Three Dimensional Model of a Single Cell in an Alginate Construct

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Summary

In cell experiments there is an interest to acquire information how a cell responds to external loads. In gel studies these cells are imbedded in gel and the gel is deformed externally. Because the gel is deformed, the cells inside the gel will deform as well. In order to capture the deformation of the cell a confocal microscope is used. A confocal microscope can create stacks of images which visualize a three dimensional environment. Due to the make up of most gelling materials the material around a cell is inhomogeneous. Because of this inhomogeneity of the gelling material there is a need to capture the deformation field around a cell. In order to capture this deformation field the gel was filled with microbeads. These microbeads need to be coated with fluorescent material. Otherwise, it would be impossible to observe them with a confocal microscope. After these images were captured, they are subjected to image analysis to convert the information from the images into data. This data is the exact location of each bead in the field of view. The position of the microbeads in the deformed state and the undeformed state can be used to calculate the displacement of each individual bead. The displacement of the microbeads can be used to create models of the displacement field. The displacement field is used to derive the deformation field. The results of these models depends on the mathematics used to calculate these models. In order to quantify these models, the estimate of the standard deviation is used to calculate the accuracy of these models. However, these results leads to the idea there is an inaccuracy, which in itself is reason for further investigation. This investigation focuses on where a confocal microscope would create its greatest error. In order to calculate this error a matlab model was created which simulates a microbead moving through a sensor field, using this method the error of a microbead displacing through such a field can be quantified. The results of this matlab model showed that the error was significantly higher in one direction (z-direction which corresponds to the depth of the experimental sample) compared to the other directions. Due to this error a new model is used in which all of the displacements from this direction are discarded and all the locations of this dimension are projected into one plane.
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1 Introduction

A chondrocyte is a cell which is located in articular cartilage. Articular cartilage is a tissue located in arthrodial joints. This tissue allows for almost frictionless motion between joint surfaces. Cartilage consist of a matrix of collagen fibers, chondrocyte’s are the cells that create and maintain these fibers.

Osteoarthritis is a very common disease amongst elderly people which has his origin in the degeneration of cartilage. Tissue degeneration due to osteoarthritis is assumed to have a mechanical origin. Since chondrocytes are the cells that maintain cartilage studying the mechanical response of these cells can lead to new insight into the disease.

In order to understand why certain diseases like osteoarthritis occur and what the effect of such a disease is, the cell itself must be studied. Chondrocytes can respond differently to different loading conditions. Several studies examine the deformation of a chondrocyte (Trickey et al. (2004), Guilak and Mow (2000)). However, there is hardly any emphasis on the deformation field around the studied cells. In order to see what the effect of osteoarthritis is on a cellular level, the cell itself must be studied.

In construct studies the material that surrounds a chondrocyte is inhomogeneous. Due to this inhomogeneity chondrocytes are not uniformly loaded. Capturing the deformation field around a chondrocyte shows the deformation applied to a single chondrocyte. If the deformation is known chondrocyte deformation can be compared from one cell to another. Comparing cellular deformation around a cell can be a vital tool in order to investigate the mechanical properties of produced cartilage.

The objective of this thesis is to develop a testing procedure to monitor the deformation around a cell. In order to monitor the deformation field around a cell microbeads are implanted in the material surrounding the cell. Before and after deformation, a confocal microscope is used to track the locations of the microbeads. The location is used to calculate the displacement of the microbeads. These displacements are the information that is necessary to create models that calculate the deformation around a cell.

This thesis starts with a small introduction in the field of chondrocytes and their experiments (Chapter 2). It continues to introduce the various materials needed for the experiment such as the construct material and the microspheres (Chapter 4). After the materials several mathematical methods are procedures are explained such as least mean square fitting and Kriging (Chapter 4.3). Following these procedures are the first results of the confocal microscope test, following the experimental results the results of the mathematical models are shown (Chapter 5). After these models are shown they are evaluated on their accuracy (Chapter 6), this evaluation leads to a simulation of a microbead moving through a sensor-field (Chapter 6.1). The results of this simulation is that current confocal microscopes create a large error in one of their dimensions, with these results new models are created which are again evaluated with their mathematical characteristics.
2 Chondrocyte deformation a literature study

Chondrocytes are responsible for the maintenance of articular cartilage. The degeneration of articular cartilage is believed to be one of the main causes of osteoarthritis (Trickey et al. (2004)).

2.1 Cartilage

Articular cartilage can be considered to be a solid matrix of collagen fibers in which water is able to move and in this water several ions are present. Within this solid matrix chondrocytes are embedded in an extracellular matrix. This matrix consists of the collagen fibers and proteoglycans.

Proteoglycans are large complex molecules that are negatively charged. A proteoglycan group gives a strong negative charge because of the strong negative compared to the synovial fluid (fluid surrounding a joint), the cartilage will swell due to osmotic pressure. The second part of the matrix are the collagen fibers articular cartilage consists mostly out of collagen type II. Collagen fibers are good at resisting tension however they are bad at resisting compression.

Articular cartilage is divided in to three distinct regions see Figure 1. These regions are:

- Deep zone
- Transitional zone
- Superficial zone

The deepzone is the part in which collagen fibers have the largest diameter (compared to the two other zones) and the fibers are arranged perpendicular to the subchondralbone. In the transitional zone the collagen fibers have a smaller diameter then in the deepzone and the fibers are arranged more randomly. Collagen fibers have the smallest diameter in the superficial zone and the fibers are densely packed together and arranged parallel towards the joint surface.

![Cartilage Regions Diagram](image-url)
2.2 Chondrocytes

In the three regions described above, chondrocytes exist and they function to maintain the network of collagen fibers. Due to the location of the chondrocytes they do not receive any nutrients from blood, instead they receive these nutrients from the synovial fluid either due to diffusion and/or due to confection. A chondrocyte consists out of several parts from which it gets its distinct mechanical response:

- Cytomembrane
- Nucleus
- Cytoplasm
- Cytoskeleton

These parts have their individual functions and will now explained more extensively.

Cytomembrane: The Cytomembrane is the outer layer of the cell. It is a phospholipid bilayer (see Figure 2) which is approximately 6 to 10 nm thick. A phospholipid is long molecule which has at one side a hydrophobic part and at the other side a hydrophilic part. A phospholipid bilayer emerged in water will move its hydrophobic heads towards each other and move the hydrophilic heads towards the water. This membrane has the ability to transport various ions and molecules from outside the cell to the inside of the cell and vice versa.

![Phospholipid bilayer](Phospholipid_bilayer.png)

Figure 2: A phospholipid bilayer is the basis for all cell membranes, from (Lodish et al. (2003)).

