFACE</td>
<td>$N \times \mathcal{D}(V)$</td>
<td>Matrix with pointers for each vertex to its adjacent faces (face index is row number in FACES)</td>
</tr>
<tr>
<td>pfEDGE</td>
<td>$N^f \times \mathcal{D}(FACES)$</td>
<td>Matrix with pointers for each face to its adjacent edges (edge index is row number in EDGES)</td>
</tr>
<tr>
<td>pVERTEX</td>
<td>$N \times \mathcal{D}(V)$</td>
<td>Matrix with pointers for each vertex $v_i$ to its adjacent vertices $\mathcal{N}(v_i)$</td>
</tr>
<tr>
<td>pTRIANGLE</td>
<td>$N \times 2 \max \mathcal{D}(V)$</td>
<td>Matrix with pointers for each vertex to its adjacent triangles (triangle index is row number in FACEstr)</td>
</tr>
<tr>
<td>pfTRIANGLE</td>
<td>$N^f \times \mathcal{D}(FACES)$</td>
<td>Matrix with pointers for each face to its adjacent triangles (triangle index is row number in FACEstr)</td>
</tr>
</tbody>
</table>
4-3 Data structure and algorithms

This section derives a new data structure to host modeling and simulations of irregularly packed cellular networks with the proposed MSD framework. This way any cellular network can be described and saved in terms of its cell junctions (vertices), cell boundaries (edges), and cell areas (faces).

Cell boundaries between pairs of cell junctions can be approximated by straight lines. Given a data set with the raw network (thin red graph in Figure 4-5), which was extracted with the image processing algorithm, a straightening algorithm is applied to prune this data set and filter all the cell junctions. It implements a straight boundary element between each pair of adjacent cell junctions. Hence, the resulting blue graph in Figure 4-5 is a straightened version of the overlying thin red graph. The straightened network is delivered as a graph $G = (V, E)$, i.e. a set $V$ with $N$ vertices, and a set $E$ with $N_e$ straight edges.

The following explains the data structure. Each vertex $v_i$, where $i \in \{1, 2, \ldots, N\}$ is stored with its index $i$ in a $N \times 1$ vector ID. The degree $D(v_i)$ of vertex $v_i$ is its number of adjacent vertices, and is stored in vector DEGREE. For each vertex $v_i$, the set of its adjacent vertices $\mathcal{N}(v_i)$ is stored in matrix pVERTEX. All the border vertices, i.e. vertices laying on the border of the network, are identified in the vector BORDER (1 for border vertices, 0 elsewhere). If the network is square, it is possible to include the orientation (dorsal, ventral, anterior, or posterior) of border vertices in the matrix BORDERdir.

Vertices are connected through edges. Each edge $e = \{v_i, v_j\}$ is stored in a $N_e \times 2$ matrix EDGES. The row number in matrix EDGES denotes the edge index. For each vertex pointers to all it is adjacent edges are stored in a row of matrix pEDGE.

Graph $G$ describes the full topology of the network, but does not contain all necessary information to build and simulate MSD models directly. In order to model all the different force profiles and mechanical properties, the simulation algorithm needs access to information about the faces of the network, and the identity of special elements (e.g. groove cell elements, slit elements, or border elements), etc. Hence, the data structure has to be extended through algorithms.

Firstly, the network can be cleaned from undesired vertices and edges. Secondly, one algorithm can add extra vertices in the corners of the network, and another can reconstruct the borders of the network by implementing extra border edges. Subsequently, a detection algorithm identifies all the faces $f$ of an individual graph $G$, based on the orientation and known adjacency of its vertices and its edges. This algorithm uses vector algebra to determine the orientation of vertices in a two-dimensional Euclidean space. See Algorithm 1 on page 47 and Figure 4-6 for a further algorithmic and pictorial explanation.

Each face $f$ is stored as a row in the matrix FACES with the set of vertices $\mathcal{N}(f)$ that it consists of. The face degree $D^f(f)$ is the number of vertices that a face consists of, and is stored in the vector pfDEGREE. Moreover, a data structure to determine a triangular mesh of the network is derived. If the barycenter of a face is known, the face can be split up into triangles. Each triangle consists of 2 adjacent vertices and the barycenter, which are stored in the matrix FACEStr. Finally, this algorithm also computes adjacency pointers between faces and edges (pfEDGE), vertices and faces (pFACE), vertices and triangles (pTRIANGLE), and faces and triangles (pfTRIANGLE).
Algorithm 1 Face detection algorithm for an individual graph $G$. See Figure 4-6 for a pictorial presentation of the algorithm.

**Input:** $X, Y, Z, ID, EDGES, DEGREE, BORDER, pVERTEX, bEDGES

**Initialization:**
Set a maximum face degree $D_{\text{f,max}}$
Number of found faces $N_f = 0$

**Main Loop:**
for $i = 1, 2, \ldots, N$ do
  Pick parent vertex $v^1 = v_i$.
  for $j \in \mathcal{N}(v^1)$ do
    Pick start vertex $v^2 = v_j$.
    for $p \in \mathcal{N}(v^1) \{v^2\}$ do
      Determine target vertex $v_{\text{target}}$. This is the vertex with the smallest negative angle $(\bigcirc)$ between the edge $\{v^1, v^2\}$ and the edge $\{v^1, v^p\}$.
    end for
  end for
  for $k = 2, \ldots, D_{\text{f,max}}$ do
    Pick current vertex $v^k$.
    for $l \in \mathcal{N}(v^k) \{v^{k-1}\}$ do
      Call the adjacent vertex $v^{(k,l)}$.
      Determine the angle between the edge $\{v^{k-1}, v^k\}$ and the edge $\{v^k, v^{(k,l)}\}$.
      Save angle in vector $\vec{\phi}(k)$, together with the vertex index of $v^{(k,l)}$.
    end for
    The smallest positive angle $(\bigcirc)$ in $\vec{\phi}$ determines the next vertex in the cycle $v^{k+1}$.
    if target vertex $v_{\text{target}}$ is adjacent to next vertex $v^{k+1}$ then
      Face $f = [v^1 \ v^2 \ v^3 \ \cdots \ v^{k+1} \ v_{\text{target}}]$ is detected, denoted by the orange arrow hitting the red circle in Figure 4-6.
      Check face $f$ with previously found faces in FACES.
      if $f \not\in$ FACES. New face found then
        Update number of found faces $N_f = N_f + 1$.
        Save $f$ as a face in face matrix as FACES($N_f,:)$.
      end if
    end if
  end for
  BREAK for loop $k$. Go to next adjacent start vertex $v_j$ of parent vertex $v^1 = v_i$ and start new face search.
end for

**Output:** FACES, fDEGREE, pFACE, pEDGE (See Table 4-1)

The resulting data structure is flexible for mechanistic modeling of MSD networks, and gives the possibility to change element parameters and assign forces per cell or per group of cells.

Table 4-1 gives all the entities of the data structure, with a clear description of their dimension and meaning.
4-4 Model building and calibration

Section 4-3 extracted all the necessary information from a 4D confocal microscopy image, to build and simulate a model of the embryonic epithelium around a single groove. This section describes the last five stages to build a model that is ready for simulation.

Firstly, Subsection 4-4-1 presents the three-dimensional folding of the network. Secondly, Subsection 4-4-2 implements the Central Nervous System (CNS) in the model. Thirdly, Subsection 4-4-3 lists the model parameters and explains how to assign values. Fourthly, Subsection 4-4-4 explains how boundary conditions can be imposed on the boundaries of the network. Finally, Subsection 4-4-5 implements different force mechanisms that were derived from the biological hypotheses.

4-4-1 Folding to three dimensions

The processed network represents the epithelial area around one of the eventual grooves in the embryo, but before SGF occurs, as depicted in Figure 4-7. Now that the data structure is available, the MSD modeling framework can be imposed to build a mechanical model of the
network. This subsection describes the algorithm which takes the processed two-dimensional network, assumes the body to have a cylindrical shape, and builds up the model in three dimensions. This algorithm consists of three important stages: calibration, back projection, and folding. Figure 4-8 and Figure 4-9 depict these stages in respectively two and three dimensions, and the following elaborates on them. The enumeration in the text matches the enumeration in Figure 4-8.

1. *calibration*:
   Using the microscopy settings and orientation, the processed network is calibrated with respect to a cylindrical body resembling the form of the embryo. The radius of the embryo is a fixed parameter, which is set as $R^{body} = 100\mu m$. Figure 4-8 shows the processed network in red and the cylindrical embryo in black. The calibration thus shifts and rotates the network such that the two-dimensional projection matches the area on the cylinder (indicated by the dashed grey lines).

![Figure 4-8: A pictorial longitudinal view of the embryo with indication of: 1. calibration, 2. back projection, and 3. folding procedure, to build a three-dimensional model of the epithelium.](image)

2. *back projection*:
   The red two-dimensional projection is not a one-on-one projection of the three-dimensional cylindrical epithelium. The cylindrical shape has to be back projected in the calibrated network, indicated in magenta in Figure 4-8 and Figure 4-9. This means the networks stretches out. Notice that the stretching is not uniform along the arc of the cylindrical surface. Further away from the longitudinal center line, the red network is more slanted, which means it has to be compensated more.

3. *folding*:
   Finally, with the actual network dimensions, the algorithm can project the magenta network on a cylinder with radius $R^{body}$. The result is a three-dimensional model of the epithelium around one of the later developing grooves, indicated in blue in Figure 4-8 and Figure 4-9.

### 4-4-2 Interaction with the Central Nervous System

One of the various forces experienced by the epidermal cells comes from the developing Central Nervous System (CNS). The ventral epithelium closely wraps around the CNS, as depicted in Figure 3-12. It is therefore stated that the epithelium experiences the CNS as a physical...
constraint. This subsection explains the mathematical formulation and implementation of this constraint.

Relatively little is known about the mechanical characteristics of the CNS [5]. Experiments tell that it is well positioned in the embryo and significantly stiffer with respect to the epithelium and other surrounding tissue [43]. Modeling the CNS as a hard physical constraint (blue in Figure 4-10) is therefore biologically unrealistic (organs are not infinitely stiff) and practically undesired (causes stiff equations and thus computational problems).

Consider Figure 4-10 and Figure 4-11. The CNS is represented by the green lined cylinders.

---

**Figure 4-9:** A three-dimensional view of the folding procedure. From left to right: the red network is 2D projection taken from microscopy data, the magenta network is stretched network compensated for curvature in the embryo, and the blue network is folded in 3D on a cylinder with the radius of the embryo.

**Figure 4-10:** Three-dimensional model of the epithelium of the embryo around one groove. The green cylinders denote the CNS. Notice that part of the ventral epithelium has not been captured by the microscope and hence has not been modeled.
The radius of the CNS, $R_{\text{CNS}}$, is approximated from cross section experiments (recall Figure 3-4) at 1/5 of the radius of the embryo, i.e. $R_{\text{CNS}} \approx \frac{R_{\text{body}}}{5} = 20\mu m$. The CNS is in direct contact with the ventral epithelium.

The constraint is implemented as a force vector. The constraint models an interaction in the radial direction of the CNS, i.e. normal to the CNS cylinder. The radius of the cylinder is $R_{\text{CNS}}$ and the distance vector from the axis of the CNS to a particular mass is $\vec{r}_i$. Ideally the CNS only acts on mass elements which are in contact. Once contact is made, the force depends on how far the epithelium indents the CNS. The implementation uses a polynomial formulation, with which the mechanical characteristic of the CNS can be tuned with one parameter $u$, i.e.

$$F^{\text{CNS}}_i(\vec{r}_i) = \begin{cases} \left( \frac{||\vec{r}_i|| - 2R_{\text{CNS}}}{R_{\text{CNS}}} \right)^2 u \frac{\vec{r}_i}{||\vec{r}_i||}, & \text{for } ||\vec{r}_i|| < 2R_{\text{CNS}} \\ 0, & \text{otherwise,} \end{cases}$$

in which $u \in \mathbb{N}$. Greater values of $u$ bring the polynomial closer to the hard discrete constraint, as depicted in Figure 4-12. This way the interaction of the epithelium with the CNS can be tuned, and instability of computations, due to stiff equations, can be prevented. During the simulations $u$ is selected once and left the same throughout the hypotheses testing. The CNS interactions is best resembled with $u = 13$.

Figure 4-11: A longitudinal view of the three-dimensional model. The amnioserosa and DC are indicated in dashed magenta, the CNS constraint in solid green, and the missing ventral epithelium in dotted red. The two plus signs indicate the dorsal(magenta) and ventral(red) line.

Figure 4-12 shows the magnitude of $F^{\text{CNS}}$ as a function of $r$ in case of a hard constraint.

This method of dealing with the CNS differs greatly from the method used in the hexagonal simulator, described in Appendix A. In this previous simulator, the position of an entering point mass was changed artificially to place it outside the CNS, without imposing any additional force. The polynomial constraint enables the implementation of a continuous force...
Figure 4-12: Three different force magnitude profiles for CNS force \( F_{CNS}^i(\vec{r}_i) \): hard discrete constraint (blue solid), polynomial constraint with \( u = 5 \) (green dashed), and idem but \( u = 13 \) (magenta dotted).

The force profile \( F_{CNS}^i \) acting on all mass elements, which is a cleaner, more realistic approach to study the effects of the CNS.

### Table 4-2: Model parameters.

<table>
<thead>
<tr>
<th>Entity</th>
<th>Dimension</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( M )</td>
<td>( N \times 1 )</td>
<td>Mass matrix with masses of all ( N ) point mass elements on diagonal</td>
</tr>
<tr>
<td>( \vec{k}^0 )</td>
<td>( N^e \times 1 )</td>
<td>Vector with constant stiffness parameters for all boundary spring-damper elements.</td>
</tr>
<tr>
<td>( \vec{k}^1 )</td>
<td>( N^e \times 1 )</td>
<td>Vector with affine stiffness parameters (nonlinear elasticity) for all boundary spring-damper elements.</td>
</tr>
<tr>
<td>( \vec{\nu} )</td>
<td>( N^e \times 1 )</td>
<td>Vector with damping parameters for all boundary spring-damper elements.</td>
</tr>
<tr>
<td>( \vec{d} )</td>
<td>( N^e \times 1 )</td>
<td>Vector with resting length parameters for all boundary spring-damper elements.</td>
</tr>
<tr>
<td>( \vec{k}^{0,\text{int}} )</td>
<td>( N^{\text{int.e}} \times 1 )</td>
<td>Vector with constant stiffness parameters for all intermediate spring-damper elements.</td>
</tr>
<tr>
<td>( \vec{k}^{1,\text{int}} )</td>
<td>( N^{\text{int.e}} \times 1 )</td>
<td>Vector with affine stiffness parameters (nonlinear elasticity) for all intermediate spring-damper elements.</td>
</tr>
<tr>
<td>( \vec{\nu}^{\text{int}} )</td>
<td>( N^{\text{int.e}} \times 1 )</td>
<td>Vector with damping parameters for all intermediate spring-damper elements.</td>
</tr>
<tr>
<td>( \vec{d}^{\text{int}} )</td>
<td>( N^{\text{int.e}} \times 1 )</td>
<td>Vector with resting length parameters for all intermediate spring-damper elements.</td>
</tr>
<tr>
<td>( \vec{\lambda} )</td>
<td>( N \times 1 )</td>
<td>Vector with friction parameter for all ( N ) point masses.</td>
</tr>
</tbody>
</table>
4-4-3 Assigning model parameters

Recall the dynamical equation in Eq. (2-5). Now that the cell shape and embryo geometry of the model is set up properly, parameter values can be assigned to the mass-, spring-, and damper-elements. Table 4-2 gives the parameters which were gathered in the model derivation, as they are implemented in the model building algorithm.

