TRACTION FORCES FROM
PHASE-CONTRAST MICROSCOPY

AN IMAGE REGISTRATION APPROACH FOR THE
RECONSTRUCTION OF CELLULAR TRACTION

MASTER THESIS
submitted in fulfillment of the requirements for the degree of
MASTER OF SCIENCE

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Understanding the cellular ability to migrate is the key to a better comprehension of many biological processes including wound healing and the formation of tumor metastases. However, cellular motility results from a highly complex system of molecular mechanisms among which forces generated by contractions of the cytoskeleton are of vital importance. To study these forces, cells are cultured on an elastic substrate providing an in vitro model of the migration process. As cellular tractions cause the underlying substrate to deform, they can be reconstructed by means of an inverse problem, which however requires an estimate of the corresponding displacement field: The most prominent way of providing this estimate is the elastic substratum method where beads are firmly embedded into the substrate and tracked over time. This is the basis of so-called Traction Force Microscopy. In this thesis we overcome the need for these beads by introducing a novel approach for the reconstruction of cellular traction forces from usual phase-contrast microscopy images of sufficient resolution. Based on a sound mathematical model of the experimental setting, we obtain the displacement field and thus the cellular traction forces by combining linear elasticity theory and a variational framework of image registration. This leads to two alternative minimization problems for which we also show the existence of a solution. Finally, we provide an implementation of the new approach and present first numerical results.
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# Glossary of Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Quantity / Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Omega \subset \mathbb{R}^3$</td>
<td>domain in the reference configuration</td>
</tr>
<tr>
<td>$\Omega^d_t \subset \mathbb{R}^3$</td>
<td>domain in the current/deformed configuration</td>
</tr>
<tr>
<td>$\Gamma = \partial \Omega$</td>
<td>boundary of the domain $\Omega$ in the reference configuration (two-dimensional manifold)</td>
</tr>
<tr>
<td>$\Gamma^d_t = \partial \Omega^d_t$</td>
<td>boundary of the domain $\Omega$ in the current/deformed configuration (two-dimensional manifold)</td>
</tr>
<tr>
<td>$\Gamma_1 \subset \Gamma$</td>
<td>portion of the boundary of the domain $\Omega$ at which surface forces are applied in the reference configuration</td>
</tr>
<tr>
<td>$\Gamma^d_{1,t} \subset \Gamma^d_t$</td>
<td>portion of the boundary of the domain $\Omega^d_t$ at which surface forces are applied in the current deformed configuration</td>
</tr>
<tr>
<td>$\Gamma_0 = \Gamma \setminus \Gamma_1$</td>
<td>portion of the boundary of the domain $\Omega$ at which Dirichlet boundary conditions are applied in the reference configuration</td>
</tr>
<tr>
<td>$\Gamma^d_{0,t} = \Gamma^d_t \setminus \Gamma^d_{1,t}$</td>
<td>portion of the boundary of the domain $\Omega^d_t$ at which Dirichlet boundary conditions are applied in the current/deformed configuration</td>
</tr>
<tr>
<td>$x \in \bar{\Omega}$</td>
<td>point in the reference configuration</td>
</tr>
<tr>
<td>$y = x^d_t \in \Omega^d_t$</td>
<td>point in the current/deformed configuration</td>
</tr>
<tr>
<td>$dx$</td>
<td>volume element in the reference configuration</td>
</tr>
<tr>
<td>$dx^d_t$</td>
<td>volume element in the current/deformed configuration</td>
</tr>
<tr>
<td>$da$</td>
<td>area element in the reference configuration</td>
</tr>
<tr>
<td>$da^d_t$</td>
<td>area element in the current/deformed configuration</td>
</tr>
<tr>
<td>$n$</td>
<td>normal at $x$ in the reference configuration</td>
</tr>
<tr>
<td>$n^d_t$</td>
<td>normal at $y$ in the current/deformed configuration</td>
</tr>
<tr>
<td>$y_t(x)$</td>
<td>deformation $y_t : \bar{\Omega} \rightarrow \bar{\Omega}^d_t$</td>
</tr>
<tr>
<td>$u_t(x) = y_t(x) - x$</td>
<td>displacement field $u_t : \bar{\Omega} \rightarrow \bar{\Omega}^d_t$</td>
</tr>
<tr>
<td>$v(x,t)$</td>
<td>motion</td>
</tr>
<tr>
<td>$\nabla(x,t)$</td>
<td>velocity</td>
</tr>
<tr>
<td>$a(x,t)$</td>
<td>acceleration</td>
</tr>
<tr>
<td>$F = Dy = Dy_t(x)$</td>
<td>deformation gradient</td>
</tr>
<tr>
<td>$J = \det Dy$</td>
<td>determinant of the deformation gradient</td>
</tr>
<tr>
<td>Symbol</td>
<td>Quantity / Variable</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------</td>
</tr>
<tr>
<td>$C$</td>
<td>right Cauchy-Green tensor</td>
</tr>
<tr>
<td>$C_{lin}$</td>
<td>linearized right Cauchy-Green tensor</td>
</tr>
<tr>
<td>$B$</td>
<td>left Cauchy-Green tensor</td>
</tr>
<tr>
<td>$\lambda_1, \lambda_2, \lambda_3$</td>
<td>principal stretches of the Cauchy-Green tensors</td>
</tr>
<tr>
<td>$I_C, II_C, III_C$</td>
<td>matrix invariants of the Cauchy-Green tensors</td>
</tr>
<tr>
<td>$E = \frac{1}{2}(C - I)$</td>
<td>Green-St Vernant strain tensor</td>
</tr>
<tr>
<td>$E_{lin}$</td>
<td>linearized Green-St Vernant strain tensor</td>
</tr>
<tr>
<td>$b(x)$</td>
<td>body force per unit volume in the reference configuration</td>
</tr>
<tr>
<td>$b^d(x)$</td>
<td>body force per unit volume in the current/deformed configuration</td>
</tr>
<tr>
<td>$s(x)$</td>
<td>surface force per unit area in the reference configuration</td>
</tr>
<tr>
<td>$s^d(x)$</td>
<td>surface force per unit area in the current/deformed configuration</td>
</tr>
<tr>
<td>$l^d_t(b^d_t, s^d_t)$</td>
<td>load</td>
</tr>
<tr>
<td>$\sigma^d_t(y, n^d_t)$</td>
<td>Cauchy stress vector</td>
</tr>
<tr>
<td>$\sigma(x, n)$</td>
<td>(first) Piola-Kirchhoff stress vector</td>
</tr>
<tr>
<td>$p(x, t)$</td>
<td>momentum</td>
</tr>
<tr>
<td>$\rho(x)$</td>
<td>mass density of the body in the reference configuration</td>
</tr>
<tr>
<td>$\rho^d_t(y)$</td>
<td>mass density of the deformed body</td>
</tr>
<tr>
<td>$T^d_t(y)$</td>
<td>Cauchy stress tensor</td>
</tr>
<tr>
<td>$T(x)$</td>
<td>first Piola-Kirchhoff stress tensor</td>
</tr>
<tr>
<td>$S(x)$</td>
<td>second Piola-Kirchhoff stress tensor</td>
</tr>
<tr>
<td>$S_{lin}(x)$</td>
<td>linearized second Piola-Kirchhoff stress tensor</td>
</tr>
<tr>
<td>$\hat{T}^d_t$</td>
<td>response function for the Cauchy stress</td>
</tr>
<tr>
<td>$\hat{T}$</td>
<td>response function for the first Piola-Kirchhoff stress</td>
</tr>
<tr>
<td>$\hat{S}$</td>
<td>response function for the second Piola-Kirchhoff stress</td>
</tr>
<tr>
<td>$\lambda, \mu$</td>
<td>Lamé constants</td>
</tr>
<tr>
<td>$\nu, \varepsilon$</td>
<td>Poisson’s ratio and Young’s modulus</td>
</tr>
</tbody>
</table>

**Image Registration**

$\Omega \subset \mathbb{R}^d$ rectangular domain of the d-dimensional image

$I$ d-dimensional image $I : \Omega \rightarrow \mathbb{R}$

$Img(d)$ set of all d-dimensional images

$\mathcal{R}$ reference image $\mathcal{R} : \Omega \rightarrow \mathbb{R}$

$\mathcal{T}$ template image $\mathcal{T} : \Omega \rightarrow \mathbb{R}$
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Quantity / Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>$y$</td>
<td>transformation $y: \mathbb{R}^d \rightarrow \mathbb{R}^d$</td>
</tr>
<tr>
<td>$u$</td>
<td>displacement field $u: \mathbb{R}^d \rightarrow \mathbb{R}^d$</td>
</tr>
<tr>
<td>$J$</td>
<td>energy functional</td>
</tr>
<tr>
<td>$D$</td>
<td>distance measure $D: \text{Img}(d)^2 \rightarrow \mathbb{R}$</td>
</tr>
<tr>
<td>$D_{SSD}$</td>
<td>sum of squared differences distance measure (SSD)</td>
</tr>
<tr>
<td>$M$</td>
<td>admissible set</td>
</tr>
<tr>
<td>$S$</td>
<td>smoother</td>
</tr>
<tr>
<td>$P_d$</td>
<td>$d$-dimensional elastic potential</td>
</tr>
<tr>
<td>$P$</td>
<td>penalty (soft constraint)</td>
</tr>
<tr>
<td>$C$</td>
<td>(hard) constraint</td>
</tr>
</tbody>
</table>

**Mathematical Modeling of Cellular Traction Forces**

- $\mathcal{E} \subset \mathbb{R}^3$: three-dimensional extracellular matrix
- $x \in \mathcal{E}$: point in the three-dimensional extracellular matrix $\mathcal{E}$
- $\mathcal{E}_{us} \subset \mathcal{E} \subset \mathbb{R}^3$: upper surface of the three-dimensional extracellular matrix $\mathcal{E}$
- $\mathcal{E}_{us,c} \subset \mathcal{E}_{us} \subset \mathbb{R}^3$: portion of the upper surface of the three-dimensional extracellular matrix $\mathcal{E}_{us}$, where the cell is located
- $\mathcal{S} \subset \mathbb{R}^2$: two-dimensional (upper) surface of the extracellular matrix
- $\mathcal{S} = \{ \hat{x} \in \mathbb{R}^2 : x \in \mathcal{E} \}$
- $\mathcal{S}_c \subset \mathcal{S} \subset \mathbb{R}^2$: portion of the two-dimensional surface $\mathcal{S}$, where the cell is located
- $f$: applied cellular traction force
- $p$: concentrated surface force applied at a single point of the surface such that $s = p\delta(x_1)\delta(x_2)$
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1. Introduction

Any child who once tumbled and grazed his or her knee could afterwards experience the fascinating process of wound healing. This is just one example of a biological process in which cell locomotion plays a decisive role (c.f. [43], p.788). Other prominent examples include embryological morphogenesis, inflammation, immune response and formation of tumor metastases (c.f. [19], p.41). Taking a closer look at the latter, the high medical relevance of a better understanding of the migration of cells becomes particularly obvious: In December 2013, the International Agency for Cancer Research (IACR) reported that in 2012 the number of new cancer cases had globally risen to 14.1 million and that in the same year an estimated number of 8.2 million people died from cancer or its direct consequences (c.f. [40]). Regarding the fact that metastasis is responsible for 90% of deaths from solid tumors (c.f. [22], p.679), the comprehension of cellular motility is of utmost importance in cancer research.

However, cell migration is characterized by complex interactions of numerous molecular mechanisms including the protrusion of the cell’s leading edge for an adhesive cell-substratum interplay and contractions of regions of this leading edge or the entire cell body. These contractions give rise to forces that result in a detachment of the rear of the cell and in a gradual forward gliding of the entire cell body (c.f. [19], p.41 and [20], p.362). Consequently, the determination of these cellular traction forces seems to be crucial to a better perception of the underlying processes leading to cellular locomotion.

To study these forces, cells are most commonly cultured on an elastic substrate providing an in vitro model of the migration process (c.f. [30], p.62). This experimental setting is illustrated by the subsequent figure:

Figure 1.1.: Illustration of the experimental setting for considering migrating cells in vitro (graphic taken from [44], p.228)
1. Introduction

In the light of the above considerations, the present thesis deals with the reconstruction of cellular traction forces. Thereby, our major contribution is the development and the presentation of a novel approach for the reconstruction of cellular traction forces from ordinary phase-contrast microscopy images of sufficient resolution based on the combination of linear elasticity theory and a flexible variational image registration framework.

This thesis is organized as follows:
In the first part of this thesis we provide the reader with some mathematical background knowledge. Considering Figure 1.1 once again, it can be recognized that the cellular traction forces are transmitted to the underlying elastic substrate via the focal adhesion sites resulting in displacements, which can be detected. Taking this observation into account, we at first give an introduction to the theory of elasticity allowing for a mathematical description of this relation. Furthermore, we present some fundamentals of image registration as this will be our method of choice for the reconstruction of the cellular traction forces from the phase-contrast microscopy data.

In Chapter 3 we continue by presenting a sound mathematical model of the experimental setting described above. We introduce a forward problem characterizing the effect of the occurring traction forces to the underlying substrate mathematically. Besides, we deduce the famous Boussinesq solution as well as the so-called plane stress approximation providing two possibilities of making the forward problem determinate.

Chapter 4 deals with a summary of major contributions to the development of modern cellular traction force determination since the 1980s. We will see that nowadays the so-called elastic substratum method is the most prominent way of reconstructing the exerted traction forces and we will present four state of the art techniques that are based on this method. Subsequently, we use the gained insights to motivate our novel approach by emphasizing that its major contribution is the utilization of usual phase-contrast microscopy images and thus overcoming the need for beads being firmly embedded in the substrate.

Based on all these considerations Chapter 5 finally addresses the presentation of the underlying idea of our completely new approach as well as its mathematical realization by means of a variational model. We also provide some analytic results yielding the existence of solutions of the resulting optimization problems.

Chapter 6 is devoted a description of the numerical realization of our new method and to the presentation and discussion of first reconstruction results.
We complete this thesis with a brief conclusion and an outlook to possible areas of future research.
2. Mathematical Background

In this chapter we present some basic principles of the theory of elasticity, which in the following will enable us to thoroughly understand the mathematical model of the experimental setting described in the previous chapter. Furthermore, we give an introduction to image registration as this will be our method of choice for the calculation of the displacement field of the underlying substrate and thereby for the reconstruction of the traction exerted by the cell.

2.1. Introduction to the Theory of Elasticity

In everyday language a body is called to behave elastically if it "returns to its original shape and size when the forces causing the deformation are removed" [5]. Using this as a starting point, this section aims at setting up a mathematical framework of elasticity, which is part of the field of continuum mechanics in physics.

We therefore begin with the mathematical description of the deformation and motion of a solid material being the scientific object of kinematics. Furthermore, this section deals with dynamics including the mathematical characterization of the associated external forces causing the deformation of the body and the resulting internal forces. We also state some fundamental balance laws and constitutive equations and in the end give an introduction to linear elasticity.

This brief overview is mainly based on the works of P. G. Ciarlet [10]. Besides, [15], [29] and [45] provided many valuable insights and additional information.

2.1.1. Kinematics

Before we deal with the causes and effects of deformations we shall at first define the term deformation in a mathematical context and introduce some means for its characterization.

Having a closer look at the common perception of elasticity, we first have to identify an 'original shape' (see above) for comparison with the deformed state. We call the earlier one reference configuration, while the later one is the so-called current or deformed configuration at time $t$. Often the initial or unloaded configuration is chosen to be the reference configuration, but actually the reference configuration does not need to be occupied by the body at any time (see [45]). From now on,
2. Mathematical Background

we assume that in the reference configuration the body occupies the closure $\bar{\Omega}$ of a domain $\Omega \subset \mathbb{R}^3$, i.e. of a sufficiently smooth, open and connected region $\Omega$ with a Lipschitz-continuous boundary $\partial \Omega$, such that the interior of the set $\bar{\Omega}$ is just the set $\bar{\Omega}$ (cf. [10], p. 36). Concerning the denotation of points within the body we distinguish between Eulerian or spatial coordinates and Lagrangian or material coordinates. In the case of **Eulerian coordinates** a point in space is fixed and then the changes of physical quantities over time are addressed. Note that in this situation the point is occupied by different particles. In the situation of **Lagrangian coordinates**, which is typically used more frequently in elasticity (see [15], p. 256), we in contrast fix one particle and track it over time. Here the particle is labeled by its position in the reference configuration. (see [45])

The subsequent drawing illustrates the reference configuration denoted by $\bar{\Omega}$ and the current configuration denoted by $\bar{\Omega}^d_t$ at time $t$ as well as the notation of points in Lagrangian and Eulerian coordinates, where in the first situation the particle is marked with $x \in \bar{\Omega} \subset \mathbb{R}^3$ while in the later one it is marked with $y = y_t(x) = x^d_t \in \mathbb{R}^3$:

![Figure 2.1.: Reference and Deformed Configuration](own representation based on [15], p.256)

The above graphic also includes a mapping $y$ transferring the reference configuration to the current configuration, at which we now take a closer look: We define (see [10], p. 27 and [29], p.2):

**Definition 2.1.**

A **deformation** of the reference configuration $\bar{\Omega}$ is a vector field $y_t : \bar{\Omega} \rightarrow \bar{\Omega}^d_t$ that is sufficiently smooth, orientation preserving and invertible on the open region $\Omega$. We write $y = y_t(x) = x^d_t$.

We point out that in general we have to restrict the invertibility of the deformation $y_t$ to the interior of the set $\bar{\Omega}$ because the map may lose its injectivity on the boundary of $\Omega$ as ‘self-contact’ has to be allowed (cf. [10], p.27). However, if a deformation
2.1. Introduction to the Theory of Elasticity

is injective on $\bar{\Omega}$, which we will henceforward assume, $y_t(\bar{\Omega})$ equals to the closure of $y_t(\bar{\Omega})$, the interior of $y_t(\bar{\Omega})$ is just $y_t(\Omega)$ and $y_t(\partial \Omega)$ is the same as $\partial y_t(\Omega)$ and we can thus justify to write:

$$\bar{\Omega}_t^d = y_t(\bar{\Omega}), \quad \Omega_t^d = y_t(\Omega), \quad \partial \Omega_t^d = y_t(\partial \Omega)$$

for the current configuration, its interior and its boundary (cf. [10], p. 36).

As in some applications it is more convenient to consider the difference between points in the current and the reference configuration rather than describing the points in the current configuration itself, we also introduce (see [10], p.28 f.):

**Definition 2.2.**

The **displacement field** is a mapping

$$u_t : \bar{\Omega} \rightarrow \bar{\Omega}_t^d$$

defined by the relation $u_t(x) = y_t(x) - x$.

We emphasize that if we want to analyze a deformation or a displacement we only consider a current deformed configuration at some fixed time $t$ and the reference configuration at some fixed time $t_0$. Consequently, the sequence of configurations between those two has no impact on the result. However, we will also need time-dependent concepts such as velocity to derive an elastic model for the experimental setting described before. Therefore we additionally introduce (see [29], p.2):

**Definition 2.3.**

A **motion** of $\bar{\Omega}$ is a time-dependent family of configurations, written $y = y(x,t)$.

The **velocity** of the material point $x$ is defined by

$$v(x,t) = \dot{y}(x,t) = \frac{\partial y}{\partial t}(x,t)$$

and the **acceleration** of a motion $y(x,t)$ is given by

$$a(x,t) = \ddot{y}(x,t) = \frac{\partial v}{\partial t}(x,t) = \frac{\partial^2 y}{\partial^2 t}(x,t)$$

For the analysis of deformations we will furthermore need the associated concept of **strain** describing the "relative [...] change in shape and size of elastic [...] materials under applied forces" [6].

By the construction of the domain $\Omega$ there exists $\rho > 0$ such that the open ball

$$B_\rho(x_0) := \{x \in \mathbb{R}^3 : |x - x_0| < \rho\}$$

is a convex subset of $\Omega$. 

2. Mathematical Background

We recall that if the deformation $y$ is differentiable we can write by means of the mean value theorem:

$$\frac{|y(x_1) - y(x_2)|}{|x_1 - x_2|} \leq \sup_{z \in [x_1, x_2]} |Dy(z)|, \quad \forall x_1, x_2 \in B_\rho(x_0)$$

Therefore it seems reasonable that a description of strain locally is given by (see [10], p. 27 and [29], p.3):

**Definition 2.4.**

Given a deformation $y : \Omega \subset \mathbb{R}^3 \rightarrow \bar{\Omega}^d \subset \mathbb{R}^3$ we define at each point of the set $\Omega$ the matrix of partial derivatives of $y$:

$$Dy = Dy_t(x) = \left( \frac{\partial y_i}{\partial x_j}(x) \right)_{ij}, \quad \text{for } i,j = 1,...,3$$

The matrix $Dy$ is called the **deformation gradient**.

Since $y_t$ is by definition assumed to preserve orientation, $\det Dy > 0 \quad \forall x \in \bar{\Omega}$.

For the sake of readability we introduce the following notations commonly used in the literature (cf. [10], p.28):

$$F := Dy$$

$$J := \det Dy$$

To provide a better insight into the physical perception of deformations we furthermore state (see [10], p.94 and [29], p.3):

**Definition 2.5.**

As the deformation gradient $F$ is a real invertible matrix, $F$ can be factorized in a unique fashion by the polar decomposition theorem from linear algebra as:

$$F = RU = VR,$$

where $R$ is a proper orthogonal matrix called the **rotation**, and $U$ and $V$ - called the **right** and **left stretch tensors** - are positive-definite and symmetric.

Indeed, $U = \sqrt{F^T F}$ and $V = \sqrt{FF^T}$, where $F^T$ is the transpose of $F$.

The matrices $C = F^T F = U^2$ and $B = FF^T = V^2$ are called the **right** and **left Cauchy-Green tensor**, respectively.

Consequently, from a physical point of view, the deformation of a body is locally given to first order by a rotation followed by a change of distances due to deformation or vice versa (see [29], p.4).
2.1. Introduction to the Theory of Elasticity

This stretching or compression applies along the orthogonal eigenvectors of \( U \) and \( V \), respectively. To measure the amount of stretching or compression we consider the corresponding eigenvalues. From the identity:

\[
RU = VR \iff U = R^{-1}VR
\]

we can easily see that \( U \) and \( V \) are similar. Therefore their eigenvalues coincide and we define (see [29], p.4):

\textbf{Definition 2.6.}

The eigenvalues of the stretch tensors denoted by \( \lambda_1, \lambda_2 \) and \( \lambda_3 \) are called \textit{principal stretches}.

As stated in Definition 2.5 \( U \) and \( V \) are positive-definite and symmetric and hence the principal stretches are real and positive. The amount of strain can then be characterized by the deviation of these principal stretches from unity.

On the basis of these principal stretches we now introduce matrix invariants, which are measures of the change of length, area and volume, respectively (see [45]):

\textbf{Definition 2.7.}

The \textit{matrix invariants} of the Cauchy-Green tensors are given by:

\[
\begin{align*}
I_C &= \lambda_1^2 + \lambda_2^2 + \lambda_3^2 = \text{tr}(C) = \|F\|^2_2 \\
II_C &= \lambda_1^2\lambda_2^2 + \lambda_2^2\lambda_3^2 + \lambda_3^2\lambda_1^2 = \frac{1}{2} [\text{tr}(C)^2 - \text{tr}(C^2)] = \|\text{Cof} F\|^2_2 \\
III_C &= (\lambda_1\lambda_2\lambda_3)^2 = \det C = |\det F|^2
\end{align*}
\]

Now being able to describe deformations of bodies in mathematical terms, we continue with the characterization of the associated forces.

\subsection*{2.1.2. Dynamics}

Considering once again our first approach towards the term elasticity, we stated that the deformation is caused by forces (cf. p.3). Regarding this, the current passage aims at setting up a mathematical description of these applied external forces as well as the resulting internal ones.

To meet this target we will have to calculate volumes and areas not only in the reference configuration, but also in the deformed configuration. At the beginning of this paragraph we therefore aim at expressing these quantities over the current configuration in terms of the same quantities over the reference configuration.

Before we deal with this objective we shall once and for all introduce an appropriate notational device to point out the importance of the distinction between both types of quantities: Every quantity defined over the deformed configuration is systematically provided with the superscript \( d \), while the respective quantity over the reference
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configuration is denoted by the same letter, but without the superscript. We already used this kind of notation when we introduced the designation of the reference and the current configuration as well as points therein (cf. Figure 2.1 on p. 4) and therefore this notation is consistent with the previous paragraph. The correspondence between a quantity defined as a function of the Lagrange variable \( x \) in the reference configuration and a similar quantity defined as a function of \( x = y_t(x) = y \) in the deformed configuration here established can be extended to divergences of tensor fields and to applied forces discussed later on (cf. [10], p.31).

Now returning to the task of defining a volume element \( dy = dx^d_t \) in the deformed configuration that corresponds to a volume element \( dx \) in the reference configuration, we recall (see [10], p. 31):

**Theorem 2.8. Change of Variables Formula in Multiple Integrals**

Let \( \Theta \) denote a measurable subset of a sufficiently smooth, open and connected region \( \Omega \), and let \( y : \Theta \rightarrow y_t(\Theta) = \Theta^d_t \) be an injective, continuously differentiable mapping with a continuous inverse \( y_t^{-1} : \Theta^d_t \rightarrow \Theta \).

Then a function \( \varphi : x^d \in \Theta^d_t \rightarrow \mathbb{R}^3 \) is \( dx^d_t \) integrable over the set \( \Theta^d_t \) and if this is the case, the following equality holds:

\[
\int_{\Theta^d_t = y_t(\Theta)} \varphi(x^d) \, dx^d = \int_{\Theta} (\varphi \circ y_t)(x) |\text{det}Dy(x)| \, dx
\]

As a direct consequence we can conclude (cf. [10], p.31):

**Corollary 2.9.**

Let \( \Theta \) be a measurable subregion of the interior of the reference configuration \( \Omega \) and let \( y_t : \Theta \rightarrow y_t(\Theta) = \Theta^d_t \) be a deformation of \( \Theta \).

Then the volume elements \( dx \) and \( dy \) at the points \( x \in \Omega \) and \( y \in \Omega^d_t \) are related by:

\[
dy = dx^d_t \quad \text{det} \, Dy(x) > 0 \quad \Rightarrow \quad \text{det} \, Dy(x) \, dx = \text{det} \, F(x) \, dx = J(x) \, dx
\]

Next, we try to find a representation of an area element in the deformed configuration in terms of an area element in the reference configuration. As the deformation \( y_t \) is sufficiently smooth by definition, the set \( \Omega^d_t \) is also a domain and therefore we can define an area element \( da^d_t \) along the boundary \( \partial \Omega^d_t \) of the deformed configuration with a unit outer normal vector \( n^d_t \) \( da^d_t \)-almost everywhere along \( \partial \Omega^d_t \) (cf. [10], p.37).

Now we can state (see [10], p.39):

**Theorem 2.10.**

The area elements \( da \) and \( da^d_t \) at the points \( x \in \partial \Omega \) and \( y = y(x) = x^d_t \in \partial \Omega^d_t \) with unit outer normal vectors \( n \) and \( n^d_t \) respectively, are related by:

\[
n^d_t \, da^d_t = (\text{det} \, Dy(x)) Dy(x)^{-T} \cdot n \, da = J(x) F(x)^{-T} \cdot n \, da = \text{Cof} \, F(x) \cdot n \, da
\]
2.1. Introduction to the Theory of Elasticity

To show the above statement we will need the subsequent lemma:

Lemma 2.11.

The following equation called **Piola’s identity** holds:

\[ \nabla \cdot \text{Cof} \ F = \nabla \cdot (J(F^{-1})^T) = 0 \]

*Proof.*

The following equation holds by definition of the divergence of a tensor field:

\[ \nabla \cdot ((JF^{-T})_i:) \]

Consider \((*)\) separately. Then the following equation clearly holds:

\[ I = FF^{-1} \]

If we derive this equation with respect to \(x_j\) we get:

\[ \begin{align*}
\nabla \cdot ((JF^{-T})_i:) \quad &\xrightarrow{\text{Product rule}} \quad \sum_{j=1}^{3} \partial_j J(F^{-T})_{ij} + J \partial_j (F^{-T})_{ij} \\
\nabla \cdot ((JF^{-T})_i:) \quad &\xrightarrow{\text{Jacobi's Formula}} \quad \sum_{j=1}^{3} \left[ \text{Cof} F : \frac{\partial F}{\partial x_j} \right] (F^{-T})_{ij} + J \partial_j (F^{-T})_{ij} \\
\n\nabla \cdot ((JF^{-T})_i:) \quad &\xrightarrow{\text{Product rule}} \quad \sum_{j=1}^{3} \partial_j J(F^{-T})_{ij} + J \partial_j (F^{-T})_{ij} \\
\n\nabla \cdot ((JF^{-T})_i:) \quad &\xrightarrow{\text{Product rule}} \quad \sum_{j=1}^{3} \left[ \text{Cof} F : \frac{\partial F}{\partial x_j} \right] (F^{-T})_{ij} + J \partial_j (F^{-T})_{ij} \\
\end{align*} \]

Inserting this result for \((*)\) yields:

\[ \nabla \cdot ((JF^{-T})_i:) = \sum_{j,k,l=1}^{3} J(F^{-T})_{kl}(\partial_j F)_{kl}(F^{-T})_{ij} - J(F^{-T})_{ik}(\partial_j F)_{kl}(F^{-T})_{lj} \]

Note that \((\partial_j F^T)_{kl} = (\partial_j F)_{lk} = (\partial_k F)_{ij} \]

\[ \nabla \cdot ((JF^{-T})_i:) = 0 \quad \forall i \]

\[ \square \]
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Using Piola’s identity, we are now able to prove Theorem 2.10.

Proof. (of Theorem 2.10)

Let \( \varphi : \Omega \rightarrow \mathbb{R}^3 \) be a vector field and \( \Theta \subset \Omega \) be a subregion with \( x \in \Theta \) and \( y_t(x) = x^d_t \in \Theta^d_t \). Then we have:

\[
\int_{\partial \Theta} \varphi(x) \Cof F(x) \cdot n \, da = \int_{\Theta} \nabla \cdot \left( \Cof F^T \varphi(x) \right) \, dx
\]

\[
\text{Product Rule}
\]

\[
= \int_{\Theta} \sum_{j=1}^3 (\partial_j \Cof F_j^T(x)) \varphi(x) + \Cof F^T_j(x)(\partial_j \varphi(x)) \, dx
\]

\[
\text{Divergence Thm}
\]

\[
= \int_{\Theta} \sum_{j=1}^3 (\partial_j \Cof F_j(x))^T \varphi(x) + \sum_{j=1}^3 \Cof F^T_j(x)(\partial_j \varphi(x)) \, dx
\]

\[
= \int_{\Theta} \left( \nabla \cdot \Cof F(x) \right)^T \varphi(x) + \text{tr}(D\varphi(x)(\Cof F^T(x))) \, dx
\]

\[
\text{Change of Variables Thm}
\]

\[
= \int_{\Theta} \text{tr}(D\varphi(y^{-1}(y))(F^{-1}(y))) \, dy
\]

\[
\text{Divergence Thm}
\]

\[
= \int_{\Theta} \varphi(y^{-1}(y)) \cdot n^d \, da^d
\]

\[
\Rightarrow n^d_t \, da^d_t = \Cof F \cdot n \, da
\]

As in the case of Corollary 2.9, the assertion of Theorem 2.10 still holds true, if the set \( \Omega \) is replaced by any subregion \( \Theta \subset \Omega \). In this situation the respective area elements and unit outer normal vectors naturally are to be understood as being defined along the boundaries of \( \Theta \).

From the above theorem we can furthermore directly conclude that the unit outer normal vectors are related by:

\[
n^d_t = \frac{\Cof F(x) \cdot n}{|\Cof F(x) \cdot n|}
\]

Now we have everything at our disposal to continue with the discussion of the external forces causing the body of interest to deform. In continuum mechanics two kinds of forces characterizing the action of the outside world on the solid are differentiated: Firstly we have (see [45]):

**Definition 2.12.**

Let \( \Theta \) be a subset of the region \( \Omega \) occupied by the body in the reference configuration and let \( \Theta^d_t \) be this subset after time \( t \). A **body force** \( b^d_t(y) \in \mathbb{R}^3 \) is a force per unit volume exerted by the external world in the current configuration at time \( t \).

Then the total force \( F^d_t \) exerted on \( \Theta^d_t \) at time \( t \) is calculated by:

\[
F^d_t(t) = \int_{\Theta^d_t} b^d_t(y) \, dy
\]
And secondly we define (see [45] and [10], p.58):

**Definition 2.13.**

Let $\Gamma_1$ be a $\sigma$-measurable subset of the two-dimensional manifold $\Gamma := \partial \Omega$ of the domain $\Omega$ with normal $n$ and let $\Gamma_{1,t}$ be this subset after time $t$ with normal $n_{\Gamma_{1,t}}$. A **surface force** $s_{\Gamma}^d(y) \in \mathbb{R}^3$, $y \in \Gamma_{1,t}$, is a force per unit area of $\Gamma_{1,t}$ acting along the portion $\Gamma_{1,t}$ of the boundary $\Gamma_{1,t}$ at time $t$.

Then the total force $F_{\Gamma}^d(t)$ exerted on $\Gamma_{1,t}$ is calculated by: $F_{\Gamma}^d(t) = \int_{\Gamma_{1,t}} s_{\Gamma}^d(y) \, dy$.

We point out that body forces such as gravitational force are exerted per unit volume at each point of the current configuration while in contrast surface forces as for example frictional force are exerted per unit area at each point of $\Gamma_{1,t}$. Such surface forces typically represent the action of another body (whatever its nature may be) on the portion $\Gamma_{1,t}$ of the boundary of the object (see [10], p.59). The pair of this two forces $l_{\Gamma}^d = (b_{\Gamma}^d, s_{\Gamma}^d)$ is called the **load**, which is often given in advance (see [29], p.4).