Nucleus: The nucleus of a chondrocyte contains the genetic material of the cell. This part of the cell is responsible for the mitosis (the dividing of a cell into two cells). The nucleus has its own membrane which is known as the Nuclear membrane.

Cytoplasm: The cytoplasm is composed of the interstitial fluid within the cytomembrane called the cytosol and a number of different organelles. One of these organelles is the cell’s nucleus and other organelles are for instance the Golgi-apparatus and the mitochondria. There are a number of free ions which can give the cytoplasm either a positive potential or a negative potential.
Cytoskeleton: A number of different filaments give the cell its rigidity to mechanical loading. The cytoskeleton is comprised of three different elements:

- Micro tubules
- Micro filaments
- Intermediate filaments

These different parts are interconnected with each other and as such they create the biggest load bearing part of the cell.

Micro tubules: The microtubules are the only component of the cytoskeleton which can resist compression. This is explained by their relatively large outer (30 nm*) and inner diameter (18 nm*). The micro tubules are also assumed to be almost inextensible (Ingber (2003)). The micro tubules originate from the micro tubule organizing center and they radiate outwards (Lodish et al. (2003)).

*The inner and outer diameter obtained from Gittes et al. (1993)
Micro filaments: The micro filaments are actin filaments which have the smallest diameter (7-9 nm). They are located mostly at the cortex which is just beneath the cytomembrane. The micro filaments are known to be under a certain prestress (Kumar et al. (2006)). Because these filaments have a small diameter they extend much easier than the thicker (Ingber (2003)) micro tubules.

Intermediate filaments: The intermediate filaments are slightly larger than the micro filaments. They are about 10 nm in diameter. The structure of the intermediate filaments looks like that of a twisted cables (see Figure 3). The intermediate filaments are located throughout the cytosol. They form networks that connect the cytoskeleton to various organelles. They can also be attached to adjacent cells through certain molecules attached to the cytomembrane.

2.3 Osteoarthritis

Osteoarthritis is a painful joint disease that is found mostly in elderly people. This disease progressively degenerates articular cartilage. Mechanical factors are believed to play a significant role in the start and progression of this disease (Shieh and Athanasiou (2003)). Though the processes which starts this disease are not known. Several studies have tested both healthy and osteoarthritic chondrocytes to see the difference in their mechanical properties of these cells (Trickey et al. (2000)).

2.4 Deformation studies

There are several methods to see what effects of mechanical loads have on cartilage. These can be categorized as:

- Explant studies
- Monolayer studies
- Construct studies

These studies are explained in detail in the following sections

Explant studies: In explant studies a piece of articular cartilage is removed from a joint. This piece of cartilage is called the explant. In these studies the explant is deformed after these deformations are applied the explant is reviewed to see its chemical content. This chemical content can be the amount of proteoglycans or the amount of created cartilage. Due to the changes in this content a better insight in the metabolism of the cell can be acquired. One of the major drawbacks of using this technique is that the mechanical environment of a cell is difficult to characterize. Another drawback of using explants is that a removed piece of cartilage can react differently than cartilage in a joint.

†From Lodish et al. (2003)
Monolayer studies: In monolayer studies, the cells are harvested from cartilage. After the cells are harvested, they are kept in a monolayer solution. A monolayer solution is a liquid in which cells get their nutrients and divide. Because this monolayer is a liquid, the environment can be controlled much more reliably than a piece of cartilage. However, cells in a monolayer flatten in course of time. Cells are normally surrounded by cartilage tissue; this helps to maintain the cells' original shape. In a liquid, this surrounding tissue is absent, thus there is no material to help the cells retain their original shape. A typical monolayer study is micropipette aspiration (see Figure 4). In micropipette aspiration, a cell is placed in front of a pipette opening and a negative pressure is applied at the opening. The negative pressure will cause the cell to deform inside the pipette. The length of the deformed cell can be related to the mechanical properties of a cell.

Figure 4: Microaspiration technique in which the length of the sucked in part, and the pressure to suck it in relate mechanical properties adapted from Trickey et al. (2004)

Construct studies: The main problem with monolayer studies is that cells lose their original shape. The problem with explant studies is that the environment in which the chondrocytes are deformed is uncontrollable. If cells are seeded in a construct material, they will retain their original shape, since the construct has approximately the same rigidity as normal cartilage. Deformation studies of cells in construct materials deform the construct material and examine the deformation of the cell by means of a confocal microscope (cite LeeKnight2000, Knight et al. (2002)). In most deformation studies, a shape index is used to quantify strain. If a piece of construct material is compressed, the cell inside the material will also compress. The shape of a compressed cell resembles an ellipsoid. Ellipsoids have two major axes if the undeformed axis are compared with the deformed axis an index can be found which can be used to quantify strain.

2.5 Models

The mechanical response of the chondrocyte is usually considered to be at least viscoelastic. This is due to the large part of the component of water in the cell. Some studies use a Biphasic model to model the cell (Guilak and Mow (2000)),
Figure 5: Confocal images of chondrocytes that are seeded in an agarose construct which is deformed and the diameters are used to calculate the strain.

here the assumption is made that a cell consists of a homogeneous mixture of a solid and a fluid part. The solid part resembles the cytoskeletal elements and other proteins, the fluid part consists of the cytoplasm (fluid contained inside the chondrocyte).

There are several studies which examine the complex behavior of a cell by assuming complex material properties. Usually, these studies (Guilak and Mow (2000)) resort to finite element modelling to calculate the response of the material. In order to create finite element models, material properties and boundary conditions have to be known. Since not all properties are known assumptions will be a necessity. These assumptions are:

- material properties
- geometric assumptions
- boundary condition

These assumptions will determine the complexity of the model and its effectiveness. A finite element model will give detailed information on the cell itself as well as the deformation-field around the cell.

Another method used frequently is a shape index. A Shape index uses only the shape of the cell to determine changes. A frequently used shape index function is, both diameters of an ellipsoid (however, other indexes can be used). Diameters are used because a compressed cell resembles an ellipsoid. Using a shape index, assumptions are not as strict as in the finite element models making them much easier to implement. This does, however, come with a penalty the information gained is only viable to examine the difference between cells themselves, a benefit of these studies is that they can be used to quickly examine large amount of cells.
3 Problem statement

In most chondrocyte deformation studies there is little or no information about the deformation field around a cell. However, in construct studies this information is vital for investigating the deformation of a cell. Construct materials are known to be inhomogeneous, this is due to the fact that these materials have large molecules. In construct studies the construct material is deformed externally. Due to the inhomogeneity of the construct material and the manner in which the construct material is loaded not all the cells experience the same deformation. In order to find the deformation applied to a specific cell much more information is needed than a shape index. If the deformation around a cell is known, then the deformation applied to the cell is known. Using this deformation, instead of the deformation applied to the construct material, the inhomogeneity of the construct material will no longer influence the results of comparing the deformation of different cells. The deformation around a cell can also lead to the design of a new experiment, for instance to study what the effect of the creation of collagen has on the deformation field around a cell.
4 Methods

In order to visualize the complete deformation, a three dimensional image around the cell is needed. A confocal microscope was chosen to capture the image of the cell before and after a deformation step. One of the drawbacks of choosing a confocal microscope is that it cannot show the deformation field around a cell. In order to show the deformation fluorescent microbeads were used to identify local deformations around the cell. Using the displacement data obtained from the microbeads several models were created to calculate the deformations around a cell. After these models were analyzed, an indication of where the problems arise with the experiments and the models can be made.