The implementation of model parameters is twofold. The first step is to assign the same parameter value for all similar elements. The second step is to vary the parameter value for specific areas in the network. The algorithm offers the possibility to identify the groove cells (red in Figure 4-13), the slit cells (in yellow), and cells on all the boundaries. The data structure enables the user to adjust the different accompanying elements. For instance, the stiffness in the boundary elements of the groove cells will be adjusted to test a specific hypothesis in Chapter 5. Similarly, for each cell category the model parameters can be adjusted according to a desired simulation plan or hypothesis.

Model parameters for specific elements or areas can be adjusted accordingly. For instance, in Chapter 5 the stiffness of all the spring elements in groove cells is varied with respect to the rest of the network with a factor \( r_{NUS} \) (Non-Uniform Stiffness (NUS)), as \( \frac{k_{groove}(l)}{k(l)} = r_{NUS} \).

4-4-4 Boundary conditions

The considered network has a border, that consists of four sides, all denoted with their orientation (dorsal, ventral, anterior, posterior). The leading edge vertices (dorsal) will be
imposed with a DC force profile in Subsection 4-4-5. The other three sides (ventral, anterior, and posterior) are treated with boundary conditions.

The ventral vertices are the most ventral vertices of the network. Most ideally, these vertices would lay on the ventral line (depicted by the red plus sign in Figure 4-11 and Figure 4-15). Assuming symmetry between both halves of the embryo (as shown in Figure 4-14), the ventral vertices could be constrained to only move along the ventral line, i.e. in $x$-direction. Unfortunately, a few rows of ventral cells cannot be modeled, due to the limited range of the microscope. This missing ventral epithelium is indicated by the red dashed line in Figure 4-11. Nevertheless, the same boundary condition is imposed on the most ventral vertices in the network. This means that the dynamics in the $y$- and $z$-direction are set to zero, and these vertices are only allowed to move in the $x$-direction.

The boundary conditions for anterior and posterior sides can be set in four different ways.

1. **No conditions, free movement.** In this case the reaction forces along these boundaries are neglected.

2. **Border edges with higher stiffness.** This is an artificial way to prevent vertices from diverging in the radial direction of the embryo. This problem was encountered and
explained by the pressure reaction forces (explained in Section 4-4-5). These forces push the epithelium in radial direction.

3. Constrain acceleration in radial direction, normal to the epithelium (cylindrical constraint). Theoretically this is the cleanest method found to impose proper boundary conditions. This method projects the acceleration on the tangential and normal axis of the cylindrical surface. Then the normal projection is zeroed, and the tangential projection is transformed to the cartesian space.

4. Impose a trajectory (if known) on the vertices. When trajectory data is available on the boundary conditions, this could be imposed, to constrain the vertices. This way reaction forces do not need to be derived. A downside is that this boundary condition rules out endogenous properties of the boundary elements, which this way can not be estimated from simulations.

In the eventual simulations method 1 and 3 are the preferred ones. These do not introduce any artificial dynamics, and show the most preferable behavior on a network level. Both methods have a down side, regarding the dynamics on cellular level. They show collapse of side border cells in longitudinal direction, since they do not impose any conditions on the dynamics in $x$-direction.

![Figure 4-15](image.png)

**Figure 4-15:** MSD model of a cellular network on one half of the embryo. Magenta dots represent leading edge vertices, magenta plus line represents dorsal line, red plus line represent ventral line, green cylinders represent Central Nervous System, black dots and red faces represent groove cells, black arrows and yellow faces represent slit cells, red circles represent side boundary vertices, red circles with black crosses represent ventral border vertices.

### 4-4-5 Force mechanisms

This subsection explains three different force mechanisms, built in the simulator: Dorsal Closure (DC), Mesodermal Forces (MF), and reaction forces.
1. **Dorsal Closure (DC) forces:**

   DC is driven by the leading edge cells, most dorsally in the epithelium, as discussed in Subsection 3-3-1. The leading edge cells are propelled locally, travel over the cylindrical surface up to the dorsal line, and propagate this movement through the rest of the epithelium. See Figure 4-11 for a pictorial cross-section.

   In order to implement this phenomenon in the model, three different solutions were considered, one force driven and two trajectory driven solutions. All three solutions are imposed on the boundary vertices of the leading edge cells. These vertices are depicted with magenta dots in Figure 4-15.

   (a) **Manual force-driven DC:**

   This method implements a constant force tangentially to the epithelium. Big disadvantage is that the epithelium does not necessarily follow the cylindrical surface, and drifts off.

   To impose a force, one needs to deal with two properties: the magnitude and the direction. Due to limited data, only the final position of the leading edge vertices can be retrieved. When the leading edge trajectory would be known, an Model Predictive Controller, could be an alternative to govern the force input. Moreover, it has been proposed to implement an extra controller to control the radial distance of the leading edge cells, with respect to the center line of the cylindrical embryo, to keep the vertices on the cylindrical surface.

   (b) **Automatic DC profile, \( C^2 \) trajectory without acceleration/jerk peaks:**

   This method simulates DC by imposing a \( C^2 \) continuous trajectory on all leading edge vertices, between their initial and final configuration. The trajectory is only imposed on the dynamics in \( y \)- and \( z \)-direction, the \( x \)-direction is left undisturbed.

   The \( C^2 \) trajectory can be represented as a second-order polynomial in velocity. The second-order \( C^2 \) trajectory provides complete flexibility in the control of profiles for smoothing motion and eliminating jerk from mechanical systems [41]. This principle is applied to the magenta leading edge vertices in the following way:

---

**Figure 4-16:** \( C^2 \) trajectory imposed on the leading edge vertices. Top down displacement profile \( p^{LE}(t) \), velocity profile \( v^{LE}(t) \), and acceleration profile \( a^{LE}(t) \).
i. It is assumed the leading edge vertices will travel over a cylindrical surface from initial condition to the final dorsal position.

ii. For each vertex the difference $p$ between final and initial position on the cylindrical surface is calculated.

iii. The shape of the $C^2$ trajectory is set as:
   - Set a time span $[0,t_{DC}]$ for DC to take place
   - Accelerate symmetrically to steady velocity $v^{LE, steady}$ at 15% of $t_{DC}$
   - Travel at steady velocity for 70% of $t_{DC}$
   - Decelerate symmetrically to zero velocity in the last 15% of $t_{DC}$
   - Arrive at final position.

iv. From these settings the velocity profile $v^{LE}(t)$ and acceleration profile $a^{LE}(t)$ are derived and imposed as boundary conditions in the simulation.

The $C^2$ trajectory and the velocity and acceleration profiles are depicted in Figure 4-16. Notice that indeed there are no sudden jumps in the acceleration, thus no jerk peaks.

(c) automatic DC profile, constant velocity $v^{LE}(t)$:
   This is a variant of method 2, using a constant velocity profile. The downside of this method is the existence of jerk peaks (jumps in acceleration), which slow down computations and cause instability in the model. Therefore the method is briefly mentioned, but not used in simulations.

Note that all methods introduce exogenous dynamics. For the sake of simplicity the DC settings are set initially and left the same for all simulations. When higher sampled data becomes available, it will be possible to extract the leading edge trajectories from the data itself, and impose these on the leading edge vertices.

2. Mesodermal Forces (MF):
   To resemble slit invagination (see Subsection 3-2-3), the epithelium is perturbed at the slit. Since little is known about the connection to the mesoderm, the timing, magnitude, and direction of the mesodermal force are set with assumptions, as described in the following.

   (a) Identify the slit cells and label them as discussed in Subsection 4-4-3
   (b) Assume a constant force magnitude $||F^{MSD}||$
   (c) Assume a set force direction, i.e. the forces act towards the center line of the embryo
   (d) Impose the force $F^{MSD}$ on the slit cells

The timing of the MF can be set and analyzed. Figure 4-15 and Figure 4-17 clearly depict the MF with thin black arrows, perturbing the yellow slit cells.

3. reaction forces
   The inner body of the embryo can be seen as a fluid which exerts a pressure force on the ectoderm, whenever there is a pressure difference between the interior and exterior. This phenomenon can be modeled using the Laplace-Young law [32].
Assume the embryo body to be a cylindrical vessel, as depicted in Figure 4-18. The larger the cylindrical radius $R_{\text{body}}$, the larger the wall tension $T$ (dashed red arrows in Figure 4-18) required to withstand a given pressure difference $\Delta p$ (solid cyan arrows in Figure 4-18) over the wall. This property can be derived from the Laplace-Young equation. It relates the pressure difference to the shape of the surface, and it is fundamentally important in the study of static capillary surfaces and cell membrane mechanics. It is a statement of normal stress balance for static fluids meeting at an interface, where the interface is treated as a surface. For an ellipsoidal body with radii $R_1$ and $R_2$, this relation is $\Delta p = T \left( \frac{1}{R_1} + \frac{1}{R_2} \right)$. For a cylindrical surface with radius $R_{\text{body}}$ this equation turns into

$$\Delta p = \frac{T}{R_{\text{body}}}.$$ 

The algorithm implements this phenomenon over the whole network, as:

(a) assume a constant pressure difference $\Delta p$

(b) triangularize the network and compute the area $A^{\text{tr}}$ and normal $\vec{n}^{\text{tr}}$ direction for all triangles

(c) compute the reaction force per triangle, i.e $F_{\text{RF, tr}}^{\text{tr}} = \Delta p A^{\text{tr}} \vec{n}^{\text{tr}}$

(d) for each triangle $j$ split out $F_{\text{RF, trj}}^{\text{trj}}$ over the two vertices on the cell boundary side of the triangle

(e) add up all the reaction forces for all vertices, and implement the force in the dynamical integration. For one vertex $v_i$, the reaction force is computed as $F_{i}^{\text{RF, tr}} =$
Figure 4-18: The Laplace-Young Law relates the pressure difference over a surface $\Delta p$ to the surface tension $T$.

$$\sum_{j \in N_{\text{adj}}(v_i)} 0.5 F_{RF,1rj},$$

where $N_{\text{adj}}(v_i)$ denotes the set of triangles adjacent to vertex $v_i$. The pressure $\Delta p$ is implemented as an extra input parameter. For each time step the reaction forces are computed, depending on the new configuration. The reaction forces are illustrated by the small cyan arrows in Figure 4-17. Notice that the arrows are not all the same length, which denotes the difference in area per vertex of its adjacent triangles.

4-4-6 Implementation and reproduction of simulations

Simulations are run in MATLAB, on a personal computer with a Pentium 2.66 GHz Intel Core 2 Duo processor and 4 GB of memory at 1067 MHz DDR3. The MSD model in Figure 4-14 is used throughout all simulations. The Runge-Kutta 4.5 method is used to numerically integrate the dynamics [17]. This model has 253 vertices and thus 253 Ordinary Differential Equations (ODEs). With all the force mechanisms explained in this chapter activated, the average integration time for a time horizon $t \in [0, 1]$, is 9.3 minutes. Due to the nonlinearities in the equations, the scalability on the mentioned system is limited. One bigger data set with 1505 vertices has been tested as comparison. The execution of a similar simulation on the same system took approximately 10 hours.

Results are saved as Visualization ToolKit (VTK) files and exported to the Paraview software environment [25]. In order to save and export the simulation results, three algorithms have been developed to represent edges, faces, and triangles. These algorithms use the data structure and time trajectory data, to save a single VTK file for each time step, for all elements of one type. This way a VTK file contains all the spatial and temporal information of a simulation.

Paraview is an open-source software system for 3D computer graphics, image processing and visualization. It can import VTK files and compile them. It provides an interface which makes 3D and temporal analysis easy and gives the ability to adjust colors and lighting to enhance analysis. Finally, it can store screenshots and animations for static analysis and representation.
4-5 Concluding remarks

This chapter presented a consecutive procedure to use 4D confocal microscopy data of embryonic epithelium of *Drosophila melanogaster*, and built up a mechanical model that resembles the exact same epithelium. The experimental procedure based on microscopy was explained. A model-driven image processing algorithm was discussed, and its application in the procedure was mentioned. It was shown that this image processing algorithm is able to extract the cellular geometry of a two-dimensional microscopy image. Taking this extracted data, a data structure was built to represent the epithelial network according to the Mass-Spring-Damper (MSD) modeling framework. Through three steps the two-dimensional network was calibrated, back projected and folded, ending up with a three-dimensional model of the epithelium. The assignment of model parameters, constraints, boundary conditions and force mechanisms were explained. Finally, an elegant method to reproduce and analyze simulation results was presented. This chapter gave all the tools to do simulations and analysis in Chapter 5.
Figure 4-19: Result of a Dorsal Closure simulation imported in Paraview: (a) Initial and (b) final frame.
Chapter 5

Simulations and Analysis

This chapter presents simulation results for the model of the epithelium of the *Drosophila melanogaster* embryo. The overarching goal of these simulations is to gain insight into the qualitative characteristics, as observed in the dynamics of the epithelium during Segmental Groove Formation (SGF). Hence, Section 5-1 introduces the analysis of the both the model as well as the real system. It lists ten qualitative characteristics, which serve as measure to assess the capabilities of the model.

Subsequently, two specific aims are addressed that relate the overarching goal. The first aim is to gain insight in the physical meaning of model parameters. Since the model is a mechanistic equivalent that resembles the epithelial cellular geometry and structure, each parameter group has a specific identity and contribution to the observed qualitative dynamics. Appendix B presents an elaborate version of the parameter selection process. Section 5-2 gathers the most important observations done during the parameter selection, which are necessary for the later simulations.

The second aim is to test different biological hypotheses concerning SGF, the studied phenotype in the development of the epithelium. This is done in three steps. Firstly, Section 5-3
formulates different simulation scenarios, based on the main biological hypothesis, which was derived earlier in Section 3-4. Secondly, Section 5-4 assesses all simulation scenarios based on a subset of the qualitative characteristics. Thirdly, Section 5-5 reports important observations and formulates specific suggestions for future research, based on all ten qualitative characteristics.

In conclusion, Section 5-6 summarizes this chapter.

5-1 Introduction to system analysis

The Mass-Spring-Damper (MSD) model of a single-layered epithelial cellular network, as built up in Chapter 4, represents a high level abstraction of the physical system under consideration. Nevertheless, the model still has a complex structure, which is not directly amenable to analyze. Moreover, the studied phenotype SGF is affected and built up by many different mechanisms and physical components. These aspects make the intended simulations and analyses inherently complex tasks.

Hence, Subsection 5-1-1 describes model properties, and motivates an appropriate analysis method. Subsequently, Subsection 5-1-2 derives qualitative characteristics, which relate to SGF. These characteristics will be used later on to assess simulations and parameter selection.

5-1-1 Model properties

Recall the mathematical formulation of the MSD model in Eq. (2-5). The model has three important properties. The models are:

- **high-dimensional:**
  Each cell vertex, i.e. each point mass, has its own three second-order equations, one for each dimension. The networks under consideration have in the order of 250 vertices, meaning a model with in the order of 1500 state variables (3 dimension × 2 state variables × 250 vertices).