Being now capable of describing the external forces, we introduce the concept of stress: In a physical context, stress locally describes the "force per unit area within materials that arises from externally applied forces" [7]. Hence we now continue with the investigation of the internal forces of stress the body generally experiences across any given real or imaginary surface (see [29], p.5). Therefore we assume that a two-dimensional manifold $\Upsilon \subset \Omega$ with normal $n$ separates the region $\Omega$ into two subregions $\Theta_1$ and $\Theta_2$. To describe the internal force along the deformed manifold $\Upsilon_{1,t}$ acting between the deformed subregions $\Theta_{1,t}$ and $\Theta_{2,t}$ the French mathematician Augustin-Louis Cauchy (1789 - 1857) presented the following hypothesis (see [45] and [10], p.60 and 62):

**Hypothesis 2.14. Cauchy’s Hypothesis**

Consider a body occupying a current configuration $\tilde{\Omega}_{1,t}$ at time $t$ and subjected to applied forces $F_b^d(t)$ and $F_s^d(t)$.

Then there exists a scalar function:

$$\sigma_{\Upsilon}^d : \tilde{\Omega}_{1,t} \times \Sigma_1 \longrightarrow \mathbb{R}^3; (y, n_{\Upsilon}^d) \mapsto \sigma_{\Upsilon}^d(y, n_{\Upsilon}^d),$$

where $\Sigma_1 = \{ v \in \mathbb{R}^3 : |v| = 1 \}$ such that for any smooth real or imaginary surface $\Upsilon_{1,t} \subset \tilde{\Omega}_{1,t}$ with normal $n_{\Upsilon}^d$ at $y = y(x) = x_{\Upsilon}^d$, $\sigma_{\Upsilon}^d(y, n_{\Upsilon}^d)$ is the surface force exerted by the material on one side of $\Upsilon_{1,t}$ onto the material on the other side.

The vector $\sigma_{\Upsilon}^d(y, n_{\Upsilon}^d)$ is called the **Cauchy stress vector** across an oriented surface element with normal $n_{\Upsilon}^d$.

For a better perception of the previous hypothesis the subsequent graphic depicts
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the two-dimensional manifold $\Sigma^d_t$ separating the deformed region $\Omega^d_t$ and illustrates the unit normal vector $n^d_t$ as well as the Cauchy stress vector $\sigma^d_t$:

![Figure 2.2: 2D manifold $\Sigma^d_t$ with normal $n^d_t$ separating $\Omega^d_t$ and the Cauchy stress vector (own representation based on [15], p.258)]

To establish a direct connection between the surface force $s^d_t(y)$ applied along a portion of the boundary of the object and the Cauchy stress vector $\sigma^d_t(y,n^d_t)$ we also allow $\Sigma$ to be a portion of the boundary of the domain $\Omega$ (cf. [10], p.60):

**Remark 2.15.**

In the situation of hypothesis 2.14, where we set $\Sigma = \Gamma_1 \subset \Gamma = \partial \Omega$, the identity:

$$\sigma^d_t(y,n^d_t) = s^d_t(y)$$

holds for any subdomain $\Theta^d_t$ of $\bar{\Omega}^d_t$ and at any point $y \in \Sigma^d_t \cap \partial \Theta^d_t$, where the unit outer normal vector on $\Sigma^d_t \cap \partial \Theta^d_t$ exists.

Here we emphasize that in both cases the Cauchy stress vector is associated with a surface $\Sigma^d_t$ with normal $n^d_t$ and thus does not only depend on the position of the material point $y \in \Sigma^d_t$ but also on the orientation of the surface represented by its normal vector $n^d_t$. However, it does not depend on the curvature or other geometrical properties of the associated surface element. (cf. [10], p.60)

Because in elasticity the Lagrangian presentation of coordinates is widely-used, we also state (see [45]):

**Definition 2.16.**

The (first) Piola-Kirchhoff stress vector $\sigma(x,n)$ is parallel to the Cauchy stress vector $\sigma^d_t(y,n^d_t)$, but measures the surface force per unit area in the reference configuration, acting across the surface $\Sigma$, having normal $n$ in the reference state.

Let $da^d_t$ be a surface element in the deformed configuration and $da$ the corresponding surface element in the reference configuration, then:

$$\sigma(x,n) \, da = \sigma^d_t(y,n^d_t) \, da^d_t$$

Having gained some insight into the description of forces in the context of elasticity, we are now able to discuss some conservation principles.
2.1. Introduction to the Theory of Elasticity

2.1.3. Balance Laws

As we aim to derive a mathematical model for the behavior of an elastic body under applied forces we not only need to define means to describe the deformation and forces, but we also have to figure out a system of differential equations describing how these are connected and interact over time. To meet this target the current paragraph deals with the introduction of some fundamental balance laws of physics, which can be regarded as the axioms of elasticity. Using these conservation principles we derive Cauchy’s theorem, one of the most famous results in elasticity.

We assume that we are in the situation of a system of constant mass, which means (cf. [29], p.4):

**Axiom 1. Conservation of Mass**

Given a deformation \( y_t(x) \) of a solid, let \( \rho^d_t(y) \) be the mass density of the deformed body at time \( t \) and let \( \rho(x) \) be the mass density in the reference configuration. Furthermore, let \( \Theta \) be a subset of the region \( \Omega \) and let \( \Theta^d_t \) be the subset after time \( t \).

Then conservation of mass states that \( \text{mass}(\Theta) = \text{mass}(\Theta^d_t) \), that is

\[
\int_{\Theta} \rho(x) \, dx = \int_{\Theta^d_t} \rho^d_t(y) \, dy, \quad \text{where } \rho(x) = \det D_y \rho^d_t(y) = J \rho^d_t(y) \tag{2.1}
\]

Because the total mass of the deformed body stays constant over time, we can apply Newton’s second law of motion, which the English physicist and mathematician Isaac Newton published in his "Mathematical Principles of Natural Philosophy" in 1687. This law states that the sum of the forces \( F \) on a body is equal to the product of the mass \( m \) of this body and the corresponding acceleration \( a \), i.e.:

\[
F = ma = \frac{d}{dt}mv = \frac{d}{dt}p = \dot{p},
\]

where \( p \) is a physical quantity called the momentum.

As the second axiom of elasticity we therefore introduce the continuum analogon of Newton’s second law (cf. [29], p.5):

**Axiom 2. Balance of Momentum**

In the situation of axiom[1] the following identity holds for all subsets \( \Theta^d_t \) of \( \Omega^d_t \):

\[
\frac{d}{dt} \int_{\Theta^d_t} \rho^d_t(y) v(y,t) \, dy = \int_{\partial \Theta^d_t} \sigma^d_t(y,n^d_t) \, da^d + \int_{\Theta^d_t} b^d_t(y) \, dy, \tag{2.2}
\]

where \( v \) is the velocity and \( b^d_t \) denotes the applied body force per unit volume at time \( t \).

This assertion is called balance of (linear) momentum.
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Following this second axiom Cauchy introduced one of the most popular results in continuum mechanics in the 1820s, namely (see [10], p.63ff. and [29], p.127):

**Theorem 2.17. Cauchy’s Theorem**

Assume that the applied body force density per unit volume $b^d_t : \Omega^d_t \rightarrow \mathbb{R}^3$ is continuous and that the Cauchy stress vector $\sigma^d_t : \Omega^d_t \times \Sigma_1 \rightarrow \mathbb{R}^3$; $(y, n^d_t) \mapsto \sigma^d_t(y, n^d_t)$ is continuously differentiable with respect to the variable $y \in \Omega^d_t$ for each $n^d_t \in \Sigma_1$ and continuous with respect to the variable $n^d_t \in \Sigma_1$ for each $y \in \Omega^d_t$.

Then the balance of momentum implies that there exists a continuously differentiable tensor field

$$T^d_t : \Omega^d_t \rightarrow \mathbb{M}^3;$$

$$y \mapsto T^d_t(y)$$

such that the Cauchy stress vector satisfies

$$\sigma^d_t(y, n^d_t) = T^d_t(y) \cdot n^d_t \quad \forall \ y \in \Omega^d_t, \forall \ n^d_t \in \Sigma_1$$

(2.3)

and such that

$$\rho^d_t(y) a(y, t) = \nabla^d \cdot T^d_t(y) + b^d_t(y) \quad \forall \ y \in \Omega^d_t$$

$$T^d_t(y) \cdot n^d_t = s^d_t(y) \quad \forall \ y \in \Gamma^d_{1,t}$$

(2.4)

holds. This system of equations is called **Cauchy’s equation of motion**, where $\rho^d_t$ is the mass density in the deformed configuration at time $t$, $n^d_t$ is the unit outer normal vector along $\Gamma^d_{1,t}$, and $s^d_t$ is the applied surface force per unit area of $\Gamma^d_{1,t}$.

The tensor $T^d_t(y)$ is called the **Cauchy stress tensor** at the point $y \in \Omega^d_t$.

**Proof.**

At first we prove the proposition:

$$\sigma^d_t(y, n^d_t) = T^d_t(y) \cdot n^d_t$$

For this purpose let $y_0$ denote an arbitrary but fixed point in $\Omega^d_t$. Because the set $\Omega^d_t$ is open by construction, there exists a neighborhood of $y_0$ for every choice of $y_0$. If we now consider a positive oriented Cartesian coordinate system $\{e_1, e_2, e_3\}$ such that its origin coincides with $y_0$, we can draw, as a particular subdomain of $\Omega^d_t$, a tetrahedron $\Delta^d_t$ in this coordinate system. We create the tetrahedron $\Delta^d_t$ such that three faces are oriented in the coordinate planes and with the fourth one denoted by $\Phi$ oriented such that all components of the associated normal vector $n^d_t$ are positive, i.e. $(n^d_t)_j = n^d_t \cdot e_j > 0$. For the sake of readability we denote $(n^d_t)_j$ by $n^d_j$. 

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Now we can choose the position of the remaining three vertices \( a, b \) and \( c \) such that 
\[
\begin{align*}
\mathbf{n}_t \cdot \mathbf{a} &= e_1, \\
\mathbf{n}_t \cdot \mathbf{b} &= e_2 \quad \text{and} \\
\mathbf{n}_t \cdot \mathbf{c} &= e_3,
\end{align*}
\]
i.e. \( a = \left( \frac{1}{n_1}, 0, 0 \right), \quad b = \left( 0, \frac{1}{n_2}, 0 \right) \) and \( c = \left( 0, 0, \frac{1}{n_3} \right) \).

Next, we consider the balance of momentum equation:
\[
\begin{align*}
\frac{d}{dt} &\int_{\Delta^d_t} \rho_t^d(y) \mathbf{v}(y,t) \, dy = \int_{\partial \Delta^d_t} \sigma_t^d(y,n^d_t) \, da_t^d + \int_{\Delta^d_t} b_t^d(y) \, dy & (2.5) \\
\iff \int_{\Delta^d_t} \rho_t^d(y) \mathbf{a}(y,t) \, dy &= \int_{\partial \Delta^d_t} \sigma_t^d(y,n^d_t) \, da_t^d + \int_{\Delta^d_t} b_t^d(y) \, dy & (2.6) \\
\iff \int_{\Delta^d_t} \rho_t^d(y) \mathbf{a}(y,t) - b_t^d(y) \, dy &= \int_{\partial \Delta^d_t} \sigma_t^d(y,n^d_t) \, da_t^d, & (2.7)
\end{align*}
\]
and fix the variable \( t \).

Due to the continuity of the Cauchy stress vector with respect to the second variable, we can consider the case \( |n^d_t| \to 0 \). Then by construction,
\[
\lim_{|n^d_t| \to 0} \text{volume } \Delta^d_t = \lim_{|n^d_t| \to 0} \int_{\Delta^d_t} dy = 0
\]
and hence Equation (2.7) reads:
\[
\lim_{|n^d_t| \to 0} \int_{\partial \Delta^d_t} \sigma_t^d(y_0,n^d_t) \, da_t^d = 0 \quad (2.8)
\]
Let the three coordinate faces of the tetrahedron $\Delta^d_t$ be labeled by $\Phi_i$ (and recall that the fourth is labeled by $\Phi$). Then the following equality holds true:

$$\int_{\partial \Delta^d_t} \sigma_t^d(y_0, n^d_t) \, da_t^d = \left( \sigma_t^d(y_0, n^d_t)(\text{area } \Phi) + \sum_{i=1}^{3} \sigma_t^d(y_0, -e_i^d)(\text{area } \Phi_i) \right) \tag{2.9}$$

where $-e_i$ is the unit outward normal vector to $\Phi_i$ at $y_0$.

By construction of the tetrahedron the area of the faces $\Phi_i$ can be calculated by:

$$\text{area } \Phi_i = \frac{n_i^d}{2n_1^d n_2^d n_3^d}$$

For the calculation of the area of the face $\Phi$ we recall the geometric interpretation of the cross product of two vectors: The result of this product can be understood as the positive area of the parallelogram that is held by these two vectors. As the calculation of areas is translationally invariant, we translate the tetrahedron by $(0, 0, -\frac{1}{n_3})$.

![Figure 2.4: Cauchy's tetrahedron translated by $(0, 0, -\frac{1}{n_3})$ (own representation)](image)

Then the area of $\Phi$ is given by:

$$\text{area } \Phi = \frac{1}{2} \begin{vmatrix} \frac{1}{n_1^d} & 0 & 0 \\ 0 & \frac{1}{n_2^d} & n_3^d \\ 0 & -\frac{1}{n_3^d} & 1 \end{vmatrix} = \frac{1}{2} \begin{vmatrix} \frac{1}{n_1^d n_2^d} & 0 & 0 \\ 0 & \frac{1}{n_2^d n_3^d} & 0 \\ 0 & 0 & \frac{1}{n_1^d n_3^d} \end{vmatrix} = \frac{1}{2} \begin{vmatrix} \frac{n_i^d}{2n_1^d n_2^d n_3^d} \\ 0 \\ 0 \end{vmatrix} = \frac{1}{2n_1^d n_2^d n_3^d}$$

Inserting these results into Equation (2.9), the Identity (2.8) yields:

$$0 = \lim_{|n_t^d| \to 0} \int_{\partial \Delta^d_t} \sigma_t^d(y_0, n_t^d) \, da_t^d = \lim_{|n_t^d| \to 0} \left( \sigma_t^d(y_0, n_t^d) \frac{1}{2n_1^d n_2^d n_3^d} + \sum_{i=1}^{3} \sigma_t^d(y_0, -e_i) \frac{n_i^d}{2n_1^d n_2^d n_3^d} \right)$$
And from this we can finally conclude:

$$\sigma^d_t(y_0, n_t^d) = [\sigma(y_0, e_1)\vert\sigma(y_0, e_2)\vert(y_0, e_3)] \cdot n_t^d$$  \hspace{1cm} (2.10)

Thus, as \( y_0 \) was chosen arbitrarily, defining \( T^d_t(y) = [\sigma(y_0, e_1)\vert\sigma(y_0, e_2)\vert(y_0, e_3)] \) we have shown the first proposition for all \( y \in \Omega^d_t \). Due to the continuity assumptions regarding the Cauchy stress vector similar reasoning yields the proposition for all \( n_t^d \in \Sigma_1 \).

Inserting this into Remark 2.15 as well as into the balance of momentum equation and recalling that due to the differentiability of \( T^d_t(y) \) we can apply the divergence theorem for tensor fields, this now implies the validity of the following equations:

$$\rho^d_t(y)a(y, t) = \nabla \cdot T^d_t(y) + b^d_t(y) \quad \forall \, y \in \Omega^d_t \quad n_t^d \cdot s^d_t(y) \quad \forall \, y \in \Gamma^d_{1,t},$$

where \( n_t^d \) is the unit outer normal vector along \( \Gamma^d_{1,t} \).

We emphasize the interpretation of the elements \((T^d_t)^{ij}_t\): Since \( \sigma^d_t(y, e_i) = (T^d_t(y))^{ij}_t e_i \), the elements of the \( i \)-th row of the tensor \( T^d_t(y) \) represent the components of the Cauchy stress vector \( \sigma^d_t(y, n_t^d) \) at the point \( y \) corresponding to the particular choice \( n_t^d = e_i \) (see [10], p.66).

In this context we shall also introduce an analogon of the Cauchy stress tensor \( T^d_t \) with respect to the reference configuration. According to the above explanations the following equality holds true (cf. Definition 2.16):

$$\begin{align*}
\text{Cauchy’s Thm.} & \iff \\
\sigma(x, n) \, da & = \frac{\sigma^d_t(y, n_t^d) \, da_t^d}{\sigma(x, n) \, da} = T^d_t(y) \cdot n_t^d \, da_t^d
\end{align*}$$

According to Theorem 2.10 \( n_t^d \, da_t^d = \text{Cof} \, F(x) \cdot n \, da \) and thus we get:

$$\sigma(x, n) \, da = T^d_t(y) \text{Cof} \, F(x) \cdot n \, da$$

Hence we define:

**Definition 2.18.**

The tensor field \( T : \Omega \rightarrow \mathbb{M}^3; \, x \mapsto T(x) \) such that

$$T(x) = T^d_t(y) \text{Cof} \, F(x)$$

is called the first Piola-Kirchhoff stress tensor.

Returning to the Cauchy stress tensor \( T^d_t \) we would like to show one important
Using this assertion we are now able to show (cf. [10], p.63 and [45]):

Due to the bilinearity of the cross product we can rewrite this as:

\[
\frac{d}{dt} \int_{\Theta_t^d} y \times \rho_t^d(y) v(y, t) \, dy = \int_{\partial \Theta_t^d} y \times \sigma_t^d(y, n_t^d) \, da_t^d + \int_{\Theta_t^d} y \times b_t^d(y) \, dy,
\]

where \( v \) is the velocity and \( b_t^d \) denotes the applied body force in the current configuration.

Using this assertion we are now able to show (cf. [10], p.63 and [45]):

**Lemma 2.19.**

If the balance of angular momentum holds, then in the situation of Theorem 2.17 the continuously differentiable tensor field \( T_t^d : \Omega_t^d \rightarrow \mathbb{R}^3; y \mapsto T_t^d(y) \) is symmetric.

**Proof.**

The balance of angular momentum states:

\[
\frac{d}{dt} \int_{\Theta_t^d} y \times \rho_t^d(y) v(y, t) \, dy = \int_{\partial \Theta_t^d} y \times \sigma_t^d(y, n_t^d) \, da_t^d + \int_{\Theta_t^d} y \times b_t^d(y) \, dy
\]

Due to the bilinearity of the cross product we can rewrite this as:

\[
0 = \int_{\Theta_t^d} y \times (\rho_t^d(y) a(y, t) - b_t^d(y)) \, dy - \int_{\partial \Theta_t^d} y \times \sigma_t^d(y, n_t^d) \, da_t^d
\]

**Divergence Thm**

\[
0 = \int_{\Theta_t^d} y \times (\nabla \cdot T_t^d(y)) \, dy - \int_{\partial \Theta_t^d} y \times T_t^d(y) \cdot n_t^d \, da_t^d
\]

**Product Rule**

\[
0 = \int_{\Theta_t^d} y \times (\nabla \cdot T_t^d(y)) \, dy - \left( \int_{\Theta_t^d} y \times (\nabla \cdot T_t^d(y)) + \nabla y \times T_t^d(y) \right) \, dy
\]

\[
\iff
0 = - \int_{\Theta_t^d} \left( (T_t^d)_{3} \nabla y_2 - (T_t^d)_{2} \nabla y_3 \right) \, dy
\]

\[
\iff
0 = - \int_{\Theta_t^d} \left( (T_t^d)_{1} \nabla y_3 - (T_t^d)_{3} \nabla y_1 \right) \, dy
\]

\[
\iff
0 = - \int_{\Theta_t^d} \left( (T_t^d F^T)_{32} - (T_t^d F^T)_{23} \right) \, dy
\]

where \( i \) denotes the \( i \)-th row of the tensor \( T_t^d \).

\[
\Rightarrow 0 = \int_{\Theta_t^d} \left( (T_t^d F^T)_{32} - (T_t^d F^T)_{23} \right) \, dy \quad \forall \Theta \subset \Omega
\]
2.1. Introduction to the Theory of Elasticity

From this we can conclude that $T^d_l F^T$ is symmetric and thus we have shown that $T^d_l$ is indeed also symmetric.

Consequently, if the balance of angular momentum holds, the number of unknown entries of the Cauchy stress tensor reduces from nine to six. Nevertheless, due to these remaining six unknowns, Cauchy’s equation of motion (2.4) cannot be solved unless we integrate additional information about the dependency of the stress tensor on a motion $y$ (cf. [29], p.8). This additional information also specifies the material of the body as until now all results hold true for all materials for which Cauchy’s hypothesis applies, be it viscous, elastic, hyperelastic, viscoelastic or multipolar (cf. [45]). In the next paragraph we will therefore deal with the characterization of elastic materials and in this context discuss some frequently assumed material properties.

At the end of this paragraph we remark that in contrast to the Cauchy stress tensor $T^d_l$ the first Piola-Kirchhoff stress tensor $T$ is not symmetric. Having discussed the advantages of the symmetry of stress tensors with respect to the determination of the unknowns, we therefore also introduce an adapted obviously symmetric version of the Piola-Kirchhoff stress tensor:

\begin{definition}
The second Piola-Kirchhoff stress tensor $S : \bar{\Omega} \rightarrow \mathbb{M}^3; x \mapsto S(x)$ is given by

\[ S(x) = F^{-1} T(x) = F^{-1} T^d_l (y) \text{Cof} F(x) = J F^{-1} T^d_l (y) F^{-T}. \]
\end{definition}

2.1.4. Elastic Materials and Their Constitutive Equations

So far, everything we discussed applies to any solid exposed to external forces no matter the material the solid is made of. In the following we now incorporate material specific information into our model. At first we state a mathematical characterization of an elastic material (see [10], p.91):

\begin{definition}
A material is \textbf{elastic} if there exists a mapping $\tilde{T}^d_l : (x,F) \in \tilde{\Omega}^d_l \times \mathbb{M}^3 \rightarrow \tilde{T}^d_l (x,F) \in \mathbb{S}^3$ called the \textbf{response function for the Cauchy stress} such that in any deformed configuration that a body made of this material occupies the Cauchy stress tensor $T^d_l (y)$ at any point $y = y(x)$ of the deformed configuration is related to the deformation gradient $Dy(x)$ at the corresponding point $x$ of the reference configuration by the equation

\[ T^d_l (y) = \tilde{T}^d_l (x, Dy(x)), \quad \forall \ y = y_l (x) \in \tilde{\Omega}^d_l. \]

This relation is called the \textbf{constitutive equation} of the material.
\end{definition}
2. Mathematical Background

With respect to the notation in the reference configuration, we also state:

**Remark 2.22.**
Equivalent formulations in terms of the reference configuration of the constitutive equation for an elastic material are given by:

\[ T(x) = \tilde{T}(x, Dy(x)) \text{ and } S(x) = \tilde{S}(x, Dy(x)), \quad \forall \ x \in \Omega, \]

where \( \tilde{T} \) or \( \tilde{S} \) are the so-called response function for the first and second Piola-Kirchhoff stress, respectively.

Before we continue, we remark that the definition of elasticity presented here is controversial among physicists, but because the use of this model has led to many advances in the analysis of structures it is still widely used and regarded as "one of the major achievements of continuum mechanics" ([10], p.93).

In preparation for the introduction of a precise constitutive equation to make the above equations determinate, we next discuss three properties of the response function of an elastic material. Firstly, we have (see [10], p.92):

**Definition 2.23.**
A material in the reference configuration \( \Omega \) is called homogeneous if its response function is independent of the particular point \( x \in \Omega \) considered; otherwise the material is said to be nonhomogeneous.

Thus, in the case of a homogeneous material the constitutive equation of an elastic material reads:

\[ T^d_t(y) = \tilde{T}_t^d(Dy(x)), \quad \forall \ y = y(x) \in \Omega_t^d. \]

Secondly, we will need an adapted version of the general physical concept of material frame-indifference stating that "any 'observable quantity', i.e. any quantity with an intrinsic character [..], must be independent of the particular orthogonal basis in which it is computed." ([10], p.100 f.) If we apply this idea to the Cauchy stress vector, we get (see [10], p. 101):

**Axiom 4. Axiom of material frame-indifference for the Cauchy stress vector**
Let the deformed configuration \( \tilde{\Omega}_t^d \) be rotated into another deformed configuration \( \Omega_t^{d'} \), where the corresponding deformation of the later configuration is accordingly given by \( y' = Qy \) for some rotation \( Q \), i.e. an orthogonal matrix with \( \det Q = 1 \).

Then

\[ \sigma_t^{d'}(x_t^{d'}, Qn) = Q\sigma_t^{d'}(x_t^{d'}, n), \quad \forall \ x \in \tilde{\Omega}, n \in \Sigma_1, \]

where \( \sigma_t^{d'} \) and \( \sigma_t^{d} : \tilde{\Omega}_t^d \times \Sigma_1 \rightarrow \mathbb{R}^3 \) denote the Cauchy stress vectors in the deformed configurations \( \Omega_t^{d'} \) and \( \Omega_t^d \), respectively, and \( x_t^{d'} = y_t'(x) = Qy_t(x), \ x_t^d = y_t(x) \).
If we assume that the Cauchy stress vector is indeed material frame-indifferent, we can confine the set of admissible response functions for the Cauchy stress (see [10], p.101 f.):

**Theorem 2.24.**

The response function $\tilde{T}^d_t : \tilde{\Omega} \times \mathbb{M}^3_+ \rightarrow \mathbb{S}^3$ for the Cauchy stress satisfies the axiom of material frame-indifference for the Cauchy stress vector if and only if for all $x \in \tilde{\Omega}$

$$\tilde{T}^d_t(x, QF) = Q\tilde{T}^d_t(x, F)Q^T \quad \forall \ F \in \mathbb{M}^3_+, \ Q \in \mathbb{O}^3_+.$$  

**Proof.**

Assuming that the axiom of material frame-indifference for the Cauchy stress vector holds, where $x^d = y_t(x)$ and $x'^d = y'_t(x) = Qy_t(x)$ we have:

$$\sigma^d_t(x^d_n, Qn) = Q\sigma^d_t(x^d_n, n) \quad \forall \ x \in \tilde{\Omega}, n \in \Sigma_1$$

$$T^d_t(x'^d_n) \cdot Qn = QT^d_t(x^d_n) \cdot n \quad \forall \ x \in \tilde{\Omega}, n \in \Sigma_1$$

$$\tilde{T}^d_t(x, Dy'(x)) \cdot Qn = Q\tilde{T}^d_t(x, Dy(x)) \cdot n \quad \forall \ x \in \tilde{\Omega}, n \in \Sigma_1$$

$$y' = Qy \iff \tilde{T}^d_t(x, QF(x)) \cdot Qn = Q\tilde{T}^d_t(x, F(x)) \cdot n \quad \forall \ x \in \tilde{\Omega}, n \in \Sigma_1$$

As this holds true for all $n \in \Sigma_1$, we can rewrite the above equation as:

$$\tilde{T}^d_t(x, QF(x)) = Q\tilde{T}^d_t(x, F(x))Q^T,$$

for all $Q \in \mathbb{O}^3_+$ and all $F \in \mathbb{M}^3_+$, such that there exists a deformation $y$ with $Dy = F$.  

Naturally, we can transfer this theorem to the response functions for the first and the second Piola-Kirchhoff stress by simple calculations:

$$\tilde{T}(x, QF) \stackrel{\text{Def}}{=} (\det QF)\tilde{T}^d_t(x, QF)(QF)^{-T}$$

$$\stackrel{\text{2.21}}{=} (\det Q)\tilde{T}(x, F)(Q^TQ^{-T})F^{-T}$$

$$\tilde{S}(x, QF) \stackrel{\text{Def}}{=} (\det Q)^{-1}\tilde{T}^d_t(x, QF)(QF)^{-T}$$

$$\stackrel{\text{2.24}}{=} (\det Q)(\det F)^{-1}(Q^{-1}Q)\det F\tilde{T}^d_t(x, F)(Q^TQ^{-T}F^{-T})$$

By assumption of a physical axiom for the Cauchy stress vector we set up conditions for the response functions of elastic materials and thus restricted their form. To
2. Mathematical Background

confine the set of admissible response functions even further, we thirdly introduce a material property (see [10], p.106):

**Definition 2.25.**

An elastic material is **isotropic at a point** \( x \) if its response function for the Cauchy stress satisfies

\[
\tilde{T}_t^d(x,FQ) = \tilde{T}_t^d(x,F) \quad \forall \ F \in \mathbb{M}_+^3, \ Q \in \mathbb{O}_+^3,
\]

i.e. if the Cauchy stress tensor is left unaltered when the reference configuration is subjected to an arbitrary rotation around the point \( x \).

*If this is not the case, i.e., if the above relation remains valid only for matrices \( Q \) in a strict subset \( \mathbb{G}_x \) of the group \( \mathbb{O}_+^3 \), the material is said to be **anisotropic at** \( x \).*

We can extend this definition of isotropy to the response functions for the first and second Piola-Kirchhoff stress by easy calculations using again the definition of these response functions as well as the definition of an isotropic material. This yields:

\[
\tilde{T}(x,FQ) = \tilde{T}(x,F)Q \quad \forall \ F \in \mathbb{M}_+^3, \ Q \in \mathbb{O}_+^3
\]

\[
\tilde{S}(x,FQ) = Q^T\tilde{S}(x,F)Q \quad \forall \ F \in \mathbb{M}_+^3, \ Q \in \mathbb{O}_+^3
\]

Now we have everything at our disposal to state a famous theorem, which Rivlin and Ericksen published in their article "Stress-deformation relations for isotropic materials" in 1955 (see [10], p.109ff.):

**Theorem 2.26.** **Rivlin-Ericksen representation theorem**

A mapping \( \tilde{T}_t^d : \mathbb{M}_+^3 \rightarrow \mathbb{S}^3 \) satisfies the following two identities

\[
\tilde{T}_t^d(x,QF) = Q\tilde{T}_t^d(x,F)Q^T
\]

\[
\tilde{T}_t^d(x,FQ) = \tilde{T}_t^d(x,F)
\]

for all \( F \in \mathbb{M}_+^3, \ Q \in \mathbb{O}_+^3 \), if and only if

\[
\tilde{T}_t^d(x,F) = \tilde{T}_t^d(x,FF^T) \quad \forall \ F \in \mathbb{M}_+^3,
\]

where the mapping \( \tilde{T}_t^d : \mathbb{S}_+^3 \rightarrow \mathbb{S}^3 \) is of the form

\[
\tilde{T}_t^d = \beta_0(\iota_B)B^0 + \beta_1(\iota_B)B^1 + \beta_2(\iota_B)B^2 \quad \forall \ B \in \mathbb{S}_+^3,
\]

\( \beta_0, \beta_1, \beta_2 \) being real-valued functions of the principal invariants \( \iota_B = (I_B, II_B, III_B) \) of the matrix \( B \).

We will not give a proof of this theorem here as this would go beyond the scope of this mathematical introduction. Instead we refer to e.g. [10], p.110ff.
As a conclusion we get the following result in the terms of elasticity (see [10], p.115):

**Corollary 2.27.**

Let there be given an elastic material whose response function is material frame-indifferent and isotropic at a point \( x \in \bar{\Omega} \).

Given an arbitrary deformation \( y_t : \bar{\Omega} \rightarrow \mathbb{R}^3 \), the Cauchy stress tensor at the point \( y = y_t(x) = x^d_t \) is given by

\[
T^d_t(y) = \tilde{T}^d_t(x, Dy) = \tilde{T}^d_t(x, F F^T),
\]

where the response function \( \tilde{T}^d_t(x, \cdot) : S^3_{>0} \rightarrow S^3 \) is of the form

\[
\tilde{T}^d_t(x, B) = \beta_0(x, \iota_B) B^0 + \beta_1(x, \iota_B) B^1 + \beta_2(s, \iota_B) B^2 \quad \forall \ B \in S^3_{>0},
\]

\( \beta_0, \beta_1, \beta_2 \) being real-valued functions of the principal invariants \( \iota_B = (I_B, II_B, III_B) \) of the matrix \( B \).

The second Piola-Kirchhoff stress tensor at the point \( x \) is given by

\[
S(x) = \tilde{S}(x, Dy) = \tilde{S}(x, F) = \tilde{S}(x, F^T F),
\]

where the response function \( \tilde{S}(x, \cdot) : S^3_{>0} \rightarrow S^3 \) is of the form

\[
\tilde{S}(x, C) = \gamma_0(x, \iota_C) C^0 + \gamma_1(x, \iota_C) C^1 + \gamma_2(x, \iota_C) C^2 \quad \forall \ C \in S^3_{>0},
\]

\( \gamma_0, \gamma_1, \gamma_2 \) being real-valued functions of the principal invariants \( \iota_C = (I_C, II_C, III_C) \) of the matrix \( C \).

If we now assume that all three properties introduced above hold true and additionally the corresponding reference configuration is a natural state, i.e. that the response functions vanish for \( F = I \), we get a fairly precise form of the constitutive equation for an elastic material (see [10], p.120f.), which we will not prove, for the same reason as before:

**Theorem 2.28.**

Let there be given a homogeneous, isotropic, elastic material whose reference configuration is a natural state. If the functions \( \gamma_0, \gamma_1, \gamma_2 \) of Theorem 2.27 are differentiable at the point \( \iota_I = (3, 3, 1) \), there exist two constants \( \lambda \) and \( \mu \) such that the response function \( \tilde{S} : M^3_+ \rightarrow S^3 \) is of the form

\[
\tilde{S}(F) = \tilde{S}(C) = \lambda(\text{tr} \ E) I + 2\mu E + o(E), \quad (2.12)
\]

where \( C = F^T F = I + 2E \Longleftrightarrow E = \frac{1}{2} (C - I) \).