4.1 Cell culture

Chondrocytes used in construct studies are usually harvested from slaughter cows. Cells needed for this experiment are approximately 14 million cells. Typically the amount of harvested cells is not sufficient to start the experiment. In order to create sufficient cells, cells are cultured in a monolayer. Culturing cells in a monolayer will allow cells to divide which increases the amount of cells. Cells where cultured in flasks after a certain amount of time (dependent on the initial cell count) cells will no longer divide, simply because there is no more room to divide. When this occurs cells are collected from these flasks and counted if the number of cells is still insufficient then the culturing process is repeated. This process is often called making a passage.

The chondrocytes were harvested from bovine articular cartilage. Bovine articular chondrocyte’s were harvested from the metacarpal phalangeal joint. The chondrocytes where seeded in alginate after three passages. During passages the cells where kept in a fetal calf solution.

4.1.1 Alginate

If cells are not grown in a construct material they will lose their spherical shape and tend to flatten. Thus in order to culture spherical cells a construct material is needed. Alginate as a construct material has several benefits, it is a cold process so no additional heat is required in contrary to agarose (if in the process the heat required is to high the cells will die, if it is to low the agarose will coagulate to early giving a inhomogeneous mix). In this study 2% alginate was used as a construct material to ensure that our cells retained their original spherical shape (which is the same as in Knight et al. (2002)).

Alginate is a material which is derived from seaweed it is a gelling compound which is used in chondrocyte cultures as a construct material. Alginate itself gels by adding di or tri-cova lent ions, calcium chloride was used to achieve gelling in our experiments. The calcium ions will force Alginate to create cross-links and as such become a gel. In our experiment a mold was used to create alginate of a certain thickness (2.5 mm respectively). The mold allowed us to create a large alginate disk. From this disk several smaller disks (6 mm diameter) were
punched out. These smaller disks were used to culture the cells. After culture these disks were cut in half to obtain the largest possible area to inspect under the confocal microscope.

4.1.2 Micro Spheres

In order to track the displacement within the alginate gel microspheres were added to serve as markers. These markers must be coated with a fluorescent die in order to track them with a confocal microscope. The markers are approximately 1 $\mu$m in diameter (as specified by manufacturer). The size was chosen such that these markers can be easily recognized (relative accuracy of the confocal microscope in xy plane is 0.24 - 0.32 $\mu$m) and that they would be small enough not to have an effect on the displacement field itself. The micro spheres used where melamine F.I.T.C. (fluorescein isothiocyanate) micro spheres form microspheres-nanospheres having a 490 nm excitation and 525 nm emission wavelength. These micro spheres are supplied in a 2.5 % solution in 5 ml deionized water. Since there are approximately 14 million cells and want to have 10 microbeads around each cell, 140 million microbeads are needed, this demands we have at least 4.66 $\mu$l solution. However experiments with this amount of bead suspension did not show the amount of microbeads expected less microbeads were found in the final gel. The loss of microbeads can be attributed to the fact that the filters used in the hardening process of the alginate have a relatively large pore size. Due to the large pore size microbeads can travel from inside the gel towards the calcium chloride suspension. In order to deduce the proper amount of bead suspension several samples where made which resulted in the deduction that an amount of 200 $\mu$l suspension to give a sufficient number of microbeads around a cell.
4.2 Image processing

4.2.1 Confocal microscopy

A confocal microscope uses a laser light to excite electrons in a biological sample. Due to the excitation of the electrons light is reflected at a different wavelength than that of the laser (this phenomena is called fluorescence). A confocal microscope illuminates a single point of the sample, due to the samples fluorescent properties light will be reflected, by placing the pinhole at an optically conjugate plane near the detector only information from the focal plane is recorded. If a scanning motion is used we will get information of the an entire 2 dimensional plane. If the microscope stage is displaced to a higher position, information from a different depth can be captured. Thus a confocal microscope is able to capture a three dimensional image of a fluorescent sample. A picture of the confocal principle is in Figure 6.

Confocal microscopes needs fluorescence to create images thus our cells where stained using a calcine staining.
Figure 6: The confocal principle: The red lines start from the light source, which is a laser. This light passes through an optical system consisting of lenses and filters, after which the light hits the focal plane where the sample is illuminated and the fluorescent dye reflects light at a different wavelength. This reflected light is split due to the dichromatic mirror and eventually passes through a pinhole after which the light is captured.
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4.2.2 Fluorescence bleaching

Fluorescence bleaching (also called photo bleaching) is an effect which occurs when after exciting electrons the intensity of the fluorescent dye will diminish. If a sample is scanned intensely the captured image will slowly fade.

In this experiment the photo bleaching was found to be the most extensive within the calcine staining of the cells. This can be resolved by shielding the sample from ambient light as well as scanning the images in the dark. The fluorescence of the microbeads was constant throughout the experiments.

Fluorescence bleaching can be used to quantify how active a cell's metabolism is however the fluorescence bleaching referred to here is only the negative effect which scanning multiple samples has.

4.2.3 Deformation device

In this experiment a deformation device was used which consists of two linear motors. These motors can be used to compress the alginate disk from one side or from both sides (Figure 7 shows the setup of the deformation device). Before the alginate disk can be compressed contact must be made without compressing the alginate (this process must be fast in order to minimize fluorescent bleaching), this is achieved by using the normal microscope on the confocal microscope. After this contact a deformation of 1%, 2%, 5% is applied on the alginate. After each deformation is applied the sample is scanned in three dimensions after approximately 2 minutes (which is similar to Trickey et al. (2000)) of application of deformation. The reason for waiting 2 minutes is to minimize time related responses of the alginate disk.

During the test the field of view of the microscope remained constant in order to ensure that the deformation captured is not affected by the inaccuracy of the actuators from the microscope.

During the scanning of the alginate sample the sample is kept wet with physiological salt in order to keep the sample from dehydrating (dehydrating occurs due the heat generated by the laser).