- **nonlinear in state variables:**
  The Euclidean distances between all adjacent vertices is necessary to compute the length of springs, to compute elastic forces. Also the time derivative of the spring length is necessary, to compute the damping forces. Moreover the elastic force $F_{\text{elastic}}$ can be defined as quadratically proportional with the length of the spring. In conclusion, this model is nonlinear and cannot be analyzed using linear techniques.

- **linear in parameters:**
  This is an advantage. Assuming that the resting lengths $d_{ij}$ are known, all other parameters appear linear in the equations making this model amenable for parameter selection and also different estimation techniques. Proper parameter estimation does rely on data, containing persistent excitation of the system dynamics.

The high-dimensionality and nonlinearities of the model restrict its analysis. Since at this point, no focused temporal data is available, data-driven parameter estimation could easily
results in over-fitting, or local minima. Such results do not work to explain behavior found in other experimental data, neither it is likely that they provide insight in the mechanistic meaning of model parameters.

Hence, it is chosen to do manual parameter selection, with the primary aim to understand the effect of parameters on the model dynamics and characteristics, as explained in the next subsection. Quantitative parameter estimation is intended as a future avenue, i.e. outside the scope of this thesis. Appendix C investigates the Adjoint-Based Parameter Estimation (ABPE) technique as an appropriate method to consider, which is motivated in Chapter 6.

5-1-2 Observed qualitative characteristics in Segmental Groove Formation

Based on the biological knowledge and experimental observations, this subsection gathers observed qualitative characteristics that explain the epithelial morphogenesis of SGF. These characteristics will be used to assess the performance of simulations.

Table 5-1 lists ten different characteristics. The first five are used to select parameter values for all model elements in the network in Section 5-2 and Appendix B. The bottom five characteristics relate to the biological hypotheses, and serve as means to test these in Section 5-4.

<table>
<thead>
<tr>
<th>Qualitative characteristics in SGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>E</td>
</tr>
<tr>
<td>F</td>
</tr>
<tr>
<td>G</td>
</tr>
<tr>
<td>H</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>J</td>
</tr>
</tbody>
</table>

5-2 Parameter selection

This section collects the most important observations from a parameter selection procedure. This procedure investigates the physical meaning of model parameters. Appendix B gives an extensive elaboration on the procedure, and lists all the observations.

Constant stiffness $k^0$ (linear elasticity):

- Stiffness in the range $k^0 \in [200, 300]$ results in best propagation of DC. The ventral epithelium shows insufficient propagation
- With only linear elasticity, dorsal cells either elongate too much, or squeeze in x-direction

Friction $\lambda$:
- Friction in the range $\lambda \in [5, 10]$ results in a smoother network trajectory, and thus in better cell shape stability

Damping $\nu$:
- Without damping dorsal cells deform unrealistically during acceleration and deceleration, i.e. damping contributes to cell shape stability
- Damping in the range $\nu \in [5, 10]$ gives most realistic behavior

Reaction force pressure difference $\Delta p$:
- The pressure difference in the range $\Delta p \in [0.1, 0.4]$ contributes to the preservation of the volume of the embryo body
- The cylindrical shape is slightly distorted by pressure difference $\Delta p$. Reason might be the calibration of the epithelium, or the neglected role of applied (pre)stress,

  Suggestion: Incorporate prestress in the model. This has implications for the boundary conditions, since these need to bear extra loads. To properly implement this idea, one should incorporate prestress information from biological experiments. This is possible by deducing elasticity and prestress from laser ablation experiments [21].

Constant stiffness of intermediate elements $k^{0,\text{int}}$:
- Without stiffness of intermediate elements, cells squeeze due to overstretching in dorsal-ventral direction.
- The stiffness ratio range $r^K = \frac{k^{0,\text{int}}}{k^0} \in [50, 100]$ gives realistic results, showing cell shape stability,

  Suggestion: This suggests that the circumferential actin belts sustain most of the propagation loads in the epithelium, with respect to the intermediate cytoskeleton. Can we show this experimentally by laser ablation of these belts?

Nonlinear elasticity in boundary elements $k(l) = k^1 \frac{(l-d)}{d} + k^0$, where $k^0$ is the nominal stiffness and $k^1$ adapts the stiffness due to applied (pre)stress:
- By selecting the nominal and nonlinear contribution to the stiffness coefficient $k(l)$, the linear regime of the elastic force can be set, as shown in Figure 2-4. A proper selection allows the model to have sufficient capacity to strain the network in the linear regime (range $k^0 \in [80, 150]$), before the nonlinear regime takes over to govern cell elongation and propagation by introducing cell stiffening (range $k^1 \in [1800, 2000]$)
• DC is propagated throughout the whole network. This means that the epithelium can deform to stretch over the cylindrical surface. This effect solves the rigid body rotation issue, which was encountered with (too high) linear elasticity.

• Studying the local effects of nonlinear elasticity, that is on cellular level, was a complex task to do on a larger network, 
  **Suggestion:** Apply the MSD modeling framework to a smaller epithelial structure to study more local phenotypes and gain more insight into the exact nonlinear elasticity characteristics of epithelial cells and structures.

• Nonlinear elasticity caused smaller cells with less than five vertices to collapse. This is likely due to the structure of intermediate elements. These are built up in between non-adjacent vertices within a cell. Cells built up from more vertices thus have more intermediate elements,
  **Suggestion:** reconsider the model structure of intermediate elements.

• The leading edge cells still deform in an unrealistic way. This is primarily due to the introduced artificial dynamics by the imposed DC profile on the leading edge vertices. But on top of that the leading edge cells might also have different mechanical properties, which is supported by observations of actin accumulation and myosin activity [43]. Therefore two suggestions are done,
  **Suggestion:** trace the DC trajectories of leading edge cells from 4D confocal microscopy data and impose these on the model,
  **Suggestion:** Study the mechanical properties of leading edge cells to incorporate these in the model.

5-3 Simulations for hypotheses testing in Segmental Groove Formation

This section tests the plausibility of the biological hypotheses, derived in Section 3-4. Model simulations and parameter selection are done to understand different force mechanisms and the non-uniformity of mechanical properties throughout the network, and their role in Segmental Groove Formation (SGF).

Recall that the main hypotheses consists of three parts, as depicted in Figure 3-12. It states that SGF relies on three mechanisms, given in Table 5-2.

<table>
<thead>
<tr>
<th>Mechanisms</th>
<th>Abbreviation</th>
<th>Full description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. DC</td>
<td>Dorsal Closure: forces pulling the leading edge dorsally</td>
<td></td>
</tr>
<tr>
<td>II. MF</td>
<td>Mesodermal Forces: forces pulling the slit cells inwards</td>
<td></td>
</tr>
<tr>
<td>III. NUS</td>
<td>Non-Uniform Stiffness: the non-uniformity of actin organization in groove cell columns, with respect to the rest of the epithelium</td>
<td></td>
</tr>
</tbody>
</table>

The three mechanisms are tested in a knockout fashion, i.e. by switching them on and off. This results in the formulation of different simulation scenarios. Taking three mechanisms
and switching them on and off results in eight different scenarios. Note that two scenarios, in which both DC and Mesodermal Forces (MF) are switched off, do not show any dynamics, and thus are not interesting to consider. The other six scenarios do show dynamics and are therefore considered, as listed in Table 5-3.

**Table 5-3:** Simulation scenarios derived from biological hypotheses, and their abbreviated description.

<table>
<thead>
<tr>
<th>Simulation scenarios</th>
<th>Abbreviated description</th>
<th>Full description</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1. noDC MF no NUS</td>
<td>no Dorsal Closure, Mesodermal Forces, no Non-Uniform Stiffness,</td>
<td></td>
</tr>
<tr>
<td>S2. noDC MF NUS</td>
<td>no Dorsal Closure, Mesodermal Forces, Non-Uniform Stiffness,</td>
<td></td>
</tr>
<tr>
<td>S3. DC no MF no NUS</td>
<td>Dorsal Closure, no Mesodermal Forces, no Non-Uniform Stiffness,</td>
<td></td>
</tr>
<tr>
<td>S4. DC no MF NUS</td>
<td>Dorsal Closure, no Mesodermal Forces, Non-Uniform Stiffness,</td>
<td></td>
</tr>
<tr>
<td>S5. DC MF no NUS</td>
<td>Dorsal Closure, Mesodermal Forces, no Non-Uniform Stiffness,</td>
<td></td>
</tr>
<tr>
<td>S6. DC MF NUS</td>
<td>Dorsal Closure, Mesodermal Forces, Non-Uniform Stiffness.</td>
<td></td>
</tr>
</tbody>
</table>

The simulation results are exported from MATLAB and imported in Paraview [25]. This way the dynamics of the model and the final state can be thoroughly inspected. The final states for all different scenarios are plotted and given in Figures Figure 5-2 through 5-7.

5-4 Qualitative analysis of the simulation scenarios

Scenario by scenario, this section briefly discusses the simulation results, guided by the qualitative characteristics set in Table 5-1.

The bottom five qualitative characteristics presented in Table 5-1 are exploited one-by-one to assess the simulations for each scenario. For each criterium a score is awarded between 0 and 5, and listed in Table 5-4. 0 means the scenario shows no resemblance on the criterium, 5 means the scenario resembles it well.

Subsections 5-4-1 through 5-4-6 present the discussion for respectively simulation scenario S1 through S6.

5-4-1 Scenario S1. no Dorsal Closure, Mesodermal Forces, no Non-Uniform Stiffness

See Figure 5-2. Based on the bottom five qualitative characteristics, this subsection analyses scenario S1, see Table 5-3. It gives a score and explanation for the resemblance of each characteristic.
Table 5-4: Scores for all six simulation scenarios on the resemblance of the bottom five characteristics of Table 5-1. Score 0/5 means no resemblance, a score 5/5 means resemblance of observations in reality.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Characteristics (see below)</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>S1.</td>
<td>noDC MF no NUS</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>F2</td>
</tr>
<tr>
<td>S2.</td>
<td>noDC MF NUS</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>F3</td>
</tr>
<tr>
<td>S3.</td>
<td>DC no MF no NUS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>F4</td>
</tr>
<tr>
<td>S4.</td>
<td>DC no MF NUS</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>F5</td>
</tr>
<tr>
<td>S5.</td>
<td>DC MF no NUS</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>F6</td>
</tr>
<tr>
<td>S6.</td>
<td>DC MF NUS</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>F7</td>
</tr>
</tbody>
</table>

Characteristics

F Slit invagination at the start of SGF: **Score 5/5**
   Mesodermal forces on the slit cells is the only activated mechanism. The result show an accurate resemblance of slit invagination, as observed in experimental data.

G The propagation of slit invagination to the rest of the groove column: **Score 0/5**
   The slit invagination does not propagate in a specific direction, but like a circular wave. Only at the side of the CNS this wave is counteracted.

H The depth of the groove with respect to the rest of the network: **Score 0/5**
   No groove formation occurs.

I The compliance with the CNS cylinder: **Score 5/5**
   The longitudinal view shows that the epithelium wraps around the CNS, indicated by the circular curve underneath the slit (see longitudinal view).

J The ladder configuration in the groove column: **Score 0/5**
   There are no clear signs of a ladder configuration, i.e. no significant differences with the initial frame which already shows a line up of groove cells in dorsal-ventral direction. This property can therefore not be related to MF. The lateral view shows how cell boundaries around the slit line up to facilitate propagation of the MF to the rest of the epithelium.
5-4-2 Scenario S2. no Dorsal Closure, Mesodermal Forces, Non-Uniform Stiffness

See Figure 5-3. Based on the bottom five qualitative characteristics, this subsection analyses scenario S2, see Table 5-3. It gives a score and explanation for the resemblance of each characteristic.

F Slit invagination at the start of SGF: **Score 5/5**
Similar to Scenario S1, an accurate resemblance of slit invagination at the start of SGF, as observed in experimental data.

G The propagation of slit invagination to the rest of the groove column: **Score 1/5**
The slit invagination slightly propagates in the direction of the groove column. Consider the lateral view. Compared to the scenario without NUS, there is a clear line up of groove cells. Moreover, the form of the slit is sharper (see 3D view), and more ellipsoidal in the direction of the groove.

H The depth of the groove with respect to the rest of the network: **Score 0/5**
Apart from the slight propagation of slit invagination towards the groove column, no other signs of groove formation occur.

I The compliance with the CNS cylinder: **Score 5/5**
The longitudinal view shows that the epithelium wraps around the CNS, indicated by the circular curve underneath the slit.

J The ladder configuration in the groove column: **Score 3/5**
Clear signs of a starting ladder configuration show up, propagating from the slit into the groove as mentioned earlier. The lateral view shows how cell boundaries around the slit line up to facilitate propagation of the MF to the rest of the epithelium. There already seems to be a bias of alignment in the dorso-ventral direction, around the groove. Since this bias is already apparent at the initial condition, this property cannot be related to MF.

5-4-3 Scenario S3. Dorsal Closure, no Mesodermal Forces, no Non-Uniform Stiffness

See Figure 5-4. Based on the bottom five qualitative characteristics, this subsection analyses scenario S3, see Table 5-3. It gives a score and explanation for the resemblance of each characteristic.

F Slit invagination at the start of SGF: **Score 0/5**
No occurrence of slit invagination.

G The propagation of slit invagination to the rest of the groove column: **Score 0/5**
No occurrence of slit invagination, thus not applicable.

H The depth of the groove with respect to the rest of the network: **Score 0/5**
No groove formation occurs.
I The compliance with the CNS cylinder: **Score 0/5**
No interaction with the CNS.

J The ladder configuration in the groove column: **Score 2/5**
The dorsal and lateral view clearly indicate that cell boundaries line up in columns in dorso-ventral direction. The groove column and its right neighbor column show slight indications of a ladder configuration, although these are not as crisp as observed in reality.

5-4-4 Scenario S4. Dorsal Closure, no Mesodermal Forces, Non-Uniform Stiffness

See Figure 5-5. Based on the bottom five qualitative characteristics, this subsection analyses scenario S4, see Table 5-3. It gives a score and explanation for the resemblance of each characteristic.

F Slit invagination at the start of SGF: **Score 0/5**
No slit invagination occurs.

G The propagation of slit invagination to the rest of the groove column: **Score 0/5**
No slit invagination, thus not applicable.

H The depth of the groove with respect to the rest of the network: **Score 2/5**
Clear signs of groove formation. The depth of the eventual groove is half the depth of a real groove.

I The compliance with the CNS cylinder: **Score 0/5**
The indenting groove approaches the CNS cylinder, but does not interact with it.

J The ladder configuration in the groove column: **Score 4/5**
The dorsal and lateral view clearly indicate that cell boundaries line up in columns in dorso-ventral direction. The groove column lines up in an almost perfect ladder configuration.

5-4-5 Scenario S5. Dorsal Closure, Mesodermal Forces, no Non-Uniform Stiffness

See Figure 5-6. Based on the bottom five qualitative characteristics, this subsection analyses scenario S5, see Table 5-3. It gives a score and explanation for the resemblance of each characteristic.

F Slit invagination at the start of SGF: **Score 5/5**
The slit invaginates at the start. Thereafter, the tension in the network builds up through DC, which pulls the slit outwards again. The lateral view indicates that the eventual state shows slight slit invagination.