\( E \) is the so-called **Green-St Vernant strain tensor**.
2. Mathematical Background

In this situation, and only in this situation, the above constants $\lambda$ and $\mu$ are called the Lamé constants of the material (cf. [10], p.121).

In the light of these results the constitutive equation connects the strain tensors as a measure of deformation with the stress tensor as a measure of stress. For this reason the term stress-strain law is just another widely-used expression for constitutive equations.

In this section we introduced important concepts and theorems of the theory of elasticity. Before we conclude our considerations on this topic we now deviate a linear version of this theory enabling us to finally present a system of equations for the calculation of traction forces exerted by the cells.

2.1.5. Linear Elasticity

In a situation where the deformation $y_t$ under consideration is small, linear elasticity models have proven to yield suitable approximations of the relevant quantities (cf. [29], p.9, [15], p.261 and [45]). Hence, the last pages of this section will be dedicated to the derivation of such a linear model of elasticity.

To develop a linear model of the behavior of an elastic material under applied forces we basically have to execute two steps of linearization, namely we have to introduce a linear version of the right Cauchy-Green tensor $C$ and accordingly a linear stress-strain law. We use the assumption that the deformation of interest is small as a starting point. In Paragraph 2.1.1 on kinematics we introduced the principal stretches and the matrix invariants of the Cauchy-Green tensors (cf. p.7). We recall that the amount of strain can be read off the principal stretches by their deviation from unity. Since the deformation is considered to be small, we can conclude that the principal stretches should be close to one. Furthermore, the matrix invariants can be characterized as measures of the change of length, area and volume, respectively. Now we recall that the first matrix invariant is given by:

$$I_C = \lambda_1^2 + \lambda_2^2 + \lambda_3^2$$

Taking the above considerations into account, the first matrix invariant should be close to 3. An alternative description of the first matrix invariant is given by:

$$I_C = tr(C)$$

where $C$ is once more the right Cauchy-Green tensor

$$C = F^TF$$
We rewrite this identity in terms of $Du$, where $u$ denotes the displacement field:

$$
C = F^T F = D_y^T D_y = (Du(x) + I(x))^T (Du(x) + I(x)) = Du(x) + Du(x)^T + Du(x)^T Du(x) + I(x)
$$

Knowing that

$$
\text{tr}(C) = \text{tr}(Du(x) + Du(x)^T + Du(x)^T Du(x) + I(x))
$$

should be close to 3 we can in particular conclude that $\text{tr}(Du(x)^T Du(x))$ and hence the entries of $Du(x)$ should be close to zero. We will come back to this point in the course of this paragraph. For now we aim at linearizing the above identity with respect to $Du$, yielding:

$$
C_{\text{lin}} = Du(x) + Du(x)^T + I
$$

As a result we introduce:

$$
E_{\text{lin}} = \frac{1}{2} (C_{\text{lin}} - I) = \frac{1}{2} (Du(x) + Du(x)^T + I - I) = \frac{1}{2} (Du(x) + Du(x)^T)
$$

$$
(E_{\text{lin}})_{ij} = \frac{1}{2} (\partial x_i u_j + \partial x_j u_i)
$$

Next, we introduce a linear stress-strain law by neglecting the higher order terms in the formulation of the response function for the second Piola-Kirchhoff stress in Theorem 2.28, yielding the following constitutive equation:

$$
S(x) = \tilde{S}(F) = \tilde{S}(C) = \lambda (\text{tr} E) I + 2\mu E
$$

Of course, we can also use a componentwise notation (cf. [10], p.131):

$$
S_{ij}(x) = \tilde{S}_{ij}(C) = \lambda \sum_{k=1}^{3} E_{kk} \delta_{ij} + 2\mu E_{ij}, \quad E_{ij} = \frac{1}{2} (C_{ij} - I_{ij})
$$

or equivalently:

$$
S_{ij}(x) = \tilde{S}_{ij}(C) = a_{ijkl} E_{kl}, \quad a_{ijkl} := \lambda \delta_{ik} \delta_{jl} + \mu (\delta_{ik} \delta_{jl} + \delta_{il} \delta_{jk}),
$$

where the last equation is the famous **Hooke’s Law** for elastic materials.
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We point out that this formulation of the constitutive equation for an elastic material is linear with respect to $C$ and $E$, respectively, but not linear with respect to the displacement field $u$. However, we can slightly modify the above equation by combining the two linearization steps and thus replacing the tensor $E$ by its linearized version $E_{\text{lin}}$ resulting in the following equations, which are now also linear with respect to $u$:

\[
(S_{\text{lin}})_{ij} = \tilde{S}_{ij}(C_{\text{lin}}) = \lambda(\sum_{k=1}^{3}(E_{\text{lin}})_{kk})\delta_{ij} + 2\mu(E_{\text{lin}})_{ij},
\]

\[
(E_{\text{lin}})_{ij} = \frac{1}{2}(\partial x_i u_j + \partial x_j u_i).
\]

(2.13)

From now on, we assume that we are in the situation of \textbf{elastostatics}, i.e. we consider an elastic material under the conditions of static equilibrium, which means that the displacement field is not a function of time and hence all time derivatives of $u$ and $y$, respectively, equal to zero (cf. [15], p.262). Consequently, the balance of linear momentum reads:

\[
0 = \int_{\partial\Omega_t} \sigma^d(y, n^d_t) \, da^d_t + \int_{\partial\Gamma_t} b^d_t(y) \, dy, \quad \forall \Theta \subseteq \Omega
\]

\[
\iff \quad 0 = \nabla^d \cdot T^d_t(y) + b^d_t(y) \quad \forall \ y \in \Omega^d_t
\]

\[
T^d_t(y) \cdot n^d_t = s^d_t(y) \quad \forall \ y \in \Gamma^d_{1,t}
\]

Next, we express this problem in terms of the first Piola-Kirchhoff stress tensor, where we will use the following identities: $\nabla \cdot T(x) = J(\nabla^d \cdot T^d_t(y))$, $b(x) = Jb^d_t(y)$ and $s(x) = Js^d_t(y)$, where $b(x)$ and $s(x)$ are the densities of the applied body force per unit volume and the applied surface force per unit area in the reference configuration:

\[
0 = J(\nabla \cdot T(x)) + Jb(x) \quad \forall \ x \in \Omega
\]

\[
JT(x) \cdot n = Js(x) \quad \forall \ x \in \Gamma_1
\]

\[
\iff \quad 0 = \nabla \cdot T(x) + b(x) \quad \forall \ x \in \Omega
\]

\[
T(x) \cdot n = s(x) \quad \forall \ x \in \Gamma_1
\]

Of course, we can rewrite this equations also in terms of the second Piola-Kirchhoff stress tensor, yielding:

\[
0 = \nabla \cdot ((Du(x) + I(x))S(x)) + b(x) \quad \forall \ x \in \Omega
\]

\[
(F(x)S(x)) \cdot n = s(x) \quad \forall \ x \in \Gamma_1
\]

\[
\iff \quad 0 = \nabla \cdot ((Du(x) + I(x))S(x)) + b(x) \quad \forall \ x \in \Omega
\]

\[
((Du(x) + I(x))S(x)) \cdot n = s(x) \quad \forall \ x \in \Gamma_1
\]
As stated before, the entries of $Du(x)$ should be close to zero. Hence we can neglect the term $Du(x)S(x)$ and end up with the following system of equations:

\[
\begin{align*}
0 &= \nabla \cdot S(x) + b(x) \quad \forall \ x \in \Omega \\
S(x) \cdot n &= s(x) \quad \forall \ x \in \Gamma_1 \\
-\nabla \cdot S(x) &= b(x) \quad \forall \ x \in \Omega \\
S(x) \cdot n &= s(x) \quad \forall \ x \in \Gamma_1
\end{align*}
\]

(2.14)

If we now combine this system of equations with equation (2.13), i.e. substituting $S$ by $S_{\text{lin}}$, we finally end up with the following boundary problem:

**Definition 2.29.**

Let $\Omega$ again denote a domain in $\mathbb{R}^3$ and $\Gamma_0$ and $\Gamma_1$ be disjoint open subsets of $\Gamma = \partial \Omega$ such that the da-measure of $\Gamma \setminus (\Gamma_0 \cup \Gamma_1)$ equals to 0.

The popular so-called linearized displacement-traction problem of three-dimensional elasticity under the assumptions of:

(a) a small deformation $y_t$ and

(b) a homogeneous, isotropic, elastic material whose reference configuration is a natural state

reads:

Given the densities of the applied body force per unit volume $b(x)$ and of the applied surface force per unit area $s(x)$ in the reference configuration, find $u \in W(\Omega) = \{ v \in W^{2,p}(\Omega); \ v = 0 \ on \ \Gamma_0 \subset \partial \Omega \}$ such that

\[
\begin{align*}
-\nabla \cdot (\lambda(\text{tr}(E_{\text{lin}}))I + 2\mu E_{\text{lin}}) &= b \quad \forall \ x \in \Omega, \\
(\lambda(\text{tr}(E_{\text{lin}}))I + 2\mu E_{\text{lin}}) \cdot n &= s \quad \forall \ x \in \Gamma_1, \\
u &= 0 \quad \forall \ x \in \Gamma_0, \\
E_{\text{lin}} &= \frac{1}{2}(Du^T + Du)
\end{align*}
\]

\[
\begin{align*}
-\nabla \cdot (\nabla \cdot u) - \mu \Delta u &= b \quad \forall \ x \in \Omega, \\
\lambda(\nabla \cdot u)I + \mu((\nabla u)^T + \nabla u) \cdot n &= s \quad \forall \ x \in \Gamma_1, \\
u &= 0 \quad \forall \ x \in \Gamma_0,
\end{align*}
\]

where we used the widely-used notation $\nabla u = Du$ in the second alternative.

The identity $-(\lambda + \mu)\nabla(\nabla \cdot u) - \mu \Delta u = b$ are the so-called *Navier-Lamé equations*.

So at the end of this section we found a linear mathematical model to describe the experimental setting introduced in the last chapter. This will enable us to calculate the traction exerted by the cells in the next chapters.
2. Mathematical Background

2.2. Introduction to Image Registration

To motivate the current section, we once again consider the experimental setting described in the introductory chapter: Given a cell on an underlying substrate, the task was to reconstruct the traction forces exerted by the cell on the substrate during locomotion.

State of the art traction force microscopy techniques, which will be described in detail in Chapter 4, obtain the traction forces indirectly by a partial direct measurement of the displacement field of the underlying substrate. By contrast, our novel approach is based on the idea of reconstructing this displacement from ordinary phase-contrast microscopy images by means of image registration. Therefore, we now give a short introduction to this area of image processing.

We start our considerations with a short description of the objective of image registration, followed by a discussion of the main ingredients of the registration problem. Afterwards, we will derive a definition of the optimization problem serving as a general mathematical setting for the different registration techniques, present the elastic registration as an example of a particular optimization problem and in the end we will comment on optimization approaches for the determination of a numerical solution of the problem.

The subsequent explanations mainly follow the two contributions [18] and [31] written by B. Fischer and J. Modersitzki and J. Modersitzki, respectively.

2.2.1. The Objective

Image registration is an image processing tool used to spatially align two or more images exhibiting essentially the same scene or object, but with slight differences with respect to position or shape, because the image is recorded from different devices and/or viewpoints and/or at different times as in our case (cf. [18], p.1 and [31], p.21). The features contained in the separate images can thus be combined or compared to extract additional information. In this context, the reconstruction of the displacement field from images of a phase-contrast microscope seems to be a classical application of image registration.

The objective of image registration can be phrased by (see [18], p.1 and [31], p.21):

Definition 2.30.

*Given a reference image \( R \) and a transformable template image \( T \), the goal of image registration is to find a suitable transformation \( y \) such that the transformed template \( T(y) \) is similar to the reference image.***

The registration problem described above is illustrated in the subsequent figure, including two magnetic resonance scans of the same knee in different positions before
and after a surgical intervention as well as the second image with a grid visualizing the transformation:

(a) Reference image $R$
(b) Template image $T$
(c) Template image with grid
(d) Transformed template image $T(y)$ with grid

Figure 2.5.: Example of a registration problem (adapted version of a graphic in [32], p.1)

### 2.2.2. The Main Ingredients of the Registration Problem

In the context of image processing or more precisely of image registration a rigorous characterization of the term ‘image’ seems to be of paramount importance. Before we continue with the definition of an image from a mathematical point of view, we would like to point out that we decided to use a continuous setting throughout this thesis. Following the ideas of J. Modersitzki in [32], p.9 we give two reasons for our decision on this issue: Generally speaking all common imaging devices return discrete images but actually the image values depict the naturally discrete measurement of an underlying continuous property of an object as for example the photon density. Using a continuous setting enables us to rather model the underlying continuous property than the discrete measurement. Beside this fairly philosophical argument our reasons are of practical nature: In a continuous setting, separate discrete images depicting the same scene but with different resolutions can be understood as being two discretizations of the same continuous image. This is advantageous e.g. with respect to an efficient numerical solution of the registration problem. Consequently, we now define (see [31], p.14):

**Definition 2.31.**

Let $d \in \mathbb{N}$, then a function $I : \mathbb{R}^d \rightarrow \mathbb{R}$ is called a **d-dimensional image** $I$, if

1. $I$ is compactly supported
2. $0 \leq I(x) < \infty \ \forall \ x \in \mathbb{R}^d$
3. $\int_{\mathbb{R}^d} I(x)^k \ dx$ is finite for $k > 0$,

where $I(x)$ gives the intensity of the image at a spatial position $x \in \mathbb{R}^d$. 
2. Mathematical Background

As mentioned above, all relevant imaging devices return discrete images. Therefore, some kind of interpolation algorithm has to be applied in order to get a continuous data set. Consequently, we can justify to assume that the images are sufficiently smooth because we can just choose the interpolation algorithm accordingly. Hence, in the following, we assume that we are given one reference image $\mathcal{R}$ and at least one template image $\mathcal{T}$, which are smooth, compactly supported, bounded, locally $k$-integrable functions on a rectangular domain $\Omega \subset \mathbb{R}^d$, where $d$ denotes the data dimensionality (f. [18], p.6).

Recalling the definition of the goal of image registration on page 28 we do not consider the template image itself but a transformed version of this image. Consequently, the forward problem reads: Given a template image $\mathcal{T} : \Omega \rightarrow \mathbb{R}$ and a suitable transformation $y : \mathbb{R}^d \rightarrow \mathbb{R}^d$, calculate the transformed template image $\mathcal{T}(y)(x)$, where we use the so-called Eulerian framework for the representation of the transformed image. Accordingly, the inverse problem is to find a suitable transformation $y$ such that the transformed template $\mathcal{T}(y)$ is similar to the reference image $\mathcal{R}$ in a sense we are going to specify in the following. As we will see later on, the image registration problem is ill-posed, which is a typical issue in the field of inverse problems.

For now we continue with the discussion of the term of image similarity (cf. [31], p.14):

**Definition 2.32.**

Let $\text{Img}(d)$ designate the set of all $d$-dimensional images and let $D : \text{Img}(d)^2 \rightarrow \mathbb{R}$ denote some particular distance measure.

We will call two images $\mathcal{R}$ and $\mathcal{T}(y)$ similar if the following equality holds true:

$$D(\mathcal{R}, \mathcal{T}(y)) \rightarrow \min_y$$

In the literature plenty distance measures have been proposed, which according to [18], p.7 can be classified into measures based on:

1. image features (e.g. markers, i.e. tags attached to the object before imaging)
2. landmarks (tags deduced after imaging)
3. moments (statistical quantities)
4. substructures (e.g. surfaces or level-sets)
5. volumetric data

Introducing examples of all these groups of distance measures goes beyond the scope of this thesis. The interested reader may for example be referred to [32], where many
2.2. Introduction to Image Registration

of the most popular distance measures are briefly discussed. Here, we focus on one of the most frequently used volumetric distance measures, which (in an adapted version) will be our image similarity measure of choice when designing our novel reconstruction approach (q.v. Chapter 5) because it has an easy interpretation and furthermore it has proven to be very effective for registering images of the same imaging modality (see [31], p.56):

Definition 2.33.

Let \( d \in \mathbb{N} \) and \( R, T \in \text{Img}(d) \).

The **sum of squared differences (SSD)** distance measure \( D^{\text{SSD}} \) is defined by

\[
D^{\text{SSD}}(\mathcal{R}, \mathcal{T}) := \frac{1}{2} \| \mathcal{T} - \mathcal{R} \|^2_{L^2(\Omega)} = \frac{1}{2} \int_{\Omega} |\mathcal{T}(x) - \mathcal{R}(x)|^2 dx
\]

(2.15)

For a transformation \( y : \mathbb{R}^d \rightarrow \mathbb{R}^d \) we also define

\[
D^{\text{SSD}}(\mathcal{R}, \mathcal{T}; y) = D^{\text{SSD}}(\mathcal{R}, \mathcal{T}(y))
\]

(2.16)

If we now insert the definition of the sum of squared differences distance measure \( D^{\text{SSD}} \) into Definition 2.32 we have a criterion for the choice of the mapping \( y \).

Let us consider an example: Given the subsequent reference and template images \( \mathcal{R}, \mathcal{T} \in \text{Img}(d) \) exhibiting a dark square on a white background, find a transformation \( y \) minimizing \( D^{\text{SSD}}(\mathcal{R}, \mathcal{T}(y)) \):

![Reference image \( \mathcal{R} \) and Template image \( \mathcal{T} \)](taken from [18], p.9)

Obviously, one possible transformation is a translation from the upper right corner to the lower left one. However, there exists more than one solution to the problem as for example rotating the image about 180° around the center of the domain gives another equally feasible solution, where both mappings are rigid transformations. If we do not only consider rigid transformations, but approve more complex ones as well, the mapping \( y \) is even indefinite in areas of constant grey values. (cf. [18], p.9)
The presented drawbacks seem to be inherent to the problem of image registration as we are searching for a vector-valued transformation \( y(x) \in \mathbb{R}^d \) for all \( x \in \Omega \subset \mathbb{R}^d \), where typically only scalar information in terms of intensity values is provided (cf. [32], p.118). In the presence of noise, the problem is even more severe as the following situation illustrates: If we add one black dot to either of the four corners of the reference or the template image considered on the previous page, we will find a unique rigid solution to the problem, which however is completely determined by a very small disruption of the original situation. Fortunately, for data with more structure the problem is less prone to the effects of noise. (cf. [18], p.9 f.) Nevertheless, we in this context introduce (cf. [23]):

**Definition 2.34.**

According to J. Hadamard, a problem is **well-posed** if:

1. there exists a solution to the problem
2. the solution is unique and
3. the solution depends continuously on the data,

otherwise the problem is called **ill-posed**.

From the above observations we can thus conclude that the image registration problem is ill-posed, as we already mentioned earlier. This calls for the incorporation of additional a-priori information about a desirable solution, which for example can be realized via a **regularization** of the problem.

There mainly exist two approaches of regularization: The first possibility is to restrict the search-space explicitly. This means that the transformation \( y \) has to be in a particular admissible set \( M \). Typical choices for \( M \) are on the one hand rigid or affine linear transformations, which can be characterized by only a few parameters, as well as potentially very high-dimensional spline-based registration techniques (cf. [18], p.5). On the other hand \( M \) can be specified to be a more general function space as for example the group of all diffeomorphisms on \( \text{Diff}(\mathbb{R}^d) \). Accordingly, registration approaches can be classified into the two groups of **parametric image registration** and **non-parametric image registration**. A second possibility of regularization is the implicit restriction of the search-space via the simultaneous minimization of a smoother \( S(y) \), where \( S \) is a Sobolev-semi-norm, that should be chosen with respect to the particular application. In general, the smoother \( S(y) \) is a bilinear form of the shape:

\[
S(y) = \frac{1}{2} a(y, y) = \frac{1}{2} \|B(y)\|_{L^2(\Omega)}^2 = \frac{1}{2} \int_\Omega (B(y), B(y)) dx,
\]

where \( B \) is a differential operator.
2.2. Introduction to Image Registration

One simple example of such a smoother $S$ is given by (see [32], p.122):

Let $B(y) = \nabla y$.

$$S(y) = \frac{1}{2} \int_\Omega |\nabla y|^2 dx$$

Again the discussion of all major regularization approaches lies beyond the scope of this chapter, especially as this field is continuously increasing. Hence we once more refer to the books of J. Modersitzki [31] and [32] for further information. Nevertheless, a prominent second example, which we will need for our calculations in the course of this thesis, will be presented in paragraph 2.2.4.

According to [18], p.10, regularization mainly guarantees the existence of a solution of the registration problem, while the uniqueness of a solution can be achieved by the introduction of so-called soft constraints or penalties $P(y)$. Penalties are means for the incorporation of additional user knowledge and are constructed in such a way that unwanted solutions are penalized as their name already implies (cf. [18], p.10). If for example the volume of the object of interest should be preserved, the following term should be small (see [18], p.10):

$$P^{VP}(y) = \int_\Omega \log(\det \nabla y)^2 dx$$

Instead of only penalizing unwanted solutions it is also possible to completely rule out inadmissible transformations by means of so-called hard constraints $C(y)$ (cf. [18], p.11). For example, the corresponding hard constraint to guarantee the incompressibility is then given by:

$$C^{VP}(y) = \det \nabla(y)(x) - 1 = 0, \quad \forall x \in \Omega$$

This way the admissible set $M$ is further restricted.

Now we have everything at our disposal to introduce an optimization problem, which combines all major ingredients of the registration problem.

2.2.3. The Optimization Problem

In the light of the previous paragraph, the introduction of one variational-based optimization problem, which allows for the simultaneous realization of all terms and constraints presented before, seems to be reasonable. We decided to use a variational approach as this allows us to access the corresponding well-known standard methods proposed in the literature. Furthermore, this way most state of the art registration approaches can be expressed in one general setting allowing for the comparison of these techniques (cf. [18], p.1).
Hence we define (see [18], p.6):

**Definition 2.35.**

Using an appropriate distance measure $D$ and a smoother $S$ the desired transformation $y$ solving the registration problem as described in Definition 2.30 is a solution of the optimization problem

$$J(y) = D(R, T(y)) + \alpha S(y) + \beta P(y) \rightarrow \min_{y \in M} \text{ subject to } C(y), \quad (2.18)$$

where $\alpha$ is a regularization parameter, $P$ is a penalty or soft constraint, $\beta$ is a penalty parameter, $M$ is a set of admissible transformations and $C$ is an additional hard constraint.

This functional $J$ commonly is called the **energy**.

A solution of this optimization problem has to fulfill the following necessary condition: The Gâteaux derivative $dJ(y; v)$ of $J$ vanishes for all suitable perturbations $v$. Note that this derivative is sometimes also called the first variation of $J$ in the direction of $v$. (cf. [31], p.78) The identity arising from this necessary condition is the so-called **optimality condition**.

In the next paragraph we present one special example of an optimization problem of the form (2.18), which in the course of this thesis will be of particular importance. Furthermore, we will calculate the corresponding optimality condition, thereby following the ideas outlined above.

### 2.2.4. Elastic Registration

In his dissertation 'Optimal Registration of Deformed Images' (1981) C.Broit proposed a non-parametric image registration approach, which he called **elastic registration**. In dependence on the physical theory of elasticity, which we briefly introduced in the first section of this chapter, this approach combines a measure for the similarity of two images exhibiting an elastic object subject to deformation and a measure for the likelihood of the corresponding deformation (cf. [31], p.77/83).

Before we continue with the discussion of this particular kind of non-parametric image registration, we remark that in this group of registration approaches it is common practice to consider the displacement $u$ rather than the transformation (or in this context the deformation) $y$, i.e. $y = x + u(x)$. Accordingly, the template image $T(y(x))$ can be rewritten as (cf. [31], p.77):

$$T_u(x) := T(x + u(x))$$
2.2. Introduction to Image Registration

Next, we relate the elastic registration problem to the optimization framework (2.18). With respect to the displacement $u$ this can be rewritten as:

$$J(u) = D(R, T_u) + \alpha S(u) + \beta P(u) \longrightarrow \min_{u \in \mathcal{M}} \text{ subject to } C(u) \quad (2.19)$$

Following the ideas of Broit we choose the sum of squared differences distance measure (cf. Definition 2.33) to be the first term of the above optimality problem:

$$D(R, T_u) := D^{SSD}(R, T_u) = \frac{1}{2} \| T_u - R \|_{L^2(\Omega)}^2 \quad (2.20)$$

To find a measure for the likelihood of the deformation or the displacement, respectively, we continue with the construction of a functional $K$, such that the required displacement $u$ can be characterized as its minimum. We make the following ansatz:

$$K(u) = \frac{1}{2} a(u, u) + b(u), \quad (2.22)$$

where $a(u, u)$ is a bilinear form serving as a regularizer, which incorporates information about the material properties and the inner forces, and $b(u)$ is a linear term reflecting the outer forces (cf. [31], p.98).

From now on we assume for the current section that $\Omega = [0, 1[^d \subset \mathbb{R}^d$ and we recall that the linearized Green - St.Vernant strain tensor is given by (cf. p.26)

$$E_{\text{lin}} = \left[ \frac{1}{2} \left( \partial x_j u_k + \partial x_k u_j \right) \right]_{j,k} \in \mathbb{R}^{d \times d}$$

Now we choose $a(u, v)$ to be the following positive semi-definite bilinear form (cf. [31], p.99), where $\mu$ and $\lambda$ are the Lamé constants:

$$a(u, v) : = \int_{\Omega} 2\mu \text{tr}(E_{\text{lin}}(u(x))^T E_{\text{lin}}(v(x))}$$

$$+ \lambda \text{tr}(E_{\text{lin}}(u(x))) \text{tr}(E_{\text{lin}}(v(x))) \ dx$$

$$= \int_{\Omega} 2\mu \sum_{j,k=1}^{d} (E_{\text{lin}}(u(x)))_{j,k} (E_{\text{lin}}(v(x)))_{j,k}$$

$$+ \lambda(\nabla \cdot u(x))(\nabla \cdot v(x)) \ dx \quad (2.23)$$

$$= \int_{\Omega} \mu \sum_{j=1}^{d} \left( \nabla u_k(x) + \partial x_j u_k(x) \right) \left( \nabla v_k(x) + \partial x_j v_k(x) \right)$$

$$+ \lambda(\nabla \cdot u(x))(\nabla \cdot v(x)) \ dx$$

$$= \int_{\Omega} \mu \sum_{k=1}^{d} \left( \nabla u_k(x) + \partial x_k u(x), \nabla v_k(x) \right)_{\mathbb{R}^d}$$

$$+ \lambda(\nabla \cdot u(x))(\nabla \cdot v(x)) \ dx,$$
2. Mathematical Background

Furthermore, we choose \( b(v) \) to be the linear form:

\[
 b(v) := - \int_{\Omega} \langle b(x), v(x) \rangle_{\mathbb{R}^d} \, dx,
\]

(2.24)

where \( b \) is the body force that we introduced in the previous section. To show that this choice of the functional \( K \) indeed yields an incorporation of information about the material properties of the deformed elastic body, we recall that for any minimizer of \( K \) with Dirichlet, Neumann or periodic boundary conditions for \( u \) the Gâteaux derivative \( dK(u, v) \) of \( K \) has to be equal to zero for all appropriate \( v \) (cf. [31], p.99).

Hence we firstly have to calculate this derivative (see [31], p.99f.):

**Theorem 2.36.**

Let \( K(u) = \frac{1}{2} a(u, u) + b(u) \), where \( a \) be defined by equation (2.23) and \( b \) be defined by equation (2.24), respectively. Moreover let \( u \in (C^2(\mathbb{R}^d))^d \).

For the perturbation \( v \in (C(\mathbb{R}^d))^d \), the Gâteaux derivative of \( K \) is given by:

\[
 dK(u, v) = \int_{\Omega} \langle -b - \mu \Delta u - (\mu + \lambda) \nabla (\nabla \cdot u), v \rangle_{\mathbb{R}^d} \, dx \tag{2.25}
\]

**Proof.**

\[
 dK(u, v) \overset{\text{Def}}{=} \lim_{h \to 0} \frac{1}{h} (K(u + hv) - K(u))
\]

\[
 \overset{\text{Def} a, b}{=} \int_{\Omega} \mu \sum_{k=1}^{d} \langle \nabla u_k(x) + \partial x_k u(x), \nabla v_k(x) \rangle_{\mathbb{R}^d}
 + \lambda (\nabla \cdot u(x)) (\nabla \cdot v(x)) - \langle b(x), v(x) \rangle_{\mathbb{R}^d} \, dx
\]

\[
 \overset{\text{Part. Integr.}}{=} \int_{\partial \Omega} \mu \sum_{k=1}^{d} v_k \langle \nabla u_k + \partial x_k u, n \rangle_{\mathbb{R}^d} + \lambda (\nabla \cdot u) \langle v, n \rangle_{\mathbb{R}^d} \, da
 - \int_{\Omega} \mu (\Delta u + \nabla (\nabla \cdot u), v)_{\mathbb{R}^d} - \lambda (\nabla (\nabla \cdot u), v)_{\mathbb{R}^d} - \langle b, v \rangle_{\mathbb{R}^d} \, dx
\]

\[
 = \int_{\partial \Omega} \mu \sum_{k=1}^{d} v_k \langle \nabla u_k + \partial x_k u, n \rangle_{\mathbb{R}^d} + \lambda (\nabla \cdot u) \langle v, n \rangle_{\mathbb{R}^d} \, da
 + \int_{\Omega} \langle -b - \mu \Delta u - (\mu + \lambda) \nabla (\nabla \cdot u), v \rangle_{\mathbb{R}^d} \, dx,
\]

where \( n \) denotes the outer normal vector on \( \partial \Omega \).

Exploiting the implicit boundary conditions \( \nabla \cdot u = \langle \nabla u_k + \partial x_k u, n \rangle_{\mathbb{R}^d} = 0 \) on \( \partial \Omega \), the boundary integral vanishes, which yields the proposition. \( \square \)

If we now set this Gâteaux derivative of \( K \) to zero, we for all suitable \( v \) end up with:

\[
 dK(u, v) = \int_{\Omega} \langle -b - \mu \Delta u - (\mu + \lambda) \nabla (\nabla \cdot u), v \rangle_{\mathbb{R}^d} \, dx = 0
\]

\[
 \iff -\mu \Delta u - (\mu + \lambda) \nabla (\nabla \cdot u) = b
\]
2.2. Introduction to Image Registration

This last term is nothing else than the Navier-Lamé equations (cf. Definition 2.29) and thus we can now conclude that any minimizer of the functional $K$ fulfills this linear equation of elasticity and hence $K$ indeed incorporates information about the material properties of the deformed object.

Furthermore, we point out that thus the above theorem provides a weak formulation of the linearized displacement-traction problem.

Using this particular bilinear form $a$ we define (see [31], p.100):

**Definition 2.37.**

Let $d \in \mathbb{N}$, $u : \mathbb{R}^d \rightarrow \mathbb{R}^d$, $u_j \in C^2(\mathbb{R}^d)$, $j = 1, ..., d$.

The **elastic potential** $P_d$ of the displacement $u$ is defined by:

\[
P_d(u) = \frac{1}{2} a(u, u) = \int_\Omega \mu \text{tr}(E_{lin}(u)^T E_{lin}(u)) + \frac{\lambda}{2} \text{tr}(E_{lin}(u))^2 \, dx \]

\[
= \int_\Omega \frac{\mu}{4} \sum_{j,k=1}^d (\partial x_j u_k + \partial x_k u_j)^2 + \frac{\lambda}{2} (\nabla \cdot u)^2 \, dx
\]

For the most frequently used cases $d = 2$ and $d = 3$, respectively, the above equation (2.28) provides the following explicit formulas:

\[
P_2(u) = \int_{\mathbb{R}^2} \mu \left[ (\partial x_1 u_1)^2 + \frac{1}{2} (\partial x_1 u_2 + \partial x_2 u_1)^2 + (\partial x_2 u_2)^2 \right] \\
+ \frac{\lambda}{2} (\partial x_1 u_1 + \partial x_2 u_2)^2 \, dx
\]

\[
P_3(u) = \int_{\mathbb{R}^3} \mu \left[ (\partial x_1 u_1)^2 + (\partial x_2 u_2)^2 + (\partial x_3 u_3)^2 \right] \\
+ \frac{\mu}{4} \left[ (\partial x_1 u_2 + \partial x_2 u_1)^2 + (\partial x_1 u_3 + \partial x_3 u_1)^2 + (\partial x_2 u_3 + \partial x_3 u_2)^2 \right] \\
+ \frac{\lambda}{2} (\partial x_1 u_1 + \partial x_2 u_2 + \partial x_3 u_3)^2 \, dx
\]

Choosing the regularization term $S(u)$ in the optimization framework with respect to the displacement $u$ (2.19) to be the elastic potential $P(u)$, the elastic registration according to Broit can be expressed by:

\[
J(u) = \frac{1}{2} \| \mathcal{T}_u - \mathcal{R} \|_{L^2(\Omega)}^2 + \int_\Omega \frac{\mu}{4} \sum_{j,k=1}^d (\partial x_j u_k + \partial x_k u_j)^2 + \frac{\lambda}{2} (\nabla \cdot u)^2 \, dx \rightarrow \min_{u \in (C^2(\mathbb{R}^d))^d}
\]

Having formulated this particular example of registration in terms of the presented optimization framework, we conclude this section with a short comment on two contrary philosophies of finding a solution of such an optimization problem.
2. Mathematical Background

2.2.5. Optimization Approaches

In this chapter we are not going to present any particular numerical optimization method (for a general survey on this topic see e.g. [34]). Instead, we introduce two different ways to approach the optimization problem, more precisely we will discuss:

1. the **first-optimize-then-discretize approach** and
2. the **first-discretize-then-optimize technique**

The first of these two classes is characterized by the calculation of an optimality condition according to the necessary condition for an extremum of the energy functional $J$. This yields a nonlinear system of partial differential equations, which then has to be equipped with suitable boundary conditions to be subsequently numerically solved by means of some kind of discretization technique. (cf. [18], p.11)

As the name already indicates, the second class inverts the order of the two major ingredients of the optimization scheme introduced before. Approaches belonging to this more recent second class can be characterized as (see [18], p.12):

**Definition 2.38.**

The **first-discretize-then-optimize technique** is based on a sequence of nested discretizations of the optimization problem

\[
J(y) = D(R, T(y)) + \alpha S(y) + \beta P(y) \rightarrow \min_{y \in M} \text{ subject to } C(y)
\]

Each discretization leads to a finite-dimensional optimization problem. Starting with a coarse discretization, a minimizer is computed, which then serves as a starting point for the optimization problem associated with the finer discretization.