Figure 7: The setup of the deformation device. The two blocks A and B deform the construct material C. by compressing it.
4.2.4 Image tracking

In order to calculate the deformation around the cell the microbeads in the alginate must be tracked. In the study of Verhulp et al. (2004) they adapt a digital image correlation algorithm to automatically track the deformation field. However, for this algorithm to work properly images need to be averaged to get better results with the algorithm. A downside of using the algorithm is that bleaching would occur even faster when an image scan more times (in order to average). Bleaching of the cells was found to be so severe when scanning the same cell multiple times, that such an algorithm would bleach the cell so severely that the cell itself is no longer visible.

The resolution of the images is 512x512x20 which corresponds to the actual measured resolution of 230x230x20 in µm.

4.2.5 Data mining

The images created by the confocal microscope must be edited to give us an accurate description of the displacement of the microbeads. As stated previously in the images several stray pixels were found. These pixels can be a source of inaccuracy thus must be filtered. These stray pixels are filtered by using a low level blur with an image editing program (standard blur in paintshop pro).

In order to calculate the displacements, a program is needed that can locate each bead/cell. Matlab was chosen due to its already available image-processing toolbox. Matlab has a built in algorithm (regioprops) to calculate the area and centroid of enclosed white areas in a picture. Before the regionprops command can be used the image must be thresholded to create a binary image (black and white). Thresholding the image was done using Otsu’s method (Otsu (1979)). Otsu’s method assumes that the image itself is bimodal and the ideal threshold can be found minimizing the between class variance (this is also a standard function in matlab).

After the image is thresholded a number of anomalies can still be found in the images. These anomalies are single pixels due to noise in the image capturing process. In order to filter out most of these anomalies only areas with an additional area in a different stack were stored. If the absolute difference between the x and y coordinate of the centroid in one stack lower is than 2 pixels with the x and y coordinate of the centroid in another stack then the area is noted to be a bead or a cell instead of an anomaly. In order to visually check if these computations where correct a plot was made of each of these stacks in matlab (which can be seen in Figure 8). In order to calculate the volume of the microbeads the areas were added simply something which can be explained as a Riemann sum. The microbeads where tracked visually using the plot function afterwards the position was noted of the un-deformed state and the displaced state. The displacements found contained a large rigid body mode. In order to calculate the displacement of the microbeads relative to the cell the rigid body movement is subtracted from the microbeads and the cell.

In order to show that the cell is deformed the cell deformation calculation
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Figure 8: On the left side the images taken by the confocal microscope on the right the visual inspection of the image tracking

according to Knight et al. (2002) is used. This deformation is defined as:

$$\varepsilon_x = \frac{X_s - X_u}{X_u}$$  \hspace{1cm} (1)

In the Y direction the deformation is defined as:

$$\varepsilon_y = \frac{Y_s - Y_u}{Y_u}$$  \hspace{1cm} (2)

These calculations showed an approximately 5% deformation of the cell with 5% deformation of the alginate.
4.3 Mathematical models

In this section the deformation field will be calculated with various models based on the microbeads displacement input. If the same cartesian coordinate frame is used in both the deformed and the un-deformed state the strain can be calculated as:

\[ \varepsilon_{ij} = \frac{1}{2} \left[ \frac{\partial u_j}{\partial x_i} + \frac{\partial u_i}{\partial x_j} + \frac{\partial u_a}{\partial x_i} \frac{\partial u_a}{\partial x_j} \right]. \]  

(3)

In the above equation Einstein notation is used\(^2\). In which \( u_i \) is the displacement in \( x \ y \) and \( z \) direction, and \( x_i \) is the coordinate in \( x \ y \) and \( z \). In order to calculate the deformation, the displacement as a function of the \( x \ y \ z \) coordinates must be known. In order to find the right interpolation between our displacements different models were used. These models are:

- Least Squares Method
- Weighted Least Squares Method
- Kriging

The calculation of the models was done using the actual scale. This will allow for the accurate modelling of the strains.

4.3.1 Least Squares method

Least squares method is a mathematical procedure in order to find the best line given a set of points. This procedure tries to minimize the squared distance between the points and a to be fitted line. It can be assumed that this line has a certain error with regards to the output which can be composed as:

\[ y = f(x, C_1...C_n) + \varepsilon. \]  

(4)

In this equation the function \( f(x, a) \) is the function that is to be determined in which \( a \) is a constant to be determined, \( \varepsilon \) is the error and \( y \) is the output. If the function is assumed to be a linear function then

\[ f(x, C_1, C_2) = C_1 x + C_2. \]  

(5)

If Equation 5 is substituted in 4 the result will be

\[ y = C_1 x + C_2 + \varepsilon. \]  

(6)

Since, the output \( y \) is known (the displacements) and the input (the location of the microbeads) \( x \) the only thing that needs to be determined are the value of the constants \( C_1 \) & \( C_0 \). Because, there are several points of input and output, it is possible to calculate the sum of squared error which these constants create.

\(^2\)In Einstein notation a repeated index is implicitly summed over
To minimize the summed squared error for all the points the following relation is minimized:

$$\sum \varepsilon^2 = \sum (y - C_1 x + C_2)^2 .$$

(7)

To minimize Equation 7 the partial derivative with respect to the constants is taken and set it to zero:

$$\frac{\partial \sum \varepsilon^2}{\partial C_1} = 0 = \sum 2x_i (y_i - C_1 x_i + C_2)$$

(8)

$$\frac{\partial \sum \varepsilon^2}{\partial C_2} = 0 = \sum 2 (y_i - C_1 x_i + C_2) .$$

(9)

This results in two equations for the two unknowns which can be solved. The solution of the equation is:

$$C_1 = \frac{n \sum x_i y_i - \sum x_i \sum y_i}{n \sum x_i^2 - (\sum x_i)^2}$$

(10)

$$C_2 = \frac{n \sum x_i^2 y_i - \sum x_i \sum x_i y_i}{n \sum x_i^2 - (\sum x_i)^2} .$$

(11)

These equations give us a direct method of calculating the best linear line for the given data points. However, the best fit can still be a wrong line.

In this experiment there is data from three dimensions and the same method of derivation is used. However, the functions used in this study are a linear, quadratic and a cubic polynomial. The equations used to fit the displacement field where:

$$u = C_1 x + C_2 y + C_3 z + C_4 .$$

(12)

for the linear least squares,

$$u = C_1 x^2 + C_2 xy + C_3 xz + C_4 y^2 + C_5 yz + C_6 z^2 + C_7 x + C_8 y + C_9 z + C_{10} .$$

(13)

for quadratic least squares, and

$$u = C_1 x^3 + C_2 x^2 y + C_3 x^2 z + C_4 y^3 + C_5 x y^2 + C_6 y^2 z + C_7 z^3 + C_8 x z^2 + C_9 y z^2 + C_{10} x y z + C_{11} x^2 + C_{12} x y + C_{13} x z + C_{14} y^3 + C_{15} y z + C_{16} z^2 + C_{17} x + C_{18} y + C_{19} z + C_{20} .$$

(14)

for cubic least squares.