G The propagation of slit invagination to the rest of the groove column: **Score 1/5**
DC pulls the slit invagination upwards and outwards. This way the slit invagination slightly propagates through the groove column, as can be seen from the dorsal view.
H The depth of the groove with respect to the rest of the network: **Score 0/5**
The slight propagation of slit invagination creates a minor indentation throughout the
groove column. This indentation lacks the sharp form seen in reality.

I The compliance with the CNS cylinder: **Score 1/5**
At the start of simulation the slit invagination is significant and interaction with the
CNS occurs. Later on the propagation of DC pulls the slit back out. Eventually the final
configuration shows contact between the epithelium and half of the bottom quadrant
($\frac{1}{2}$) of the CNS.

J The ladder configuration in the groove column: **Score 3/5**
the dorsal and lateral view clearly indicate that cell boundaries line up in columns in
dorso-ventral direction. The groove column and its right neighbor both line up in a
ladder configuration which is clear but not as crisp as observed in real.

---

**5-4-6 Scenario S6. Dorsal Closure, Mesodermal Forces, Non-Uniform Stiffness**

See Figure 5-7. Based on the bottom five qualitative characteristics, this subsection analyses
scenario **S6**, see Table 5-3. It gives a score and explanation for the resemblance of each
characteristic.

F Slit invagination at the start of SGF: **Score 5/5**
The slit invaginates at the start and wraps around the Central Nervous System (CNS).

G The propagation of slit invagination to the rest of the groove column: **Score 3/5**
The slit invagination propagates through the groove. But as DC builds up extra tension
in the groove column, the slit is pulled back out.

H The depth of the groove with respect to the rest of the network: **Score 5/5**
A clear groove forms, indenting sharply with respect to the rest of the epithelium.

I The compliance with the CNS cylinder: **Score 3/5**
At the start of simulation the slit invagination is significant and interaction with the
CNS occurs. Eventually the final configuration shows contact between the epithelium
and the bottom quadrant of the CNS. This is likely due to the fact that the tension
built up in the groove is too high.

J The ladder configuration in the groove column: **Score 5/5**
The dorsal and lateral view clearly indicate that cell boundaries line up in columns in
dorso-ventral direction. The groove column lines up in a perfect ladder configuration.

---

**5-5 Resemblance of qualitative characteristics**

This section explains per characteristic the importance of different model parameters and
mechanisms. It also points at characteristics which have not been resembled with the simu-
lations, and gives suggestions for improvements.
A The propagation of DC through the whole network:

(a) Linear elasticity was insufficient to propagate DC to the ventral epithelium.
(b) Nonlinear elasticity enabled full propagation of DC.
(c) Damping was necessary to secure propagation during acceleration and deceleration of DC.
(d) The damping was dependent on the leading edge dynamics which were imposed artificially.

**Suggestion:** deduce leading edge DC trajectory from temporal data and impose this on the model, as mentioned before.

B The smoothness of the surface of the network:

(a) Friction was introduced to resemble the connection of the epithelium to the Extracellular Matrix (ECM). This feature improved the smoothness of surface of the network significantly.

C The cell shape stability:

(a) Nonlinear elasticity was necessary to guarantee cell shape stability, more insight can be won from simulations and biological experiments.

**Suggestion:** study the nonlinear elasticity characteristics in epithelial cellular networks in more detail. Use a smaller network and a simpler phenotype, and incorporate insight from laser ablation experiments.

(b) As mentioned earlier, introducing nonlinear stiffness unveiled a model inadequacy, concerning the structure of intermediate elements.

(c) Damping was necessary to prevent oscillations and over-stretching in cell deformation during acceleration.

D The alignment of cell boundaries:

(a) Cell boundary elements tended to line up to propagate forces. This phenomena was observed in dorsal-ventral direction, as well as around the groove slit.

(b) The cellular geometry of the initial framework already showed alignment of cells (and cell boundaries) in dorsal-ventral direction, most apparent in the groove column. This suggests that the initial cellular geometry might have an important contribution to the propagation of DC.

**Suggestion:** Study the cell motility before the start of groove formation. Do cells already line up in columns? Is there already tension in the network in dorsal-ventral direction?

E The preservation of the cylindrical shape of the embryo body:

(a) The reaction forces, resembled by a pressure difference $\Delta p$ over the epithelium, were necessary to guarantee the volume and cylindrical shape of the embryo body.
(b) Networks with linear elasticity needed too much stiffness to propagate DC through the whole network. This resulted in rigid body rotation around the ventral line, which disrupted the cylindrical shape of the embryo body.

(c) Nonlinear elasticity was necessary to enable more strain capacity and propagation of DC in the network, such that the epithelium could transverse over the cylindrical surface.

(d) Depending on the boundary condition used for the side border vertices (anterior and posterior), different issues showed up that distorted the cylindrical shape at the borders of the network. The boundary conditions either introduced undesired exogenous dynamics, or did not satisfy the desired behavior. A possible reason is the lack of knowledge about the actual boundary conditions in the cut epithelium. There are two suggestions to deal with this lack of knowledge,

Suggestion: Simulate networks with multiple grooves (minimum of three) to be able to neglect undesired boundary effects,

Suggestion: Study the effect of prestress, and deduce prestress from laser ablation experiments to pose proper boundary conditions.

F Slit invagination at the start of SGF:

Consider Table 5-4.

(a) Mesodermal forces on the slit cells were sufficient for slit invagination at the start of SGF.

(b) The amplitude of the mesodermal force on a single vertex in the range \(|\vec{F}_{MSD}|| \in [9000, 11000]\) resembled the depth of slit invagination, as observed in reality. As a comparison, the average amplitude of the reaction force on a single vertex necessary to keep the network on the cylindrical surface was in the range \(|\vec{F}_{RF}|| \in [3.7, 7.4]\). This suggested that the local perturbation of MF might be a necessary condition for slit invagination,

Suggestion: Study the local connection of the groove slit cells to the underlying mesoderm, which physical mechanism enables this connection and how are the MF generated and imposed on the epithelium?

G The propagation of slit invagination to the rest of the groove column:

Consider Table 5-4.

(a) The propagation of slit invagination was slightly determined by DC or NUS only, but significant propagation only occurred when both mechanisms were introduced.

(b) The depth of the initial slit invagination was quenched by DC. The tension built up in the groove column pulled the slit back out of its invaginated state,

Suggestion: The MF were assumed to be constant throughout the simulations. To secure slit invagination, study the introduction of extra features in the constitution of the MF, such as time varying forces, or a locking condition after the slit has invaginated.

Suggestion: Investigate how the tension in the groove column builds up. It could be the network has to stretch too much. Improving the boundary conditions, or the nonlinear elasticity characteristic in the groove cell elements could have a positive effect on this issue.
H The depth of the groove with respect to the rest of the network:
Consider Table 5-4.

(a) The groove only indented along the whole groove column in the scenarios with DC and NUS switched on (S4 and S6).
(b) Addition of MF (scenario S6) was sufficient to create a groove that resembles the actual depth, as observed in reality.
(c) The NUS in the boundary and intermediate elements in the range $\frac{\kappa_{\text{groove}}(l)}{\kappa(l)} \in [75, 100]$ resembled the right groove depth.

I The compliance with the CNS cylinder:
Consider Table 5-4.

(a) The compliance with the CNS cylinder was perfectly resembled, when DC was not exerted.
(b) Without MF, no slit invagination occurred, and therefore the epithelium did not come in contact with the CNS.
(c) DC pulled out the initial slit invagination caused by MF, suggestions for this issue were done at characteristic G.

J The ladder configuration in the groove column:
Consider Table 5-4.

(a) The ladder configuration appeared whenever DC was exerted. Without NUS or MF (scenario S3) a ladder configuration was observed in multiple cell columns, but most apparent in the groove column. This suggests that cellular geometry plays an important role in this characteristic, see suggestion at characteristic D.
(b) Variational stiffness in the groove column results in a ladder configuration that is always crisper than with no NUS.

Suggestion: This insight suggests that NUS is necessary for forming a ladder configuration as crisp as observed in reality. Laser ablation experiments can be done to determine the elasticity inside and outside of the groove, to determine an elasticity profile over the epithelium.

5-6 Concluding remarks

This chapter presented simulations and system analysis. It first interpreted the applicability of analysis methods to the MSD modeling framework. Manual parameter selection was proposed to gain further insight into all the model parameters. Before starting the selection procedure, the real system was analyzed. Ten important qualitative characteristics were derived in discussion with biologists, to serve as measures to assess simulation efforts.

Subsequently, two analysis efforts were executed. Firstly, Appendix B and Section 5-2 showed that DC closure can be resembled by selecting seven different model parameters. The first five characteristics were used to assess the parameter selection efforts. It gave value ranges for all parameters and listed important observations. For instance, it showed that linear elasticity
is insufficient to resemble the dynamics of DC over the whole epithelium and indicated that nonlinear (quadratic) elasticity does a significantly better job. Moreover, it suggested to reconsider some assumptions made in the modeling process.

Secondly, the gained insight into the parameters was then used in Sections 5-3 to test the biological hypotheses which claimed the necessity of three mechanisms in the phenotype SGF. Six simulation scenarios were formulated and run to test the plausibility of all mechanisms. The second set of five characteristics was now used to assess the contribution of the mechanisms to the qualitative dynamic behavior. Section 5-4 then analyzed the simulation results, and showed that three mechanisms together form a sufficient basis to resemble the desired qualitative behavior. The knockout analysis enabled gaining insight into the contributions and sufficiency of the mechanisms to resemble the epithelial dynamics observed in SGF.

Section 5-5 gathered all the observations, from both parameter selection as well as hypotheses testing, with respect to each characteristic. Important observations were followed up by suggestions, which pointed at new insight, and recommendations for model improvements, biological experiments, and image processing. This analysis acts as an input for both the overall study and discussion on SGF and the general field of modeling epithelial cellular networks.
Figure 5-2: Simulation results for scenario **S1**: no DC, MF, no NUS. Cartesian axes are $x$ red, $y$ yellow, and $z$ green.
Figure 5.3: Simulation results for scenario S2: no DC, MF, NUS. Cartesian axes are x red, y yellow, and z green.

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Figure 5-4: Simulation results for scenario S3: DC, no MF, no NUS. Cartesian axes are $x$ red, $y$ yellow, and $z$ green.
Figure 5-5: Simulation results for scenario S4: DC, no MF, NUS. Cartesian axes are $x$ red, $y$ yellow, and $z$ green.

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Figure 5-6: Simulation results for scenario S5.: DC, MF, no NUS.
Figure 5-7: Simulation results for scenario S6: DC, MF, NUS. Cartesian axes are $x$ red, $y$ yellow, and $z$ green.
This chapter analyzes the simulation results, presents conclusions for the MSc work, and provides recommendations for future research. Following the philosophy of the Kitano cycle [23], questions are raised to extract the implications for the biological knowledge and other insights enabled by the modeling efforts and system analysis, as depicted by the cyclic nature of Figure 6-1.

Firstly, Section 6-1 lists the key implications with respect to the MSc objective and challenges, which were set in Chapter 1. Subsequently, Section 6-2 proposes a number of recommendations to increase the accuracy and applicability of the Mass-Spring-Damper (MSD) modeling framework. Thereupon, Section 6-3 gives further recommendations concerning additional initial efforts undertaken in this project, dealing with image processing and with wet lab biological experiments.

This systems biology research project consisted of many different disciplines coming together and overlapping each other, both in terms of its challenges and its members. Section 6-4 zoom out and summarizes this thesis while collecting different key contributions.

![Figure 6-1: Project overview, indicating the steps addressed in Chapter 6.](image-url)
6-1 Key biological implications

The MSD framework was derived in Chapter 2, following biological knowledge on epithelial cellular networks. The application on the epithelium of *Drosophila melanogaster* embryo provided feedback on its ability to resemble qualitative characteristics of epithelial dynamics, as observed from biological experiments. This section connects the insight drawn from modeling and simulations with the three addressed biological challenges in Table 1-1, which relate to the MSc objective, which is to develop a quantitative mathematical model for the dynamics of the epithelium, that helps to understand the role played by forces and non-uniformity of mechanical properties.

1. **Investigate the mechanical structure and connections in epithelial cells**

The MSD modeling framework incorporated insights from biological literature and experimental observations to formulate mechanistic models that can describe the cytoskeleton, cell-cell connections and the connection of cells to the Extracellular Matrix (ECM), and that are adapted to cellular geometry. Simulations of Segmental Groove Formation (SGF) gave suggestions to how all these components are resembled by specific mechanistic modeling elements.

Specifically, the role of circumferential actin belts in sustaining and propagating loads throughout a cellular network was emphasized, and was resembled with spring-damper elements. Simulations showed that epithelial dynamics can be resembled significantly more accurately by replacing the linear elasticity characteristic in spring-damper elements with a nonlinear (quadratic) version. More experiments and modeling efforts are needed to determine the exact nonlinear elasticity characteristics, and their time-varying properties that could be linked with currently investigated cellular processes. Similar to the circumferential actin belts, the intermediate cytoskeleton can be modeled with spring-damper elements, albeit it being a lumped representation of different components. An issue in modeling the intermediate cytoskeletons of smaller cells was encountered. The corresponding modeling assumptions should be reconsidered to improve the accuracy of the model in resembling deformation of individual (small) cells.

The efforts in image processing, data structure design, and model building enabled modeling of the actual geometry of cellular networks. Synthetic experiments in SGF suggested that the cellular geometry is a critical factor in the morphogenesis of grooves in the epithelium of the *Drosophila melanogaster* embryo, and shed light on the ratios of mechanical properties of the cytoskeleton on the cell boundaries over its intermediate structure. These experiments showed that the groove column shows more alignment, without any variation of mechanical properties that would suggest to do so. Since this alignment seems to be beneficial for the ladder configuration observed in groove cells, biological experiments could address this observation and be aimed to study cellular geometry in earlier development to find out what mechanism regulates this phenotype.

2. **Investigate the origin and contribution of force mechanisms active in the development of the epithelium of the Drosophila melanogaster embryo**

A number of suggestions from biological observations enabled the formulation of a main hypothesis on the mechanical working of SGF. The hypothesis considered the epithelium as a mechanical system, and identified different force mechanisms which are claimed to perturb the system.
All this insight was incorporated in the model building algorithm, to test the mechanisms in a knockout fashion, i.e. by switching force mechanisms and non-uniformity of mechanical properties on and off. Different simulation scenarios were assessed on their resemblance of a set of qualitative characteristics derived from observations of SGF. This assessment led to conclusion that a combination of three mechanisms, i.e. Dorsal Closure (DC), Mesodermal Forces (MF), and Non-Uniform Stiffness (NUS), is sufficient to resemble SGF in the epithelium of the *Drosophila melanogaster* embryo, when exerted on a MSD model.

In the assessment of the simulations, three issues were encountered, which relate to the three mechanisms. Firstly, more knowledge about the dynamics of the leading edge cells during DC could help to eliminate the artificial dynamics around the leading edge, as observed in simulations. Secondly, the constitution of the MF in the model needs to be reconsidered to improve the resemblance of slit invagination. Thirdly, as mentioned before, a deeper understanding of the nonlinear elasticity and its time-varying properties could help to further explain the tension that is built up in the groove column.

Notice that these three suggestions all point at possible modeling efforts to improve the understanding of the dynamics and timing of the different mechanisms. These modeling efforts would be most successful if accompanied by extended focused experimental data that is collected over three spatial dimensions as well as over (developmental) time. Hence, Section 6-3 lists a number of specific recommendations for experimental design and image processing.