Under the assumption that the solution with respect to the coarse discretization is a reasonable starting guess for the next level, the advantage of this class of algorithms is that the finer discretization can be interpreted as a correction step and hence the computational calculations are expected to be less costly. Furthermore, the risk of falling into the trap of a local minimum can thus be reduced.
3. Mathematical Modeling of Cellular Traction Forces

The reconstruction of cellular traction forces is crucial to a better understanding of cell locomotion, providing new insights into biological processes like for example wound healing, embryonic morphogenesis and the formation of new vessels in tumors and metastases (comp. [43], p.788 and [44], p.227). To address this problem from a mathematical point of view, a sound mathematical model of cellular traction force and its effect to the underlying substrate is required.

In the first part of this chapter we therefore introduce a three-dimensional model of cellular traction assuming the fundamentals of elasticity presented in Chapter 2.1 to be known. Against this background, we state the forward problem allowing for a prediction of the behavior of the extracellular matrix for given applied forces. Under certain conditions the so-called Boussinesq solution provides one way to specify this prediction in terms of explicit formulas. Thus, the second part of this chapter is devoted to the detailed deduction of these famous equations. As we will see in Chapter 4, many of the nowadays prevailing approaches for the reconstruction of cellular traction forces are based on this Boussinesq solution. Besides, we will discuss some techniques, which are based on a two-dimensional approximation of the underlying three-dimensional model. At the end of this chapter we consequently introduce this so-called plane stress approximation including a presentation of the conditions of its applicability.

3.1. A Mathematical Model of Cellular Traction Forces

For the detection and reconstruction of cellular traction forces, cells are studied in vitro. To set up a mathematical model of these forces, we consider the case of cells being cultured on an extracellular matrix, which is made up of an elastic material.\(^1\)

\(^1\)We remark that in 1997, C. G. Galbraith and M. P. Sheetz (cf. [21]) proposed to measure cellular traction forces directly by means of a microneedle device, where the cells are not cultured on an extracellular matrix, but instead are laid on the pad of the device. This approach and its later improvements allow for dynamic measurements of subcellular tractions without the influence of other regions of the cell. Nevertheless, we decided to concentrate on the more popular elastic substrate methods, as the microneedles seem to be a less natural environment for the cells (c.f. [1], p.2049). This observation is of particular importance as R. J. Pelham and Y.-L. Wang showed that cells are able to respond to their surrounding environment (cf. [36], p.13661).
3. Mathematical Modeling of Cellular Traction Forces

After choosing a cell to be observed, in the case of 2D data, the setting is recorded from above. Typically, the imaging device of choice is a phase-contrast microscope. An example of the resulting data including the characteristic white halo is given below:

![Image](a) Human melanoma (MV3) cell on a collagen I-coated elastic matrix recorded by a phase-contrast microscope

![Image](b) Same setting recorded ten minutes later

**Figure 3.1:** Human melanoma (MV3) cell on a collagen I-coated elastic extracellular matrix recorded by a phase-contrast microscope. (q.v. [39])

Recorded by Christian Stock, made available by Albrecht Schwab (Institute of Physiology II, University of Münster).

As we can see, the boundaries at all sides of the image are of artificial nature rather introduced by the limited field of view of the objective of the image device than being the border of the substrate. We will come back to this observation when we finally introduce the mathematical boundary value problem.

The following sketch emphasizes the three-dimensional structure of the setting and thus allows for an intuitively accessible description of the corresponding mathematical model:

![Sketch](a) Human melanoma (MV3) cell on a collagen I-coated elastic extracellular matrix recorded by a phase-contrast microscope

![Sketch](b) Same setting recorded ten minutes later

**Figure 3.2:** Sketch depicting a cell exerting traction forces to an underlying substrate,

(own representation)

In the above drawing the black cuboid stands for the section of the extracellular matrix that is located within the field of view of the imaging device. From now on we shall denote this three-dimensional extracellular matrix by $\mathcal{E} \subset \mathbb{R}^3$ and points therein by $x$. Furthermore, the red item represents the cellular body, in which the
3.1. A Mathematical Model of Cellular Traction Forces

cell nucleus (drawn in blue) is embedded. The cell rests on the upper surface of the extracellular matrix which we from now on denote by $E_{us} := \{ x \in E : x_3 = 0 \}$ provided that this surface is assumed to be in the $x_1x_2$-plane such that the extracellular matrix is located in $x_3 < 0$. Alternatively, we can define the projection $P_{pt,1} : \mathbb{R}^3 \rightarrow \mathbb{R}^2; \ x \mapsto P_{pt,1}(x) = (x_1, x_2)^T =: \hat{x}$, i.e. the third component of all points $x \in \mathbb{R}^3$ is simply cut off. Then we can identify the surface of the extracellular matrix with the set $S := \{ \hat{x} \in \mathbb{R}^2 : x \in E \} \subset \mathbb{R}^2$. Finally, we can observe that the cell is connected to the substrate via the cellular focal adhesion sites, which are depicted by the green dots. As stated before, we aim at reconstructing the exerted cellular traction forces from the induced displacements of the underlying substrate. In this context, from a physical point of view, it seems to be reasonable to assume that the cellular traction force is only exerted at those points of the upper surface of the extracellular matrix, where the cell is located. We denote the set of these points by $E_{us,c} \subset E_{us}$ and $S_c \subset S$, respectively.

Having introduced some notations for the description of the different portions of the substrate, we continue with a closer inspection of the applied forces: Recalling Chapter 2.1 we differentiate between body forces $b$ and surface forces $s$. In the above setting the body force $b$ typically is gravity, which in the case of an extracellular matrix of a usual thickness of $10\text{-}300\ \mu\text{m}$ can be expected to be small. In our setting, the applied surface force $s$ is just the cellular traction force that we from now on denote by $f$. Now our first major assumption states that the cellular traction force normal to the surface of the underlying matrix can be neglected (cf. [37], p.208), i.e. $f = (f_1, f_2, 0)^T$. As the cellular traction is only exerted via the focal adhesion sites at the upper surface of the substrate, we can conclude that the applied surface force only depends on $x_1$ and $x_2$.

The second major assumption is that the extracellular matrix is made up of a homogeneous, isotropic and fully elastic material. As experimental evidence suggests that the applied cellular traction forces $f$ as well as the induced displacements $u = (u_1, u_2, u_3)^T$ of the underlying substrate are small, we can apply the small strain linear elasticity theory. Consequently, we are in the situation of the displacement traction problem of three-dimensional elasticity (q.v. Definition 2.29 on p.27).
3. Mathematical Modeling of Cellular Traction Forces

Before we can eventually introduce the corresponding boundary value problem relating \( f \) and \( u \), we have to adjust the notation used in the previous chapter to our experimental setting for the sake of a consistent notation throughout the remaining chapters of this thesis: The domain in the reference configuration, which we denoted by \( \Omega \), corresponds to the elastic extracellular matrix \( \mathcal{E} \). Accordingly, its boundary is \( \partial \mathcal{E} \). The portion of the domain where the surface force is applied, formerly labeled by \( \Gamma_1 \), obviously becomes \( \mathcal{E}_{us,c} \). Furthermore, it seems to be reasonable to assume the displacement to be zero at the lower surface of the extracellular matrix if the substrate is sufficiently thick. As explained above, the rim of the substrate is introduced artificially. Hence, in principle, it was feasible to either assume that at these portions of the boundary a given force is applied or to suppose that the displacement at these boundary points equals to zero. There exist arguments in favor of any alternative. However, we decided for the latter option because, if we once again consider the images of Figure 3.1, we can argue that there seem to be only very little displacements at the border of the images. As we will see in the second section of this chapter, this choice is also more convenient, if the Boussinesq solution is to be applied.

Taking all these observations into account, the effect of cellular traction can thus be modeled by the following boundary value problem:

\[
\begin{align*}
-(\lambda + \mu)\nabla(\nabla \cdot u) - \mu \Delta u &= b \quad \forall \, x \in \mathcal{E}, \\
\lambda (\text{tr} \, \nabla u) I + \mu ((\nabla u)^T + \nabla u) \cdot n &= f \quad \forall \, x \in \mathcal{E}_{us,c}, \\
u &= 0 \quad \forall \, x \in \mathcal{E}_0 := \partial \mathcal{E} \setminus \mathcal{E}_{us,c} 
\end{align*}
\] (3.1)

To get a better feeling for the effects of cellular traction forces, Figures 3.4 to 3.7 on the subsequent pages depict the results of the computation of the induced displacements for five different versions of given applied forces. To calculate the displacement fields, we used the MATLAB® Partial Differential Equation Toolbox.

At first, we set up a rectangular geometry representing a vertical cross section through that part of the elastic extracellular matrix, which is located within the field of view of the imaging device.

As mentioned above, the thickness of the substrate usually ranges from 10 \( \mu \text{m} \) to 300 \( \mu \text{m} \). In this context, we decided that a substrate thickness of 100 \( \mu \text{m} \) seems to be a feasible choice. Following the previous descriptions, the upper surface of the substrate should be in the \( x_1x_2 \)-plane. On a scale of 1:100 \( \mu \text{m} = 10^{-4} \text{ m} \), the geometry in \( x_3 \)-direction consequently is in \([-1, 0]\). Typically, the thickness of the extracellular matrix is much smaller than its horizontal expansion. Hence, we chose the geometry to be ten times as wide as it is high. As we consider a vertical cross section of \( \mathcal{E} \), only the \( x_1 \)-direction of the geometry is of interest. Thus, the geometry with respect to \( x_1 \) reaches from \(-5 \) to \( 5 \).
3.1. A Mathematical Model of Cellular Traction Forces

Next, we shall assign boundary conditions to the borders of our geometry. Assuming the cell exerting the traction forces to lie in the middle of the upper surface of the substrate, i.e. a little left and right of the origin, we label this section of the geometry by $\mathcal{E}_{us,c}$ while all the others are labeled by $\mathcal{E}_0$. The result of this modeling can be seen below:

![Figure 3.3: Vertical cross section of the elastic substrate](image)

After constructing the geometry depicted above, we shall continue with the definition of the applied forces: Above, we already mentioned that the applied body force $b$ is typically small. Here we simply set $b$ equal to zero. Concerning the traction force $f$, we already pointed out that the force application points should coincide with the cell’s location. Taking into account that plant, animal and human cells typically measure between 1 µm and 100 µm, we decided the traction force to be applied at $x_1 \in [-0.1, 0.1]$. Besides, previous results (c.f. e.g. [14]) indicate that the forces are strongest at the rim of the cellular body, point at different directions and that a maximum force intensity of $2 \times 10^3$ pN/µm² seems to be a reasonable choice (c.f. [1], p.10). Finally, we set the two Lamé constants $\mu$ and $\lambda$ characterizing the elastic material to 2000 pN/µm² (c.f. [1], p.11).

Taking all these assumptions into account, we chose $f_1$ to be given by:

$$f_1(x) := \begin{cases} 
0.2, & -0.1 \leq x_1 \leq -0.05, \ x \in \mathcal{E}_{us,c} \\
0.002, & -0.05 < x_1 \leq 0, \ x \in \mathcal{E}_{us,c} \\
-0.002, & 0 < x_1 \leq 0.05, \ x \in \mathcal{E}_{us,c} \\
-0.2, & 0.05 < x_1 \leq 0.1, \ x \in \mathcal{E}_{us,c} \\
0, & x \in \mathcal{E}_0 
\end{cases}$$

where in this case we assumed that the net force exerted by an isolated adherent cell equals to zero, as it is sometimes claimed in the literature (c.f. [8], p.C597). Then the solution of the boundary value problem (3.1) corresponding to $b = 0$ and
3. Mathematical Modeling of Cellular Traction Forces

\( f = f_1 \) is depicted in the subsequent Figure 3.4 (a), where we used the 'quiver' command in MATLAB \(^\text{R}^\text{��} \) to plot the induced displacement field \( u(f_1) \). Figure 3.4 (b) is just an close up of the area of the substrate where the relevant displacements occur. Note that enlarged views of all images of the current section can be found in Appendix I.

![Figure 3.4: Experiment 1: Displacements calculated from \( f_1 \)](image)

For the sake of clarity, we decided to use the standard deviation of the length of the vectors representing the local values of \( u(f_1) \) as a threshold for displaying a vector. Besides, we scaled the arrows such that a length of shaft of 0.1 corresponds to a displacement of 2.5 \( \mu \text{m} \).

From this first numerical experiment we can conclude that the cellular traction forces cause the elastic substrate to deform in a section that in all spacial directions goes far beyond the area where the forces are applied. On the other hand, we can see that the induced displacements are the largest close to this area of force application and vanish towards the borders of the extracellular matrix if it is thick and broad enough. The maximal value of \( u(f_1) \) equals to 2.37 \( \mu \text{m} \), which is in the same order as reference values published in the literature (c.f. for example [1], p.2055). Furthermore, the displacement field seems to be axially symmetric with respect to the...
3.1. A Mathematical Model of Cellular Traction Forces

Taking into account the symmetric character of $f_1$, it can be stated that in summary these observations meet our expectations.

We shall now investigate how the induced displacements change if we vary the applied forces:

Therefore, we at first halve the originally applied force $f_1$, i.e.:

$$ f_2(x) := \frac{1}{2} f_1(x) $$

Then a comparison of the resulting displacement fields $u(f_1)$ and $u(f_2)$ yields:

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig3_5a}
\caption{Enlarged display of the relevant image section for $f = f_1$}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig3_5b}
\caption{Enlarged display of the relevant image section for $f = f_2$}
\end{figure}

**Figure 3.5.:** Experiment 2: Halving the applied forces

To facilitate the comparison of both displacement fields, we again used the standard deviation of the length of the vectors representing the local values of $u(f_1)$ as a threshold for displaying a vector and scaled the arrows such that a length of shaft of 0.1 corresponds to a displacement of 2.5 $\mu$m. For the next examples, this will be our standard procedure.

As our underlying model is linear, we in this case expected the arrows representing the induced displacements to point into the same direction, but to be half as long, which is exactly what we can observe.
In this context, a maximal value of the displacement field $u(f_2)$ of 1.18 $\mu$m fits in perfectly.

We continue with an investigation of the impact of the ratio of forces being applied at the rim and under the interior of the cell. Therefore, we define:

$$f_3(x) := \begin{cases} 
0.102, & -0.1 \leq x_1 \leq -0.05, \quad x \in \mathcal{E}_{us,c} \\
0.1, & -0.05 < x_1 \leq 0, \quad x \in \mathcal{E}_{us,c} \\
-0.1, & 0 < x_1 \leq 0.05, \quad x \in \mathcal{E}_{us,c} \\
-0.102, & 0.05 < x_1 \leq 0.1, \quad x \in \mathcal{E}_{us,c} \\
0, & x \in \mathcal{E}_0 
\end{cases}$$

The subsequent figure is a comparison of the displacements resulting from the originally applied forces $f_1$ and applied forces given by $f_3$, respectively. While in the first case, the portion of the force being applied at the rim of the cell was very dominant (ratio 100:1), the force magnitude at the rim of the cell and the magnitude of the forces applied under its interior are now near-balance (ratio 51:50).

\textbf{Figure 3.6.:} Experiment 3: Changing the ratio of the forces applied at the rim and under the interior of the cell
Having a closer look at both displacement fields, we can observe that this ratio seems to affect the size of the portion of the elastic material where the displacements occur: a highly unbalanced ratio seems to induce displacements at bigger distances. Furthermore, we calculated the total amount of induced displacements: \( f_1 \) caused displacements of 294.8 \( \mu m \) while \( f_3 \) brought about displacements of only 200.7 \( \mu m \).

Finally, we consider two cases where the applied force field is no longer symmetric. Taking into account previous results (c.f. for example [13], p.2310), this seems to be the canonical situation. Hence we determine \( f_4 \) to be given by:

\[
\begin{align*}
    f_4(x) := & \begin{cases} 
    0.2, & -0.1 \leq x_1 \leq -0.05, \quad x \in \mathcal{E}_{us,c} \\
    0.002, & -0.05 < x_1 \leq 0, \quad x \in \mathcal{E}_{us,c} \\
    -0.1, & 0 < x_1 \leq 0.05, \quad x \in \mathcal{E}_{us,c} \\
    -0.102, & 0.05 < x_1 \leq 0.1, \quad x \in \mathcal{E}_{us,c} \\
    0, & x \in \mathcal{E}_0
    \end{cases}
\end{align*}
\]

Besides, we study a situation, where the applied force field is not only asymmetric, but additionally the assumption of a zero net force is breached:

\[
\begin{align*}
    f_5(x) := & \begin{cases} 
    0.2, & -0.1 \leq x_1 \leq -0.05, \quad x \in \mathcal{E}_{us,c} \\
    0.002, & -0.05 < x_1 \leq 0, \quad x \in \mathcal{E}_{us,c} \\
    -0.0015, & 0 < x_1 \leq 0.05, \quad x \in \mathcal{E}_{us,c} \\
    -0.15, & 0.05 < x_1 \leq 0.1, \quad x \in \mathcal{E}_{us,c} \\
    0, & x \in \mathcal{E}_0
    \end{cases}
\end{align*}
\]

The displacements induced by these applied forces are depicted in the subsequent figure on the next page. Thereby we again used the displacement field corresponding to the originally applied surface force \( f_1 \) as a reference value to discuss the arising differences.

Concerning the resulting displacements of the first of these asymmetric applied forces we have to state that only minor discrepancies can be observed. However, if we concentrate our attention to the area close to the origin, we can realize slight differences with respect to the direction the arrows are pointing at and this seems to be a little less symmetric. Nevertheless, this effect is somewhat less visible than we expected. Furthermore, we can recognize that the displacements in this case occur in a smaller portion of the elastic material. However, this portion still appears to have a quite symmetric shape.
3. Mathematical Modeling of Cellular Traction Forces

Figure 3.7: Experiment 4: Canceling the symmetric shape of the applied force and neglecting the assumption of zero net force

By contrast, in the case of applied forces defined by $f_5$ the portion where the induced displacements occur looks highly asymmetric. Besides, we can see that most of the arrows point from left to right, which seems to be reasonable as the total net force is positive.

Having discussed some examples of applied forces $f_i$ and their resulting displacements $u(f_i)$, we shall conclude this section by introducing the forward problem
3.2. The Boussinesq Solution

corresponding to the boundary value problem (3.1): Given an applied traction force \( \mathbf{f} = (f_1, f_2, 0)^T \) the induced displacement \( \mathbf{u} = (u_1, u_2, u_3)^T \) of the underlying substrate can be calculated by the following Fredholm integral equation of the first kind:

\[
    u_i = \int \int G_{ik}(x_1, x_1', x_2, x_2', x_3) f_k(x_1', x_2') \, dx_1' \, dx_2', \quad \text{for } i, k = 1, \ldots, 3 \tag{3.2}
\]

where the \( G_{ik} \) represent so-called Green’s functions with a weak singularity at the origin. To make this equation determinate, these Green’s functions have to be ascertained. Therefore, the remaining part of this chapter deals with the presentation of two alternative approaches for the deduction of explicit formulas for these functions.

3.2. The Boussinesq Solution

In this section we deduce the well-known Boussinesq solution providing explicit formulas for the Green’s functions \( G_{ik} \) in the integral equation (3.2), which was originally published by the French scientist J. V. Boussinesq [4]. Here we will follow the work of L. D. Landau and E. M. Lifshitz [27], pp.22-25.

In the setting of the small strain linear elasticity theory (q.v. Chapter 2.1.5), the deduction of the Boussinesq solution is based on three major assumptions:

- Firstly, we suppose that the elastic medium of interest occupies a half-space, that is the elastic medium is on one side bounded by an infinite plane, while surface forces \( s \) are applied to its free surface (comp. [27], p.22). We define this bounding surface to be the \( x_1x_2 \)-plane, such that the elastic medium is located in \( x_3 > 0 \). For a better perception of this setting Figure 3.8 illustrates Boussinesq’s problem. To obviate misapprehension we would like to point out that the half-space actually is of infinite thickness and extensiveness, but for obvious reasons this could not be depicted.

![Figure 3.8: Illustration of Boussinesq’s problem](image-url)
3. Mathematical Modeling of Cellular Traction Forces

• Secondly, we assume that the elastic material is not subject to any body forces (comp. [27], p.16), i.e. \( b = 0 \). This means in particular that gravity is neglected. Hence the Navier-Lamé equations (comp. definition [2.29]) become:

\[
-(\lambda + \mu) \nabla (\nabla \cdot u) - \mu \Delta u = 0 \tag{3.3}
\]

• Thirdly, the applied (surface) force is required to vanish at infinity such that there is no deformation at infinity (comp. [27], p.22).

In this situation we can find explicit formulas for the calculation of the displacement field \( u \).

To present these formulas in their most common form we have to mildly adjust our notation introduced in Chapter 2.1 including the Lamé constants \( \mu \) and \( \lambda \), which characterize the elastic material. Beside these Lamé constants two other constants are likewise widely-used, namely the so-called Poisson’s ratio \( \nu \) and Young’s modulus \( \varepsilon \). All these constants have to be determined experimentally from a given elastic material. However, it is sufficient to either determine the Lamé constants \( \mu \) and \( \lambda \) or Poisson’s ratio \( \nu \) and Young’s modulus \( \varepsilon \) as they are related by (see [10], p.128):

\[
\nu = \frac{\lambda}{2(\lambda + \mu)} \quad \varepsilon = \frac{\mu(3\lambda + 2\mu)}{\lambda + \mu} \tag{3.4}
\]

\[
\lambda = \frac{\varepsilon \nu}{(1 + \nu)(1 - 2\nu)} \quad \mu = \frac{\varepsilon}{2(1 + \nu)}
\]

We are now able to rewrite Equation (3.3), which holds true throughout the space occupied by the elastic material (comp. [27], p.22):

\[
-(\lambda + \mu) \nabla (\nabla \cdot u) - \mu \Delta u = 0
\]

\[
\iff \left( \frac{\varepsilon \nu}{(1 + \nu)(1 - 2\nu)} + \frac{\varepsilon}{2(1 + \nu)} \right) \nabla (\nabla \cdot u) + \frac{\varepsilon}{2(1 + \nu)} \Delta u = 0 \tag{3.5}
\]

\[
\iff \left( \frac{2\nu + (1 - 2\nu)}{2(1 - 2\nu)(1 + \nu)} \right) \nabla (\nabla \cdot u) + \frac{1}{2(1 + \nu)} \Delta u = 0
\]

\[
\iff \nabla (\nabla \cdot u) + (1 - 2\nu) \Delta u = 0
\]

\[\text{In the literature Young’s modulus is often denoted by } E, \text{ but to obviate confusion with the Green-St Vernant strain tensor } E, \text{ we decided to use } \varepsilon \text{ instead.}\]
3.2. The Boussinesq Solution

We make the following ansatz for a solution $u$ of the last equation of (3.5) (see [27], p.22):

$$u = h + \nabla \phi, \quad (3.6)$$

where $\phi$ is some physical scalar and the vector $h$ satisfies Laplace’s equation $\Delta h = 0$.

If we now insert the Identity (3.6) into Equation (3.5), we get:

$$\nabla (\nabla \cdot (h + \nabla \phi)) + (1 - 2\nu) \Delta (h + \nabla \phi) = 0$$

$$\iff \nabla (\nabla \cdot h + \Delta \phi) + (1 - 2\nu) \Delta (h + \nabla \phi) = 0$$

$$\iff \nabla (\nabla \cdot h) + 2(1 - \nu) \Delta (\nabla \phi) + (1 - 2\nu) \Delta h = 0$$

$$\iff 2(1 - \nu) \nabla (\Delta \phi) = -\nabla (\nabla \cdot h)$$

Hence we can conclude:

$$2(1 - \nu) \Delta \phi = -\nabla \cdot h \quad (3.7)$$

Next, we choose $h_1$ and $h_2$ to be the derivative with respect to $x_3$ of some function $g_1$ and $g_2$, respectively (comp. [27], p.23), i.e.:

$$h_1 = \frac{\partial g_1}{\partial x_3} \quad h_2 = \frac{\partial g_2}{\partial x_3} \quad (3.8)$$

According to our assumptions $h_1$ and $h_2$ satisfy Laplace’s equation and hence we can always choose $g_1$ and $g_2$ such that $\Delta g_1 = 0 = \Delta g_2$.

In combination with Equation (3.8) the Identity (3.7) reads:

$$2(1 - \nu) \Delta \phi = - \frac{\partial}{\partial x_3} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) \quad (3.9)$$

$$\iff 2(1 - \nu) \Delta \phi = - \frac{\partial}{\partial x_3} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) \quad (3.10)$$

We continue by claiming that the following equation provides a solution of (3.10):

$$\phi = -\frac{x_3}{4(1 - \nu)} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) + \psi, \quad (3.11)$$

where $\psi$ is again some harmonic function which fulfills $\Delta \psi = 0$. 

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3. Mathematical Modeling of Cellular Traction Forces

The validity of this proposition can be noticed easily from the following calculations:

\[ 2(1 - \nu) \Delta \phi = 2(1 - \nu) \nabla \cdot (\nabla \phi) \]

\[ = 2(1 - \nu) \nabla \cdot \left( \nabla \left[ -\frac{x_3}{4(1 - \nu)} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) + \psi \right] \right) \]

\[ \text{prod. rule} \quad \nabla \cdot \left( \begin{array}{c}
-\frac{x_3}{2} \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 + \frac{\partial \psi}{\partial x_1} \\
-\frac{x_3}{2} \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 + \frac{\partial \psi}{\partial x_2} \\
-\frac{1}{2} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) - \frac{x_3}{2} \frac{\partial}{\partial x_3} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) + \frac{\partial \psi}{\partial x_3}
\end{array} \right) \]

\[ \text{prod. rule} \quad = -\frac{x_3}{2} \left[ \frac{\partial^2}{\partial x_1^2} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) + \frac{\partial^2}{\partial x_2^2} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) \right] \\
- \frac{x_3}{2} \left[ \frac{\partial^2}{\partial x_3^2} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) \right] + \frac{\partial^2 \psi}{\partial x_1^2} + \frac{\partial^2 \psi}{\partial x_2^2} + \frac{\partial^2 \psi}{\partial x_3^2} \\
- \frac{1}{2} \frac{\partial}{\partial x_1} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) - \frac{1}{2} \frac{\partial}{\partial x_2} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) \]

\[ = -\frac{x_3}{2} \left( \frac{\partial \Delta g_1}{\partial x_1} \right)_{x=0} + \frac{\partial \Delta g_1}{\partial x_2} \left. \frac{\Delta g_2}{\partial x_2} \right|_{x=0} + \frac{\Delta g_3}{\partial x_3} - \frac{\partial }{\partial x_3} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) \]

Hence, if we succeed in finding the harmonic functions \( g_1, g_2, h_3 \) and \( \psi \) we have an explicit formula for the calculation of the displacement \( u \).

The next step is the incorporation of the boundary conditions on the free boundary, where the surface force is applied, i.e., we consider the \( x_1 x_2 \)-plane at \( x_3 = 0 \). Thus we know that the unit outward normal vector \( N \) points exactly in the negative \( x_3 \)-direction, that is \( N = (0, 0, -1)^T \). Now we recall Equation (2.14), stating that at the boundary the identity \( S(x) \cdot n = s(x) \) holds true for all \( x \in \Gamma_1 \), where \( S \) is the second Piola-Kirchhoff stress tensor and \( s \) is the applied surface force in the reference configuration. Thus substituting \( N \) for \( n \) yields:

\[ S_{i3} = -s_i \] (3.12)

Furthermore, in the context of the linear elasticity theory for the second Piola-Kirchhoff stress tensor the subsequent stress-strain law holds (see Equation (2.13)):

\[ (S_{\text{lin}})_{ij} = \tilde{S}_{ij}(C_{\text{lin}}) = \lambda \sum_{k=1}^{3} (E_{\text{lin}})_{kk} \delta_{ij} + 2\mu (E_{\text{lin}})_{ij}, \]

\[ (E_{\text{lin}})_{ij} = \frac{1}{2} (\partial x_i u_j + \partial x_j u_i) \]
3.2. The Boussinesq Solution

Before we proceed we again adjust our notation to the notation used throughout this section. Replacing the Lamé constants once more by Young’s modulus and Poisson’s ratio we end up with (see Equations (3.4)):

\[
(S_{lin})_{ij} = \frac{\varepsilon}{1+\nu} \left( (E_{lin})_{ij} + \frac{\nu}{1-2\nu} \sum_{k=1}^{3} (E_{lin})_{kk} \delta_{ij} \right)
\]

(3.13)

Thus, the boundary condition now reads:

\[
(S_{lin})_{i3} = \frac{\varepsilon}{1+\nu} \left( (E_{lin})_{i3} + \frac{\nu}{1-2\nu} \sum_{k=1}^{3} (E_{lin})_{kk} \delta_{i3} \right) = -s_i
\]

We continue with a case analysis with respect to \(i\):

**case \(i = 1\):**

\[
\frac{\partial}{\partial x_1} \left( \frac{\partial}{\partial x_3} \left[ -\frac{x_3}{4(1-\nu)} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) + \psi \right] \right)
\]

\[
+ \frac{\partial}{\partial x_3} \left[ \frac{\partial g_1}{\partial x_3} + \frac{\partial}{\partial x_1} \left[ -\frac{x_3}{4(1-\nu)} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) + \psi \right] \right]
\]

\[
+ \frac{2\nu}{1-2\nu} \sum_{k=1}^{3} \frac{\partial}{\partial x_k} \left[ h_k + \frac{\partial}{\partial x_1} \left[ -\frac{x_3}{4(1-\nu)} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) + \psi \right] \delta_{i3}
\]

\[
= -\frac{2(1+\nu)}{\varepsilon} s_1
\]

At the boundary of the half-plane holds \(x_3 = 0\).

This yields:

\[
\left. \left[ \frac{\partial^2 g_1}{\partial x_3^2} \right]_{x_3=0} + \left[ \frac{\partial}{\partial x_1} \left( -\frac{1}{2(1-\nu)} + \frac{2(1+\nu)}{2(1-\nu)} h_3 \right) \right]_{x_3=0} + \left[ \frac{\partial}{\partial x_1} \left( -\frac{1}{2(1-\nu)} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) + \frac{\partial \psi}{\partial x_3} \right) \right]_{x_3=0} = -\frac{2(1+\nu)}{\varepsilon} s_1
\]

Hence, we finally end up with the following first boundary condition:

\[
\left. \left[ \frac{\partial^2 g_1}{\partial x_3^2} \right]_{x_3=0} + \left[ \frac{\partial}{\partial x_1} \left( -\frac{1}{2(1-\nu)} h_3 - \frac{1}{2(1-\nu)} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + \frac{2}{\partial x_3} \right) \right) \right]_{x_3=0} = -\frac{2(1+\nu)}{\varepsilon} s_1
\]

(3.14)
This last case again requires some work: 

\[ i = 54 \]

By analogous proceeding we get the second boundary condition:

\[ i = 3. \]

This last case again requires some work:

\[ 2 \left[ \frac{\partial^2 g_k}{\partial x_3^2} \right] x_3 = 0 + \left[ \frac{\partial}{\partial x_3} \left( \frac{1}{1-\nu} \right) h_3 - \frac{1}{2(1-\nu)} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + 2 \frac{\partial \psi}{\partial x_3} \right) \right] x_3 = 0 \]

\[ = - \frac{2(1+\nu)}{\varepsilon} s_2 \]

\[ i = 3 : \]

Two fold application of the product rule yields:

\[ 2 \left[ \frac{\partial}{\partial x_3} h_3 - \frac{1}{4(1-\nu)} \left( \frac{\partial^2}{\partial x_3^2} \right) \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) \right] + \left[ \frac{\partial}{\partial x_3} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) \right] + \left[ \frac{\partial^2}{\partial x_3^2} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) \right] \]

\[ = - \frac{2(1+\nu)}{\varepsilon} s_3 \]

Setting \( x_3 = 0 \) and taking into account Equation (3.13) as well as the fact that \( g_1, g_2, h_3 \) and \( \psi \) are harmonic functions, the above equation becomes:

\[ 2 \left[ \left( \frac{1}{2(1-\nu)} + \frac{\nu}{1-2\nu} \right) \frac{\partial}{\partial x_3} \right] x_3 = 0 \]

\[ = - \frac{2(1+\nu)}{\varepsilon} s_3 \]

\[ \iff 2 \left[ \left( \frac{(1-\nu)(1-2\nu)}{2(1-\nu)} \right) \frac{\partial}{\partial x_3} h_3 + \left( \frac{(1-\nu)(1-2\nu)}{2(1-\nu)(1-2\nu)} \right) \frac{\partial}{\partial x_3} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} \right) \right] x_3 = 0 \]

\[ = - \frac{2(1+\nu)}{\varepsilon} s_3 \]
Thus, the third boundary condition reads:

\[
\frac{\partial}{\partial x_3} \left( h_3 - \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} \right) \right)_{x_3=0} = - \frac{2(1+\nu)}{\varepsilon} s_3 \tag{3.16}
\]

Even though Equations (3.14)-(3.16) impose conditions for the choice of \(g_1, g_2, h_3\) and \(\psi\), these functions are not defined uniquely yet. Consequently, we can introduce an arbitrary additional constraint for the determination of these auxiliary functions. According to [27], p.24, an adequate constraint is given by:

\[
(1 - 2\nu)h_3 - \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} \right) + 4(1 - \nu) \frac{\partial \psi}{\partial x_3} = 0, \tag{3.17}
\]

i.e. we set the bracketed term, that is the second summand, of Equations (3.14) and (3.15) to zero. Pursuant to [27], p.24 the admissibility of this condition can be inferred from the absence of contradiction in the further considerations and the final result.