For least square fits there is a method which can calculate the range of the fitted constants e.g. $C_1$ due to the assumption of an confidence interval. The deviation of the constants can be calculated as:

$$\Delta \beta_j = t_{\alpha/2, n-p} \sqrt{\hat{\sigma}^2 C_{jj}} .$$

(15)
In this equation $\Delta \beta_j$ is the deviation of the constant with index $j$, $t$ is the $t$-distribution and $\alpha$ is the confidence interval $n$ is the number of datapoints in the data and $p$ is the number of constants we fitted. $\hat{\sigma}^2$ is the estimate of the variance of the datapoints which is calculated by:

$$\hat{\sigma}^2 = \frac{SS_E}{n-p}. \quad (16)$$

In which $n$ is the number of datapoints and $p$ the number of fitted constants and $SS_E$ is the sum of the squares of the residuals, which is calculated by:

$$SS_E = \sum_{i=1}^{n} (u_i - \hat{u}_i)^2. \quad (17)$$

In Equation 17 the matrix $C_{jj}$ is the covariance matrix which is the inverse of the matrix used to calculate the fit.

### 4.3.2 Weighted least squares method

One of the drawbacks of Least squares method is that it is sensitive to points that are called outliers. Outliers are points that differ significantly from the rest of the data. A method of reducing the influence of these outliers is weighted least squares method. If an outliers was first multiplied by a value close to 0 its influence is diminished. The weights are used to diminish its effect on the overall fit,

$$\sum \varepsilon^2 = \sum w_i (y_i - C_1 x_i + C_2)^2. \quad (18)$$

In which $w_i$ is the weighing factor for each data point.

In this experiment the distance of the bead from the cell is used as a weighing factor. Because the focus is on the deformation field around a cell, thus information closer to the cell will be more important than information further away. This can be seen in equation:

$$w = e^{-C|x_c - x_b|}. \quad (19)$$

In this equation $x_c$ is the coordinate of the cell and $x_b$ is the coordinate of the bead. The constant $C$ is used to modify the equation in such a way that the weights are representative for the field of view in the experiment. To determine this constant the weight of a point located at the largest possible distance from the cell to be 0.1.

### 4.3.3 Kriging

Kriging is another method used to interpolate between samples however the following relation is used:

$$y = f(x) + Z(x). \quad (20)$$

In this equation $f(x)$ is a polynomial and $Z(x)$ is a functional departure from that polynomial. The difference between least squares algorithm and Kriging
4 METHODS

lies in $Z(x)$. This functional departure is a stochastic Gaussian process that is the uncertainty of the mean value of $y$ with an expected value of $E(Z(x))$. The covariance between two points ($x_1$ and $x_2$) is:

$$\text{cov}(Z(x_1), Z(x_2)) = \sigma^2 R(x_1, x_2).$$

(21)

In which $\sigma^2$ is the process variance and $R(x_1, x_2)$ is the spatial correlation function. The spatial correlation function determines how the different points influence the actual interpolation. A spatial correlation function can be:

$$R(x_1, x_2) = e^{-\theta|x_1 - x_2|^2}.$$  

(22)

In the above equation $\theta$ is a positive real number, it is also one of the parameters that needs to be determined just as the values of the constants in least square fitting. The other values that need to be determined are the constants in the polynomial from Equation 20. For the Kriging model a Kriging toolbox was used namely the D.A.C.E. package which easily allows mathematical modelling using different spatial correlation functions/different polynomials. The spatial correlation functions used are Gaussian.

4.3.4 Calculation of the derivatives

In order to calculate the deformation, the derivatives of the displacement are needed. For least mean squares the derivatives can be calculated using derivatives of the to be fitted function, this leads to:

$$\frac{du}{dx} = C_1$$  

(23)

$$\frac{du}{dy} = C_2$$  

(24)

$$\frac{du}{dz} = C_3.$$  

(25)

For linear least squares fitting. These values can directly be substituted into equation 3 For quadratic least squares fit the derivatives become:

$$\frac{du}{dx} = 2C_1x + C_2y + C_3z + C_7$$  

(26)

$$\frac{du}{dy} = C_2x + 2C_4y + C_5z + C_8$$  

(27)

$$\frac{du}{dz} = C_3x + C_5y + 2C_6z + C_9.$$  

(28)

And for cubic least square fitting these become:

$$\frac{du}{dx} = 3C_1x^2 + 2C_2xy + 2C_3xz + C_5y^2 + C_8z^2 + C_{10}yz + 2C_{14}x + C_{12}y + C_{13}z + C_{17}.$$  

(29)
\[
\frac{du}{dy} = C_2 x^2 + 3C_4 y^2 + 2C_5 xy + 2C_6 yz +
C_9 z^2 + C_{10} xz + C_{12} y + 2C_{14} y +
C_{15} z + C_{18} \tag{30}
\]

\[
\frac{du}{dz} = C_3 x^2 + C_6 y^2 + 3C_7 z^2 + 2C_8 xz +
2C_9 yz + C_{10} xy + C_{13} x + C_{15} y +
2C_{16} z + C_{19}. \tag{31}
\]

The Kriging toolbox directly gives us derivatives, so only the strain needs to be computed according to Equation 3.
5 Results

In the early experiments the amount of microbeads was insufficient to create models which can be seen in Figure 9.

Figure 9: One of the first bead experiment the amount of microbeads was found to be insufficient to create models, in this figure 3 cells and 4 microbeads are visible.

From these early experiments the amount of microbeads was increased which gave the following result which can be seen in Figure 10. After the first deformation (1% compression on the alginate) is applied. The deformed and the undeformed images can be combined to show the actual movement of the microbeads and the cell. This movement is something which in mechanics is referred to as a rigid body mode, however, microbeads do move relative towards each other. This movement is much smaller compared to the rigid body mode.
Figure 11: The deformed and the undeformed state of the alginate sample, in red the un-deformed state, in grey the deformed state. Clearly, most microbeads shift to the right.
The response of 2 cells from different alginate samples have been calculated. The results are shown in three different views these are the mid-planes from the 230x230x20 block. Thus the z midplane corresponds to the z coordinate being fixed (z = 10). In this view all the strains are calculated. Since there are 3 deformation (1%,2%,5%) steps and at least 6 quantities (if only the strains are shown) and 3 views(each midplane) this would lead to 36 different views for just one cell and one fit method. In order to keep this section clear of abundant pictures this section contains only images of the first successful cell experiment are shown. For quadratic least squares the results are show in Figure 12 For Kriging the results are shown in Figure 13