3. **Investigate the non-uniformity of mechanical properties over the epithelium**

Next to the investigation of NUS in groove cells, both synthetic and biological experiments further addressed and formulated other questions about the non-uniformity of mechanical properties, both spatially over the epithelium as well as over time.

The leading edge cells seem to contain more actin accumulation [43]. This was not yet incorporated in the model, and could be another explanation for the artificial dynamics in the leading edge observed in simulations.

So far, the MSD framework neglected the existence of adherens junction rearrangements. Different cellular phenotypes observed in reality depend on these rearrangements, such as cell motility, cell proliferation, and apical constriction (cell shrinkage). Hence, to model these phenotypes, adherens junction rearrangements should be incorporated. The current framework contains a data structure, which enables the rearrangements of cell boundaries and vertex adjacencies. Since the dynamics of adherens junction rearrangements still form an area of intensive biological studies, elaborate discussions with biologists should be done first, before incorporating corresponding behavior in the model.

The claimed connection of groove slit cells to the mesoderm has not yet been corroborated by biological experiments. Synthetic experiments provided support for the claim by indicating the importance of MF. Since 4D confocal microscopy shows specific cellular behavior in the groove slit, it is advisable to further investigate the dynamics and mechanical properties of groove slit cells. Again, simulations could serve as a prediction to explain observed phenotypes.
6-2 Recommendations for modeling efforts

Throughout the analysis of simulations and derivation of conclusions, a number of suggestions for model improvements was done. This section briefly lists all these suggestions. Subsection 6-2-1 mentions model improvements which can be addressed in the short term, and Subsection 6-2-2 lists more ambitious and/or challenging ideas for the long term.

6-2-1 Recommendations for modeling efforts for the short term

- Reconsider the model structure of intermediate spring-damper elements:
  Intermediate (spring-damper) elements are assigned in between vertices, which belong to the same cell, but which are not adjacent to each other. One of the effects of this, is that the more vertices a cell consists, the more intermediate elements are assigned to it. Especially, for smaller cells that consist of three or four vertices, and thus have zero or two intermediate elements, this results in inaccurate resemblance of the intermediate cytoskeletal structure. This conclusion is supported by simulation results, showing the collapse of smaller cells. The introduction of nonlinear elasticity characteristics emphasized this problem.

- Trace the DC trajectories of leading edge cells from 4D confocal microscopy data and impose these on the model:
  For the sake of tractability of parameter analysis, the DC profile of the leading edge vertices was selected once and held fixed throughout all simulations. The implementation of a $C^2$ continuous trajectory resembled an approximation of the actual DC trajectory, which still led to artificial dynamics in the simulations. When time trajectory data becomes available, the DC profile can be derived and imposed on the model as an input. It could also serve as a reference trajectory, to do output tracking using dynamic inversion over nonlinear (time-varying) models [14, 10]. In this case, one could model a DC force on the leading edge cells and determine the desired force inputs to steer the system such that the reference trajectory is followed. This opportunity enables the study of the origin of DC forces.

- Incorporate data from laser ablation experiments:
  Laser ablation enables the study of viscoelasticity in epithelial cells [24]. Within cellular networks one can sever either individual cytoskeletal components, as well as cut whole lines or arrays through the network. By measuring the response and deformation of the severed components, relations can be deduced, which relate endogenous forces to the deformation of the locally severed cytoskeletal components, using control-theoretic concepts [38]. This can shed light on viscoelastic properties and stress-strain relation in the epithelial structures.

Regarding the MSD modeling framework, this type of data is amenable to validate model parameters, or to incorporate deducible features in the model, such as stiffness, damping, and prestress. Selected model parameter values can be validated in the embryo in different ways. One could deduce values for stiffness and damping from the response of ablated actin belts, and compare the non-uniformity of these properties over the network with the distributions found in parameter selection. Or vice versa, one could
use the model as a predictor, by first simulating laser ablation experiments on the model. This way, one could compare and validate the simulated network response with the response measured in real laser ablation experiments.

Moreover, boundary conditions for the side borders of the modeled network can be formulated with experimentally deduced prestress data. This could help to eliminate the artificial dynamics due to boundary conditions used so far.

6-2-2 Recommendations for modeling efforts for the long term

- **Adjoint-Based Parameter Estimation (ABPE):**
  The ABPE method addresses complex estimation problems for systems which either nonlinear, high-dimensional, non-convex, non-smooth, or under-sampled, or a combination thereof. This parameter estimation technique was intended to quantify model parameters and force mechanism inputs, based on experimental data. To implement this technique, one needs focused experimental data, i.e. which means with persistent excitation, and sufficient resolution both in space and in time. Since in parallel to the modeling and simulation, efforts are still done to collect this type of data, the technique is already considered in this thesis but not yet executed. Appendix C explains the theoretical framework and specifies how it could be applied to the MSD modeling framework.

- **Modeling adherens junction rearrangements:**
  Adherens junction rearrangements are an apparent phenotype in any kind of (epithelial) cellular structure. Their exact effect on the dynamics of epithelial structures is an area of extensive studies. New insight might enable the incorporation of this phenotype in the MSD modeling framework. This could enable the study of higher-level phenotypes as cell motility, cell proliferation and apical constriction, to understand their relations to the dynamics and mechanics of epithelial cellular networks.

- **Application of MSD modeling framework in other studies:**
  Apart from the current academic use of the model, there are other applications for which the modeling framework could be useful.

  - **Any general epithelial modeling study:**
    Epithelial cellular networks are found in a wide variety of geometry and structure. Nevertheless, within the class of single-layered epithelia, many similar structures are found back across the animal kingdom [5]. The MSD modeling framework can be easily adapted to study other structures within this class of epithelia. For instance, to study morphogenesis of the planar epithelium of the wing of the *Drosophila melanogaster*. During the development of this epithelium most of the cells adapt a perfect hexagonal shape. Modeling efforts with the MSD framework could be done to study the mechanisms that regulate this phenotype, and what its structural implications are for the functioning of the wing.

  - **Biomaterials sciences:**
    A biomaterial is a material that is used and adapted for a medical application, such as wound healing or artificial organ development. Often these materials are developed empirically, until the desired functioning is achieved. The MSD modeling
Conclusions and Recommendations

Framework can be employed to structure the development and analysis of epithelial biomaterials.

6-3 Further project recommendations

This section gathers recommendations for related efforts within the project. The insight from the modeling efforts has enabled the further exploitation of experimental techniques, and can serve as prediction to do (often expensive) biological experiments these in an effective way. Moreover, encountered issues in efforts to resemble real dynamical behavior has triggered the demand for more experimental data to improve the accuracy of the model.

Subsection 6-3-1 lists how image processing could be used to collect more temporal and spatial information from 4D confocal microscopy data. Subsequently, Subsection 6-3-2 lists all the suggestion done exploit three different experimental techniques.

6-3-1 Recommendations on image processing

Two general recommendations are done, which aim at enabling quantitative analysis of simulation efforts.

- Collect time trajectory data:
  
  In Section 4-2 efforts on image processing were mentioned. The dynamic model generator enables extraction of cellular geometry of consecutive microscopy images, by segmentation, morphing, and registration. The latter two stages are in development, and will enable the collection of data on network dynamics in two spatial dimensions and in time. This data can be used to analyze two-dimensional structures, such as planar cell arrays. Moreover, it can serve to do quantitative parameter estimation and model validation, e.g. through application of the ABPE method mentioned in Appendix C.

- 3D segmentation:
  
  To also quantitatively analyze three-dimensional epithelial structures with the MSD modeling framework, the 4D confocal microscopy data should be processed in three dimensions to extract focused data in three spatial dimensions and time. This is a challenging objective, which gets more attention from the computer science community. An example of 3D segmentation of confocal microscopy data can be found in [22].

6-3-2 Recommendations on experimental design

Chapter 5 and Section 6-2 often mentioned the possibilities to improve the accuracy of the modeling process by incorporating more experimental data and considering novel experimental techniques. This section discusses the potential of three different experimental techniques, i.e. 4D confocal microscopy, actin staining, and laser ablation, and gives suggestions on how to use the collected data.

- 4D confocal microscopy:
  
  The necessary details of this method were already discussed in Section 4-1. In the
following, three suggestions are done to further exploit this experimental technique with an eye to MSD modeling.

- **Optimize the area covered in dorsal-ventral direction:**
  In the synthetic model a number of ventral cell rows were missing, since these were not covered by the microscope, as indicated in Figure 4-11. This issue was partially solved by calibrating the network. Nevertheless it still caused some artifacts in the dynamics of the model. To have a more realistic model, new data should cover the whole dorsal-ventral arc from leading edge to ventral line. This is a complex task, especially when one also wants to cover this area throughout DC, since then the arc of the epithelium covering the embryo increases significantly.

- **Cover two or three groove columns with their neighboring non-groove epithelium on sufficient resolution:**
  In simulations of MSD models with multiple grooves, the effect of the artificial dynamics due to ill-posed boundary conditions is likely to be relatively smaller. This could improve the analysis of the overall dynamics.

- **Measure groove slit invagination on high resolution, to study the dynamics and possible connection to the mesoderm.**

**Actin staining:**
Actin staining images turned out to be very insightful to understand the mechanical structure of epithelial cells and networks. It gave insight to interpret the non-uniformity of properties within the segmental grooves. Besides the study of actin organization in the groove, actin staining can be used to study other phenotypes as

- the structure of the groove slit cells,
- the temporal variation and thus development of actin organization, or
- the actin organization around the slit, to study the connection to the mesoderm.

**Laser ablation experiments:**
This type of experiments is promising and could yield new insight to improve the model accuracy, as motivated in Section 6-2-1. The following two suggestions for specific laser ablation experiments build upon this motivation.

- **Cut of a disc over the groove column and its adjacent cell columns:**
  Do this at different time instances during SGF. The deformation of the disc reveals the stored potential energy and its distribution. This way the difference in elasticity and internal (pre)stress between the groove and non-groove columns can be deduced.

- **Severe individual actin cables with temporal and spatial resolution to deduce an elasticity distribution, both over the network and over time.**

### 6-4 Key contributions

The main objective of this thesis was to develop a mathematical modeling framework for single-layered epithelial cellular structures. Chapter 2 identified different structural components of epithelial cells and networks, founded on a literature survey \[16\] and communication
with biologists. The gathered biological knowledge made it possible to derive criteria for the development of a modeling framework. A comparison based on these criteria motivated the selection of a modeling method: the Discrete Element Method (DEM). With this method, Chapter 2 derived the Mass-Spring-Damper (MSD) modeling framework. The MSD framework was motivated by identifying different analogies between cellular components and classical mechanical elements. The mathematical formulation of the modeling framework was founded with a graph-theoretic notation, and the mathematical equations were set up using a vertex discretization, which resembles the geometry of cell boundaries and cell junctions.

The initial need for a modeling framework arose from the biological study of Segmental Groove Formation (SGF) in the embryonic development of *Drosophila melanogaster*. Therefore the plausibility of the MSD modeling framework was assessed by applying it to this case study. Chapter 3 introduced SGF and derived biological hypotheses about the dynamics of the epithelium, and interpreted these from an engineering perspective.

Chapter 4 derived a procedure to represent the epithelium of the embryo with a mechanistic model. This procedure took image data of the epithelium obtained with 4D confocal microscopy, and built up a mechanical MSD model. The MSD model resembled the same epithelium and approximated its cellular geometry. Image processing algorithms extracted the cellular geometry from a two-dimensional microscopy image. Taking the extracted data, a data structure and some algorithms were developed to mathematically represent the epithelial network. The data structure is generally applicable for the MSD framework. A three-stage process calibrated the two-dimensional network, projected it back, and folded it on a three-dimensional cylindrical surface that resembles the embryo just before SGF. The assignment of model parameters, constraints, boundary conditions and force mechanisms were the last steps to prepare the model for simulations. Export algorithms were developed to save simulation results for reproduction and system analysis.

Through simulations and system analysis, Chapter 5 (and Appendix B) selected feasible parameters to resemble specific mechanical and dynamical characteristics observed in SGF. Interpretation of the hypotheses led to the formulation of six different simulation scenarios and ten qualitative characteristics. These characteristics formed the basis for two important assessments on the model.

The first simulations shed light on the contributions of mechanistic model parameters on the model dynamics, and how these relate to dynamic behavior observed in the epithelium of the embryo. The second assessment round focused on biological hypotheses testing. Analysis of these simulations provided insights into the contribution of different force mechanisms that are claimed to be of evident importance in the phenotype SGF. The synthetic experiments showed that a combination of three mechanisms, i.e. Dorsal Closure (DC), Mesodermal Forces (MF), and Non-Uniform Stiffness (NUS), is sufficient to resemble most of the dynamic behavior observed in SGF in a MSD model. Alongside, some issues regarding the resemblance of local cellular dynamics and specific characteristics in SGF were identified. Throughout the two assessments, a number of suggestions were done to improve the model, and to further exploit biological experiments and image processing efforts.

The new insights and the derived suggestions show that the MSD modeling framework is a plausible tool to study epithelial morphogenesis in the epithelium of the *Drosophila melanogaster* embryo, and support its application in the study of other single-layered epithelial cellular networks.
Continuing this systems biological line of research, many new directions are opening up and opportunities are enabled to increase the understanding of epithelial morphogenesis and its importance in nature.
Appendix A

Hexagonal Mass-Spring-Damper Modeling

In an early phase of this systems biology project the first models were derived using a Mass-Spring-Damper modeling framework. Since the geometry of actual epithelia could not yet be retained from the microscopy data, it was chosen to build the first models assuming a regularly packed hexagonal two-dimensional configuration, as depicted in Figure A-1. In the sequel the algorithm developed in this effort is explained. This brief explanation forms the basis for an extensive elaboration on the eventual data-driven model building algorithm, explained in Chapter 4.

In order to simulate Segmental Groove Formation in three dimensions, the following algorithm was applied.

1. Determine dimensions of the considered network and build a 2D hexagonal network of cells. Note that only a limited number of modeling elements can be defined because of computational tractability.

2. Fold the network three-dimensionally to resemble the cylindrical form of the embryo before SGF. The constructed model is shown in Figure A-2 in its initial state. Notice that the model only represents half of the segment of the embryo. This is done to speed because of symmetry (with respect to the $x,y$-plane), and speeds up the simulations.

3. Implement the Central Nervous System as a physical constraint, depicted as the brown colored cylinders in Figure A-2.

4. Manually set the different modeling parameters for stiffness, damping, friction, resting lengths, etc.

5. Impose boundary conditions on the boundaries of the network.

6. Choose and set force profiles: Dorsal Closure, Mesodermal Forces, reaction forces, etc.
7. Set the time horizon for simulation

8. Integrate the dynamics

9. Plot the outputs

Figure A-3 shows the output of a simulation on which Dorsal Closure, Mesodermal Forces and a higher stiffness along the groove column is applied.

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These first modeling efforts were done to come up with the hypotheses given in Chapter 3 and gain first insight in the potential of the Mass-Spring-Damper (MSD) modeling framework. A number of first observations were done and led to some recommendations and new research questions, which have inspired both the literature survey [16], as well as the later derivation of the eventual data-driven MSD modeling framework.