Therefore the above boundary conditions now read:

\[
\left[ \frac{\partial^2 g_1}{\partial x_3^2} \right]_{x_3=0} = - \frac{2(1+\nu)}{\varepsilon} s_1 \]

\[
\left[ \frac{\partial^2 g_2}{\partial x_3^2} \right]_{x_3=0} = - \frac{2(1+\nu)}{\varepsilon} s_2 \tag{3.18}
\]

\[
\left[ \frac{\partial}{\partial x_3} \left( h_3 - \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} \right) \right) \right]_{x_3=0} = - \frac{2(1+\nu)}{\varepsilon} s_3
\]

By now we have everything at our disposal to completely determine the four required harmonic functions \(g_1, g_2, h_3\) and \(\psi\).

For the sake of simplicity, we now assume that the surface of the elastic half-space is subject to a concentrated force. This means that we suppose that the area where the force is applied is so small that it can be regarded as one single point of this surface. We denote this concentrated force acting on a single point of the surface by \(p\).

This concentrated force \(p\) has the same impact on the elastic substrate as the surface force \(s\), if \(s\) and \(p\) are related by:

\[
s = p\delta(x_1)\delta(x_2), \tag{3.19}
\]

where the origin is located at the point of application of \(p\).

In this situation, the displacement \(u\) induced by an applied concentrated force \(p\)
3. Mathematical Modeling of Cellular Traction Forces

can be computed by:

\[ u_i = G_{ik}(x_1, x_2, x_3) p_k, \quad (3.20) \]

However, instead of calculating the displacement \( u \) via this product in dependence on an applied concentrated force \( p \), the displacement can equivalently be determined from the following integral equation establishing the connection to Equation (3.2):

\[ u_i = \int \int G_{ik}(x_1 - x'_1, x_2 - x'_2, x_3) s_k(x'_1, x'_2) \, dx'_1 \, dx'_2 \quad (3.21) \]

where \( s \) and \( p \) are related by (3.19).

Next, we try to determine the quantities \( g_1, g_2, h_3 \) and \( \psi \) for a known concentrated force \( p \). For this purpose we state that according to [27], p.24 a harmonic function \( \xi \), which is zero at infinity and has a given normal derivative \( \frac{\partial \xi}{\partial x_3} \) on the plane \( x_3 = 0 \), is given by the formula:

\[ \xi(x_1, x_2, x_3) = -\frac{1}{2\pi} \int \int \frac{[\partial \xi(x'_1, x'_2, x'_3)]}{\partial x_3} \bigg|_{x_3=0} \frac{dx'_1 \, dx'_2}{r}, \]

where

\[ r = \sqrt{(x_1 - x'_1)^2 + (x_2 - x'_2)^2 + x_3^2}. \]

By construction we know that \( \frac{\partial g_1}{\partial x_3} \) and \( \frac{\partial g_2}{\partial x_3} \) as well as \( g_1, g_2, h_3 \) and \( \psi \) are harmonic functions. Consequently, it is clear that the function \( h_3 - \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} \right) + \frac{\partial \psi}{\partial x_3} \) also satisfies Laplace’s equation. Besides, the values of the normal derivatives of \( \frac{\partial g_1}{\partial x_3}, \frac{\partial g_2}{\partial x_3} \) and this last function on the plane \( x_3 = 0 \) are given by the conditions (3.18).

Therefore we can conclude that the following identities hold true:

\[ h_3 - \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} \right) + \frac{\partial \psi}{\partial x_3} = -\frac{1}{2\pi} \int \int -\frac{2(1+\nu)}{\varepsilon} s_3(x'_1, x'_2) \frac{dx'_1 \, dx'_2}{r} \]

\[ = \frac{1+\nu \varepsilon}{\pi \varepsilon} \frac{p_3}{r} \quad (3.22) \]

And analogously we get:

\[ \frac{\partial g_1}{\partial x_3} = \frac{1+\nu \varepsilon}{\pi \varepsilon} \frac{p_1}{r} \]

\[ \frac{\partial g_2}{\partial x_3} = \frac{1+\nu \varepsilon}{\pi \varepsilon} \frac{p_2}{r}, \]

where now \( r = \sqrt{x_1^2 + x_2^2 + x_3^2} \).
3.2. The Boussinesq Solution

As we know (comp. Equations (3.6) and (3.11)) that the displacement \( u \) is given by:

\[
\mathbf{u} = h + \nabla \left[ -\frac{x_3}{4(1-\nu)} \left( \frac{\partial g_1}{\partial x_1} + \frac{\partial g_2}{\partial x_2} + h_3 \right) + \psi \right],
\]

we can conclude that besides \( h_3 \) and \( \psi \) we have to determine \( \frac{\partial g_1}{\partial x_1} \) and \( \frac{\partial g_2}{\partial x_2} \), but not \( g_1 \) and \( g_2 \) themselves. As the next step we therefore first derive \( \frac{\partial g_1}{\partial x_3} \) and \( \frac{\partial g_2}{\partial x_3} \) with respect to \( x_1 \) and \( x_2 \), respectively and afterwards integrate over \( x_3 \) from \( \infty \) to \( x_3 \).

Derivation yields:

\[
\frac{\partial^2 g_1}{\partial x_1 \partial x_3} = \frac{\partial}{\partial x_1} \left( \frac{1+\nu}{\pi \varepsilon} p_1 \frac{1}{\sqrt{x_1^2 + x_2^2 + x_3^2}} \right)
= \frac{1+\nu}{\pi \varepsilon} \left( -\frac{1}{2} \frac{p_1}{\sqrt{x_1^2 + x_2^2 + x_3^2}} 2x_1 \right) \quad (3.23)
= -\frac{1+\nu}{\pi \varepsilon} \frac{p_1 x_1}{r^3}
\]

By analogous calculations we get:

\[
\frac{\partial^2 g_2}{\partial x_2 \partial x_3} = -\frac{1+\nu}{\pi \varepsilon} \frac{p_2 x_2}{r^3} \quad (3.24)
\]

Then the integral reads:

\[
\int_{\infty}^{x_3} -\frac{1+\nu}{\pi \varepsilon} \frac{p_1 x_1}{\sqrt{x_1^2 + x_2^2 + x_3^2}} = -\frac{1+\nu}{\pi \varepsilon} p_1 x_1 \int_{\infty}^{x_3} \frac{1}{\sqrt{x_1^2 + x_2^2 + x_3^2}} dx_3 \quad (3.25)
\]

We consider the integral on the r.h.s. separately where we set \( x_1^2 + x_2^2 = c \), as this term is independent of \( x_3 \):

\[
I = \int_{\infty}^{x_3} \frac{1}{\sqrt{c+x_3^2}} dx_3
\]

We apply the Eulerian Substitution, i.e.:

\[
t - x_3 = \sqrt{c+x_3^2} \iff t = \sqrt{c+x_3^2} + x_3 = r + x_3
\]

\[
I = \frac{t^2 - c}{2t} \iff x_3 = \frac{t^2 - c}{2t} = \frac{t}{2} + \frac{c}{2t}
\]

\[
\frac{dx_3}{dt} = \frac{1}{2} + \frac{c}{2t^2} \iff dx_3 = \frac{1}{2} + \frac{c}{2t^2} dt
\]

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Hence we can determine \( I \) by:

\[
I = \int_{\frac{t^2 - c^2}{2t}}^{\frac{1}{t} \sqrt{t} + \frac{c \sqrt{t}}{2t}} \left( t^2 - c^2 \right) \left( \frac{1}{t^2} + \frac{c}{2t} \right) \left( t^2 + \frac{c^2}{2t} \right)^2 dt
\]

\[
= \int_{\frac{t^2 - c^2}{2t}}^{\frac{t^2 - c^2}{2t} + \frac{c^2}{t}} \left( t^2 - c^2 \right) \left( \frac{1}{t^2} + \frac{c}{2t} \right) \left( t^2 + \frac{c^2}{2t} \right)^2 dt
\]

\[
= \int_{\frac{t^2 - c^2}{2t}}^{\frac{t^2 - c^2}{2t} + \frac{c^2}{t}} \left( \frac{t \sqrt{t} + c \sqrt{t}}{2t} \right)^{-2} dt
\]

\[
= \int_{\frac{t^2 - c^2}{2t}}^{\frac{t^2 - c^2}{2t} + \frac{c^2}{t}} \left( \frac{t^2 + c}{2 \sqrt{t}} \right)^{-2} dt
\]

\[
= \int_{\frac{t^2 - c^2}{2t}}^{\frac{t^2 - c^2}{2t} + \frac{c^2}{t}} \frac{4t}{(t^2 + c)^2} dt = \int_{\frac{t^2 - c^2}{2t}}^{\frac{t^2 - c^2}{2t} + \frac{c^2}{t}} \frac{1}{(t^2 + c)^2} dt
\]

\[
s = c + t^2
\]

\[
= \int_{\frac{1}{\sqrt{t^2 + c}}}^{\frac{1}{\sqrt{t^2 + c}}} \left( \frac{t^2 - c^2}{2t} \right) \left( \frac{t^2 + c}{2 \sqrt{t}} \right)^{-2} dt = \int_{\frac{1}{\sqrt{t^2 + c}}}^{\frac{1}{\sqrt{t^2 + c}}} \left( \frac{t^2 + c}{2 \sqrt{t}} \right)^{-2} dt
\]

\[
\frac{2}{s} \left( \frac{t^2 - c}{\sqrt{t^2 - c}} \right) \left( \frac{t^2 - c^2}{\sqrt{t^2 - c}} \right) = \frac{2}{s} \left( \frac{t^2 - c}{\sqrt{t^2 - c}} \right)
\]

\[
= \frac{2}{c + (r + x_3)^2} \left|_{x_3}^{\infty} \right. - \frac{2}{r^2 + 2rx_3 + x_3^2 + c} \left|_{x_3}^{\infty} \right. = \frac{2}{r^2 + 2rx_3 + x_3^2 + c} \left|_{x_3}^{\infty} \right. - \frac{2}{r^2 + 2rx_3 + x_3^2 + c} \left|_{x_3}^{\infty} \right. = \frac{1}{r(r + x_3)}
\]

If we insert this result in Equation (3.25), we finally end up with:

\[
\frac{\partial g_1}{\partial x_1} = \frac{1 + \nu}{\pi \tau} \frac{p_1 x_3}{r(r + x_3)}
\]
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And by analogous reasoning we get:

\[
\frac{\partial g}{\partial x_2} = \frac{1 + \nu}{\pi \varepsilon} \frac{p_2 x_2}{r(r+x_3)}
\]  

(3.27)

Using these results, we are now able to determine \( h_3 \) and \( \frac{\partial \psi}{\partial x_3} \).

Therefore we insert the Identities (3.26) and (3.27) into the Equations (3.17) and (3.22). Then we can calculate \( h_3 \) and \( \frac{\partial \psi}{\partial x_3} \) by solving the system of equations.

Furthermore, the quantities \( \frac{\partial \psi}{\partial x_1} \) and \( \frac{\partial \psi}{\partial x_2} \) can be obtained from \( \frac{\partial \psi}{\partial x_3} \) by a similar procedure as used for the determination of \( \frac{\partial g}{\partial x_1} \) and \( \frac{\partial g}{\partial x_2} \), respectively. This means we integrate the known function with respect to \( x_3 \) and afterwards differentiate the resulting function with respect to \( x_1 \) and \( x_2 \).

As these calculations are laborious while not providing any new ideas and insights, we decided to omit them.

Here, we just state the resulting explicit formulas for the displacement \( u \):

\[
u_1 = \frac{1 + \nu}{2\pi \varepsilon} \left[ \left( \frac{x_1 x_3}{r^3} - \frac{(1 - 2\nu)x_1}{r(r+x_3)} \right) p_3 + \left( \frac{2(1 - \nu)r + x_3}{r(r+x_3)} \right) p_1 \right]
+ \frac{1 + \nu}{2\pi \varepsilon} \left[ \left( \frac{(2r(\nu r + x_3) + x_3^2)x_1}{r^3(r+x_3)^2} \right) (x_1 p_1 + x_2 p_2) \right]
\]

\[
u_2 = \frac{1 + \nu}{2\pi \varepsilon} \left[ \left( \frac{x_2 x_3}{r^3} - \frac{(1 - 2\nu)x_2}{r(r+x_3)} \right) p_3 + \left( \frac{2(1 - \nu)r + x_3}{r(r+x_3)} \right) p_2 \right]
+ \frac{1 + \nu}{2\pi \varepsilon} \left[ \left( \frac{(2r(\nu r + x_3) + x_3^2)x_2}{r^3(r+x_3)^2} \right) (x_1 p_1 + x_2 p_2) \right]
\]

\[
u_3 = \frac{1 + \nu}{2\pi \varepsilon} \left[ \left( \frac{2(1 - \nu)}{r} + \frac{x_3^2}{r^3} \right) p_3 \right]
+ \frac{1 + \nu}{2\pi \varepsilon} \left[ \left( \frac{1 - 2\nu}{r(r+x_3)} + \frac{x_3}{r^3} \right) (x_1 p_1 + x_2 p_2) \right],
\]

where

\[
r = \sqrt{x_1^2 + x_2^2 + x_3^2}
\]

We recall the Relation (3.20):

\[
u_i = G_{ik}(x_1, x_2, x_3)p_k
\]
Then equating coefficients finally yields the subsequent explicit formulas for the wanted Green’s functions in the integral equation (3.21):

\begin{align*}
G_{11} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{2(1 - \nu)r + x_3}{r(r + x_3)} + \frac{(2r(\nu r + x_3) + x_3^2)x_1^2}{r^3(r + x_3)^2} \right] \\
G_{21} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{(2r(\nu r + x_3) + x_3^2)x_1x_2}{r^3(r + x_3)^2} \right] \\
G_{31} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{(1 - 2\nu)x_1}{r(r + x_3)} + \frac{x_1x_3}{r^3} \right] \\
G_{12} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{(2r(\nu r + x_3) + x_3^2)x_1x_2}{r^3(r + x_3)^2} \right] \\
G_{22} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{2(1 - \nu)r + x_3}{r(r + x_3)} + \frac{(2r(\nu r + x_3) + x_3^2)x_2^2}{r^3(r + x_3)^2} \right] \\
G_{32} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{(1 - 2\nu)x_2}{r(r + x_3)} + \frac{x_2x_3}{r^3} \right] \\
G_{13} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{x_1x_3}{r^3} - \frac{(1 - 2\nu)x_1}{r(r + x_3)} \right] \\
G_{23} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{x_2x_3}{r^3} - \frac{(1 - 2\nu)x_2}{r(r + x_3)} \right] \\
G_{33} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{2(1 - \nu)}{r} + \frac{x_3^2}{r^3} \right]
\end{align*}

At the end of this section we shall relate the underlying setting of the derivation of the Boussinesq solution with our experimental setting for the reconstruction of cellular traction forces. We assume that the elastic material is the extracellular matrix on which the cell is cultured. Furthermore, the applied surface force \( s \) is just the cellular traction force \( f \). As the cell is located on the upper surface of the extracellular matrix and this is the place where the traction force is exerted, this is the free surface of the elastic material occupying a half-space. If we again decide for this surface to be the \( x_1x_2 \)-plane, the elastic material in this case is in \( x_3 < 0 \). Thus the corresponding drawing is given by:

\[ \text{Figure 3.9.: Detection of cellular traction forces with respect to the Boussinesq solution} \]
3.2. The Boussinesq Solution

Hence the setting for the reconstruction of cellular traction forces is turned upside-down in comparison with the situation of the deduction of the Boussinesq solution. Therefore the signs in the Green’s functions have to be adjusted accordingly. Thus, in this situation the Green’s functions read:

\[
\begin{align*}
G_{11} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{2(1 - \nu)r - x_3}{r(r - x_3)} + \frac{(2r(\nu r + x_3) + x_3^2)x_1^2}{r^3(r + x_3)^2} \right] \\
G_{21} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{(2r(\nu r - x_3) + x_3^2)x_1 x_2}{r^3(r - x_3)^2} \right] \\
G_{31} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{(1 - 2\nu)x_1}{r(r - x_3)} + \frac{x_1 x_3}{r^3} \right] \\
G_{12} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{(2r(\nu r - x_3) + x_3^2)x_1 x_2}{r^3(r - x_3)^2} \right] \\
G_{22} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{(1 - 2\nu)x_2}{r(r - x_3)} + \frac{x_2 x_3}{r^3} \right] \\
G_{32} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{(1 - 2\nu)x_2}{r(r - x_3)} + \frac{x_2 x_3}{r^3} \right] \\
G_{13} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{x_1 x_3}{r^3} - \frac{(1 - 2\nu)x_1}{r(r - x_3)} \right] \\
G_{23} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{x_2 x_3}{r^3} - \frac{(1 - 2\nu)x_2}{r(r - x_3)} \right] \\
G_{33} &= \frac{1 + \nu}{2\pi \varepsilon} \left[ \frac{2(1 - \nu)}{r} + \frac{x_3^2}{r^3} \right]
\end{align*}
\]

Hence, we can conclude that we are now able to determine the displacement field \( u \) for known cellular traction forces \( f \) at every point \( x \) of the elastic extracellular matrix \( \mathcal{E} \).

However, we would like to point out that this Boussinesq solution only provides an approximate solution to the problem of the determination of the traction forces exerted by a cell to the underlying substrate as the extracellular matrix is neither of infinite extensiveness nor of infinite thickness. In fact, the thickness of the substrate usually is in the order of 10-300 \( \mu \)m.

To get a feeling for the effect of this deviation from the assumption of an infinite substratum thickness, we follow the explanations of U. S. Schwarz and coworkers \[38\], p.1384:

As experimental evidence suggests, we assume

\[
d = \sqrt{\frac{s}{\varepsilon}} \quad (3.28)
\]

to be the typical displacements induced by an applied surface force \( s \).
3. Mathematical Modeling of Cellular Traction Forces

Considering the forward problem under the assumptions of the Boussinesq problem, given by:

\[
\mathbf{u}_i = \int \int G_{ik}(x_1 - x'_1, x_2 - x'_2, x_3) s_k(x'_1, x'_2) \, dx'_1 \, dx'_2,
\]

the Boussinesq solution predicts that the displacement \( u \) at a distance \( r \) from the origin scales as

\[
u = \frac{s}{\varepsilon r}.
\]

By contrast, the real displacement \( \tilde{u} \) scales as

\[
\tilde{u} = cr,
\]

where \( c \) is a dimensionless factor that has to be determined from the boundary conditions. Then the Boussinesq solution is an appropriate approximation, if

\[
u = \frac{s}{\varepsilon r} \approx cr = \tilde{u} \quad \& \quad \tilde{u} \approx 0 \quad (3.29)
\]

hold at \( r = h \), where \( h \) is the thickness of the substrate.

The first part of \((3.29)\) yields the condition:

\[
c = \frac{s}{\varepsilon h^2} \quad \frac{d^2}{h^2} \quad (3.28)
\]

If we combine this condition with the second part of \((3.29)\) we end up with:

\[
\tilde{u} = ch = \frac{d^2}{h} \quad \approx \quad 0
\]

Hence we reason that the Boussinesq solution seems to be a proper approximation of the real displacements, if these are much smaller than the thickness of the substrate \((d^2 \ll h)\). As the substrate is not only of finite thickness, but also of finite extensiveness, similar reasoning yields the second condition stating that the length and width of the area where the force is applied has to be much smaller than the thickness of the substrate (cf. \([8]\), p.C598 and \([38]\), p.1384). Furthermore, we can conclude that if the substrate is not sufficiently thick and hence one of these conditions is violated, the Boussinesq solution predicts larger displacements than one can actually observe in reality.

Having introduced the Boussinesq solution as one common way to specify the Green’s functions in the forward problem \((3.2)\) and having discussed the limits of its applicability, we present a second possibility for the explicit calculation of the displacement \( u \) for given applied forces in the next section.
3.3. The Plane Stress Approximation

In this section we deduce a two-dimensional approximation of the underlying three-dimensional model of cellular traction. Thereby the book 'Theory of Elasticity' by L. D. Landau and E. M. Lifshitz [27] (p.38 and p.46 f.) serves as our key source. This two-dimensional approximation, known as the plane stress approximation, is based on the following three major assumptions:

- Firstly, the approximation should be applied to thin plates only, where we regard an elastic medium to be a thin plate, if its thickness is small compared with its dimensions in the other two directions (see [27], p.38). Note that this condition is contrary to the assumption of an infinite thickness of the half-plane applied in the previous section. If the thickness of the substrate is sufficiently small, pursuant to [27], p.46, the deformation may be considered to be uniform over the thickness of the substrate. If we again choose the lower surface of the elastic substrate to be the $x_1x_2$-plane, such that the substrate is in $x_3 > 0$, the strain tensor consequently only depends on $x_1$ and $x_2$.

- Secondly, we again assume the occurring displacements to be small such that the theory of linear elasticity (q.v. Chapter 2.1.5) can be applied. In a similar manner as before we call the displacements of points in the plate small, if they are small in comparison with the thickness of the substrate.

- Thirdly, we only consider longitudinal deformations occurring in the plane of the plate (see [27], p.46). In the majority of cases this kind of deformation occurs due to forces applied at the rim of the substrate or alternatively due to body forces acting in its plane. In this situation the boundary condition on both surfaces of the thin plate are assumed to be given by:

$$S_{ij} \cdot n_j = 0 \quad \forall \ x \in \Gamma_1$$

As we have chosen the coordinate system such that the surfaces of the elastic material are perpendicular to the $x_3$-axis, the normal vector at these surfaces is just parallel to this axis and thus the above equation yields:

$$S_{i3} = 0 \quad \text{for } i = 1...3 \quad (3.30)$$

We emphasize that according to [27], p.46, these conditions still hold true in the subsequent considerations, if the forces causing the longitudinal deformation are applied to the surfaces of the plate, because these forces are still small in comparison with the induced longitudinal internal stresses $S_{11}, S_{12}$ and $S_{22}$ in the plate.
3. Mathematical Modeling of Cellular Traction Forces

Taking all these assumptions into account, we have everything at hand to deduce the corresponding equations of equilibrium.

First of all, from the third assumption we infer that $S_{13}, S_{23}$ and $S_{33}$ have to be small and hence can be considered to be approximately zero throughout the elastic material, as these quantities are zero at both surfaces of the thin substrate. Next, we recall Equation (3.13) giving the linearized version of the second Piola-Kirchhoff stress tensor $(S_{\text{lin}})_{ij}$ in terms of Poisson’s ratio $\nu$ and Young’s modulus $\varepsilon$:

$$(S_{\text{lin}})_{ij} = \frac{\varepsilon}{1+\nu} \left( (E_{\text{lin}})_{ij} + \frac{\nu}{1-2\nu} \sum_{k=1}^{3} (E_{\text{lin}})_{kk} \delta_{ij} \right),$$

$$(E_{\text{lin}})_{ij} = \frac{1}{2} (\partial x_i u_j + \partial x_j u_i)$$

Bearing our considerations concerning $S_{13}, S_{23}$ and $S_{33}$ in mind, we get:

$$(S_{\text{lin}})_{13} = \frac{\varepsilon}{1+\nu} ((E_{\text{lin}})_{13}) = 0$$

$\Rightarrow (E_{\text{lin}})_{13} = 0$ (3.31)

$$(S_{\text{lin}})_{23} = \frac{\varepsilon}{1+\nu} ((E_{\text{lin}})_{23}) = 0$$

$\Rightarrow (E_{\text{lin}})_{23} = 0$

$$(S_{\text{lin}})_{33} = \frac{\varepsilon}{(1+\nu)(1-2\nu)} \left( (1-\nu)(E_{\text{lin}})_{33} + \nu((E_{\text{lin}})_{11} + (E_{\text{lin}})_{22}) \right) = 0$$

$\Rightarrow (E_{\text{lin}})_{33} = -\frac{\nu((E_{\text{lin}})_{11} + (E_{\text{lin}})_{22})}{(1-\nu)}$

Now we can use these insights to calculate the corresponding non-zero components of the stress tensor:

$$(S_{\text{lin}})_{11} = \frac{\varepsilon}{(1+\nu)(1-2\nu)} \left( (1-\nu)(E_{\text{lin}})_{11} + \nu((E_{\text{lin}})_{22} - \frac{\nu((E_{\text{lin}})_{11} + (E_{\text{lin}})_{22})}{(1-\nu)}) \right)$$

$= \frac{\varepsilon}{(1-\nu)(1-2\nu)} \left( (1-2\nu + \nu^2 - \nu^2)(E_{\text{lin}})_{11} + (\nu(1-\nu-\nu))(E_{\text{lin}})_{22} \right)$

$= \frac{\varepsilon}{1-\nu^2} ((E_{\text{lin}})_{11} + \nu(E_{\text{lin}})_{22})$

By analogous calculations we get:

$$(S_{\text{lin}})_{22} = \frac{\varepsilon}{1-\nu^2} ((E_{\text{lin}})_{22} + \nu(E_{\text{lin}})_{11})$$

For the sake of completeness we also state:

$$(S_{\text{lin}})_{12} = \frac{\varepsilon}{1+\nu} ((E_{\text{lin}})_{12})$$
3.3. The Plane Stress Approximation

Combining all these equations and taking into account that \((E_{\text{lin}})_{ij} = (E_{\text{lin}})_{ji}\) holds for all \(i, j\), the linearized second Piola-Kirchhoff stress tensor becomes:

\[
S_{\text{lin}}(x) = \begin{pmatrix}
\frac{\varepsilon}{1-\nu^2}((E_{\text{lin}})_{11} + \nu(E_{\text{lin}})_{22}) & \frac{\varepsilon}{1-\nu^2}((E_{\text{lin}})_{12}) & 0 \\
\frac{\varepsilon}{1-\nu^2}((E_{\text{lin}})_{12}) & \frac{\varepsilon}{1-\nu^2}((E_{\text{lin}})_{22} + \nu(E_{\text{lin}})_{11}) & 0 \\
0 & 0 & 0
\end{pmatrix}
\] (3.32)

This way we eliminated the displacement \(u_3\) from the linear stress-strain law. Furthermore we can conclude that \(x_3\) can be neglected.

Therefore we can for a moment consider the elastic substrate to be a two-dimensional medium, i.e. we assume the thickness of the elastic medium to amount to zero and thus we can reduce the strain tensor to be a \(2 \times 2\) matrix depending on points \(x \in \mathbb{R}^2\) and the corresponding displacement as well as the applied force are now two-dimensional quantities of the form \(u := (u_1, u_2)^T\) and \(b := (b_1, b_2)^T\), respectively. In this situation Cauchy’s equation of motion (c.f. (2.14)) yields:

\[- \left( \frac{\partial S_{11}}{\partial x_1} + \frac{\partial S_{12}}{\partial x_2} \right) = b_1
- \left( \frac{\partial S_{21}}{\partial x_1} + \frac{\partial S_{22}}{\partial x_2} \right) = b_2\]

Now we incorporate our first assumption stating that the deformation may be considered to be uniform over the thickness of the substrate, if the elastic material is sufficiently thin. Let the thickness of the substrate again be denoted by \(h\). Then the thickness-averaged equations of equilibrium read:

\[
h \left( \frac{\partial S_{11}}{\partial x_1} + \frac{\partial S_{12}}{\partial x_2} \right) = -b_1
h \left( \frac{\partial S_{21}}{\partial x_1} + \frac{\partial S_{22}}{\partial x_2} \right) = -b_2\]

Inserting the Identity (3.32) in the above equations finally gives the result:

\[
\varepsilon h \left[ \frac{1}{1-\nu^2} \frac{\partial}{\partial x_1} ((E_{\text{lin}})_{11} + \nu(E_{\text{lin}})_{22}) + \frac{1}{1+\nu} \frac{\partial}{\partial x_2} ((E_{\text{lin}})_{12}) \right] = -b_1
\]

\[
\varepsilon h \left[ \frac{1}{1-\nu^2} \frac{\partial^2 u_1}{\partial x_1^2} + \nu \frac{\partial^2 u_2}{\partial x_2^2} + \frac{1}{2(1+\nu)} \frac{\partial^2 u_1}{\partial x_1 \partial x_2} + \frac{1}{2(1+\nu)} \frac{\partial^2 u_2}{\partial x_1 \partial x_2} \right] = -b_1
\]

\[\iff \varepsilon h \left[ \frac{1}{1-\nu^2} \frac{\partial^2 u_1}{\partial x_1^2} + \frac{1}{2(1-\nu)(1+\nu)} \frac{\partial^2 u_2}{\partial x_2^2} + \frac{1}{2(1+\nu)} \frac{\partial^2 u_1}{\partial x_1 \partial x_2} \right] = -b_1\]

And analogously:

\[
\varepsilon h \left[ \frac{1}{1-\nu^2} \frac{\partial^2 u_2}{\partial x_2^2} + \frac{1}{2(1-\nu)} \frac{\partial^2 u_1}{\partial x_1 \partial x_2} + \frac{1}{2(1+\nu)} \frac{\partial^2 u_2}{\partial x_1 \partial x_2} \right] = -b_2
\]
3. Mathematical Modeling of Cellular Traction Forces

Pursuant to [14], p.2009, there exist elegant solutions for the above two-dimensional system of equations, if certain boundary conditions and traction distributions are given. Referring to [41], it is stated that if for example the elastic medium is assumed to be infinite and simple stress boundary conditions are applied, the corresponding Green’s functions for $i, k = 1, 2$ are given by:

$$G_{ik} = \frac{(1 + \nu)^2}{4\pi \varepsilon h} \left[ \frac{(x_i - x_i')(x_k - x_k')}{|x - x'|^2} + \delta_{ik} \frac{(3 - \nu)}{(1 + \nu)} \ln \left( \frac{1}{|x - x'|^2} \right) \right],$$

(3.33)

where $h$ again represents the thickness of the elastic substrate. Hence we introduced a second way to specify the Green’s functions for an approximate calculation of the displacement field $u$ for given applied forces $f$. Note that by averaging over the substrate thickness we transferred the originally three-dimensional forward problem (3.2) into a two-dimensional system. Consequently, whenever the plane stress approximation is applied all occurring quantities are 2D.

At the end of this section we shall again relate the underlying setting of the deduction of this plane stress approximation with our mathematical model for the reconstruction of cellular traction forces.

We regard the extracellular matrix $\bar{\mathbf{E}}$, on which the cell is cultured, to be the elastic material in the form of a thin plate. In our situation, the relevant applied force is surely just the cellular traction. As one major assumption of the plane stress approximation states that the surface force at the boundaries of the elastic substrate equals to zero, it is common usage (c.f. for example [4], p.4 and [14], p.2009) to equate the cellular traction with the applied body force, even if this traction force is applied at the upper surface of the elastic material. Then this yields the following relation of the applied traction force $f$ and the induced displacement field $u$:

$$\varepsilon h \left[ \frac{1}{1 - \nu^2} \frac{\partial^2 u_1}{\partial x_1^2} + \frac{1}{2(1 - \nu)} \frac{\partial^2 u_1}{\partial x_1 \partial x_2} + \frac{1}{2(1 + \nu)} \frac{\partial^2 u_2}{\partial x_2^2} \right] = -f_1$$

$$\varepsilon h \left[ \frac{1}{1 - \nu^2} \frac{\partial^2 u_2}{\partial x_2^2} + \frac{1}{2(1 - \nu)} \frac{\partial^2 u_1}{\partial x_1 \partial x_2} + \frac{1}{2(1 + \nu)} \frac{\partial^2 u_1}{\partial x_1^2} \right] = -f_2$$

Defining

$$\mu' = \frac{h\varepsilon}{2(1 + \nu)} \quad \text{and} \quad \lambda' = \frac{h\nu \varepsilon}{1 - \nu^2},$$

one can validate easily that the above two-dimensional system of equations can be equivalently rewritten as:

$$-\mu' \Delta u - (\mu' + \lambda') \nabla (\nabla \cdot u) = f,$$

(3.34)

which are just the Navier-Lamé equations in 2D for $\mu = \mu'$, $\lambda = \lambda'$ and $b = f$. 

66

Nowadays, the most popular way of calculating cellular traction forces is making use of the so-called Traction Force Microscopy (TFM), which is based on the observation of beads in an elastic substrate under applied traction forces. Therefore, this chapter is devoted to the presentation and discussion of this technique.

We will start our considerations on this topic by tracing early contributions to the reconstruction of cellular traction. These early publications are of special importance as they introduce the idea of regarding the reconstruction task as an inverse problem: instead of directly considering the traction forces exerted by the cell, the displacements induced by cell locomotion in an elastic medium are measured and subsequently the underlying forces are calculated from these observations. In the course of this chapter we will furthermore shortly introduce four state of the art TFM techniques, which are all based on this inverse problem formulation, even if they differ with respect to the solution. Thereby we will assume the Boussinesq solution as well as the plane stress approximation (both introduced in Chapter 3) to be known. For the sake of consistency we will adjust the notations deployed by the authors of the respective approaches to the notations used throughout this thesis.

At the end of this chapter we shall use the gained insights to derive a motivation for a new way of reconstructing cellular traction forces.