Figure 12: Strain results of QLSM, shown as a strain tensor in which blue is a 10% compressive strain and red is a 15% tensile strain
Figure 13: Strain results of Kriging, shown as a strain tensor in which blue is a 10% compressive strain and red is a 15% tensile strain.
6 Accuracy

In order to know if the models were implemented correctly, the displacement of the cross-sections was examined which can be seen in Figure 14 which has on the left side the displacements results of quadratic least mean squares and on the right the results of Kriging. When the fitted displacement against the actual displacement is examined (which can be seen in Figure 15 again on the left QLSM and right Kriging), results show the same fitted answer as the input which is to be expected and least square results are not exactly the same as the input. The fitted versus the unfitted data shows us that both methods are implemented correctly. Since both methods are implemented correctly and both of these methods show a large variation in the strains in $\varepsilon_{zz}$. This variation is unexpected due to the way the construct is deformed. In order to investigate if these deformations exist an analysis of the accuracy of the fits must be made.

In order to calculate the accuracy of the least mean square fit of the strains, the variation of the individual constants is used to calculate the variation of the strains. The error calculation can be made using the standard deviation as

\[ \text{Error} = \sigma \]
the starting point of our analysis. The analysis starts by using the standard deviation to calculate the range of the individual constants. Using this analysis gives a direct method to calculate the range of the fitted displacement and the derivative of the displacement. The variation of the derivative can be used to calculate the variation of the strain. However, there are different methods how to deal with the range of the constants, firstly a method which uses the largest absolute value possible to deal with the range of these constants.

Starting from the quadratic least squares fit:

\[ u = C_1 x^2 + C_2 xy + C_3 xz + C_4 y^2 + C_5 yz + C_6 z^2 + C_7 x + C_8 y + C_9 z + C_{10}. \]  

(32)

And assume that each constant has its \( \hat{C}_i \) as its range we can calculate the range for \( u \):

\[ u = (C_1 + \hat{C}_1)x^2 + (C_2 + \hat{C}_2)xy + (C_3 + \hat{C}_3)xz + (C_4 + \hat{C}_4)y^2 + \]

\[ (C_5 + \hat{C}_5)yz + (C_6 + \hat{C}_6)z^2 + (C_7 + \hat{C}_7)x + \]

\[ (C_8 + \hat{C}_8)y + (C_9 + \hat{C}_9)z + (C_{10} + \hat{C}_{10}). \]  

(33)

As can be seen in Equation 33 each of the added ranges is linear which gives us directly the range of \( u \) which we call \( \Delta u \):

\[ \Delta u = \hat{C}_1 x^2 + \hat{C}_2 xy + \hat{C}_3 xz + \hat{C}_4 y^2 + \]

\[ \hat{C}_5 yz + \hat{C}_6 z^2 + \hat{C}_7 x + \]

\[ \hat{C}_8 y + \hat{C}_9 z + \hat{C}_{10}. \]  

(34)

Since a constant can either be positive or negative it is likely that results from Equation 34 are lower than expected. In order to calculate the worst case scenario the absolute value of each individual part is taken and added, then Equation 34 looks like:

\[ \Delta u = |\hat{C}_1 x^2| + |\hat{C}_2 xy| + |\hat{C}_3 xz| + |\hat{C}_4 y^2| + \]

\[ |\hat{C}_5 yz| + |\hat{C}_6 z^2| + |\hat{C}_7 x| + \]

\[ |\hat{C}_8 y| + |\hat{C}_9 z| + |\hat{C}_{10}|. \]  

(35)

In order for us to calculate the range of the strain, the range of the derivatives must be calculated which is:

\[ \Delta \left( \frac{du}{dx} \right) = 2|\hat{C}_4| \]  

\[ \Delta \left( \frac{du}{dy} \right) = |\hat{C}_2| \]  

\[ \Delta \left( \frac{du}{dz} \right) = |\hat{C}_3| \]  

(36)

(37)

(38)

The equation for strain in three dimensions for \( \varepsilon_{xx} \):

\[ \varepsilon_{xx} = \frac{du}{dx} + \frac{1}{2} \left( \frac{du}{dx} \frac{dv}{dx} + \frac{dv}{dx} \frac{dw}{dx} + \frac{dw}{dx} \frac{du}{dx} \right). \]  

(39)
And for the shear strain $\varepsilon_{xy}$ the following equation exists:

$$
\varepsilon_{xy} = \frac{1}{2} \left( \frac{dv}{dx} + \frac{du}{dy} + \frac{dv}{dx} \frac{du}{dy} + \frac{dv}{dx} \frac{dv}{dy} + \frac{dw}{dx} \frac{dw}{dy} \right). 
$$

(40)

If the derivatives are written in the following manner:

$$
\frac{du}{dx} = \frac{du}{dx} + \Delta \frac{du}{dx}. 
$$

(41)

For the nonlinear parts $\frac{dw}{dx} \frac{dw}{dy}$ extra calculations are needed

$$
\frac{dw}{dx} \frac{dw}{dy} = \left( \frac{dw}{dx} + \Delta \frac{dw}{dx} \right) \left( \frac{dw}{dy} + \Delta \frac{dw}{dy} \right). 
$$

(42)

which leads to:

$$
\frac{dw}{dx} \frac{dw}{dy} = \frac{dw}{dx} \frac{dw}{dy} + \Delta \frac{dw}{dx} \frac{dw}{dy} + \Delta \frac{dw}{dx} \frac{dw}{dy} + \Delta \frac{dw}{dx} \frac{dw}{dy}. 
$$

(43)

Again this is leads to a part which is the same as in the previous calculation. This can be used to calculate the strain.

The second method that is used to calculate the difference in the strain is not just adding all the absolute values but using a squared error measure which is explained here briefly. Starting from the same quadratic formula as before:

$$
u = C_1 x^2 + C_2 xy + C_3 xz + C_4 y^2 + C_5 yz + C_6 z^2 + C_7 x + C_8 y + C_9 z + C_{10}. 
$$

(44)

Again assume that each constant has its $\hat{C}_i$ as its range. The range of $u$ can be calculated, however now the squared response is used with the range of the constants squared.

$$
u^2 = ((C_1 + \hat{C}_1) x^2)^2 + ((C_2 + \hat{C}_2) xy)^2 + ((C_3 + \hat{C}_3) xz)^2 + ((C_4 + \hat{C}_4) y^2)^2 + ((C_5 + \hat{C}_5) yz)^2 + ((C_6 + \hat{C}_6) z^2)^2 + ((C_7 + \hat{C}_7) x)^2 + ((C_8 + \hat{C}_8) y)^2 + ((C_9 + \hat{C}_9) z)^2 + ((C_{10} + \hat{C}_{10}))^2. 
$$