- First guess of the magnitude of different mechanical parameters and force profiles
- If so, then what is the exact location where the mesoderm(al muscles) pull(s) the epithelium inwards? At this stage it was not yet clear how the slit invaginates, as is hypothesized in Chapter 3. This hypothesis was a result of the discussion initiated by these simulations.
- Is the Central Nervous System (CNS) really a critical constraint in modeling Segmental Groove Formation (SGF)? No hard evidence has been found yet.
- Cells over-stretch in the simulations, i.e. the Dorsal Closure (DC) perturbation does not propagate through the network sufficiently causing extra strain in the dorsal epithelium, visible in Figure A-3 and indicated by the red side of the strain spectrum. Nonlinear elasticity was considered in the form of a locking criterium. A hard constraint preventing springs from further stretching after a set strain value. This locking condition resulted caused the forces to better propagate, but in an artificial way. The most dorsal cells still stretched first, and when hitting the locking deformation limit suddenly locked. This resulted in a propagation of DC forces that resembled a domino effect. Therefore it was recommended to consider either softer constraints or other smoother modeling techniques.
- The groove column shows alignment during Segmental Groove Formation. This alignment is also observed in reality. It could be due to the symmetric properties of the modeling framework (hexagonal cells). Irregularly packed networks, resembling real cell geometry, should be considered to gain further insight in the alignment effect.
• Reaction forces are modeled using a volume preservation assumption on the volume of the modeled part of the embryo. This assumption is per definition not valid for all segments that can be considered. A more realistic reaction force mechanism should be considered and implemented.

In the following section, a precise explanation is given of the eventual data-driven MSD modeling framework. In this derivation all the different model elements, force mechanisms, data structures, simulation algorithms, and other essential feature will be explained in detail.
Appendix B

Parameter Selection

This chapter presents simulation efforts done to resemble Dorsal Closure, as a single imposed phenotype. It uses the first five qualitative characteristics from Table 5-1 as a measure to select parameter values for all model elements. These parameter values are used throughout Section 5-3 to test a number of biological hypotheses.

First, Section B-1 introduces the method to do parameter selection. Subsequently, Section B-2 presents simulation results and observations about all different parameters. These observations are interpreted in Section 5-5 to form recommendations for future research.

B-1 Analysis method

The derived Mass-Spring-Damper (MSD) model of single-layered epithelial cellular networks represent a high level abstraction of the physical system under consideration. Nevertheless they are still complex and not straightforward to analyze. This section introduces and motivates a method to analyze the model and select parameter values. A short road map of five steps is defined to properly analyze and select all the different parameters, i.e.

1. determine qualitative characteristics
   Determine what qualitative behavior the simulations should resemble. Use these characteristics to assess the quality of parameter selection efforts.

2. list all the related model parameters:
   Determine which model parameters affect the dynamic behavior related to the qualitative characteristics. These parameters will be selected and analyzed.

3. make likely starting assumptions
   This procedure depends on the biological hypothesis under consideration. Examples are setting parameter values equal in regions of elements to reduce the number of parameters, or setting parameter values on/off/constant for parameters which are not interesting for analysis of the considered characteristics.
4. **select one parameter group at a time**
   To keep oversight over a high-dimensional model, it is important to not vary all parameters continually, but rather look at the effect of individual parameters. Note that this is not a method that seeks the whole parameter space thoroughly, which is not feasible for manual parameter selection anyways. This method is useful to gain qualitative insight in parameters, rather than trying to match a given data set quantitatively.

5. **iterate back and forth between different parameters to resemble the right qualitative behavior**
   Manual parameter selection is a time consuming effort, mainly because of this step. The different parameters are likely to be intimately depending on each other. Changing one parameter will affect the desired behavior which was resembled before, by selecting another parameter. Unfortunately, this is the cleanest method, at this stage of modeling and analysis.

### B-2 Simulations and parameter selection

This section simulates the model that was built in Chapter 4 to gain insight in the physical meaning of different model parameters. It uses five qualitative characteristics which are observed in the real epithelia of *Drosophila melanogaster* embryos. For each parameter group a single value is determined (equal for all similar elements), which is then used to test the different hypotheses related to Segmental Groove Formation (SGF) in the sequel. The resulting model simulation is pictured with (a) its initial and (b) its final state in Figure B-1.

1. **determine qualitative characteristics**
   A first set of parameter values is determined, by resembling Dorsal Closure (DC). The network has to resemble a stretching procedure over the cylindrical surface, without forming a groove, as indicated in Figure 4-7. Recall Section 5-1-2 for the derivation of these characteristics. The qualitative characteristics related to resembling DC are the top five in Table 5-1, for clarity again listed below.

   A The propagation of Dorsal Closure through the whole network  
   B The smoothness of the surface of the network  
   C The cell shape stability  
   D The alignment of cell boundaries  
   E The preservation of the cylindrical shape of the embryo body

2. **List all the related model parameters**
   
   (a) $k^0$, stiffness coefficient of boundary elements
Figure B-1: Result of a Dorsal Closure simulation: (a) Initial and (b) final frame.

(b) $\lambda$, friction coefficient at point mass elements
(c) $\nu$, damping coefficient of boundary elements
(d) $\Delta p$, pressure difference resembling reaction forces over whole network
(e) $k^{0,\text{int}}$, stiffness coefficient of intermediate elements
(f) $\nu^{\text{int}}$, damping coefficient of intermediate elements
(g) $k^1$, nonlinear stiffness coefficient of boundary elements

3. make likely starting assumptions

- Assume there is no Segmental Groove Formation, i.e. try to resemble Dorsal Closure of the network over a cylindrical surface.
• Assume the trajectory of the leading edge vertices is known, and thus imposed upfront as a $C^2$ continuous trajectory, as explained in Subsection 4-4-5.

• Assume the reaction force can be selected with a single parameter for the pressure difference $\Delta p$, i.e. assume the pressure difference is equal over the whole network.

• At first, assume all springs to have a linear elasticity characteristic $k^0$. Find out whether the desired behavior can be resembled this way. Then introduce nonlinear elasticity in the boundary elements, as discussed in Subsection 2-4-2, and do the same assessment.

• Assume all parameters are equal for all elements, no variation over the network.

• Do not vary masses $m_i$. The mass mathematically serve as a scale factor, since these are multiplied with the highest derivative $\ddot{x}_i$. Therefore mass does not have an interesting qualitative effect on the dynamics, in this stage of model analysis. All masses are therefore set to 1, $m_i = 1$.

• Set the final time of the simulation $t_f$ constant. It is assumed that the time frame in which the simulated phenotypes happen is constant. For sake of simplicity it is set to $t_f = 1$.

4. select one parameter group at a time / 5. iterate back and forth between different parameters to resemble the right qualitative behavior

(a) Select stiffness coefficient $k^0$:

**Observations:**

See Figure B-2 and Figure B-3.

- Stiffness in the range $k^0 \in [200, 300]$ results in best propagation of DC
- Propagation of DC does not reach the ventral epithelium
- Increasing stiffness further results in rigid body rotation around the ventral line
- Solely stiffness results in network dynamics which are not smooth, cell boundaries buckle out of the cylindrical surface, see Figure B-4
- Cell boundaries line up in dorsal-ventral direction
- With only linear elasticity, dorsal cells either elongate too much, or squeeze in $x$-direction

**Elaboration:**

The first criterium that needs to be met is the propagation of the Dorsal Closure trajectory of the leading edge vertices through the whole network. Without satisfying this criterium, nothing can be said about other criteria. Intuitively one can reason that the whole network needs enough stiffness in order to react to the velocity profile of the leading edge. Selecting $k^0$ reveals this property. Figure B-2 shows the final frame of a simulation where all $k_{i,j} = 1$. This stiffness profile clearly lacks the ability of the network to follow the leading edge. Increasing the stiffness to $k_{i,j} = 250$ gives a more satisfying first result, as depicted in Figure B-3. Nevertheless, DC still does not propagate all the way towards the ventral line. Moreover dorsal cells elongate too much, and show oscillatory behavior at the start and end of DC.
(b) Select friction coefficient $\lambda$:

**Observations:**

- Friction in the range $\lambda \in [5, 10]$ results in a smoother network trajectory, and thus in better cell shape stability
- Too high friction results in vertices getting stuck, by which dorsal cells elongate too much
- Friction without damping shows instability of cell shape, i.e. cells deform unrealistically during acceleration and deceleration

**Elaboration:**

Friction forces and (endogenous) damping forces share the property that they are both proportional to velocity. Both parameters are deliberately selected in parallel to understand their both explicit contributions more clearly. What is clear is that both parameters contribute to the smoothness of the network when perturbed by Dorsal Closure. This becomes apparent when both parameters are set to zero, which is depicted in Figure B-4. Cells flip over and are squashed as if they had no volume. When inspecting the cells close to the leading edge, one can see vertices are not decelerated enough at the end of Dorsal Closure, and therefore move on and pass the leading edge vertices. Because both damping and friction
are neglected, only elastic forces can stop them. This phenomena can be seen as a final undesired oscillatory behavior, because part of the injected energy is conserved in the network. The same undesired behavior is observed for acceleration at the start of Dorsal Closure. The cells close to the leading edge do not start in time, because there is no damping force (proportional with velocity) between them and the leading edge vertices, which makes them unable to follow the imposed Dorsal Closure trajectory. This phenomenon can only be ascribed to the damping, since the friction will only impose an extra counteracting force, in the opposite direction of the Dorsal Closure profile.

![Figure B-4: Simulating with no friction and no damping. The resulting oscillations result in flipping and squashing of cells. The yellow network is the initial configuration, the blue network is the final configuration.](image)

The friction acts directly on a vertex and represents the interaction of the network, i.e. the ectoderm, with the underlying epithelial layer, the mesoderm, see Section 2-1. This interaction causes energy dissipation and results in a sliding behavior of the ectoderm with respect to the mesoderm. This behavior contributes to the smooth motion of the network. Figure B-5 shows a simulation with sufficient friction, resulting in a smooth following trajectory of the whole network.

When the friction is too high, the propagation of Dorsal Closure is counteracted too much and vertices get stuck, as depicted in Figure B-6. In other words, the connection to the mesoderm is too strong for the ectoderm, making it unable to follow the Dorsal Closure profile of the leading edge.

(c) Select damping coefficient $\nu$:

**Observations:**

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• Without damping dorsal cells deform unrealistically during acceleration and deceleration, i.e. damping contributes to cell shape stability
• Damping in the range \( \nu \in [5, 10] \) gives most realistic behavior
• Too high damping results in rigid body motion of the individual spring-damper elements during acceleration and deceleration, which causes these elements to tumble and entangle

**Elaboration:**
The damping represents energy dissipation in the network itself, i.e. through damping interaction within the actin bundles of cell boundaries and cytoskeletal components, technically seen between two adjacent vertices. Just like the springs, a damper acts along the direction of the two adjacent vertices. Therefore it contributes to how vertices follow their neighbor vertices. The damping force on point mass \( m_i \) coming from point mass \( m_j \) is proportional to their relative velocity,

\[
\vec{F}_{\text{damping}}^{i,j} = \nu_{i,j} \frac{d l_{i,j}}{dt} \frac{\vec{x}_j - \vec{x}_i}{|| \vec{x}_j - \vec{x}_i ||}
\]

With respect to Dorsal Closure, the damping contributes to two important properties, i.e.
i. the propagation during acceleration and deceleration

At those moments the relative velocity \( \vec{x}_j - \vec{x}_i \) between different adjacent vertices changes. Figure B-8 and Figure B-9 show that higher damping results in better propagation.

ii. damping of oscillations in the network, between adjacent vertices

When damping is properly selected, these two effects both contribute to cell shape stability.

An undesired effect of high damping is that the network starts acting as a rigid body, especially during acceleration. This way the trajectory of the following vertices are distorted, such that the whole network gets stuck, because the trajectory cannot be propagated to the rest of the network anymore. Figure B-7 shows this undesired effect. The acceleration of the leading edge vertices triggers the following vertices too much, causing them to tumble over, and get stuck.

![Figure B-7: Selecting the damping coefficients \( \nu \). Too high damping results in tumbling and entanglement of the spring-damper elements that have to follow the leading edge. The yellow network is the initial configuration, the blue network is the final configuration.](image)

Selecting both the damping and friction, according to the set criteria, results in behavior depicted in Figure B-10. As a result of increasing stiffness, friction and damping, to improve the propagation, smoothness and cell shape behavior, there is also an undesired result. It is clear that the whole network now moves inwards, i.e. it shows a rigid body rotation around the ventral line. This effect will be counteracted by selecting the Laplace-Young pressure difference parameter \( \Delta p \), in the following section.

(d) Selecting the pressure difference \( \Delta p \):
**Observations:**

See Figure B-11 and Figure B-12.

- The pressure difference $\Delta p$ contributes to the preservation of the volume of the embryo body.
- When no prestress is introduced, a pressure difference range of $\Delta p \in [0.1, 0.4]$ gives desired results.
- The cylindrical shape is slightly distorted by pressure difference $\Delta p$.

**Elaboration:**

Selecting the reaction forces through the pressure difference $\Delta p$ influences the preservation of the cylindrical shape of the embryo body. See Figure B-11 and Figure B-12. Looking at these cross sections of the model at initial(yellow) and final(blue) state gives a clear view of this property. Figure B-12 shows a result where $\Delta p$ clearly is too high. Figure B-11 shows a final state which approximates the cylindrical shape. Nevertheless, one can see that the ventral epithelium tends to diverge outwards and the dorsal epithelium still comes inwards.

(e) Selecting the stiffness and damping of the intermediate elements, i.e. $k^{0,\text{int}}$ and $\nu^{\text{int}}$ with respect to the stiffness and damping of the boundary elements, i.e. $k^{0}$ and $\nu$:

**Observations:**

- Without stiffness and damping of intermediate elements, cells squeeze due to overstretching in dorsal-ventral direction.
The stiffness ratio range $r^K = \frac{k^{0, \text{int}}}{k^0} = \frac{\nu^{\text{int}}}{\nu} \in [50, 100]$ gives realistic results, showing cell shape stability.

- Too high intermediate stiffness results in rigid body rotation around the ventral line.

Elaboration:
The ratio between the intermediate element stiffness/damping and the boundary element stiffness/damping is defined as $r^K = \frac{k^{0, \text{int}}}{k^0} = \frac{\nu^{\text{int}}}{\nu}$, i.e. It is chosen to lump the study of intermediate damping and intermediate stiffness together, since the selection of intermediate damping $\nu^{\text{int}}$ alone, did not yield any interesting mechanistic insight.

Ratio $r^K$ is selected to study the contribution of the two different types of spring-damper elements to the studied phenotypes. Figure B-13 and Figure B-14 show results for respectively a low and too high ratio $r^K$. When $r^K$ is too high, the intermediate stiffness is so high, that the cells do not reshape when perturbed by Dorsal Closure, but maintain their initial, more circular shape. Moreover, the overall stiffness of both boundary and intermediate elements makes the network go into rigid body rotation around the ventral line. When $r^K$ is low, the cells change their shape and become more rectangular, as desired.

(f) Selecting nonlinear elasticity in boundary elements $k(l, d) = k^1 \frac{(l-d)}{d} + k^0$, where $k^0$ is the nominal stiffness and $k^1$ adapts the stiffness due to applied (pre)stress.

Observations:
See Figure B-15. By introducing nonlinear elasticity the model is richer and able
to incorporate both:

- The quadratic elastic force relation contributes to the stiffening effect (discussed in Subsection 3-4.2) by increasing the stiffness coefficient $k$ once the cell stretches, see Eq. (2-4). This way the elastic force increases and propagates to adjacent cells, while the cell does not stretch further.