4.1. Early Contributions

In 1980, Harris and coworkers published a seminal paper [25], in which they came up with the idea to use thin films of silicone rubber culture substrata for the detection of cellular traction forces. Observing the reactions of this elastic substratum to adhering and locomoting individual cells, they found ways of making traction forces ‘visible’ as these forces distort the substratum and cause it to wrinkle (c.f. [44], p.228). According to the authors themselves, they chose this indirect approach as the traction forces exerted by an individual cell are so weak and exerted over such a small area that conventional techniques fail to detect them (c.f. [25], p.177). Actu-
4. State of the Art Reconstruction of Cellular Traction Forces

ally, A. K. Harris published the idea to use an elastic substratum for the detection of cellular traction forces as early as 1973 (c.f. [24]). But in his former studies Harris cultured cells on clottable plasma. This clottable plasma turned out to carry several disadvantages, among which the susceptibility of protein sheets to biochemical alteration by the cells seems to be of paramount importance. This is because cells can course the plasma to shrink, which leads to misinterpretations with respect to the cellular traction forces (c.f. [25], p.177). Therefore, the authors drew the conclusion that there was the need for an elastic substratum being not only nontoxic, but also as inert as possible to biochemical change. Furthermore, they stated that, for practical reasons, this substratum should permit the application of interference reflection microscopy while showing sufficiently little strain-induced birefringence. As mentioned before, their solution was the development of thin films of silicone rubber culture substrata meeting all these conditions. (c.f. [25], p.177)

To produce these thin films, the scientists conceived the following procedure: After spreading a relatively thick layer of silicone fluid onto a cover-glass, its free surface is crosslinked by a short exposure to heat (c.f. [25], p.177 and [38], p.1380). This results in a thin elastic film of silicone rubber culture substrata over a silicone fluid, having the side effect that the un-crosslinked fluid serves as a lubricant (see [25], p.177).

For a better perception of this approach the subsequent figure depicts one of the results obtained by Harris and coworkers.

![Figure 4.1.](image)

"An individual chick heart fibroblast whose locomotion and contractility have visibly wrinkled the silicone rubber substratum upon which it is crawling (the bar is 50 µm long)". (image taken from [25], p.178)

By culturing cells on this special substrate and letting them spread, Harris and coworkers were able to introduce a reliable method for the detection of traction forces exerted by individual cells such as fibroblasts. Besides, the paper also included a first hint towards the quality of these forces, as the scientists tried to estimate the magnitude of the shear forces by distorting the elastic substrate to a similar degree with the help of calibrated flexible glass microneedles (c.f. [25], p.179). However, this estimate was still very vague and no precise relation between the wrinkling and the corresponding traction forces could be established (c.f. [35], p.225).
4.1. Early Contributions

As the approach of Harris and coworkers failed to detect the forces exerted by rapidly locomoting cells such as leucocytes (c.f. [28], p.1957), Lee and coworkers [28] published a modification of this approach in 1994. They proposed to firmly embed marker beads in a thin elastic but prestressed and thus non-wrinkling film such that the displacements of the beads reflect the cellular traction forces (c.f. [28], p.1957). The following drawing provides an easily accessible intuition of this innovation, that is known under the title elastic substratum method in the literature:

![Figure 4.2](image)

**Figure 4.2.:** Illustration of the elastic substratum method firstly proposed by Lee et al. (adjusted version of a graphic in [30], p.10)

Tracking these beads over time and plotting their displacements leads to an alternative way of depicting the applied traction forces, thereby overcoming the highly nonlinear problem of wrinkleng of the substratum.

In 1995 and 1996, M. Dembo, T. Oliver and coworkers published the two articles [35] and [14], in which they further refined the elastic substratum method as proposed by Lee et al. Firstly, the scientists designed a so-called airbrush device facilitating the uniform dispersion of the beads in the elastic film. In this vein, they realized a density of approximately $3 \times 10^4$ firmly anchored beads per mm$^2$ (c.f. [35], p.226) leading to a more detailed representation of the traction induced displacement field. The result of this alteration can be seen in the following figure:

![Figure 4.3](image)

**Figure 4.3.:** "Phase contrast image demonstrating uniform distribution of latex beads ($3 \times 10^4$ beads/mm$^2$) in traction assay films, created with airbrush device". (image taken from [35], p.228)

Beside this technical innovation the authors demonstrated the elastic behavior of the used films and reasoned from their observations that the bead displacement could be modeled by means of the small strain linear elasticity theory (c.f. [35], p.229).
4. State of the Art Reconstruction of Cellular Traction Forces

On that basis they for the first time established a rigorous framework for an analysis of the relationship between the measured displacements and the underlying traction forces. Applying the plane stress approximation (c.f. Chapter 3.3), they recalled that for given tractions \( f \) the induced displacements \( u \) can be calculated by the thickness-averaged forward problem (c.f. equation (3.33)):

\[
 u_i = \int \int G_{ik}(x_1 - x_1', x_2 - x_2') f_k(x_1', x_2') \, dx_1' dx_2',
\]

where

\[
 G_{ik} = \frac{(1 + \nu)^2}{4\pi \varepsilon h} \left[ \frac{(x_i - x'_i)(x_k - x'_k)}{|x - x'|^2} + \delta_{ik} \frac{(3 - \nu)}{(1 + \nu)} \ln \left( \frac{1}{|x - x'|^2} \right) \right].
\]

Dembo, Oliver and coworkers were the first ones to realize that the previously introduced idea to determine the sought-after traction forces indirectly from the observable measured bead displacements can be expressed by considering the inverse problem corresponding to the above forward problem. For this reason, nowadays the contributions of these authors are of major importance. In principal, the authors suggested to search for the distribution of traction densities placed at discrete locations under the cell, that provided the closest estimate of the recorded 2D bead displacements (c.f. \[35\], p.229). This means, setting

\[
 (Kf)_i := \int \int G_{ik}(x_1 - x_1', x_2 - x_2') f_k(x_1', x_2') \, dx_1' dx_2',
\]

they looked for a traction force

\[
 f^* = \min_f \|Kf - u\|_2^2.
\]

However, as the Green's functions \( G_{ik} \) typically scale as \( \frac{1}{r} \), where \( r = \|x\|_2 \), and have a singularity at the origin, the forward problem is a Fredholm integral equation of the first kind (c.f. \[26\], p.22) with a weakly singular kernel. Consequently, \( K \) is a compact linear operator (c.f. \[26\], p.29) and this problem is inherently ill-posed (c.f. \[26\], p.300).

To cure the ill-posedness, it is common practice to incorporate additional a-priori knowledge about desired properties of the solution via a regularization term \( R(f) \) into the minimization problem to guarantee the existence of a stable solution. Thus, the resulting minimization problem reads:

\[
 f^* = \min_f \{\|Kf - u\|_2^2 + \alpha R(f)\},
\]

where \( \alpha \) is the so-called regularization parameter balancing the effects of both terms. A frequent choice for the side constraint \( R \) is the so-called zero-order Tikhonov regularization, given by \( R(f) = \|f\|_2^2 \) (c.f. \[38\], p.1383). Concerning the reconstruction
4.1. Early Contributions

of cellular traction forces, this is a feasible choice as experimental evidence indicates that the exerted forces are small, which also seems to be reasonable from a biological point of view, if we assume the cell to act as efficient as possible. First- and higher-order Tikhonov regularizations involve derivatives of $f$ leading to smoother force fields.

To solve the inverse problem Dembo, Oliver and coworkers used a Bayesian approach, incorporating the zero- and first-order Tikhonov regularization, respectively, by means of the a-posteriori likelihood. A discussion of the mathematical details of this method lies beyond the scope of this thesis as this paragraph was meant to provide a brief overview of important contributions towards the development of TFM. Hence, we refer the interested reader to [14].

Subsequently, one of the results presented in the article [35], p.235, is given:

![Figure 4.4: Computing cell-substratum tractions from particle displacements in an elastic silicone rubber substratum.](image)

**Figure 4.4.** 'Computing cell-substratum tractions from particle displacements in an elastic silicone rubber substratum. a: Negative phase contrast image of a keratocyte (outlined in white) locomoting toward the right (arrow) on a silicone rubber substratum into which $1 \mu m$ beads (black spheres) have been incorporated. This cell was recorded several minutes of locomotion in a straight line. Bead locations shown here represent stressed condition of film (bead locations for unstressed film are not shown, but were recorded 3 min before the cell’s arrival). b: The bead displacement field that is generated when a cell locomotes across the field of view, with reference to bead positions recorded 3 min before the cell’s arrival. [...] c: The traction density at each of the 113 nodes of the cell mesh was determined so as to minimize the discrepancy between observed (b) and predicted (d) bead displacement. d: Bead displacements predicted by traction density distribution in c. [...] Both traction and displacement vectors have been amplified $5 \times$ for display purposes. Cell velocity is $0.3 \mu m/s$. [...]’

(image taken from [35], p.234)
As a first step towards the solution of the inverse problem introduced before, the displacements of the beads have to be determined. Therefore, the beads are directly tracked over time, which is sometimes called particle tracking velocimetry (PTV) in the literature (c.f. [37], p.207). Furthermore, a translational drift correction is carried out to account for the systematic error introduced by misalignment of the two images and a Gaussian smoothing is applied to reduce the influence of noise in the data (c.f. [14], p.2010). Afterwards, the cell in the recorded data was overlaid by a mesh. Then the traction at each node of the mesh was determined by minimizing the discrepancy between the observed displacement and the one predicted by calculation of the forward problem for a given plane stress Green’s function (c.f. [35], p.229). Basically, this was achieved by the inversion of a large system of linear equations in real space (c.f. [37], p.208). As stated before, zero-order and first-order Tikhonov regularization, respectively, was applied to guarantee a stable solution (c.f. [14], p.2012).

In summary, this technique brought about the first image of already feasible resolution, exhibiting a reliable quantitative map of the exerted cellular traction forces calculated from the displacement measurements, even if it required high computational efforts.

In 1997, R. J. Pelham and Y.-L. Wang further improved the elastic substratum approach for the reconstruction of cellular traction forces introduced so far: They designed a thin polyacrylamide-based, collagen-coated flexible substrate, whose flexibility can be varied while maintaining its chemical characteristics (c.f. [36], p.13661). Their basic idea was to keep the concentration of acrylamide constant while changing the concentration of bis-acrylamide. The authors themselves underlined the significance of this development as they showed that cells have the ability to respond to their surrounding environment. Pelham and Wang observed that cellular adhesion structures as well as the motile behavior significantly vary, if the flexibility of the extracellular matrix is altered (c.f. [36], p.13661). To prevent misinterpretations of the cellular behavior, the flexibility of the substrate should therefore be controlled in a reproducible way. According to the scientists, the polyacrylamide-based collagen-coated flexible substrate additionally has the following advantageous properties (c.f. [36], p.13664): Beside its good optical quality and minimal thickness facilitating the application of fluorescence techniques, the porous nature of the polyacrylamide gel seems to be a more physiological environment for cell culture. Finally, it could be shown that the substrate behaves like a nearly ideal elastic material. Additionally, B. Sabass and coworkers stated, that this innovation also brought about the possibility of thicker non-wrinking elastic substrates (c.f. [37], p.207) being a requirement for the application of the Boussinesq theory (c.f. Section 3.2).
4.1. Early Contributions

As a consequence of all these observations, the polyacrylamide-based, collagen-coated flexible substrate became the predominantly used material in the field of Traction Force Microscopy.

Based on these early contributions numerous different Traction Force Microscopy approaches have been proposed in the last one and a half decades. However, according to J. H.-C. Wang and his coauthors all of these TFM approaches comprise the following three major steps (see [44], p.229 ff.):

1. Fabricate elastic polyacrylamide gel substrate with a flat surface.
2. Obtain a pair of null force and force loaded microscopy images, from which the displacement field can be determined based on the movement of markers on the surface of the polyacrylamide gel substrate.
3. Use the substrate deformation to compute the cellular traction forces.

Pursuant to the same article, widely-used TFM methods have the first step and partly the second step in common, but vary concerning the determination of the displacement field from the image data as well as with respect to the third step (c.f. [44], p.230).

To fabricate the polyacrylamide gel substrate, fluorescent micro-beads are mixed with an acrylamide/bis-acrylamide mixture with a predetermined concentration of both compounds. Next, this mixture is added to a pretreated cover-glass, covered with a second cover-glass and the whole set is turned upside down, such that gravity forces the fluorescent beads to move to the surface of the second cover-glass. After some time the mixture solidifies and the second cover-glass can be removed. Then the fluorescent beads are located at the free surface of the gel disk. After several treatments of the gel disk including the exposure to ultraviolet light and washing, it is finally coated with collagen type I. (c.f. [44], p.230)

For data acquisition individual cells on the fabricated polyacrylamide gel substrate are selected and after calibration of the technical devices, they are recorded by a CCD-camera system on an inverted microscope. The result is a force loaded microscopy image. Afterwards, the cells are removed from the gel disk by trypsinization. The null force microscopy image is provided by taking an image of the same location. These images form the basis for the determination of the substrate displacement field. (c.f. [44], p.230)

The underlying idea of the common TFM approaches is to identify the marker positions in the null force as well as in the force loaded image to obtain the movement of the fluorescent micro-beads reflecting the displacement field of the substrate. The
way these positions are determined differs among the various existing approaches. Finally, the inverse problem of reconstructing the cellular traction forces from the measured bead displacements has to be solved. The biggest discrepancy between the numerous TFM techniques can be found regarding the solution of this inverse problem.

However, at the end of this section, we can conclude that modern Traction Force Microscopy methods are still closely linked to the early contributions presented in this section, where the significance of these developments is especially obvious with respect to data acquisition and to the formulation of the inverse problem.

4.2. Prevailing Traction Force Microscopy Approaches

In the following we will introduce four widely-used approaches for the reconstruction of cellular traction forces, where the cell is located on the surface of an elastic substrate. These assays are all either based on the Boussinesq solution or on the plane stress approximation introduced in Chapter 3. As there exist numerous scientific publications dealing with Traction Force Microscopy, we surely raise no claim to completeness, but rather aim at providing an insight into existing techniques.

We discuss the selected approaches in chronological order based on the date of publication. However, the chosen order might be controversial as the second and the third technique have been developed almost simultaneously.

The Boundary Element Method by Dembo and Wang

The first TFM technique we are going to discuss was published in the article [13] by M. Dembo and Y.-L. Wang in 1999. In the literature, this approach is often called the Boundary Element Method (BEM) (c.f. [30], p.2).

Basically, this technique resembles the earlier approach of Dembo, Oliver and coworkers presented in Section 4.1. However, the authors incorporated the scientific findings of Pelham and Wang [36] into this approach: Firstly, just as proposed by Pelham and Wang, they used a type I collagen-coated polyacrylamide gel as a substrate for the cells, in contrast to the liquid-supported silicone films Dembo, Oliver and coworkers deployed before (c.f. [13], p.2307 and p.2309). The second major difference can be observed with respect to the determination of the Green’s functions in the forward problem (3.2): As already stated before, the development of the type I collagen-coated polyacrylamide gel allowed for the application of thicker substrates. This seems to be advantageous, as in this way it is more likely that the condition
of the typical induced displacements being much smaller than the thickness of the substrate is met. While the earlier approach was based on the plane stress approximation assuming a thin elastic material, the scientists now applied the Boussinesq solution (c.f. [13], p.2309), which seems to be more feasible concerning the increased substrate thickness. Otherwise, using again a Bayesian approach the scientists proceeded in a similar manner as described in Section 4.1.

To get an impression of the quality of the outcome produced by an application of this method, the subsequent figure depicts a selection of results presented by the authors themselves.

![Figure 4.5.](image)

(a) Measured displacements  
(b) Predicted displacements  
(c) Artificial mesh  
(d) Calculated traction forces

**Figure 4.5.:** A selection of results presented by Dembo and Wang [13]

(a) "Displacement vectors (three times actual size). These start from the position of marker beads in the absence of the cell and point toward the corresponding position in the presence of the cell. (b) "The theoretical marker displacements computed by back substitution of the best fit tractions into’ the forward problem under application of the Boussinesq solution. (c) 'A mesh is generated to pave the projected area of the cell with quadrilaterals.’ (d) 'Traction vectors that maximize Bayesian likelihood are computed and an image is rendered by drawing a vector (whose length is proportional to the traction magnitude) in the center of each mesh quadrilateral.” In this rendering, tractions are regarded as ‘significant’ only if the vector magnitude is larger than its standard deviation (as computed by the bootstrap method).’ (see [13], p.2309 f.) (images taken from [13], p.2309 f.)
4. State of the Art Reconstruction of Cellular Traction Forces

The Fourier Transform Traction Cytometry by Butler et al.

We continue with the presentation of the so-called Fourier Transform Traction Cytometry (FTTC), which was conceived by J. P. Butler and coworkers in 2002 (c.f. [8]).

The first difference in comparison to the previously discussed Boundary Element Method can be observed with respect to the determination of the displacement field from the given image data: While Dembo and Wang tracked the beads directly, Butler and coworkers suggested to determine these displacements statistically by means of cross-correlation functions (c.f. [8], p.C598). In the literature, the term particle image velocimetry (PIV) can sometimes be found for this approach (c.f. [37], p.207). However, according to the authors themselves, this was not the main objective of their paper (c.f. [8], p.C602). Instead, they aimed at providing a simpler framework for the solution of the inverse problem than the BEM by Dembo and Wang, thereby reducing the computational costs.

Using the forward problem (3.2) in terms of the Green’s functions as given by the Boussinesq solution as a starting point, the novel idea of this approach is to transfer the forward problem introduced in terms of the integral equation (3.21) to the Fourier space. As this integral equation is just another notation for the convolution of the functions \( G_i \) and \( f \), Butler and coworkers suggested to apply the well-known convolution theorem. This theorem states that the Fourier transform of a convolution is just the product of the Fourier transforms of the convolved functions, i.e.:

\[
\text{FT}_2(G * f) = \text{FT}_2(G) \cdot \text{FT}_2(f),
\]

where \( \text{FT}_2 \) is the two-dimensional Fourier transform. Consequently, the adjusted forward problem reads:

\[
\text{FT}_2(u) = \text{FT}_2(G) \cdot \text{FT}_2(f)
\]

The authors claimed that in the FTTC approach noise only occurs during the process of the determination of the displacement field from the image data (c.f. [8], p.C602). They declared that regarding the calculation of the traction fields there was no need for any regularization as \( \text{FT}_2(G) \) was strictly diagonal in the Fourier space (see [8], p.C596). Consequently, they reasoned that \( \text{FT}_2(G) \) could be inverted easily and they presented the following solution to the inverse problem of calculating the traction forces:

\[1\] Butler and coworkers stated that they ignored displacements in the vertical direction and took the normal stress to equal to zero (see [8], p.C596).
applied traction forces from given measured displacements (c.f. \cite{8}, p.C596):

\[
FT_2(f^*) = FT_2^{-1}(((FT_2(G))^{-1}FT_2(u)),
\]

(4.3)

where $FT_2^{-1}$ denotes the two-dimensional inverse Fourier transform.\textsuperscript{2}

Based on these considerations the authors presented two versions of the Fourier Transform Traction Cytometry:

The result of the first one, which they called unconstrained FTTC, is just the inverse Fourier transform of $FT_2(f^*)$ as calculated by equation (4.3). This method is unconstrained in the sense that all displacement data of the whole image area without any modifications are used to calculate $f^*$ and no a-priori knowledge about the location of the cell is taken into account. Butler and coworkers pointed out that this method gives a traction map such that the corresponding displacements completely resemble the measured ones (c.f. \cite{8}, p.C595).

We have seen that the previously presented BEM approach by Dembo and Wang, which is based on the integral equation (3.21), automatically fulfills the biomechanical constraint of the reconstructed non-zero traction forces being located under the cell. By contrast the unconstrained FTTC results in a traction image, which might include positive tractions exterior to the cell’s boundary (c.f. Figure 4.6 on p.78 and \cite{30}, p.76).

Therefore, the authors proposed a second so-called constrained version that demands the a-priori knowledge of the cell’s boundary. This constrained FTTC is an iterative approach: Firstly, the unconstrained FTTC is carried out. Next, the traction image is redefined by setting the tractions outside the known boundary of the cell to zero. Afterwards, the displacements corresponding to this redefined traction field is computed by applying the inverse Fourier transform to Equation (4.2). Within the cell boundary the primarily measured displacement field is now replaced by these calculated displacements and thus the experimental displacements are modified (c.f. also \cite{30}, p.16). The scientists proposed to repeat these steps (with the exception of the first step) until convergence is attained. In contrast to the unconstrained version of the FTTC, this procedure provides a result, in which the tractions are restricted to the area where the cell is located (c.f. Figure 4.6 (c) on p.78). However, no convergence analysis was provided in this article and according to R. Michel and coworkers this unconstrained version is thus used less frequently (c.f. \cite{30}, p.3).

Several years later B. Sabass and coworkers however showed that in the presence of noise, e.g. due to elastic inhomogeneities or due to the optical setup of observation and recording (c.f. \cite{37}, p.208), the introduction of a side constraint such as a Wiener filter or zero-order Tikhonov regularization can remarkably improve the FTTC results (c.f. \cite{37}, p.214). Furthermore, it was shown in the same paper, that when equipped with a suitable regularization the FTTC yields results comparable to the BEM, while only demanding a fraction of the run-time (\cite{37}, p.217).
As before, we complete the introduction of the method with a selection of the results presented by the authors themselves:

![Displacement field](image1)

(a) Displacement field

![Traction field computed with the unconstrained FTTC](image2)

(b) Traction field computed with the unconstrained FTTC

![Traction field computed with the constrained FTTC](image3)

(c) Traction field computed with the constrained FTTC

Figure 4.6.: A selection of results presented by Butler et al. [8]

(a) The displacement field. "Arrows show the relative magnitude and direction of the displacement field of the gel under the adherent smooth muscle cell. Colors show the absolute magnitude of the displacements in µm (see color bar)." (b) The traction field computed from the displacement field in (a) with the use of unconstrained FTTC. [...] Also shown is the boundary of the cell, although it is important to note that this information was not used in computing the tractions. Arrows show the relative magnitude and direction of the tractions. Colors show the absolute magnitude of the traction vectors in Pa." (c) The traction field computed from the displacement field in (a) with the use of constrained FTTC." (images taken from [8], p.C600 f.)

The third TFM approach we address in this section is the so-called Traction Recovery with Point Forces (TRPF) (c.f. [37], p.208). It is based on the two articles [3] and [38] by N. Q. Balaban, U. S. Schwarz and coworkers, published in 2001 and 2002, respectively.

The key achievement of this method is the incorporation of biological findings stat-
4.2. Prevailing Traction Force Microscopy Approaches

ing that the principal type of cell-matrix adhesion are so-called focal adhesion sites (c.f. \[38\], p.1380).

There exist several different microscopy techniques, which allow for the detection of these focal adhesions: For example, interference reflection microscopy provides their positions as dark areas in the resulting images (c.f. \[38\], p.1380). Alternatively, a fluorescence microscope can be used (q.v. Figure 4.7), exploiting the fact that these focal adhesion sites carry the protein vinculin, which can be marked by green fluorescent protein also known as GFP (c.f. \[38\], p.1381). Howsoever the positions of these focal adhesions are detected, the major assumption of Balaban, Schwarz and coauthors is that the cell applies significant traction forces solely via these small areas, as the name of their method already indicates.

Like the approaches that we already discussed, this technique is based on the elastic substratum method. Therefore, the first step towards a reconstruction of the cellular traction forces is the determination of the location of the beads in the recorded images. Here, additionally, the positions of the focal adhesion sites have to be detected. To solve both of these tasks, Balaban, Schwarz and coworkers applied the so-called 'water algorithm' as published by E. Zamir et al. \[48\]. Zamir and coauthors described the idea of this algorithm by considering water that recedes from a hilly landscape after a flood. As the water stage falls, more and more peaks (local maxima) appear out of the water and already existing hills increase in size. Once two previously separated hills extend their area such that they meet, the decisive factor whether to merge them or not shall be their size. (c.f. \[48\], p.1657). Thus, \(N\) vectors representing the displacements of the beads at locations \(x_i\) and \(M\) focal adhesions at locations \(x'_i\) can be determined from the available image data. Typically, \(N\) is in the order of 1000, while \(M\) is in the order of 100. (c.f. \[3\], p.471).

Next, the inverse problem of reconstructing the cellular traction forces from these measured displacements has to be addressed. Thereto, the scientists argued that their experimental setting similar to the BEM or the FTTC allowed for an application of the Boussinesq theory as presented in Chapter 3.2 (c.f. \[3\], p.471). It is then assumed that the cellular tractions over the area of each focal adhesion can be regarded as a point force \(f(x')\) as long as no displacements occurring closer to a focal adhesion than its lateral extension are included into the reconstruction of the forces applied at this adhesion site (c.f. \[38\], p.1382). Now taking into account the assumption of the cellular traction being exerted only at the focal adhesions, i.e. only at a discrete number of locations, the reconstruction problem becomes:

\[
U = GF,
\]

where \(U = (u_1(x_1), u_2(x_1), u_1(x_2), u_2(x_2), ..., u_1(x_N), u_2(x_N))\) is a \(2N\)-vector,
4. State of the Art Reconstruction of Cellular Traction Forces

\[ F = (f_1(x'_1), f_2(x'_1), f_1(x'_2), f_2(x'_2), ..., f_1(x'_M), f_2(x'_M)) \] is a \(2M\)-vector and \(G\) is a matrix given by (c.f. [38], p.1382):

\[
G = \begin{pmatrix}
G_{11}(x_1 - x'_1) & G_{12}(x_1 - x'_1) & G_{11}(x_1 - x'_2) & G_{12}(x_1 - x'_2) & \cdots \\
G_{21}(x_1 - x'_1) & G_{22}(x_1 - x'_1) & G_{21}(x_1 - x'_2) & G_{22}(x_1 - x'_2) & \cdots \\
G_{11}(x_2 - x'_1) & G_{12}(x_2 - x'_1) & G_{11}(x_2 - x'_2) & G_{12}(x_2 - x'_2) & \cdots \\
G_{21}(x_2 - x'_1) & G_{22}(x_2 - x'_1) & G_{21}(x_2 - x'_2) & G_{22}(x_2 - x'_2) & \cdots \\
\vdots & \vdots & \vdots & \vdots & \ddots
\end{pmatrix}
\]

Then the authors suggested to reconstruct the cellular traction forces exerted at the focal adhesions by means of the following minimization problem (c.f. [3], p.471):

\[
F^* = \min_{F} \{ \|GF - U\|^2 + \alpha \|F\|^2 \},
\]

i.e. just like Dembo and Wang they applied a zero-order Tikhonov regularization to guarantee a stable solution. Furthermore, assuming the noise arising during the determination of the displacement field to follow a \(\mathcal{N}(\mu, \sigma)\)-distribution, they claimed that \(\alpha\) should be chosen such that \(\|GF - U\|^2\) attains the value \(2(N - M)\sigma^2\) (c.f. [3], p.471).

Here, too, we conclude the presentation of this approach with a selection of results:

\[\text{Figure 4.7.: A selection of results presented by Schwarz et al. [38]}\]

(a) Fluorescence image of the cell, which is transfected with GFP-vinculin. Vinculin is a major component of focal adhesions and its localization is used to identify the regions, which correspond to large forces. (b) Force reconstruction for \(\alpha = 0.01\) (too weak). (c) Force reconstruction for \(\alpha = 0.1\). In (b), regularization is too weak, and the force pattern is erratic. In (c), there is an unexplained drift in the lower part of the force pattern, but the overall force pattern is reasonable. Ellipses are fits to the focal adhesions as marked by GFP vinculin. (images taken from [38], p.1390).
4.2. Prevailing Traction Force Microscopy Approaches

As we can see, the emphasis is put on the assumption of the cellular traction being applied only at the positions of the focal adhesions. Furthermore, the previous images illustrate that a proper choice of the regularization parameter $\alpha$ is crucial to the quality of the reconstruction.

According to B. Sabass and coworkers, this is a computationally cheap and reliable method that however requires precise knowledge of the exact locations of the focal adhesion sites (c.f. [37], p.217 f.).

The Adjoint Method by D. Ambrosi et al.

At last, we provide some insights into the so-called Adjoint Method (AM) first conceived by D. Ambrosi [1] in 2006 and further discussed by D. Ambrosi and coworkers [2] in 2009.

In contrast to the previously described prevailing TFM approaches, which are all based on the Boussinesq solution, D. Ambrosi decided to apply the plane stress approximation as presented in Chapter 3.3 (c.f. [1], p.2054). Besides, according to Ambrosi and coauthors themselves, the major difference of their approach to already existing techniques can be phrased in terms of the order of solving the Navier-Lamé equations and minimizing the distance between measured and computationally predicted displacements: While in the three previously presented methods the equations of linear elasticity are solved before the difference of the computed displacements to the measured ones is minimized, they do it just the other way round (c.f. [1], p.2052).

However, we continue with a step-by-step presentation of the main ideas of this method: At first, we shall introduce the following notations: Let $u(x)$ denote the displacement vector field in $x_1$ and $x_2$ direction, $x \in \mathbb{R}^2$. Furthermore, we now have to introduce the notation $S_0$ for the subset of $S$ where the displacement field is known by measurement. Then the function $u_0(x)$ with support in $S_0$ provides the measured values of the two-dimensional beads displacements at points $x \in S_0$. Finally, we recall that the area where the cell is located and the tractions apply is denoted by $S_c \subset S$ (c.f. [1], p. 2052). The following figure provides an easily accessible intuition of these notations:

\[ \text{Figure 4.8.:} \text{ Notations used in the current paragraph (image taken from [2], p.169)} \]
4. State of the Art Reconstruction of Cellular Traction Forces

As already stated, this method is based on the plane stress approximation (c.f. [2], p.168), i.e. in accordance with Equation (3.34), the applied traction force \( f \) and the induced displacement field \( u \) are related by 

\[-\mu' \Delta u - (\mu' + \lambda') \nabla (\nabla \cdot u) = f.\]

In this context, Ambrosi introduced the following cost functional (c.f. [1], p.2053):

\[
J(f) = \int_{\Omega} \| u - u_0 \|^2_{L^2(\Omega)} dx + \alpha \int_{\Omega} \| f \|^2_{L^2(\Omega)} dx,
\]

where again the zero-order Tikhonov regularization is used as a penalty term.

By defining the projector (c.f. [1], p.2053):

\[\mathbb{P} u = \chi_0 u,\]

where \( \chi_0 \) is the indicator function on the set \( \Omega_0 \), the above Equation (4.4) can be rewritten as:

\[
J(f) = \int_{\Omega} \| \mathbb{P} u - u_0 \|^2_{L^2(\Omega)} dx + \alpha \int_{\Omega} \| f \|^2_{L^2(\Omega)} dx
\]

Next, Ambrosi suggested (c.f. [1], p.2053) to reconstruct the cellular traction force field \( f \) from the measured displacements \( u_0 \) by solving the following constrained minimization problem, where \( M_c \) is a convex and closed subset of \( L^2(\Omega) \):

\[
J(f) = \int_{\Omega} \| \mathbb{P} u - u_0 \|^2_{L^2(\Omega)} dx + \alpha \int_{\Omega} \| f \|^2_{L^2(\Omega)} dx \rightarrow \min_{f \in M_c \subset L^2(\Omega), supp(f) = \Omega_c} \quad (4.5)
\]

subject to (c.f. [1], p.2052)

\[Au := -\mu' \Delta u - (\mu' + \lambda') \nabla (\nabla \cdot u) = f \quad (4.6)\]

Recalling the forward problem we can justify to write \( u = u(f) \). Besides, we know that for any minimum \( f^* \) of the above functional the Gâteaux derivative \( dJ(f^*) \) of \( J \) necessarily has to equal to zero for all appropriate test functions \( v \). Thus the following optimality condition can be derived:

\[
\begin{align*}
    dJ(f^*, v) &= \lim_{\tau \to 0} \frac{J(f^*+\tau v) - J(f^*)}{\tau} = \left. \frac{d}{d\tau} J(f^*+\tau v) \right|_{\tau=0} = 0 \\
\iff \frac{d}{d\tau} \int_{\Omega} \langle \mathbb{P} u(f^*+\tau v) - u_0, \mathbb{P} u(f^*+\tau v) - u_0 \rangle + \alpha \langle f^*+\tau v, f^*+\tau v \rangle \big|_{\tau=0} dx = 0 \\
\iff \int_{\Omega} 2 \langle \mathbb{P} u(f^*+\tau v) - u_0, \mathbb{P} u'(f^*+\tau v)v \rangle + 2\alpha \langle f^*+\tau v, v \rangle \big|_{\tau=0} dx = 0 \\
\iff \int_{\Omega} \langle \mathbb{P} u(f^*+\tau v) - u_0, u'(f^*+\tau v)v + \alpha (f^*+\tau v, v) \big|_{\tau=0} dx = 0 \\
\iff \int_{\Omega} \langle \mathbb{P} u(f^*) - u_0, u'(f^*)v \rangle + \alpha \langle f^*, v \rangle \big|_{\tau=0} dx = 0 \\
\iff \int_{\Omega} \langle \mathbb{P} u(f^*) - u_0, \mathbb{P} u(f^*) - u_0 \rangle + \alpha \langle f^*, v \rangle \big|_{\tau=0} dx = 0,
\end{align*}
\]

where in the last step we used the fact that \( u \) and \( f^* \) depend linearly on each other.
4.2. Prevailing Traction Force Microscopy Approaches

Because this optimality condition holds true for all appropriate test functions \( v \) and \( f, f^* \in M_c \), we can choose \( v \) to equal to \( f - f^* \) leading to the equation (c.f. [1], p.2053):

\[
\int_S \langle \mathbb{P}(f^*) - u_0, u(f) - u(f^*) \rangle \, dx + \alpha \int_S \langle f^*, f - f^* \rangle \, dx = 0 \quad \forall \, f \in M_c \tag{4.7}
\]

In this situation Ambrosi introduced the following adjoint problem (c.f. [1], p.2053):

\[
A^* q = \mathbb{P} u - u_0, \quad q|_{\partial S} = 0 \tag{4.8}
\]

Now this adjoint problem can be inserted into Equation (4.7), yielding (c.f. [1], p.2053):

\[
\int_S A^* q(u(f) - u(f^*)) dx + \alpha \int_S f^*(f - f^*) dx = 0
\]

Taking into account that here \( A \) is self-adjoint, this equation becomes:

\[
\int_S q(Au(f) - Au(f^*)) dx + \alpha \int_S f^*(f - f^*) dx = 0 \quad \iff \quad Au = f^* = -\frac{\chi_c}{\alpha} q, \quad u|_{\partial S} = 0 \tag{4.9}
\]

where \( \chi_c \) is the indicator function on the set \( S_c \) and in the last step we applied the assumption that \( \text{supp}(f^*) = S_c \).