(45)

The previous analysis can be used to calculate the error that occurs in the strains.
The results of the accuracy calculation in three dimensional analysis is shown in the following tables (Table 1 for the absolute method and Table 2 for the squared method). The tables with the results show that the fits are highly unreliable. Clearly, these tables show a large amount of inaccuracy, this is also reflected the resulting range of the displacement, which can be seen in Figure 16. In Figure 16, 3 lines are shown, the blue line is the fitted response of the displacement $u$ along the x-direction, the values of y and z are chosen such that this is the middle line in the 3D environment. From Figure 16 the blue line is the fitted response of the displacement and the red line is the ranges added to the constants, the green line is the fitted response with the ranges deducted from the constants. Thus, the range of the displacements is quite large and the response of the strain (which is dependant on the derivative of the displacement) also has a large range. These large ranges show the reason for the large inaccuracy of the strain. In order to improve on the errors created here an investigation into the reasons behind these errors is needed. It is know from the confocal microscope that the error is approximately 0.24 to 0.32 $\mu$m. Another point of interest is the number of constants versus the number of datapoints which can be improved by using a two dimensional analysis. Both these problems are addressed in the following section.

<table>
<thead>
<tr>
<th>Table 1: Results of the three dimensional accuracy calculation absolute method showing the relative error</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ε</strong></td>
</tr>
<tr>
<td>$\varepsilon_{xx}$</td>
</tr>
<tr>
<td>$\varepsilon_{xy}$</td>
</tr>
<tr>
<td>$\varepsilon_{xz}$</td>
</tr>
<tr>
<td>$\varepsilon_{yy}$</td>
</tr>
<tr>
<td>$\varepsilon_{yz}$</td>
</tr>
<tr>
<td>$\varepsilon_{zz}$</td>
</tr>
</tbody>
</table>

Table 2: Results of the three dimensional accuracy calculation squared method showing the relative error

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ε</strong></td>
</tr>
<tr>
<td>$\varepsilon_{xx}$</td>
</tr>
<tr>
<td>$\varepsilon_{xy}$</td>
</tr>
<tr>
<td>$\varepsilon_{xz}$</td>
</tr>
<tr>
<td>$\varepsilon_{yy}$</td>
</tr>
<tr>
<td>$\varepsilon_{yz}$</td>
</tr>
<tr>
<td>$\varepsilon_{zz}$</td>
</tr>
</tbody>
</table>
Figure 16: Displacement $u$ as a function of $x$, in which blue is the fitted response, red is the range of the constants added by the fitted response and green is the range of the constants subtracted from the fitted response.
6.1 Accuracy confocal microscope

For construct studies a confocal microscope is generally used to view the deformation of a chondrocyte. Since the information given by the microscope is used to calculate different models, it is of great importance to evaluate the error that is made by using this information. A confocal microscope like all other equipment has a certain accuracy range, in the microscope that is used in these experiments the range is 0.24 to 0.32 µm. In order to see how such an error relates to an error in the position of the microbeads, a simulation of a microbead moving through a sensor-grid is used. This simulation creates a sphere (which resembles a microbead) and moves (movements are chosen to be comparable to the actual movements that were found in the experiments) this bead through a grid. During its movement the center of the bead is compared with its digitalized counterpart the bead in the sensorgrid. This comparison gives a direct calculation of the error that is created by the digitalization of the microbead.

![Figure 17: Digitalization of circle displacement: Red square shows real-grid and blue square shows sensorgrid](image)

In Figure 17 several (81) small rectangles are shown, one of which is colored red this is what is called realgrid. The realgrid is a grid which is used to calculate if a sensor-grid square should be filled. The sensorgrid is shown in the top left corner of Figure 18 in this corner there are 9 blue rectangles that fill up a large square, this large square is part of the sensorgrid. The program

![Figure 18: Digitalization of circle displacement: A bead in the sensorgrid](image)

starts by creating a circle just as is seen in Figure 18. After the placement of the circle the program decides which realgrid rectangles should be filled. For Figure 18 the solution of filling the squares is shown in Figure 19. In order to see which part of the sensorgrid should be filled, the program decides this on
the number of realgrid rectangles that are filled for instance in Figure 19 the top left sensor-grid rectangle has 3 blue colored squares and 6 empty squares, thus it should not be filled, because there are more empty than full squares. If the same rule is applied to fill the sensorgrid squares, then the following results are achieved, which are shown in Figure 20.

Figure 19: Digitalization of circle displacement: A bead filled out in the real grid

Figure 20: Digitalization of circle displacement: The resulting image of the digitalization of a bead
Since these calculations can be done at any displacement step, the difference between the input movement of the circle and the resulting digitalized movement can be calculated. In Figure 21 a circle moving in 2 rows is shown, the upper row shows the real simulation and the lower shows the digitalized simulation (this situation is a bit exaggerated). This simulation can be used to predict the error made in the calculation of the displacement of the microbeads due to the errors in the confocal microscope. These displacements lead to the following graphs (Figure's 22, 23, and 24) in which the error in the displacement divided by the sensorgrid versus the size of the circle divided by the sensorgrid. Both sides where divided with the sensor grid thus the results are independent of the size of the sensorgrid.
Figure 22: Results of circle displacement: On the y axis the error in displacement $u$ divided by the sensor grid and on the x axis the radius of the microbeads divided by the sensor grid.

Figure 23: Results of circle displacement: On the y axis the error in displacement $v$ divided by the sensor grid and on the x axis the radius of the microbeads divided by the sensor grid.
Figure 24: Results of circle displacement: On the y axis the displacement \( w \) divided by the sensorgrid and on the x axis the radius of the microbeads divided by the sensor grid
Looking at the possible deviation from the accuracy of the confocal microscope, the xy deviation is about 10 times smaller than the z deviation (these results are shown in Table 3). From this analysis it is clearly shown that the error is the highest in the z-direction, and that the error in the calculation in the xy plane is quite small. The inaccuracy of the z-direction can be a large source of errors when calculating a three dimensional model thus showing that a two dimensional model is currently preferred due to the limitations of the confocal microscope.

Table 3: Results of the matlab displacement program the error is the absolute error in position during displacement.