- By selecting the linear and quadratic contribution to the stiffness coefficient $k(1,d)$, the linear regime of the elastic force can be set, as shown in Figure 2-4. A proper selection allows the model to have sufficient capacity to strain the network in the linear regime (range $k^0 \in [80, 150]$), before the nonlinear regime takes over to govern cell elongation and propagation by introducing cell stiffening (range $k^1 \in [1800, 2000]$). Sufficient strain positively affects the rigid body rotation issue with linear elasticity, because the network can now deform to stretch over the cylindrical manifold.

- The linear elasticity range ($k^0 \in [80, 150]$) of the nonlinear elasticity characteristic is significantly lower than the linear elasticity range ($k^0 \in [200, 300]$) of the linear elasticity characteristic.

**Elaboration:**

The procedure was continued and finalized after introducing the nonlinear elasticity characteristic.

Apart from the findings in biology which state that cellular mechanics is nonlinear,
there are two more reasons to introduce nonlinear elasticity characteristics that result from the parameter selection with linear elasticity.

To propagate the Dorsal Closure profile through the whole network, a high linear elasticity was necessary. First, this high stiffness led to undesired local cellular dynamics. To guarantee the smoothness of the network and the cell shape stability, this was compensated with sufficient damping and friction. But introducing more damping and friction again resulted in less propagation of the Dorsal Closure profile. And second, the high stiffness resulted in rigid body rotation of the whole network around the ventral axis. This can be seen in Figure B-10. The network has rotated inwards, with respect to the actual cylindrical surface, which is resembled by the yellow network.

A model with linear elasticity is thus not able to resemble the desired behavior, by doing manual parameter selection.

After selecting the parameters assuming linear elasticity characteristics for all springs in the network, the same procedure has been done with nonlinear (quadratic) elasticity characteristics. Recall Eq. (2-4), which defined a stiffness characteristic that is dependent of the prestress $k(l, d) = k^1 \frac{(l-d)}{d} + k^0$, where $k^0$ is the nominal stiffness and $k^1$ adapts the stiffness due to applied (pre)stress. The parameter selection can thus be continued by selecting parameter values for $k^0$ and $k^1$. 

\textbf{Figure B-13:} Selecting ratio of stiffness between intermediate and boundary elements $r^K$. Lateral view of final frame, showing result for low $r^K$, resembling relatively stiffer boundary elements. The yellow network is the initial configuration, the blue network is the final configuration.

\textbf{Figure B-14:} Selecting ratio of stiffness between intermediate and boundary elements $r^K$. Lateral view of final frame, showing result for high $r^K$, resembling equal stiffness. The yellow network is the initial configuration, the blue network is the final configuration.
Figure B-15: Nonlinear elasticity incorporated. The yellow network is the initial configuration, the blue network is the final configuration.
This appendix introduces the theoretical framework of the Adjoint-Based Parameter Estimation (ABPE) method [20]. Moreover, it discusses the application of the method on Mass-Spring-Damper (MSD) models.

C-1 Theoretical framework

Consider a set of nonlinear ODEs or PDEs, denoted by

$$\mathcal{E}(\rho(t), \theta) = 0,$$  \hspace{1cm} (C-1)

where \( \rho(t) \) are the state variables and \( \theta \) is a set of unknown parameters and (control) inputs. Notice that \( \theta \) could also encompass discretized time-dependent input profiles. ABPE minimizes a given cost function \( \mathcal{J}(\rho(t), \theta) \), as

$$\min_{\theta} \mathcal{J}(\rho(t), \theta), \tag{C-2}$$

subject to \( \mathcal{E}(\rho(t), \theta) = 0. \)

In order to do so, the gradient of \( \nabla_{\theta} \mathcal{J}(\rho(t), \theta) \) needs to be considered. Consider the first order variation of the cost function \( \delta \mathcal{J}(\rho(t), \theta) = \left( \frac{\partial \mathcal{J}}{\partial \rho} \right)^T \delta \rho + \left( \frac{\partial \mathcal{J}}{\partial \theta} \right)^T \delta \theta \), where \( \delta \) is a virtual spatial deformation or virtual parameter perturbation of a function at a particular nominal state, a concept widely used in mechanics of materials and fluid dynamics. Now also consider the first order variation of the dynamical equations \( \delta \mathcal{E}(\rho(t), \theta) = \left( \frac{\partial \mathcal{E}}{\partial \rho} \right)^T \delta \rho + \left( \frac{\partial \mathcal{E}}{\partial \theta} \right)^T \delta \theta = 0. \)
C-2 Application to the specific model

Consider the dynamical equations of a general MSD model with \(N\) point masses and \(i \in \{1, 2, \ldots, N\}\), as derived in Eq. (2-5),

\[
m_{i} \ddot{x}_{i}(t) - \sum_{j \in \mathcal{N}(v_{i})} \left\{ \left( k_{i,j}(li,j) \left( l_{i,j} - d_{i,j} \right) + \nu_{i,j} \frac{dl_{i,j}}{dt} \right) \frac{\dot{x}_{j} - \dot{x}_{i}}{|\dot{x}_{j} - \dot{x}_{i}|} \right\} + \lambda_{i} \dot{x}_{i} - \sum_{\mu} F_{i}^{ex,\mu}(x, t) = 0,
\]

(C-3)

where point mass \(m_{i}\) is located at vertex \(i\), and \(\vec{x}_{i} \in \mathbb{R}^{3}\) denotes its position. \(\mathcal{N}(v_{i})\) denotes the set of point masses adjacent to \(m_{i}\). \(k_{i,j}, l_{i,j}, \) and \(d_{i,j}\) are respectively the stiffness coefficient, the length, and the resting length of a spring between point mass \(m_{i}\) and \(m_{j}\), correspondingly \(\nu_{i,j}\) is the damping coefficient. Furthermore, \(\lambda_{i}\) is the friction coefficient at \(m_{i}\), and \(\sum_{\mu} F_{i}^{ex,\mu}(t)\) is the sum of all \(\mu\) external forces perturbing \(m_{i}\). For now, linear elasticity characteristics are assumed, i.e. \(k_{i,j}(l_{i,j}) = k_{0,ij}\).

Notice that all the terms in Eq. (C-3) are brought to the left hand side to achieve the form of Eq. (C-1). The state variables \(\rho(t)\) are now the positions \(\vec{x}_{i}(t)\) and velocities \(\dot{x}_{i}(t)\) of all the \(N\) point masses, hence \(\rho(t) \in \mathbb{R}^{6N}\). The unknown parameters and control inputs are collected in \(\theta = [\vec{m}^{T} \ k^{0,\vec{v}}^{T} \ \vec{\lambda}^{T} \ \vec{\theta}^{ex,\vec{T}}]^{T}\), where \(\vec{\theta}^{ex}\) denotes the vector of parameters related to the external forces \(\sum_{\mu} F_{i}^{ex,\mu}(\rho(t), t)\).

C-2.1 Cost function

Different options can be considered regarding the cost function \(J\). In a parameter estimation scheme, the most straightforward choice is to let the cost function compare the solution of the system in Eq. (C-3) \(\rho(t; \theta)\), which depends on \(\theta\), with the available observations \(\rho^{obs}(t)\). Two options are considered. The first looks at the difference vector of simulated spring lengths \(l(\rho(t))\) with the observed quantities \(l^{obs}(t)\), where \(l(\rho(t))\) is computed by taking the Euclidean distance between the adjacent vertices with position \(\vec{x}_{i}(t)\) and \(\vec{x}_{j}(t)\), and \(l^{obs}(t)\) is a known vector of observations. This yields the optimization problem

\[
J(\rho(t), \theta) = \int_{0}^{T} \left\| l(\rho(t)) - l^{obs}(t) \right\|_{2}^{2} dt,
\]

(C-4)

where \(l, l^{obs} \in \mathbb{R}^{N_{e}}\) and \(N_{e}\) is the number of boundary elements and thus springs in the network. The second cost function applies a similar idea, but uses the positional data, which yields

\[
J_{2}(\rho(t), \theta) = \int_{0}^{T} \left\| \rho(t) - \rho^{obs}(t) \right\|_{2}^{2} dt.
\]

C-2.2 Derivation of the Adjoint-Based Parameter Estimation problem

In order to apply optimization based parameter estimation, the derivation of the gradient of the cost function \(J\), with respect to a set of parameters \(\theta\), is considered. Computing the gradient of the cost function with respect to the parameters \(\theta\) is not straightforward. In order
to tackle this problem an adjoint variable set, with its own adjoint equation, can be introduced. Gradient values with respect to a particular quantity, in this context the parameters \( \theta \) of interest to estimate, can be efficiently calculated by solving the adjoint equation [20, 34].

To arrive at the adjoint-based gradient, the first variation of the cost function \( J \) is derived. For the case of the cost function defined in Eq. (C-4), based on the lengths of boundary interest to estimate, can be efficiently calculated by solving the adjoint equation [20, 34].

For the case of the cost function defined in Eq. (C-4), based on the lengths of boundary

\[
\frac{\partial l}{\partial t} \rho(t) dt + \frac{\partial l}{\partial \theta} \rho(t) \delta \theta dt.
\]

Now subtract Eq. (C-7) from the first variation of the cost function in Eq. (C-5). This gives

\[
\delta J(\rho(t), \theta) = \int_0^T 2 \left( \frac{\partial l}{\partial t} \rho(t) - l^{\text{obs}}(t) \right) J^\top \rho(t) \delta \rho dt + \int_0^T \delta \rho(t) \delta \theta dt.
\]

Applying integration by parts on the term \(- \int_0^T p^\top(t) \delta \rho(t) dt\), one obtains

\[
- \int_0^T p^\top(t) \delta \rho(t) dt = -p^\top(T) \delta \rho(T) + p^\top(0) \delta \rho(0) + \int_0^T \delta \rho(t) \delta \theta dt.
\]

Plugging Eq. (C-9) into Eq. (C-8) yields

\[
\delta J(\rho(t), \theta) = \int_0^T \left[ \dot{\rho}^\top(t) + p^\top(t) f_\rho(\rho(t), \theta) + 2 \left( \frac{\partial l}{\partial t} \rho(t) - l^{\text{obs}}(t) \right) J^\top \rho(t) \right] \delta \rho dt + \int_0^T p^\top(t) f_\theta(\rho(t), \theta) \delta \theta dt.
\]

Now impose three ad hoc conditions on Eq. (C-10), i.e.

\[
\mathcal{P}(\rho(t), \theta, p(t)) = \dot{p}^\top(t) + p^\top(t) f_\rho(\rho(t), \theta) + 2 \left( \frac{\partial l}{\partial t} \rho(t) - l^{\text{obs}}(t) \right) J^\top \rho(t) = 0,
\]

\[
\delta \rho(0) = 0,
\]

\[
\delta \rho(T) = 0.
\]
The first constraint in Eq. (C-11) is the system of adjoint equations \( \mathcal{P}(\rho(t), \theta, p(t)) = 0 \). The other two constraints in Eq. (C-11) are conditions on \( \delta \rho(0) \), and \( \delta \rho(T) \). This yields

\[
\delta \mathcal{J}(\rho(t), \theta) = \int_0^T p^\top(t) f_\theta(\rho(t), \theta) \delta \theta dt = \left( \int_0^T p^\top(t) f_\theta(\rho(t), \theta) dt \right) \delta \theta = \nabla_\theta \mathcal{J}(\rho(t), \theta) \delta \theta,
\]

where \( \nabla_\theta \mathcal{J}(\rho(t), \theta) \) is the gradient of the cost function with respect to the parameters \( \theta \).

The gradient can be used to find solutions for the ABPE problem

\[
\min_{\theta} \delta \mathcal{J}(\rho(t), \theta) = \left( \int_0^T p^\top(t) f_\theta(\rho(t), \theta) dt \right) \delta \theta,
\]

subject to \( \mathcal{E}(\rho(t), \theta) = 0, \mathcal{P}(\rho(t), \theta, p(t)) = 0, \nabla_\theta \rho(0) = 0, \nabla_\theta \rho(T) = 0. \)

The derivation of the ABPE allows a computable way to optimize this problem with respect to the unknown parameters, given certain constraints. To implement the problem in Eq. (C-12), one should derive the linearized dynamics \( f_\rho \), and \( f_\theta \) of Eq. (C-6). More details on the implementation can be gathered from [34, 6].
Appendix D

Stability Analysis on Mass-Spring-Damper Network Models

This chapter presents analysis on stability of Mass-Spring-Damper (MSD) models. First, Section D-1 gives a mathematical introduction on MSD models. Second, Section D-2 proves Lyapunov stability for two-dimensional MSD network models.

D-1 Mathematical model formulation

Chapters 2 and 4 defined the MSD modeling framework. Now consider a network with $N$ point masses. Consider each point mass $m_i$, with $i \in \{1,2,\ldots,N\}$, representing 6 state variables, i.e. 3 position variables $\vec{x}_i$ and 3 velocity variables $\dot{\vec{x}}_i$. Using the second law of Newton one can now write down a second order differential equation for each point mass $m_i$, i.e.

\[ m_i \ddot{\vec{x}}_i = F_{\text{elastic}}^i + F_{\text{damping}}^i + F_{\text{friction}}^i + F_{\text{ex}}^i \]

\[ = \sum_{j \in \mathcal{N}(i)} \left\{ \left( k_{i,j}(l_{i,j} - d_{i,j}) + \nu_{i,j} \frac{d}{dt} (l_{i,j} - \vec{x}_i) \right) \frac{\vec{x}_j - \vec{x}_i}{||\vec{x}_j - \vec{x}_i||} \right\} - \lambda_i \vec{x}_i + \sum_{\mu} F_{\text{ex,}\mu}^i \]  \hspace{1cm} (D-1)

where $k_{i,j}$ and $\nu_{i,j}$ denote the stiffness and damping coefficients of a spring-damper element between masses $m_i$ and $m_j$, and $l_{i,j}$ is the distance between the two masses. The stiffness and damping force can only act in the direction of the element between the two masses, which is $\frac{\vec{x}_j - \vec{x}_i}{||\vec{x}_j - \vec{x}_i||}$. $\lambda_i$ is the friction coefficient at mass $m_i$ and $\sum_{\mu} F_{\text{ex,}\mu}^i$ denotes the vectorial sum of all $\mu$ external forces acting on mass $m_i$, including the different force mechanisms.
D-2 Stability analysis

A Mass-Spring-Damper network has a special property. When not perturbed by an external input, i.e. \( \sum_{i} F_i^{ext} = 0 \), it has a set of equilibria, for which holds \( l_{i,j} = d_{i,j} \) and \( \| \dot{x}_i \| = 0 \), \( \forall i \). This section proves Lyapunov stability of this set of equilibria.

For a dynamical system, a Lyapunov function \( V : \mathbb{R}^p \to \mathbb{R} \) typically is a function that maps the state variables \( \rho(t) \) to an energy-based function. E.g., consider the sum of the potential and of the kinetic energy of the MSD model, \( V(\rho(t)) = \sum_i \left( \sum_{j \in \mathcal{N}(v_i)} \frac{1}{2} k_{i,j} (x_{i,j} - x_{i,j})^2 \right) + \sum_i m_i \| \dot{x}_i \|^2 > 0 \), where \( i \) and \( j \) denote the vertex index of adjacent vertices \( v_i \) and \( v_j \), and \( \| \dot{x}_i \| \) denotes the magnitude of the velocity of point mass \( m_i \). \( \rho(t) \) is the set of transformed state variables such that the origin of \( V(0) = 0 \). This means that \( \rho(t) \) consists of \( l_{i,j} - d_{i,j} \) and \( \| \dot{x}_i \| \).