Having found a criterion for a minimum \( f^* \) of the cost functional \( J \), we insert this result into the side constraint (4.6). Then the direct and the adjoint problem lead to the following system of equations (c.f. [1], p.2054):

\[
Au = f^* = -\frac{\chi_c}{\alpha} q, \quad u|_{\partial S} = 0 \quad \iff \quad A^* q = \mathbb{P} u - u_0 = \chi_0 u - u_0, \quad \{\hat{h} a t q|_{\partial S} = 0 \tag{4.10}
\]

Pursuant to Ambrosi, the two equations of the above system are now discretized by a finite element method using linear basis functions on an unstructured mesh (c.f. [1], p.2054). Finally, the resulting linear systems are solved by a global conjugate gradient method (c.f. [1], p.2054).
4. State of the Art Reconstruction of Cellular Traction Forces

As before, we shall complete this paragraph by providing a selection of results gained with this adjoint method:

(a) Experimental displacements of the beads
(b) Computed displacement of the gel layer
(c) Computed force field

Figure 4.9.: A selection of results presented by Ambrosi [1].
(a) Experimental displacement of the beads merged in the upper layer of the gel [...]. The computational mesh is represented in grey. The mesh satisfies two constraints: it has a node in every point where displacement has been measured and a sequence of element sides coincides with the boundary of the cell. The reference vector at the bottom left corner is 6 microns long.
(b) Computed displacement of the gel layer. The reference vector at the bottom left corner is 6 microns long.
(c) Computed force field exerted by the fibroblast on the gel layer. The magnitude of the reference vector at the bottom left corner corresponds to $10^3$ Pico Newton.
(images taken from [1], p.2055 f. and p.2058)
4.3. Motivation for a Novel Reconstruction Approach

In the previous sections of the current chapter we gained some insights into the development of the reconstruction of cellular traction and presented four prevailing TFM approaches, which all provide results of feasible quality and resolution. Nevertheless, in the introductory chapter of this thesis, it has been announced that we aim at introducing a novel image registration approach for the reconstruction of these forces. Hence, one may now ask why one should think of such a new method.

Concerning existing TFM approaches, J. H.-C. Wang and coworkers stated that there is room for future improvement for example with respect to efficiency. Furthermore, pursuant to these scientists, a higher level of automation was desirable. (c.f. [44], p. 234)

These claims can be regarded as a starting point for our considerations: As we have seen on the previous pages, modern methods for the reconstruction of cellular traction forces are in the vast majority of cases based on the observation of beads being firmly embedded in the underlying elastic substrate. At the end of Section 4.1 we described the production of such substrates including the uniform dispersion of such beads. To our minds, this process seems to be laborious as well as time- and resource-consuming. Therefore, we draw the conclusion that it might be worthwhile to think of an alternative way to get a reliable estimate of the displacement of the elastic substrate due to traction forces exerted by a cell.

Based on this idea, we developed a novel method for the reconstruction of cellular traction forces from standard phase-contrast microscopy images displaying cells on usual collagen I-coated elastic polyacrylamide gel substrates.
5. An Image Registration Approach for the Reconstruction of Cellular Traction

Motivated by the goal of overcoming the need for beads being firmly embedded in the elastic polyacrylamide gel substrate to calculate the traction forces exerted by adhering cells and instead facilitating the utilization of standard phase-contrast microscopy images, we present our novel reconstruction approach in this chapter. Hence, we start by briefly introducing the basic ideas of the new method. We continue with a presentation of two versions of the resulting image registration problem, thereby assuming the reader to be familiar with the mathematical fundamentals of the theory of elasticity and of image registration, which we provided in Chapter 2 as well as with the notations introduced in Chapter 3.1. Finally, we deal with the existence of solutions for both versions of the optimization problem.

5.1. The Idea in a Nutshell

In a nutshell, one can describe the basic idea of our novel approach as follows: Consider a cell adhering to an elastic, typically collagen I-coated polyacrylamide gel substrate. We emphasize here that no beads are embedded in any layer of this substrate. Suppose that this setting is observed from above with a phase-contrast microscope over a series of time and the result is recorded, for example with a CCD-camera system. The main idea of our new method is that the displacement of the elastic polyacrylamide gel substrate due to cellular locomotion should be detectable in the resulting sequence of 2D phase-contrast microscopy images provided that these are of sufficient resolution. Thereby we regard the resolution to be sufficient if it allows for the identification of structural details of the extracellular matrix in the image background. In the following we will show that in this case the displacements caused by the applied cellular traction forces as well as these forces themselves can be reconstructed from this standard phase-contrast microscopy data by means of a specifically constructed image registration algorithm.

Before we continue with a more detailed discussion of observations, which will eventually bring about four constraints that have to be taken into account when constructing the registration problem, we would like to present one example of a data set that is convenient for our purpose. Thereby, we aim at providing a better per-
5. An Image Registration Approach for the Reconstruction of Cellular Traction

ception of what we actually mean, when requiring images of a sufficient resolution. As can be recognized by the characteristic white halo surrounding the cell, these images have been recorded by a phase-contrast microscope with some kind of image device. The resolution is 0.31 µm/pixel and an image was taken every 10 minutes. The whole image sequence actually consists of 37 images of a single moving human melanoma (MV3) cell and we here show the first three frames of the video:

![Figure 5.1](image)

**Figure 5.1.**: Phase-contrast microscopy images of a human melanoma (MV3) cell on a collagen I-coated polyacrylamide gel substrate. The resolution is 0.31µm/pixel. Recorded by Christian Stock, made available by Albrecht Schwab (q.v. [39]) (Institute of Physiology II, University of Münster).

As stated before, these images depict the experimental setting in top view. In this context, we would like to point out that we are aware of the fact that these images thus only involve two-dimensional information about the induced displacements even though we have seen in Chapter 3.1 that traction forces also cause displacements in vertical direction. We will keep this observation in mind and take it into account when constructing the registration problem.

Keeping these images as an example of a typical data set in mind, we continue with the discussion of some observations leading to side conditions characterizing the overall registration problem that we are going to introduce in the subsequent section:

- At first, we would like to emphasize that we are only interested in the displacement field of the underlying substrate, not in the movement of the cell itself as to the best of our knowledge no method exists such that inferences about the applied traction forces can be drawn from the cellular movement. Furthermore, the cellular body in the image foreground blocks our view on that part of the extracellular matrix that is located behind. Therefore, we have to take care that the deformation that results from the image registration of two consecutive images is not adulterated by the cellular movement, but instead provides a reliable estimate of the displacement field of the underlying substrate. To reach this aim, in the respective images the area where the cell (including the white halo) is located is generously replaced by a black mask.
5.1. The Idea in a Nutshell

As the image registration algorithm under some constraints minimizes the distance between a reference and a deformed template image, in this case only the image sections exterior to the union of the black masks in the reference and in the deformed template image should be considered for alignment.

The subsequent figure depicts one of the images of the sequence presented above and the same image including such a black mask:

![Image](image.png)

**Figure 5.2.** Example of a phase-contrast microscopy image in which the cell is covered by a black mask. (For details concerning the numerical implementation see Chapter 6.)

- Of course, this procedure requires the rough segmentation of the cell from the phase-contrast microscopy image. However, the result of this segmentation can be used to integrate a second side constraint into the registration approach: From a biomechanical point of view, it seems to be reasonable that non-zero traction forces only occur at those points of the image where the cell is located, i.e. inside the black area.

- Speaking of the applied cellular traction forces, we claim that these forces and the induced displacement field are related by the linearized displacement-traction problem of three-dimensional elasticity (c.f. Definition 2.29) provided that the extracellular matrix is made of an elastic material that fulfills all the requirements listed in Definition 2.29 and that the displacements are typically sufficiently small such that we can justify to apply linear elasticity models. Hence, at this point the actual three-dimensional nature of the displacement field is taken into account.

- Finally, as we have seen in the previous chapter, former results indicate that the applied cellular traction forces are in the order of a few nano newton. Furthermore, we assume the cell to act as efficient as possible. Thus we claim that the magnitude of the cellular tractions should be expected to be small.

Taking all these considerations into account, we shall now realize the presented ideas mathematically.
5.2. The Mathematical Realization of the Idea: Optimization Framework

As the underlying idea of our novel approach in short can be phrased as reconstructing the applied cellular traction forces from standard phase-contrast microscopy images by means of image registration, this section is dedicated to the introduction of the corresponding image registration problem.

Therefore, we consider two consecutive frames of a sequence of phase-contrast microscopy images and without loss of generality we call the first of these images the template image $T$ and the second one the reference image $R$. Then, using the registration framework published by B. Fischer and J. Modersitzki (c.f. e.g. [18]), which we shortly presented in Chapter 2.2, we will state the image registration task for the calculation of Traction Forces from Phase-Contrast Microscopy (TFPCM) in terms of an optimization problem of the general form:

$$J(f, u) = D(R, Tu) + \alpha S + \beta P \longrightarrow \min_{f \in M_1, \ u \in M_2}$$

subject to $C$,

where $D$ is an appropriate distance measure, $S$ is a smoother, $P$ is a so-called penalty or soft constraint, $C$ denotes some hard constraint and $M_1$ and $M_2$ are sets of admissible force fields and displacement fields, respectively.

We start by constructing an appropriate distance measure: Just as in Chapter 2.2.4 we suppose to use the sum of squared differences (SSD) distance measure (q.v. Equation (2.20)), given by:

$$D_{SSD}(R, Tu) = \frac{1}{2} \left\| Tu - R \right\|_2^2 = \frac{1}{2} \int_{S} |T(x + u(x)) - R(x)|^2 dx$$

as a starting point for the first term of the above optimality problem.

However, we have to adjust the above distance term according to our observations stated in the previous section. First of all, we have to take into account the two-dimensionality of the available data, this is $R, T \in Img(2)$. Consequently, in the distance measure we only consider points of the two-dimensional upper surface of the elastic material, which we denote by $S$ in accordance with the notation introduced in Chapter 3.1. Besides, the displacement $u$ has to be a two-dimensional vector field. Furthermore, according to our first observation, only the image sections exterior to the union of the areas where the cell is located in the reference image and in the deformed template image are considered for alignment in the registration algorithm.

Therefore, we henceforward denote the image section where the cell is located in the reference image by $S_{c, R}$ and the respective section in the template image by $S_{c, T}$. Besides, we define $\chi_{c, R}^-$ to be the characteristic function on the set $S \setminus S_{c, R}$ and $S_{c, T}$.
5.2. The Mathematical Realization of the Idea: Optimization Framework

χ_{c,T} to be the characteristic function on the set Ω \setminus Ω_{c,T}, respectively. Then we introduce the following distance measure:

\begin{align*}
D^{TFPCM}(R, T_u) &:= \frac{1}{2} \int_Ω \chi_{c,R}(x) |T(x + u(x)) - R(x)|^2 \chi_{c,T}(x + u(x)) \, dx & (5.1) \\
&= \frac{1}{2} \int_Ω \chi_{c,R} \cap \chi_{c,T}(I - u) |T(x + u(x)) - R(x)|^2 \, dx, & (5.2)
\end{align*}

where I is the identity matrix.

We continue by introducing a mathematical formulation of our fourth demand stating that the magnitude of the reconstructed cellular traction forces should be small. Here, we have to take the two-dimensionality of the area where the force is applied into account, too. Using the Traction Force Microscopy approaches that we discussed in the previous chapter as an inspiration, we decided to use the zero-order Tikhonov regularization as a smoother, i.e.:

\begin{align*}
S^{TFPCM}(f) &:= \frac{1}{2} \int_Ω |f(x)|^2 \, dx
\end{align*}

Recalling that it is common practice to assume that the cellular traction force normal to the surface of the extracellular matrix can be neglected (c.f. Chapter 3.1 and e.g. [37], p.208), we would like to point out that this smoother returns the same value for \( f = (f_1, f_2, 0) \) than for \( f = (f_1, f_2) \).

To guarantee that the maximal magnitude of the force field provided by this registration problem is in a realistic range we additionally introduce the hard constraint:

\begin{align*}
C^{TFPCM}_1(f) : \|f(x)\|_\infty \leq \gamma, \quad \forall x \in Ω & (5.3)
\end{align*}

where \( \gamma \) is a constant that has to be chosen with respect to the particular application.

At last, we have to incorporate the remaining two observations of the previous section into our optimization framework. As we will see, the two conditions corresponding to these observations can be handled simultaneously. Thereby, we can choose whether we would like to realize them as a hard or as a soft constraint. In this context, we decided to provide two alternative versions of the optimization problem: one with a hard and the other with a soft constraint. However, these two versions are not equivalent as the first one is based on the classic linearized displacement-traction problem of three-dimensional elasticity while the second one uses the plane stress approximation introduced in Chapter 3.3.

Then, in the first case, we incorporate the information about the elastic character of the underlying extracellular matrix by requiring that the three-dimensional displacement field \( u \) and the cellular traction force \( f = (f_1, f_2, 0) \) fulfill the following
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hard constraint

\[ C_2^{TFPCM}(f,u) : \begin{cases} 
-(\lambda + \mu)\nabla(\nabla \cdot u) - \mu \Delta u = b, & x \in \mathcal{E} \\
\lambda \text{tr}\nabla u I + \mu ((\nabla u)^T + \nabla u) \cdot n = (1 - \chi_{c,T}) f, & x \in \mathcal{E}_{us,c} \\
u = 0, & x \in \mathcal{E}_0, 
\end{cases} \]

where the notation is in accordance with the notations introduced in Chapter 3.1.

We would like to point out that by setting the surface force \( s \) to \( (1 - \chi_{c,T}) f \), we also meet the biomechanical condition stating that the non-zero traction forces only occur at points where the cell is located.

In the second case, we decided to apply the plane stress approximation (q.v. Chapter 3.3), yielding the two-dimensional system of equations

\[-(\lambda' + \mu')\nabla(\nabla \cdot u) - \mu' \Delta u = (1 - \chi_{c,T}) f\]

Recalling Theorem 2.36 and Definition 2.37 this condition can be realized by the following soft constraint:

\[ P^{TFPCM}(u,f) : = P_2(u) - \int_{\Gamma_1} (1 - \chi_{c,T}) f u \, dx \]

\[ = \int_{\mathcal{E}} \mu' [(\partial x_1 u_1)^2 + \frac{1}{2} (\partial x_1 u_2 + \partial x_2 u_1)^2 + (\partial x_2 u_2)^2] + \frac{\lambda'}{T} (\partial x_1 u_1 + \partial x_2 u_2)^2 - (1 - \chi_{c,T}) f u \, dx \quad (5.4) \]

Combining all terms introduced in the current section, we finally end up with the following two alternative image registration problems:

1) The **projected constrained TFPM approach**, given by:

\[ \mathcal{J}^{pTFPCM}(f,u) \longrightarrow \min_{f \in L^2(\mathcal{E}_{us}), \, u \in W^{1,2}(\mathcal{E})} \quad \text{s.t.} \quad C_1^{TFPCM}(f), \, C_2^{TFPCM}(f,u) \]

where

\[ \mathcal{J}^{pTFPCM}(f,u) = \frac{1}{2} \int_{\mathcal{E}} \chi_{c,R}(x) |T(x + \mathbb{P}_{\text{funct},j}(u(x))) - R(x)|^2 (x + \mathbb{P}_{\text{funct},j}(u(x))) \, dx \\
+ \alpha \frac{1}{2} \int_{\mathcal{E}} |f(x)|^2 \, dx \]

\[ C_1^{TFPCM}(f) : \|f(x)\|_\infty \leq \gamma, \quad \forall x \in \mathcal{E} \]

\[ C_2^{TFPCM}(f,u) : \begin{cases} 
-(\lambda + \mu)\nabla(\nabla \cdot u) - \mu \Delta u = b, & x \in \mathcal{E} \\
\lambda \text{tr}\nabla u I + \mu ((\nabla u)^T + \nabla u) \cdot n = (1 - \chi_{c,T}) f, & x \in \mathcal{E}_{us,c} \\
u = 0, & x \in \mathcal{E}_0 
\end{cases} \]
5.2. The Mathematical Realization of the Idea: Optimization Framework

Before we continue with the second version of the optimization problem we would like to emphasize that in this first case the cellular traction \( f \) as well as the displacement \( u \) are three-dimensional vector fields. However, we already explained that regarding the distance measure a two-dimensional displacement field is required as the available data are 2D images. Therefore, some kind of projection \( P_{\text{funct},j} \) reducing the dimension of \( u \) has to be applied. Thereby, the index \( j \) indicates that several choices for the definition of this projection are conceivable: For example, assuming that the images only depict the in-plane displacements of the upper surface of the substrate, we can define the projection \( P_{\text{funt},1} : L^2(\mathbb{R}^d, \mathbb{R}^3) \rightarrow L^2(\mathbb{R}^d, \mathbb{R}^2) \) simply cutting the third component of \( u \in L^2(\mathbb{R}^d, \mathbb{R}^3) \) off. Based on the assumption that the out-of-plane displacements have some effect on the resulting images, another possibility might be to define a projection \( P_{\text{funt},2} : L^2(\mathbb{R}^d, \mathbb{R}^3) \rightarrow L^2(\mathbb{R}^d, \mathbb{R}^2) \) that averages the third component of \( u \in L^2(\mathbb{R}^d, \mathbb{R}^3) \) out. As our knowledge of the effect of the out-of-plane displacements of the extracellular matrix on the resulting images was limited, we decided to leave the exact form of the applied projection open. For the sake of readability we will henceforward abbreviate \( P_{\text{funt},j} \) by \( P \). Finally, we would like to remark that this projected version of \( u \) should also only depend on \( x_1 \) and \( x_2 \).

2) The \textbf{projected unconstrained TFPCM approach}, given by:

\[
\mathbf{J}_{\text{puTFPCM}}(f, u) \rightarrow \min_{f \in L^2(\mathcal{S}), u \in W^{1,2}(\mathcal{S})} \text{ s.t. } \mathbf{C}_{1}^{\text{TFPCM}}(f)
\]

where

\[
\mathbf{J}_{\text{puTFPCM}}(f, u) = \frac{1}{2} \int_{\mathcal{S}} \chi_{c,R}(x) |\mathcal{T}(x + u(x)) - \mathcal{R}(x)|^2 \chi_{c,T}(x + u(x)) \, dx + \alpha \frac{1}{2} \int_{\mathcal{S}} |f(x)|^2 \, dx + \beta \left( P_2(u) - \int_{\mathcal{S}} (1 - \chi_{c,T}) \, f \, u \, dx \right)
\]

\[
\mathbf{C}_{1}^{\text{TFPCM}}(f) : ||f(x)||_{\infty} \leq \gamma, \quad \forall \; x \in \mathcal{S},
\]

Just like in the first case, we assumed that the displacements at the artificial sides of the substrate (c.f. Chapter 3.1) equal to zero. Besides, we would like to point out that in this case all quantities are two-dimensional as this second approach is based on the plane stress approximation.

We shall complete the current chapter by analyzing the existence of a solution of these two optimization problems.
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5.3. Existence of a Solution

As it is common practice in the context of the calculus of variations, we will show that both energy functionals introduced in the previous section indeed have a minimizer.

At first, we address this objective with respect to the projected constrained Traction Forces from Phase-Contrast Microscopy approach: We start by considering the hard constraint \( C_{TFPCM} \) relating the applied forces and the resulting displacements via the linearized displacement-traction problem of three-dimensional elasticity. Recalling Chapter 3.1, this relation can be rewritten by means of the forward problem stating that for known applied traction forces \( f((x_1, x_2, 0)^T) \) the resulting displacements \( u((x_1, x_2, x_3)^T) \) can be calculated by the equation:

\[
 u_i = \int \int G_{ik}(x_1, x_1', x_2, x_2', x_3) f_k(x_1', x_2') \, dx_1' dx_2', \quad \text{for } i, k = 1, \ldots, 3
\]

where the \( G_{ik} \) are appropriate Green’s functions.

As already reasoned in Chapters 3 and 4, this forward problem typically is a Fredholm integral equation of the first kind and hence we can justify to write:

\[
 u = Kf, \tag{5.5}
\]

where \( K \) is a compact linear operator.

Keeping this in mind, we continue by considering the energy functional:

\[
 J_{\text{pcTFPCM}}(f, u) = \frac{1}{2} \int_\Omega \chi_c - \mathcal{R}(x) \left| \mathcal{T}(x + \mathbb{P}(u(x))) - \mathcal{R}(x) \right|^2 \chi_c - \mathcal{T}(x + \mathbb{P}(u(x))) \, dx \\
 + \alpha \frac{1}{2} \int_\Omega |f(x)|^2 \, dx
\]

\[
 = \frac{1}{2} \int_\Omega \chi_c - \mathcal{R}(x) \left| \mathcal{T}(x + \mathbb{P}(u(x))) - \mathcal{R}(x) \right|^2 \, dx \\
 + \alpha \frac{1}{2} \int_\Omega |f(x)|^2 \, dx
\]

\[
 = \frac{1}{2} \int_\Omega \chi_c - \mathcal{R}(x) \left| \mathcal{T}(x + \mathbb{P}(Kf(x))) - \mathcal{R}(x) \right|^2 \, dx \\
 + \alpha \frac{1}{2} \int_\Omega |f(x)|^2 \, dx,
\]

where, according to Equation (5.5), we substituted \( u \) by \( Kf \) in the last step.

Hence we transferred the original energy functional \( J_{\text{pcTFPCM}} \) depending on \( f \) and \( u \) into an energy functional \( \tilde{J}_{\text{pcTFPCM}} \) that only depends on \( f \). Note that \( \mathbb{P}K \) is again a compact operator provided that the projection \( \mathbb{P} \) is continuous.

Next, we introduce the generally nonlinear function \( g : L^2(\mathbb{S}, \mathbb{R}^3) \rightarrow L^2(\mathbb{S}, \mathbb{R}^2) \):

\[
 g : g \mapsto \mathcal{T}(x + \mathbb{P}(g(x)))
\]
Then we get:
\[
\tilde{J}_{pcTFPCM}(f) = \frac{1}{2} \int_{\mathbb{R}^3 \setminus \{(x, y) \in \mathbb{R}^3 \mid \dot{\mathbf{r}}(Kf)\}} |g(Kf)|^2 \, dx + \alpha \frac{1}{2} \int_{\mathbb{R}^3} |f^2| \, dx
\]
\[
= \frac{1}{2} \|g(Kf) - \mathcal{R}\|_{L^2(\mathbb{R}^3 \setminus \{(x, y) \in \mathbb{R}^3 \mid \dot{\mathbf{r}}(Kf)\}}^2 + \alpha \frac{1}{2} \|f\|_{L^2(\mathbb{R}^3)}^2
\]

Finally, defining the function \( h : L^2(\mathbb{G}) \rightarrow L^2(\mathbb{G}) \) by:
\[
h : g \mapsto g(Kg),
\]
the above energy functional becomes:
\[
\tilde{J}_{pcTFPCM}(f) = \frac{1}{2} \|h(f) - \mathcal{R}\|_{L^2(\mathbb{R}^3 \setminus \{(x, y) \in \mathbb{R}^3 \mid \dot{\mathbf{r}}(Kf)\}}^2 + \alpha \frac{1}{2} \|f\|_{L^2(\mathbb{G})}^2
\] (5.6)

Thus we can easily realize that the minimization of this energy functional is a non-linear optimization problem in \( f \) with a zero-order Tikhonov regularization. Consequently, we can now show the existence of a solution of the pcTFPCM registration problem by standard arguments as provided for example by H.W. Engl, M. Hanke, and A. Neubauer in [16].

For the sake of readability we abbreviate \( \tilde{J}_{pcTFPCM}(f) \) by writing \( \tilde{J}_\alpha(f) \). Then, considering once again Equation (5.6) we can easily realize that the following observation holds true:
\[
\tilde{J}_\alpha(f) \geq 0 \quad \forall f \in L^2(\mathbb{G}, \mathbb{R}^3)
\]
and thus \( \tilde{J}_\alpha(f) \) is bounded below.

Consequently, there exists a minimizing sequence \((f_n)_n \in L^2(\mathbb{G}, \mathbb{R}^3)\) such that:
\[
\tilde{J}_\alpha(f_n) \longrightarrow \inf_f \tilde{J}_\alpha(f)
\]
and this minimizing sequence is uniformly bounded below, i.e.:
\[
\|f_n\|_{L^2(\mathbb{G})}^2 \leq C,
\]
where \( C \) is an appropriate constant.

Then there exists a subsequence \((f_{n,m})_m \) of \((f_n)_n\) that we henceforward also denote by \((f_n)_n\) such that
\[
f_n \rightharpoonup f^* \in L^2(\mathbb{G}, \mathbb{R}^3)
\]
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Now taking into account the compactness of the operator \( K \), we get:

\[
Kf_n \rightarrow Kf^* \in L^2(\mathcal{S},\mathbb{R}^3)
\]

As already stated, we assumed the projection \( \mathbb{P} \) to be continuous. Besides, we now suppose that the template image \( T \) is Lipschitz continuous. Then we can conclude, that \( g \) is a continuous function. This yields:

\[
g(Kf_n) \rightarrow g(Kf^*) \in L^2(\mathcal{S})
\]

Therefore, we finally get:

\[
\tilde{J}_\alpha(f^*) = \| h(f^*) - R \|^2_{L^2(\mathcal{S}\setminus(\mathcal{S}_c \cap \mathcal{S}_h(f)))} + \frac{\alpha}{2} \| f^* \|^2_{L^2(\mathcal{S})} = \lim_{n \to \infty} \| h(f_n) - R \|^2_{L^2(\mathcal{S}\setminus(\mathcal{S}_c \cap \mathcal{S}_h(f)))} + \frac{\alpha}{2} \| f_n \|^2_{L^2(\mathcal{S})} = \inf f \tilde{J}_\alpha(f)
\]

Hence we have shown that there indeed exists a solution of the problem:

\[
\tilde{J}_\alpha(f) = \frac{1}{2} \| h(f) - R \|^2_{L^2(\mathcal{S}\setminus(\mathcal{S}_c \cap \mathcal{S}_h(f)))} + \frac{\alpha}{2} \| f \|^2_{L^2(\mathcal{S})} \rightarrow \min_{f \in L^2(\mathcal{S},\mathbb{R}^3)}
\]

and thus there also exists a solution of the projected constrained Traction Forces from Phase-Contrast Microscopy approach.

Next, we consider the projected unconstrained Traction Forces from Phase-Contrast Microscopy approach: To prove the existence of a solution of this second image registration problem, the books 'Introduction to the Calculus of Variations' \[11\] and 'Direct Methods of the Calculus of Variations' \[12\] both written by B. Dacorogna served as our key source. However, as a first step we transfer the original minimization problem with respect to \( f \) and \( u \), given by:

\[
J^{puTFPCM}(f,u) \rightarrow \min_{f \in L^2(\mathcal{S}), u \in W^{1,2}_0(\mathcal{S})} \text{ s.t. } C_1^{TFPCM}(f)
\]

where

\[
J^{puTFPCM}(f,u) = \frac{1}{2} \int_\mathcal{S} \chi_{\overline{c}R}(x) |T(x + u(x)) - R(x)|^2 \chi_{\overline{c}T}(x + u(x)) dx + \frac{\alpha}{2} \int_\mathcal{S} |f(x)|^2 dx + \beta (P_2(u) - \int_\mathcal{S} (1 - \chi_{\overline{c}T}) f u dx)
\]

\[
C_1^{TFPCM}(f) : \| f(x) \|_\infty \leq \gamma, \quad \forall x \in \mathcal{S},
\]
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into a minimization problem $J_{\text{puTFPCM}}(u)$ that only depends on $u$. Thereto, we recall that any $f^*$ minimizing $J_{\text{puTFPCM}}(f, u)$ necessarily has to fulfill the following condition:

$$\frac{\partial J_{\text{puTFPCM}}}{\partial f}(f^*, u) = \alpha f^* - \beta (1 - \chi_{c,T}) u \overset{!}{=} 0$$

Now taking into account the side constraint $C_1^{\text{TFPCM}}(f)$, this yields:

$$f^* = \begin{cases} 
-\gamma, & u < -\frac{\alpha \gamma}{\beta(1-\chi_{c,T})} \\
\frac{\beta}{\alpha} (1 - \chi_{c,T}) u, & -\frac{\alpha \gamma}{\beta(1-\chi_{c,T})} \leq u \leq \frac{\alpha \gamma}{\beta(1-\chi_{c,T})} \\
\gamma, & \frac{\alpha \gamma}{\beta(1-\chi_{c,T})} \leq u,
\end{cases} \quad (5.7)$$

i.e. for a known displacement field $u$, the traction force field $f^*$ can be calculated by this equation. We will come back to this observation in the course of this thesis.

For now we continue by substituting $f$ in the above energy functional $J_{\text{puTFPCM}}(f, u)$ by Equation (5.7). For the sake of readability we abbreviate $\tilde{J}_{\text{puTFPCM}}(u)$ by writing $\tilde{J}_{\alpha,\beta}(u)$. Furthermore we define $\vartheta := \frac{\alpha \gamma}{\beta(1-\chi_{c,T})}$.

Then we get:

$$\tilde{J}_{\alpha,\beta}(u) = \frac{1}{2} \int_{\Theta} \chi_{c,T}^\| |T(I + u) - \mathcal{R}|^2 \chi_{c,T}^\| (I + u) \, dx$$

$$\quad + \beta \left( \int_{\Theta} \frac{\mu'}{4} \sum_{j,k=1}^2 (\partial x_j u_k + \partial x_k u_j)^2 + \lambda' (\nabla \cdot u)^2 \, dx \right)$$

$$\quad + \begin{cases} 
\int_{\Theta} \frac{\alpha^2}{2} + \beta \gamma (1 - \chi_{c,T}) u \, dx, & u < -\vartheta \\
-\frac{\beta^2}{\alpha} \int_{\Theta} |1 - \chi_{c,T}|^2 |u|^2 \, dx, & -\vartheta \leq u \leq \vartheta \\
\int_{\Theta} \frac{\alpha^2}{2} - \beta \gamma (1 - \chi_{c,T}) u \, dx, & \vartheta < u
\end{cases}$$

In this context we define (c.f. [17], p.443 and p.453):

**Definition 5.1.**

Assume that the functional $J(\cdot)$ is of the form

$$J(w) = \int_{\Theta} L(Dw(x), w(x), x) \, dx,$$

defined for appropriate functions $w : \Theta \rightarrow \mathbb{R}^2$ satisfying: $w = g$ on $\partial \Theta$.

Then $L$ is the so-called Lagrangian corresponding to $J$. 

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In our case the Lagrangian is obviously given by:

\[ \tilde{L}_{\alpha,\beta}(Du(x), u(x), x) = \frac{1}{2} \chi_{c,R} |T(I + u) - R|^2 \chi_{c,T}(I + u) \]

\[ + \beta \left( \frac{\mu'}{4} \sum_{j,k=1}^{2} (\partial x_j u_k + \partial x_k u_j)^2 + \frac{\lambda'}{2} (\nabla \cdot u)^2 \right) \]

\[ + \begin{cases} \frac{\alpha \gamma^2}{2} + \beta \gamma (1 - \chi_{c,T}) u, & u < -\vartheta \\ -\frac{\beta^2}{\alpha} |1 - \chi_{c,T}|^2 |u|^2, & -\vartheta \leq u \leq \vartheta \\ \frac{\alpha \gamma^2}{2} - \beta \gamma (1 - \chi_{c,T}) u, & \vartheta < u \end{cases} \]

Now, according to B. Dacorogna the following theorem holds true (c.f. [11], p.79):

**Theorem 5.2. (Existence of a Minimizer)**

The problem

\[ J(w) = \int_{\mathcal{G}} L(Dw(x), w(x), x) dx \rightarrow \min_w, \]

where

- \( \mathcal{G} \subset \mathbb{R}^2 \) is a bounded open set
- \( L : \mathbb{R}^{2 \times 2} \times \mathbb{R}^2 \times \tilde{\mathcal{G}} \rightarrow \mathbb{R} \)
  \( L = L(\xi, \zeta, x) \)
- \( w \in w_0 + W^{1,2}_0(\mathcal{G}, \mathbb{R}^2) \)

has a solution \( w^* \in w_0 + W^{1,2}(\mathcal{G}, \mathbb{R}^2) \) provided the two following main hypotheses are satisfied

\( (H1) \) Convexity: \( \xi \mapsto L(\xi, \zeta, x) \) is convex for every \( (\zeta, x) \in \mathbb{R}^2 \times \tilde{\mathcal{G}} \);

\( (H2) \) Coercivity: There exist \( p > q \geq 1 \) and \( c_1 > 0, c_2, c_3 \in \mathbb{R} \) such that:

\[ L(\xi, \zeta, x) \geq c_1 |\xi|^p + c_2 |\zeta|^q + c_3 \quad \forall (\xi, \zeta, x) \in \mathbb{R}^{2 \times 2} \times \mathbb{R}^2 \times \tilde{\mathcal{G}} \]

We will not give a proof of this theorem here as this would go beyond the scope of this thesis. Instead we refer to [12].