<table>
<thead>
<tr>
<th></th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>$u$</td>
<td>0.048 $\mu$m</td>
</tr>
<tr>
<td>$v$</td>
<td>0.051 $\mu$m</td>
</tr>
<tr>
<td>$w$</td>
<td>0.45 $\mu$m</td>
</tr>
</tbody>
</table>
6.2 Two dimensional analysis

In the simulation of the microbead displacement it is shown that the z direction is the direction which contains the largest amount of error. In order to minimize the influence of the z direction a two dimensional model is used. This two dimensional model uses a new data set which however this data comes from the same image’s. The difference between the data from the 3D set compared 2D set, is that in the 2D set all z displacements are removed and all z positions are fixed in one plane. One of the reasons of a new data set is that in the previous calculations the number of fitted constants versus the available datapoints is quite low. In order to increase the number of available data points only the first displacement is observed. The reason this results in more data points is quite simple due to the rigidbody movement of the gel microbeads will displace outside the field of view(microbeads outside the field of view can not be tracked since their end location is unknown). Figure 25 shows the standard deviation as well as the fitted displacement of the least mean squares method. It is clear that the fit is averaging out results just as expected with least mean squares method. Another benefit of having 2D data is that it can be shown in 3D (see Figure 26 for QLMS and Figure 27 for Kriging) From these fits the differences between the methods can be seen. The difference between Kriging and QLMS is that Kriging will fit every displacement while least mean squares fit averages out the individual displacements to a acquire a mean fit. The differences these methods have are also evident in the in the calculated strains. Note that due to the quadratic fit the strains are linear functions which are difficult to compare with the bumpy response of the Kriging fit. However the same calculations as before can be used to calculate the accuracy of the 2 dimensional quadratic fit. The results are shown in the following tables: If these numbers are compared

Table 4: Results of the two dimensional accuracy calculation absolute method, showing the relative error

<table>
<thead>
<tr>
<th></th>
<th>1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε_{xx}</td>
<td>128 %</td>
</tr>
<tr>
<td>ε_{xy}</td>
<td>447 %</td>
</tr>
<tr>
<td>ε_{yy}</td>
<td>60 %</td>
</tr>
</tbody>
</table>

with the three dimensional analysis, it is obvious that these models show less error. Since the error is reduced it would seem that the steps used to improve

Table 5: Results of the two dimensional accuracy calculations squared method, showing the relative error

<table>
<thead>
<tr>
<th></th>
<th>1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε_{xx}</td>
<td>58 %</td>
</tr>
<tr>
<td>ε_{xy}</td>
<td>60 %</td>
</tr>
<tr>
<td>ε_{yy}</td>
<td>59 %</td>
</tr>
</tbody>
</table>
6 ACCURACY

the overall accuracy work. These steps included reducing the amount of fitable constants increasing the amount of datapoints and to remove the direction in which these datapoints are inaccurate.
Figure 25: $u$ as a function of $x$ on the left and $v$ as a function of $y$ on the right, red lines are the fits and ranges are the standard deviation, both of the fits are QLMS fits.
Figure 26: Top figure $u$ as a function of $x$ and $y$ with the black dots as the datapoints, bottom figure $v$ as a function of $x$ and $y$ both are quadratic least square fits
Figure 27: Top figure $u$ as a function of $x$ and $y$ with the black dots as the datapoints, bottom figure $v$ as a function of $x$ and $y$ both are Kriging fits
Figure 28: Top figure $\varepsilon_{xx}$ as a function of $x$ and $y$ fitted with QLMS, bottom figure $\varepsilon_{xx}$ as a function of $x$ and $y$ fitted with Kriging, red is 8% tensile strain, blue is 8% compressive strain, green indicates no strain.
Figure 29: Top figure $\varepsilon_{xy}$ as a function of x and y fitted with QLMS, bottom figure $\varepsilon_{xy}$ as a function of x and y fitted with Kriging. Red is 5% tensile strain, blue is 5% compressive strain, green indicates no strain.
Figure 30: Top figure $\varepsilon_{yy}$ as a function of $x$ and $y$ fitted with QLMS, bottom figure $\varepsilon_{yy}$ as a function of $x$ and $y$ fitted with Kriging, red is 5% tensile strain, blue is 5% compressive strain, green indicates no strain.
7 Discussion

7.1 Cells culture

Cells that were used are bovine cells, which of course are only a resemblance to humanoid cells however, it is near impossible to acquire healthy humanoid cells.

Due to the creation process of the Alginate many microbeads are lost in the calcium chloride solution, this can be solved by either changing the density of the filters or choosing for another gelling material (however these have their own downsides).

The Microspheres tend to clutter around each other this can influence the deformation around a cell even though there are not a lot microspheres.

7.2 Image processing

Currently identification of microbeads between deformation steps is done manually, it might be beneficial to apply a mathematical procedure for this. One of the major flaws in these models is that they will have an amount of error due to either the fit being too inflexible, or the fit is too flexible thus creating an overfitting problem.

Due to the biphasic nature of alginate and chondrocytes and the fact that the analysis can not deal with any time related responses the timing is critical to generate unbiased results.

7.3 Mathematical modelling

A real problem with least square fitting is that the constants needed to create a fit grows fast when examining higher degree polynomials, though complex deformation fields might require this.

Another problem using interpolation methods is that they exhibit the same behavior between fitted points however their information outside the fitted points is usually very inaccurate.

Kriging always fits the exact displacements having data with contains some form of noise can lead to responses driven by this noise. Differentiating these results can lead to the noise becoming so large that it influences the results.

Using least squares method will result in the data being averaged out, due to the averaging nature of the method some results can be missed.
8 Conclusions and Recommendations

8.1 Cells and culture

It might be interesting to see how generated tissue deforms from an alginate sample using this technique and comparing this to a sample of alginate without cells and tissue.

8.2 Datamining

Having more data with more tests can lead to better analysis of current strain fields.

Waiting longer than 2 min before taking pictures can decrease the biphasic non linear response thus increase the accuracy of the responses between different samples.

It might be beneficial to do an outlier analysis on the current data points. An outlier is a datapoint, which deviates greatly from the rest of the data. Due to this deviation it will affect the models greatly. If these outliers are removed the results of these models should improve.

In the current experiment the images where taken at the same location inside the alginate. Doing so will result in microbeads moving from the field of view (which becomes an increasing problem as the strain on the cell increases). It can be beneficial to move the field of view to continuously have the cell of interest in the center of the field of view. The amount of microbeads moving from the field of view should be reduced especially with large deformation on the alginate sample.

Another method to increase the accuracy is to only take one deformation stage instead of the three stages that are currently used. This should also lead to less data points moving outside the image area.

Using more microbeads can be considered as a step to also increase the data points to constants ratio, however this can also compromise the overall response of the cell.

8.3 Mathematical modelling

The datapoints around the cell only give displacement information around the cell they give little to no information of the deformation of the cell. It is possible to combine the current fit techniques with the method to calculate cellular strains according to Lee et al. (2000).

In order to correctly model deformations around a cell higher order least square fits can be required however these do need more data to correctly fit the data, which is always a difficult choice, and can not be made on just a few data sets.
References