Taking the time derivative of \( V(\rho(t)) \) results in
\[
\dot{V}(\rho(t)) = \sum_i \left( \sum_{j \in \mathcal{N}(v_i)} k_{i,j} (l_{i,j} - d_{i,j}) \frac{d l_{i,j}}{dt} \right) + 2 m_i \| \dot{x}_i \| \frac{d \| \dot{x}_i \|}{dt}.
\]

Since \( \frac{d \| \dot{x}_i \|}{dt} = \frac{1}{\| \dot{x}_i \|} \ddot{x}_i \dot{x}_i \) and \( \frac{d l_{i,j}}{dt} = \frac{1}{l_{i,j}} (x_i - x_j) \cdot (\ddot{x}_i - \ddot{x}_j) \), one can rewrite Eq. (D-2) as
\[
\dot{V}(\rho(t)) = \sum_i \left( \sum_{j \in \mathcal{N}(v_i)} k_{i,j} (l_{i,j} - d_{i,j}) \frac{1}{l_{i,j}} (x_i - x_j) \cdot (\ddot{x}_i - \ddot{x}_j) \right) + 2 m_i \dddot{x}_i \dddot{x}_i.
\]

Plugging the dynamical equations from Eq. (D-1) into Eq. (D-3) yields
\[
\dot{V}(\rho(t)) = \sum_i \left( \sum_{j \in \mathcal{N}(v_i)} k_{i,j} (l_{i,j} - d_{i,j}) \frac{1}{l_{i,j}} (x_i - x_j) \cdot (\ddot{x}_i - \ddot{x}_j) \right) + 2 \dddot{x}_i \dddot{x}_i + \dddot{x}_i \left\{ \left( k_{i,j} (l_{i,j} - d_{i,j}) + \nu_{i,j} \frac{d l_{i,j}}{dt} \right) \| x_i - x_i \| \right\} - \lambda_i \dddot{x}_i.
\]

Now consider a general network composed of Mass-Spring-Damper elements, as depicted in Figure D-1. For sake of simplicity, let us analyze a network of two point masses \( m_1 \) and \( m_2 \), connected through one spring-damper element. Since \( \mathcal{N}(v_1) = \{ 2 \} \) and \( \mathcal{N}(v_2) = \{ 1 \} \), the dynamical equations for \( m_1 \) and \( m_2 \) are
\[
m_{1} \dddot{x}_1 = \left( k_{1,2} (l_{1,2} - d_{1,2}) + \nu_{1,2} \frac{d l_{1,2}}{dt} \right) \dddot{x}_2 - \dddot{x}_1 - \lambda_1 \dddot{x}_1,
\]
\[
m_{2} \dddot{x}_2 = \left( k_{2,1} (l_{2,1} - d_{2,1}) + \nu_{2,1} \frac{d l_{2,1}}{dt} \right) \dddot{x}_1 - \dddot{x}_2 - \lambda_2 \dddot{x}_2.
\]

Notice that \( k_{1,2} = k_{2,1} = k \), \( l_{1,2} = l_{2,1} = l \), \( d_{1,2} = d_{2,1} = d \), and \( \nu_{1,2} = \nu_{2,1} = \nu \). Moreover,
Figure D-1: A simplified diagram of a 2-dimensional Mass-Spring-Damper model.

assume \(m_1 = m_2 = m\). Following Eq. (D-4), the time derivative of \(V(\rho(t))\) is

\[
\dot{V}(\rho(t)) = 2k(l - d) \frac{1}{l} (\ddot{x}_1 - \ddot{x}_2)^\top (\dot{x}_1 - \dot{x}_2) + \\
+ 2\ddot{x}_1^\top \left\{ (k(l - d) + \nu \frac{dl}{dt}) \frac{\ddot{x}_2 - \ddot{x}_1}{l} - \lambda_1 \ddot{x}_1 \right\} + \\
+ 2\ddot{x}_2^\top \left\{ (k(l - d) + \nu \frac{dl}{dt}) \frac{\ddot{x}_1 - \ddot{x}_2}{l} - \lambda_2 \ddot{x}_2 \right\}
\]

\[
= -\frac{\nu dl}{l dt} (\ddot{x}_1 - \ddot{x}_2)^\top (\dot{x}_1 - \dot{x}_2) + \\
- \lambda_1 \ddot{x}_1^\top \ddot{x}_1 - \lambda_2 \ddot{x}_2^\top \ddot{x}_2
\]

\[
= -\frac{\nu}{l^2} (\ddot{x}_1 - \ddot{x}_2)^\top (\dot{x}_1 - \dot{x}_2) (\ddot{x}_1 - \ddot{x}_2)^\top (\ddot{x}_1 - \ddot{x}_2) + \\
- \lambda_1 \ddot{x}_1^\top \ddot{x}_1 - \lambda_2 \ddot{x}_2^\top \ddot{x}_2
\]

\[
= -\frac{\nu}{l^2} ((\ddot{x}_1 - \ddot{x}_2)^\top (\dot{x}_1 - \dot{x}_2))^2 + \\
- \lambda_1 \ddot{x}_1^\top \ddot{x}_1 - \lambda_2 \ddot{x}_2^\top \ddot{x}_2. \tag{D-6}
\]

\(S = \{\rho(t) \in \mathbb{R}^p : \dot{V}(\rho(t)) = 0\}\) is the set of states for which \(\dot{V}(\rho(t)) = 0\). \(S\) encompasses more than only the origin, i.e. all states for which holds that \(\dot{x}_1 = \dot{x}_2 = 0\). Since \(V(\rho(t)) > 0, \forall \rho(t) \neq 0\), and \(\dot{V}(\rho(t)) \neq 0, \forall \rho(t) \neq 0\), by applying the principle of LaSalle, it can be shown that \(\rho(t) = 0\) is the largest invariant set within \(S\), and hence, the origin is Lyapunov stable [35].

By induction, Eq. (D-6) proves that an unperturbed MSD network has a set of equilibria, i.e. the set defined by \(l_{i,j} = d_{i,j}, \forall \{i,j\}\) and \(\|\ddot{x}_i\| = 0, \forall i\), which is Lyapunov stable.


# Glossary

## List of Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCSC</td>
<td>Delft Center for Systems and Control</td>
</tr>
<tr>
<td>TU Delft</td>
<td>Delft University of Technology</td>
</tr>
<tr>
<td>SGF</td>
<td>Segmental Groove Formation</td>
</tr>
<tr>
<td>UC Berkeley</td>
<td>University of California, at Berkeley</td>
</tr>
<tr>
<td>EECS</td>
<td>Electrical Engineering and Computer Sciences</td>
</tr>
<tr>
<td>PDE</td>
<td>Partial Differential Equation</td>
</tr>
<tr>
<td>PDEs</td>
<td>Partial Differential Equations</td>
</tr>
<tr>
<td>ODE</td>
<td>Ordinary Differential Equation</td>
</tr>
<tr>
<td>ODEs</td>
<td>Ordinary Differential Equations</td>
</tr>
<tr>
<td>ABPE</td>
<td>Adjoint-Based Parameter Estimation</td>
</tr>
<tr>
<td>MSD</td>
<td>Mass-Spring-Damper</td>
</tr>
<tr>
<td>FEM</td>
<td>Finite Element Method</td>
</tr>
<tr>
<td>DEM</td>
<td>Discrete Element Method</td>
</tr>
<tr>
<td>DC</td>
<td>Dorsal Closure</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular Matrix</td>
</tr>
<tr>
<td>Odd</td>
<td>Odd Skipped</td>
</tr>
<tr>
<td>Crb</td>
<td>Crumbs</td>
</tr>
<tr>
<td>aPKC</td>
<td>atypical Protein Kinase C</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>Ena</td>
<td>Enabled</td>
</tr>
<tr>
<td>Arm</td>
<td>Armadillo</td>
</tr>
<tr>
<td>NUS</td>
<td>Non-Uniform Stiffness</td>
</tr>
<tr>
<td>MF</td>
<td>Mesodermal Forces</td>
</tr>
</tbody>
</table>
# List of Symbols

**Set theory, graph theory, and network notation**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mathbb{N}$</td>
<td>set of the nonnegative numbers ({0, 1, 2, \ldots})</td>
</tr>
<tr>
<td>$\mathbb{R}$</td>
<td>set of the real numbers</td>
</tr>
<tr>
<td>$i \in \mathcal{N}$</td>
<td>$i$ is in the set $\mathcal{N}$</td>
</tr>
<tr>
<td>$\mathcal{N}\setminus{n}$</td>
<td>the set $\mathcal{N}$ with exception of its subset ${n}$</td>
</tr>
<tr>
<td>$\mathcal{N} \cup \mathcal{M}$</td>
<td>union of sets $\mathcal{N}$ and $\mathcal{M}$</td>
</tr>
<tr>
<td>$i$</td>
<td>index for vertices</td>
</tr>
<tr>
<td>$v_i$</td>
<td>vertex in set $\mathcal{V}$</td>
</tr>
<tr>
<td>$v_k$</td>
<td>the $k$th vertex in the search of a face in a graph</td>
</tr>
<tr>
<td>$v^{(k,l)}$</td>
<td>one of the adjacent vertices of the $k$th vertex in the search of a face in a graph</td>
</tr>
<tr>
<td>$N$</td>
<td>number of vertices is a set $\mathcal{V}$</td>
</tr>
<tr>
<td>$\mathcal{V}$</td>
<td>set of $N$ vertices</td>
</tr>
<tr>
<td>$\mathcal{D}(v_i)$</td>
<td>degree of vertex $v_i$, i.e. its number of adjacent vertices</td>
</tr>
<tr>
<td>$\mathcal{N}(v_i)$</td>
<td>the set of indices of vertices adjacent to vertex $v_i$</td>
</tr>
<tr>
<td>$\mathcal{N}^\text{int}(v_i)$</td>
<td>the set of indices of vertices that lie in cells adjacent to vertex $v_i$ but that are not directly adjacent to $v_i$</td>
</tr>
<tr>
<td>$e_{i,j}$</td>
<td>edge between vertex $v_i$ and vertex $v_j$</td>
</tr>
<tr>
<td>$N^e$</td>
<td>number of edges in a set $\mathcal{E}$</td>
</tr>
<tr>
<td>$\mathcal{E}$</td>
<td>set of edges</td>
</tr>
<tr>
<td>$\mathcal{G} = (\mathcal{V}, \mathcal{E})$</td>
<td>a graph defined by vertices $\mathcal{V}$ and edges $\mathcal{E}$</td>
</tr>
<tr>
<td>$f$</td>
<td>face</td>
</tr>
<tr>
<td>$\mathcal{D}(f)$</td>
<td>face degree of face $f$, i.e. the number of vertices that $f$ consists of</td>
</tr>
<tr>
<td>$H$</td>
<td>incidence matrix</td>
</tr>
<tr>
<td>$L = HH^T$</td>
<td>Laplacian matrix</td>
</tr>
<tr>
<td>$\phi$</td>
<td>angle between adjacent edges</td>
</tr>
<tr>
<td>$\circlearrowleft$</td>
<td>denoting negative angle</td>
</tr>
<tr>
<td>$\circlearrowright$</td>
<td>denoting positive angle</td>
</tr>
</tbody>
</table>
### Matrices and vectors

$\vec{b} \in \mathbb{R}^n$  
\begin{align*}
  &\text{a } n \times 1 \text{ vector} \\
  ||\vec{b}|| &\text{ Euclidean distance of the vector } \vec{b} \\
  ||\vec{b}||_2 &\text{ 2-norm of the vector } \vec{b} \\
  \nabla g &\text{ gradient of a function } g \text{ with respect to } \vec{b} \\
  A \in \mathbb{R}^{n \times m} &\text{ a } n \times m \text{ matrix} \\
  A^\top &\text{ transpose of the matrix } A \\
  a_{ij} &\text{ the entry in matrix } A \text{ on the } i\text{th row and the } j\text{th column}
\end{align*}

### MSD modeling

$(x, y, z)$  
\begin{align*}
  &\text{Cartesian coordinates} \\
  t &\text{ time dimension} \\
  i &\text{ index for point masses } m_i, \text{ coinciding with indices for vertices } v_i \\
  m_i &\text{ point mass at vertex } v_i \\
  M &\text{ mass matrix} \\
  \vec{x}_i &\text{ position of point mass } m_i \\
  \dot{\vec{x}}_i &\text{ velocity of point mass } m_i \\
  \ddot{\vec{x}}_i &\text{ acceleration of point mass } m_i \\
  k_{i,j} &\text{ stiffness coefficient of spring between point masses } m_i \text{ and } m_j \\
  k(l) &\text{ stiffness coefficient of spring as a function of its length } l \\
  k^0 &\text{ nominal stiffness coefficient} \\
  k^1 &\text{ stiffness coefficient related to applied (pre)stress} \\
  \nu_{i,j} &\text{ damping coefficient of damper between point masses } m_i \text{ and } m_j \\
  l_{i,j} &\text{ length of spring between point masses } m_i \text{ and } m_j \\
  d_{i,j} &\text{ resting length of spring between point masses } m_i \text{ and } m_j \\
  \lambda_i &\text{ friction coefficient at point mass } m_i \\
  \Lambda &\text{ friction matrix} \\
  F_i &\text{ force perturbing point mass } m_i \\
  S(k, l, L) &\text{ stiffness/damping matrix} \\
  \text{int} &\text{ denotation for intermediate elements} \\
  \text{elastic} &\text{ denotation for elastic forces} \\
  \text{damping} &\text{ denotation for damping forces} \\
  \text{tr} &\text{ denotation for triangular elements} \\
  \text{friction} &\text{ denotation for friction forces} \\
  \text{RF} &\text{ denotation for reaction forces} \\
  \text{LE} &\text{ denotation for leading edge trajectories} \\
  \text{ex} &\text{ denotation for external/exogenous forces} \\
  R &\text{ radius} \\
  \vec{r}_i &\text{ distance vector of point mass } m_i \text{ with respect to the CNS} \\
  \Delta p &\text{ pressure difference over the epithelium}
\end{align*}

Roel I.J. Dobbe  
Master of Science Thesis
### Miscellaneous

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g(x(t))$</td>
<td>$g$ is a function dependent of $x$, which is function dependent of $t$</td>
</tr>
<tr>
<td>$g(x)$ is $C^p$</td>
<td>$p$th derivative of $g(x)$, i.e. $\frac{\partial^p g}{\partial x^p}$, is continuous</td>
</tr>
<tr>
<td>$g : \mathcal{D} \to \mathbb{R}$</td>
<td>function with domain $\mathcal{D}$ and range $\mathbb{R}$</td>
</tr>
<tr>
<td>$\mathcal{E}(\rho(t), \theta) = 0$</td>
<td>system of dynamical equations</td>
</tr>
<tr>
<td>$\delta$</td>
<td>virtual state deformation or virtual parameter perturbation</td>
</tr>
<tr>
<td>$V(\rho(t))$</td>
<td>Lyapunov function</td>
</tr>
</tbody>
</table>