In the light of the above theorem, the problem of proving the existence of a minimum of \( \tilde{J}_{\alpha,\beta}(u) \) reduces to showing that \( \tilde{L}_{\alpha,\beta}(Du, u, x) \) is coercive and convex with respect to \( Du \).
1) **Convexity:**

To prove the convexity of the Lagrangian with respect to $D\hat{u}$ we have to show that

$$\tilde{L}_{\alpha,\beta}(tD\hat{u} + (1-t)D\hat{u}, u, x) \leq \tilde{L}_{\alpha,\beta}(tD\hat{u}(x), u(x), x) + \tilde{L}_{\alpha,\beta}((1-t)D\hat{u}(x), u(x), x)$$

To show the validity of this inequality we will need the following lemma:

**Lemma 5.3.**

The function $g : \mathbb{R} \rightarrow \mathbb{R}; x \mapsto g(x) = x^2$ is strictly convex in $x$.

**Proof.** The claim follows directly, as $g''(x) = 2 > 0$ is a sufficient condition for convexity. \[\square\]

As a consequence of the above deliberations, we consider:

$$\tilde{L}_{\alpha,\beta}(tD\hat{u} + (1-t)D\hat{u}, u, x)$$

$$= \frac{1}{2} \chi_{c,\mathcal{R}} |\mathcal{T}(I + u) - \mathcal{R}|^2 \chi_{c,\mathcal{T}}(I + u)$$

$$+ \beta \left( \mu' \sum_{j,k=1}^2 \left( \frac{1}{2} \left[ t \left( \frac{\partial u_k}{\partial x_j} + \frac{\partial u_j}{\partial x_k} \right) + (1-t) \left( \frac{\partial \hat{u}_k}{\partial x_j} + \frac{\partial \hat{u}_j}{\partial x_k} \right) \right] \right)^2 \right)$$

$$+ \frac{\beta}{2} \left( \lambda' \sum_{j,k=1}^2 \left[ t \left( \frac{\partial u_1}{\partial x_1} + \frac{\partial u_2}{\partial x_2} \right) + (1-t) \left( \frac{\partial \hat{u}_1}{\partial x_1} + \frac{\partial \hat{u}_2}{\partial x_2} \right) \right]^2 \right)$$

$$+ \left\{ \begin{array}{ll}
\frac{\alpha^2}{2} + \beta \gamma (1 - \chi_{c,\mathcal{T}}) u, & u < - \vartheta \\
-\frac{\beta^2}{\alpha} |1 - \chi_{c,\mathcal{T}}|^2 |u|^2, & - \vartheta \leq u \leq \vartheta \\
\frac{\alpha^2}{2} - \beta \gamma (1 - \chi_{c,\mathcal{T}}) u, & \vartheta < u
\end{array} \right. \right.$$
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Then an application of the above Lemma 5.3 yields:

\[ \tilde{L}_{\alpha,\beta}(tDu + (1-t)D\hat{u}, u, x) \]

\[ < \frac{t}{2} \chi_{\mathcal{R}} |T(I + u) - \mathcal{R}|^2 \chi_{\mathcal{R}}(I + u) + \frac{1-t}{2} \chi_{\mathcal{R}} |T(I + u) - \mathcal{R}|^2 \chi_{\mathcal{R}}(I + u) \]

\[ + \beta \left( \mu' \sum_{j,k=1}^2 \left( t \left[ \frac{1}{2} \left( \frac{\partial u_k}{\partial x_j} + \frac{\partial u_j}{\partial x_k} \right) \right]^2 + (1-t) \left[ \frac{1}{2} \left( \frac{\partial \hat{u}_k}{\partial x_j} + \frac{\partial \hat{u}_j}{\partial x_k} \right) \right]^2 \right) \]

\[ + \frac{\beta}{2} \left( \lambda' \left[ t \left( \frac{\partial u_1}{\partial x_1} + \frac{\partial u_2}{\partial x_2} \right)^2 + (1-t) \left( \frac{\partial \hat{u}_1}{\partial x_1} + \frac{\partial \hat{u}_2}{\partial x_2} \right)^2 \right) \right) \]

\[ + t \left( \begin{array}{l}
\alpha \gamma^2 + \beta \gamma (1 - \chi_{\mathcal{R}}) u, \quad u < - \vartheta \\
- \frac{\beta^2}{\alpha} |1 - \chi_{\mathcal{R}}|^2 |u|^2, \quad - \vartheta \leq u \leq \vartheta \\
\alpha \gamma^2 - \beta \gamma (1 - \chi_{\mathcal{R}}) u, \quad \vartheta < u
\end{array} \right) \]

\[ + (1-t) \left( \begin{array}{l}
\alpha \gamma^2 + \beta \gamma (1 - \chi_{\mathcal{R}}) u, \quad u < - \vartheta \\
- \frac{\beta^2}{\alpha} |1 - \chi_{\mathcal{R}}|^2 |u|^2, \quad - \vartheta \leq u \leq \vartheta \\
\alpha \gamma^2 - \beta \gamma (1 - \chi_{\mathcal{R}}) u, \quad \vartheta < u
\end{array} \right) \]

\[ = \tilde{L}_{\alpha,\beta}(tDu(x), u(x), x) + \tilde{L}_{\alpha,\beta}((1-t)D\hat{u}(x), u(x), x) \]

This yields the validity of the above claim.

2) Coercivity

To show that \( \tilde{L}_{\alpha,\beta}(Du, u, x) \) is indeed also coercive, we need the subsequent well-known theorem (c.f. [10], p.291):

**Theorem 5.4. (Korn’s Inequality)**

**Let** \( \Omega \) **be a domain in** \( \mathbb{R}^d \).

Then, for all \( v \in W^{1,2}(\Omega) \) **there exists a constant** \( \kappa > 0 \) **such that**

\[ \|v\|^2_{W^{1,2}(\Omega,\mathbb{R}^d)} \leq \kappa \int_{\Omega} \sum_{j,k=1}^d |v_j(x)|^2 + \left[ \frac{1}{2} \left( \frac{\partial v_j}{\partial x_k} + \frac{\partial v_k}{\partial x_j} \right) (x) \right]^2 dx \]

The proof of this theorem lies again beyond the scope of this thesis.

We would like to remark that Korn’s Inequality is equivalent to the assertion:

\[ \sum_{j,k=1}^d \left| \frac{\partial v_j}{\partial x_k} (x) \right|^2 \leq \kappa \sum_{j,k=1}^d \left[ \frac{1}{2} \left( \frac{\partial v_j}{\partial x_k} + \frac{\partial v_k}{\partial x_j} \right) (x) \right]^2 \quad (5.8) \]
5.3. Existence of a Solution

Now we have everything at our disposal to show that \( \tilde{L}_{\alpha,\beta}(Du,u,x) \) satisfies the coercivity condition (H2). Thereeto we start by considering the term:

\[
\frac{1}{2} \chi_{c,R} |T(I + u) - R|^2 \chi_{c,T}(I + u) + \frac{1}{2} \chi_{c,R} |T(I + u) - R|^2 \chi_{c,T}(I + u)
\]

Obviously the above term is positive and consequently, the following estimate holds true:

\[
\tilde{L}_{\alpha,\beta}(Du(x), u(x), x) \geq \beta \mu' \sum_{j,k=1}^2 \left| \frac{1}{2} \left( \frac{\partial v_j}{\partial x_k} + \frac{\partial v_k}{\partial x_j} \right) \right|^2 + \frac{\beta \lambda'}{2} (\nabla \cdot u)^2
\]

\[
+ \left\{ \begin{array}{ll}
\frac{\alpha^2}{2} + \beta \gamma (1 - \chi_{c,T}) u, & u < -\vartheta \\
-\frac{\beta^2}{\alpha^2} |1 - \chi_{c,T}|^2 |u|^2, & -\vartheta \leq u \leq \vartheta \\
\frac{\alpha^2}{2} - \beta \gamma (1 - \chi_{c,T}) u, & \vartheta < u
\end{array} \right.
\]

\[
\geq \frac{\beta}{\kappa} \mu' \sum_{j,k=1}^2 \left| \frac{\partial v_j}{\partial x_k} \right|^2
\]

\[
+ \left\{ \begin{array}{ll}
\beta \gamma (1 - \chi_{c,T}) u, & u < -\vartheta \\
-\alpha^2, & -\vartheta \leq u \leq \vartheta \\
-\beta \gamma (1 - \chi_{c,T}) u, & \vartheta < u
\end{array} \right.
\]

\[
\geq \frac{\beta}{\kappa} \mu' \|Du(x)\|_F^2 - \beta \gamma (1 - \chi_{c,T}) |u(x)| - \frac{\alpha^2}{2}
\]

\[
\geq \frac{\beta}{\kappa} \mu' \|Du(x)\|_F^2 - \beta \gamma |u(x)| - \frac{\alpha^2}{2},
\]

where \( \| \cdot \|_F \) denotes the popular Frobenius norm.

Hence we can conclude, that \( \tilde{L}_{\alpha,\beta}(Du,u,x) \) is indeed coercive. Then the existence of a minimum \( u^* \) of the energy functional \( \tilde{J}_{\alpha,\beta}(u) \) follows from Theorem 5.2. Inserting this minimum \( u^* \) into Equation (5.7), we can calculate the traction forces \( f^* \) corresponding to this displacement field.

Thus we have indeed shown the existence of a minimum of both image registration problems introduced in the previous section.
6. Implementation and Results

In the previous chapters we have motivated and introduced a completely new approach for the reconstruction of cellular traction forces from common phase-contrast microscopy images by means of image registration.

At the end of this thesis we shall comment on the numerical realization of this novel method: As already announced in Chapter 5.1, the implementation of the actual image registration approach requires a preprocessing step facilitating the rough segmentation of the cell in the available data provided by C. Stock and A. Schwab, members of the Institute of Physiology II, University of Münster. To solve this segmentation task, we integrated a Chan-Vese type segmentation algorithm (c.f. [9]) for two-dimensional data made available by H. Dirks, member of the Institute of Applied Mathematics, University of Münster, into the "Sabine" framework originally conceived by M. Möller et al. (c.f. [33]). Therefore, the first part of this chapter deals with a brief summary of the main ideas of the corresponding routine. Afterwards, the displacement field of the extracellular matrix as well as the respective cellular traction forces are reconstructed from the available phase-contrast microscopy images by means of our novel puTFPCM approach. For the realization of this optimization problem we used the framework of the "Flexible Algorithms for Image Registration (FAIR)" MATLAB toolbox, which J. Modersitzki published in 2009 (c.f. [32]). In the second part of this chapter we therefore briefly explain the major steps of this implementation. The last part of this chapter finally deals with the presentation and discussion of first numerical results serving as a "proof of principle" of the TFPCM approach developed in the course of this thesis.

6.1. Preprocessing:

Creating the Required Indicator Functions

Recalling our considerations concerning the image alignment in Chapter 5.1 and considering once again the accordingly designed distance measure given by Equation (5.1), it becomes apparent that our image registration approaches not only require a reference and a template image as an input, but also the corresponding indicator functions providing information on the location of the cell in the respective images. The extraction of this information from the available phase-contrast microscopy im-
6. Implementation and Results

ages is regarded as a preprocessing step, which we address in the current section. First we have to state that the segmentation of cells from phase-contrast microscopy data is a challenging task as these images are usually characterized by a low contrast. Furthermore, the grey values of the cell can be above, below or even indistinguishable from the typical values of the image background and finally the cells are surrounded by a bright halo which might be located inside or outside of the cell (c.f. [33], p.397). To overcome these problems M. Möller et al. (c.f. [33]) introduced the 'Sabine' framework especially developed for the segmentation and tracking of cells in phase-contrast microscopy data. This recent semi-automatic tracking method, where the user is only requested to roughly determine the cell position in the first frame of the image sequence, can be described as a two step algorithm (c.f. [33], p.396): It consists of a topology preserving variational rough tracking step using normal velocity information including a volume constraint and of a refinement step, where a geodesic active contour algorithm in the style of (c.f. [47]) based on the gray level image is applied (c.f. [33], p.396). We do not go into mathematical detail here, but instead refer the interested reader to the article [33].

In the light of these observations the "Sabine" framework seemed to be a promising tool for solving our preprocessing problem. However, unfortunately we had to recognize that in our case the rough tracking step of the algorithm provided a too vague segmentation of the cell. On closer inspection, this makes indeed sense as according to [33], p.400, this first step is based on the hypothesis that cells can be identified with regions of high normal velocity, while in contrast the image background is characterized by a low normal velocity. As earlier results indicate, this assumption is widely met in phase-contrast microscopy data at usual resolution, but as we stated in Chapter 5.1 our TFPCM approach is based on the idea that the images are of such high resolution that the displacement of the substrate is visible in the image background. In our special case, the assumption of a low normal velocity of the image background is thus violated as can also be seen in the subsequent figure:

(a) Normal velocity image  
(b) Resulting contour

Figure 6.1.: Result of the rough tracking step of the "Sabine" framework (c.f. [33]) when applied to the first frames of our phase-contrast microscopy data
From the above results we could on the one hand conclude that the displacement of the extracellular matrix can indeed be detected in the phase-contrast microscopy data, but on the other hand we had to recognize that in our special case the consideration of the normal velocity image provided no benefit for the solution of our preprocessing problem. On the contrary, if we had taken this green contour line to be the basis of our indicator functions such that points in the interior of the contour return the value 0 and points exterior to it the value 1, our reconstruction approach would have neglected the principal displacements of the extracellular matrix leading to adulterated traction forces. Consequently, we skipped the rough tracking step of the "Sabine" framework and instead decided to apply a standard segmentation algorithm to the grey value image directly after the rough manual segmentation by the user. We tested the so-called "ChanVese2DImproved" routine by H. Dirks, member of the Institute of Applied Mathematics, University of Münster, which is based on [9] by T. F. Chan and L. A. Vese and part of the so-called "SegMedix Toolbox" [46] as well as the "Generalized gradient vector flow external forces for active contours approach" by C. Xu and J. L. Prince [47], which just corresponds to executing the refinement step of the "Sabine" algorithm. Due to the challenges typical for phase-contrast microscopy data that we already mentioned before, both methods could not provide an exact segmentation of the cell. While the first one tends to form holes within the cellular body, the latter one cuts off very thin parts of the cell. However, reflecting once more about the area that should be neglected in the distance measure, we came to the following conclusion: On the one hand this area should be as small as possible to include as many displacement information as possible, but on the other hand it should not only include the cell itself, but also the bright halo as otherwise this might distort the results. Hence, we reasoned that we do not need an exact segmentation, but that instead a dilated version of the (outer) contour resulting from one of the above segmentation methods might be sufficient.

In this context, we decided to apply the 'ChanVese2DImproved' routine to the available phase-contrast microscopy data. This approach is characterized by a minimization of the functional (see [9], p.268):

\[
F(c_1, c_2, C) = \mu \cdot \text{Length}(C) + \nu \cdot \text{Area}(\text{inside}(C)) \\
+ \lambda_1 \int_{\text{inside}(C)} |u_0(x, y) - c_1|^2 \, dx \, dy \\
+ \lambda_2 \int_{\text{outside}(C)} |u_0(x, y) - c_2|^2 \, dx \, dy,
\]

where \(C\) denotes the evolving curve in a domain labeled by \(\Omega\) being the boundary of an open set \(\omega \subset \Omega\), i.e. \(C = \partial \omega\). Besides, \(\text{inside}(C)\) is just the region \(\omega\) and hence \(\text{outside}(C)\) is the region \(\Omega \setminus \bar{\omega}\). Finally, \(u_0\) denotes the image and \(c_1\) and \(c_2\) are the averages of the regions \(\text{inside}(C)\) and \(\text{outside}(C)\), respectively (c.f. [9], p.267).

Afterwards, we dilated the (outer) contour by a disk of a radius of 8 pixel.
6. Implementation and Results

The result is depicted in the subsequent figure:

![Figure 6.2.](image)

**Figure 6.2.:** Left: Example of a result obtained by applying the 'ChanVese2Dimproved.m' routine subsequently dilated by a disk of radius 8. Right: The corresponding indicator function used for the further calculations.

We decided to use the images resulting from this procedure for our further calculations. Nevertheless, there seems to be some space for improvements regarding the determination of the indicator functions required for our novel TFPCM approach. Besides, it should be analyzed whether the size of the radius of the disk used for the dilation of the originally obtained contour has a significant influence on the reconstructed traction forces. However, this investigation goes beyond the scope of this thesis, but might be another area of future research.

To facilitate an easy implementation of this preprocessing step we created a so-called 'Preprocessing' MATLAB® GUI that can be found on the DVD attached to this thesis. Basically, this GUI is an adapted version of the 'Sabine' program by M. Möller, where we integrated the 'ChanVese2Dimproved.m' routine as a second possible segmentation refinement step. Furthermore, we designed the GUI such that the rough segmentation step can be skipped just as explained. We conclude this section with a screenshot displaying this GUI:

![Figure 6.3.](image)

**Figure 6.3.:** Preprocessing GUI
6.2. Numerical Realization of the Optimization Problem: Reconstructing the Cellular Traction Forces

As already announced, this section is dedicated to the implementation of our novel TFPCM approach motivated and introduced in the previous two chapters. However, in this thesis we rather focused on the discussion of the mathematical backgrounds and the development of a completely new technique for the reconstruction of cellular traction forces from standard phase-contrast microscopy images on the basis of a sound mathematical model than on the numerical realization. For this reason, we will only provide one implementation, serving as a "proof of principle" of either of the two alternative optimization problems, which we introduced at the end of Section 5.2.

As mentioned at the beginning of that section our construction of the optimization problems is guided by the general registration framework provided by B. Fischer and J. Modersitzki (c.f. [18]). Therefore, the application of the "Flexible Algorithms for Image Registration (FAIR)" MATLAB toolbox, which was published by J. Modersitzki in 2009 and is especially designed for image registration problems based on variational modeling, seems to be the logical consequence.

According to J. Modersitzki himself (c.f. [32], p.2 f.), the characteristic feature of the FAIR toolbox is its modular structure: Based on the first-discretize-then-optimize approach (c.f. Definition 2.38) and on variational modeling this does not only enable a flexible combination of different distance measures, smoothers and constraints, thus making the toolbox feasible for many different applications, but also allows for the incorporation additional features. Furthermore, the FAIR toolbox already contains several state of the art implementations of these different building blocks as well as tutorials and examples including an implementation of the elastic regularizer as introduced in Chapter 2.2.4 in two dimensions (c.f. [32], p.121). Recalling our two versions of the image registration problem (c.f. Chapter 5.2), this virtually was the reason why we decided in favor of an implementation of the projected unconstrained TFPCM approach, which we will address in the following.

To reach this aim, we recall that we transferred the optimization problem \( J_{puTFPCM}^{\alpha,\beta} \) depending on \( f \) and \( u \) into an optimization problem \( \tilde{J}_{\alpha,\beta} \) which only depends on...
6. Implementation and Results

on \( u \) in Chapter 5.3 given by:

\[
\tilde{J}_{\alpha,\beta}^{puTFPCM}(u) = \frac{1}{2} \int_{\Omega} \chi_{c,R} |T(I + u) - R|^2 \chi_{c,T}(I + u) \, dx \\
+ \beta \left( \int_{\Omega} \frac{1}{4} \sum_{j,k=1}^{2} (\partial x_j u_k + \partial x_k u_j)^2 + \frac{\lambda}{2} (\nabla \cdot u)^2 \, dx \right) \\
+ \begin{cases} 
\int_{\Omega} \frac{\alpha^2}{2} + \beta \gamma (1 - \chi_{c,T}) \, dx, & u < -\vartheta \\
-\beta^2 \int_{\Omega} |1 - \chi_{c,T}|^2 |u|^2 \, dx, & -\vartheta \leq u \leq \vartheta \\
\int_{\Omega} \frac{\alpha^2}{2} - \beta \gamma (1 - \chi_{c,T}) \, dx, & \vartheta < u
\end{cases}
\]

Then the corresponding optimal cellular traction forces \( f^* \) for a known displacement field \( u \) could be calculated by (c.f. Equation (5.7)):

\[
f^* = \begin{cases} 
-\gamma, & u < -\frac{\alpha \gamma}{\beta(1-\chi_{c,T})} \\
\frac{\beta}{\alpha} (1 - \chi_{c,T}) u, & -\frac{\alpha \gamma}{\beta(1-\chi_{c,T})} \leq u \leq \frac{\alpha \gamma}{\beta(1-\chi_{c,T})} \\
\gamma, & \frac{\alpha \gamma}{\beta(1-\chi_{c,T})} < u
\end{cases}
\]

where we already integrated the constraint

\[ C_1^{TFPCM}(f) : \|f(x)\|_{\infty} \leq \gamma, \quad \forall x \in \Omega \]

Accordingly, we will deploy the FAIR toolbox to find a minimum \( u^* \) of the functional \( \tilde{J}_{\alpha,\beta}^{puTFPCM}(u) \) and subsequently use this displacement field to calculate the sought-after cellular traction forces.

Consequently, the remaining part of this section deals with some comments on the major steps required for the numerical realization of the minimization of \( \tilde{J}_{\alpha,\beta}^{puTFPCM}(u) \) within the FAIR framework. The complete MATLAB routines can again be found on the DVD attached to this thesis.

As a first step we always have to execute the 'FAIRstartup.m' file included in the FAIR folder to guarantee that the numerous FAIR functions are added to the current MATLAB path.

Afterwards, the input data has to be read in: In this context it is important to take care that the indicator function is normalized to \([0,1]\). As the FAIR framework enables the usage of multiscale and multilevel strategies for the efficient solution of the optimization problem (c.f. [32], p.9), where we decided for the latter option, the next step is the transformation of the imported data into a multilevel representation. The FAIR toolbox comprises the function 'getMultilevel' for this purpose. In addition to a reference and a template image, this function only requires the size
of the images \( m = [m_1, m_2] \) (in our case \( m_1 = 366 \) and \( m_2 = 472 \)) and a domain specification \( \omega \) that in our case is given by \([0, 366 \times 0.31, 0, 472 \times 0.31]\) as one pixel in the image corresponds to 0.31 \( \mu \text{m} \) in reality (c.f. [32], p.41).

To guarantee the reproducibility of our numerical experiments we created a file called 'data_complete_new2.mat' containing all of these values.

Using the examples included in the FAIR toolbox as templates we continued by designing a distance measure function "CTFR_D_new2". Furthermore, we created a so-called object function "CTFR_Object_Function_new2" calling our distance measure function as well as the two-dimensional elastic regularizer already provided by the FAIR toolbox and which combines these two functions with the term

\[
\begin{cases}
\int_{\Omega} \frac{\alpha^2}{2} + \beta \gamma (1 - \chi_{c,T}) |u| \, dx, & u < -\vartheta \\
-\frac{\beta^2}{\alpha} \int_{\Omega} |1 - \chi_{c,T}|^2 |u|^2 \, dx, & -\vartheta \leq u \leq \vartheta \\
\int_{\Omega} \frac{\alpha^2}{2} - \beta \gamma (1 - \chi_{c,T}) |u| \, dx, & \vartheta < u
\end{cases}
\]

such that this object function can be regarded as our energy functional \( \tilde{J}_{\alpha,\beta}^{puTFPCM} (u) \).

Finally, we needed a function "CTFR_MLIR_new2", which on the basis of a multilevel approach minimizes our object function by means of a Broyden-Fletcher-Goldfarb-Shanno (BFGS) type optimization algorithm provided by S. Suhr, member of the Institute of Applied Mathematics, University of Münster, and of the Institute of Mathematics and Image Computing, University of Lübeck.

We do not go into further detail here, but instead refer to the complete codes that can be found on the DVD enclosed to this thesis.

To complete the current section, we would like to point out that this DVD also comprises a program called "plane_stress_approximation_new2" executing these routines and returning plots of the resulting displacement field and the corresponding forces, some of which will be presented and discussed in the subsequent section.

### 6.3. First Results Obtained with the puTFPCM Approach

We conclude this thesis with a presentation of the first results gained with our novel puTFPCM approach by means of the previously described implementation.

For the sake of reproducibility, we shall at first state that we set the two thickness-averaged Lamé constants \( \mu' \) and \( \lambda' \) both to 2000 \( \text{pN/\mu m}^2 \). Besides, we chose the constant \( \gamma \) making the hard constraint \( C_{1}^{TFPCM} (f) \) (c.f. (5.3)) determinate to equal to 2100 \( \text{pN/\mu m}^2 \). Thereby, the results presented in [1], p.10, served as a reference. Finally, we had to assign values to the regularization parameters \( \alpha \) and \( \beta \). To our

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1Note that the regularization parameters denoted by \( \alpha \) and \( \beta \) in this thesis are labeled by 'epsilon' and 'alpha', respectively, within these codes.
6. Implementation and Results

minds, it seemed to be reasonable to choose $\beta$ larger than $\alpha$ to emphasize the information about the elastic character of the underlying substrate. However, the exact ratio of $\alpha$ and $\beta$ was chosen by trial and error. Regarding the range of the absolute values of these parameters, we reasoned that the maximal value of the reconstructed traction forces should be in the range of $\gamma$. In the end, these considerations brought about the values $\alpha = 2.5 \times 10^{-7}$ and $\beta = 5 \times 10^{-4}$.

We applied the projected unconstrained Traction Forces from Phase-Contrast Microscopy approach to frames 1 and 2 of the video of the human melanoma (MV3) cell consisting of 37 high-resolution phase-contrast microscopy images. As described at the beginning of Chapter 5.2, we considered the first of these frames to be the template and the second one to be the reference image:

![First frame of the video](image1)

(a) First frame of the video (template image $T$)

![Second frame of the video](image2)

(b) Second frame of the video (reference image $R$)

**Figure 6.4.** Phase-contrast microscopy images of a human melanoma (MV3) cell on a collagen I-coated polyacrylamide gel substrate. The resolution is 0.31 $\mu$m/pixel. Recorded by Christian Stock, made available by Albrecht Schwab (q.v. [39]) (Institute of Physiology II, University of Münster).

Additional to these images we passed the corresponding indicator functions gained with the preprocessing routine described above to our puTFPCM algorithm and executed the program 'plane_stress_approximation_new2'.

First of all, this program returned a displacement field $u$, which we plotted by means of the MATLAB® 'quiver' command. For the sake of clarity, we decided to only display those vectors representing the local displacements that measured at least 0.075 times the standard deviation of the Euclidian norm of $u$. The result, or more precisely, a close-up of the area where the relevant displacements occur, is depicted in Figure 6.5 a). Image b) is the respective normal velocity image as returned by the

---

3The whole video sequence providing a better perception of the cellular locomotion is contained in the DVD attached to this thesis. In this context, we would like to point out that the displacements of the underlying substrate that can hardly be seen in the images depicted in this thesis can be recognized best by quickly switching between consecutive frames.
6.3. Results

'Sabine' framework (c.f. Section 6.1). Let us recall that in such images, high intensity values characterize areas of high normal velocities, i.e. these areas correspond to bright image sections. Then, from our point of view, the area where the major displacements occur bears enough resemblance to the bright sections of the normal velocity image to regard this result as being generally reasonable. (Note that enlarged views of the results presented in this section can be found in Appendix II.)

![Displacement field](image1)

(a) Displacement field

![Normal velocity image](image2)

(b) Normal velocity image

![Horizontal component of f](image3)

(c) Horizontal component of $f$

![Vertical component of f](image4)

(d) Vertical component of $f$

![Vector field representing f](image5)

(e) Vector field representing $f$

**Figure 6.5.:** Results of the puTFPCM approach (I):

a) and b): Comparison of the reconstructed displacement field $u$ and a close-up of the corresponding normal velocity image. c), d) and e): Different representations of the reconstructed cellular traction forces $f$

Furthermore, the program provided a reconstruction of the applied traction forces. Images c) and d) show the horizontal and the vertical component of these forces, respectively. Besides, we again used the MATLAB® 'quiver' command to provide an image of the corresponding vector field. The result can be seen in image e), which displays is a close-up of the area where nonzero traction forces occur.
6. Implementation and Results

Concerning the reconstruction of the applied cellular traction forces, we can state that the results obviously satisfy our assumption of zero traction forces exterior to the area where the cell (including the halo) is located. Besides, we can observe that at the upper-left and lower-right portion of the cell the force is directed towards the lower-left corner of the image, while it is directed towards the lower-right corner in the left and the upper-middle part of the cell. There seem to be no areas of the cell where the traction force is tending upwards. Furthermore, the reconstructed forces are strongest on the left part of the cell and close to its upper boundary where the maximal magnitude of the traction force is in the order of 2000 pN/µm². If we once more take a closer look at the respective phase-contrast microscopy images, these observations seem to be in accordance with the visible displacements of the underlying substrate in the proximity of the cellular body. In this context, we would like to present one last figure displaying a joint plot of the applied traction forces and the induced displacements, which altogether seem to be in a reasonable relation:

![Figure 6.6:](image)

**Figure 6.6.:** Results of the puTFPCM approach (II):
Joint plot of the vector fields representing the displacements $u$ of the extracellular matrix (blue) and the cellular traction forces $f$ (green), respectively.

In conclusion, the numerical results provided in this section can be regarded as a 'proof of principle' for the TFPCM approach introduced in this thesis. However, we would like to emphasize that for the time being we raise no claim to any reliable quantitative statements as a detailed quantitative analysis including the validation of the gained results by studying synthetic data lies beyond the scope of this thesis.

Nevertheless, in summary these reconstructions seem to be a promising first result of our novel TFPCM approach.
7. Conclusion and Outlook

By combining linear elasticity theory and a flexible variational image registration framework we developed a novel approach for the reconstruction of cellular traction forces from usual high-resolution phase-contrast microscopy images.

Thereby, we proceeded as follows:
In the short introductory chapter we demonstrated the high medical relevance of a better understanding of cellular migration. Furthermore, we outlined how the traction force contributes to the overall process of cell motility and provided an easily accessible intuition of the experimental setting.
Afterwards, a detailed mathematical introduction to the theory of elasticity and image registration was provided in the second chapter. These two disciplines have already been related by means of the Elastic Registration approach serving as a starting point for the later construction of our novel method for the determination of cellular tractions. Chapter 3 was dedicated to the introduction of a mathematical model of the experimental setting including a consistent notation. Besides, we presented the forward problem predicting the displacement field of the underlying elastic substrate for known cellular traction forces. In addition, two possibilities to make this forward problem determinate have been deduced, namely the Boussinesq solution and the plane stress approximation. In Chapter 4 we provided an overview of the development of the reconstruction of cellular traction forces since the 1980s and presented four state of the art methods that are based on the popular elastic substratum method. The gained insights served as a motivation for our new Traction Forces from Phase-Contrast Microscopy approach. Additional to the development of this new method in terms of two alternative minimization problems, we also proved the existence of the respective solutions in the fifth chapter. The last chapter of this thesis dealt with the numerical implementation of the introduced framework. Finally, we presented and discussed first numerical results obtained with our new approach.

Even though these first results seem to be very promising, there exist several areas that could serve as a starting point for future research, some of which we already mentioned in the course of this thesis:
7. Conclusion and Outlook

The most urgent objective for future research seems to be the validation of the gained results. To make the puTFPCM approach usable for practical applications we have to guarantee the reliability of the reconstructed forces. Therefore, a detailed quantitative analysis is required. This quantitative analysis should also involve the application of our puTFPCM method to synthetic data and besides comprise an analysis of the influence of the parameter choice.

Furthermore, we have seen that until now the indicator function resulting from the preprocessing step is far from optimal. One possibility of improving the segmentation result could be the incorporation of a topology constraint to the Chan-Vese segmentation algorithm similar to the rough segmentation step in [33], but applied to the grey value image. In addition, the influence of the radius of the disk used for dilation should be investigated.

Beside these improvements regarding the numerical implementation one could also think of future research concerning the underlying mathematical model:

Recalling the results of our calculations of the displacement fields for known cellular traction forces in Chapter 3 we could observe that the cell does not only cause horizontal displacements, but also has major effects in vertical direction. Besides, generally speaking, our TFPCM approach is not limited to two-dimensional quantities. For example, the optimization problem of the puTFPCM approach could be easily adjusted to three-dimensional data if we simply replace $\mathcal{G}$ by $\mathcal{E}$ the two-dimensional elastic potential by its three dimensional equivalent. Concerning the pcTFPCM approach, the optimization problem even simplifies as the projections $P_{\text{funct},j}(g)$ are no longer required. However, this would require the availability of a three-dimensional data set.

Finally, recalling Footnote 3 on p.41 we shall point out that one might consider replacing the linearized displacement-traction problem of three-dimensional elasticity by its nonlinear counterpart in the future.
A. Appendix I

Figure A.1: Enlarged view of Experiment 1: Displacements calculated from $f_1$ (first image)
Figure A.2.: Enlarged view of Experiment 1: Displacements calculated from $f_1$ (second image)
Figure A.3.: Enlarged view of Experiment 2: Halving the applied forces (displacements calculated from $f_2$)
Figure A.4: Enlarged view of Experiment 3. Changing the ratio of the forces applied at the rim and under the interior of the cell (displacements calculated from $f_3$)
Figure A.5.: Enlarged view of Experiment 4 (part I): Canceling the symmetric shape of the applied force (displacements calculated from $f_4$)
Figure A.6.: Enlarged view of Experiment 4 (part II): Canceling the symmetric shape of the applied force and neglecting the assumption of zero net force (displacements calculated from $f_5$)
B. Appendix II

Figure B.1.: Enlarged view of Results of the puTFPCM approach (I) a): Displacement field

Figure B.2.: Enlarged view of Results of the puTFPCM approach (I) b): Normal velocity image
B. Appendix II

Figure B.3.: Enlarged view of Results of the puTFPCM approach (I) c):
Vertical component of the reconstructed applied cellular traction forces

Figure B.4.: Enlarged view of Results of the puTFPCM approach (I) d):
Horizontal component of the reconstructed applied cellular traction forces

Figure B.5.: Enlarged view of Results of the puTFPCM approach (I) e):
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Bibliography


Eidesstattliche Erklärung


Alle auf der DVD beigefügten Programme sind von mir selbst programmiert oder durch Angabe von Herkunft kenntlich gemacht worden.


Eva-Maria Brinkmann