Quantification of cytoskeletal deformation in living cells

Quantification of Cytoskeletal deformation in Living Cells

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In order to get a better insight in the mechanisms causing tissue damage there is an interest from within the biology community to quantify cellular deformations upon external loading. The cytoskeleton plays an important role in the transmission of forces throughout the cell. This study aims to quantify deformation by applying image matching algorithms on confocal microscopy images.

Introduction

In biology, various cell types are sensitive to changes in mechanical loading. On the short term physiological mechanical loading leads to cell response. For instance upon increased load in the heart, muscle cells immediately increase their contractile force. In blood vessels, increased shear stress on endothelial cells leads to cellular deformation and subsequent vasodilation, mediated by chemical signals from the endothelial cells to the underlying smooth muscle cells. On the long term, increased mechanical loading leads to changes in cellular gene expression, resulting in a more adequate function. Prolonged pathological mechanical loading can also be harmful for living cells, which lose their ability to adapt to the changed environmental conditions. For instance, prolonged external mechanical loading on skeletal muscle can lead to pressure sores, characterized by necrosis of the tissue [1].

External forces can be transduced mechanically to the cell nucleus via an intracellular network of proteins, composing the cytoskeleton [2] (Figure 1). The cytoskeleton is the internal framework of the cell, composed of three different types of molecular polymers (Figure 2). The first type are the thick filaments or microtubules. They are relatively thick structures with one end anchored in the centrosome near the nucleus and the other free in the cytosol; this end can lengthen and shorten very quickly. The second type of filaments are the thin filaments, composed of actin. They can be found mainly near the cell membrane. The third type of filaments are the intermediate filaments which extend from the cell nucleus to the cell periphery. Several types of intermediate filaments exist, such as vimentin.

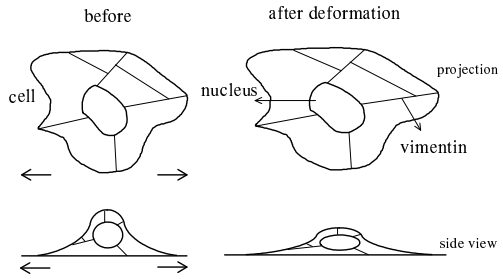

Figure 1: The cytoskeleton is involved in the transmission of forces throughout the cell.

In order to get a better insight in the mechanisms which actually cause cellular adaptation or damage it is necessary to get access to strains and stresses inside the tissue as a whole, and thus in its composing cells. Deformation of the cytoskeleton caused by mechanical loading can be measured in living cells. The aim of this study is to quantify the deformation of the cytoskeleton by applying image analysis techniques on confocal microscopy images. In this study the intermediate filament vimentin was

chosen as a representative for cytoskeletal deformation. Vimentin filaments were selected because they are in close contact with the cell membrane as well as with the nucleus. In order to visualize the displacements and deformations of the vimentin filaments, cultured living Chinese Hamster Ovary (CHO) cells were used. In these cells, the vimentin filaments were fluorescently labelled through the coupling with the Green Fluorescent Protein (GFP). Natural spontaneous movements of these cells, and thus of the cytoskeleton inside, were followed using confocal microscopy [3].

The dynamic behaviour of the intermediate filament network in living cells has been studied before using GFP [4, 5]. However these studies were limited to the calculation of displacements of vimentin dots and end points of vimentin filaments. In contrast, this study aims at the development of a method which can be used to quantify the deformations of the entire vimentin cytoskeleton.

To quantify deformations of the cytoskeleton, the applied image analysis should meet certain criteria. The most important requirements are that the deformation of the entire cytoskeleton should be quantified and that it should be possible to quantify deformations up to maximal physiological deformation. The Hierarchical Feature Vector Matching (HFVM) program was applied to determine the displacements and deformations of the cytoskeleton.

Hierarchical Feature Vector Matching

Hierarchical Feature Vector Matching (HFVM) was developed at the Institute of Digital Image Processing of Joanneum Research in Graz, Austria. Originally, it was developed to create a 3-dimensional model of a scenery like, for instance, a planetary surface. Two 2-dimensional images generated by CCD cameras are processed; a so-called *disparity map* is computed. The disparity map indicates for each pixel of one of the input images, which pixel of the other image represents the same spot in the scenery. From the disparity map, the *Digital Elevation Model* can be computed, if the exact positions of the cameras and their optical properties are known. HFVM is a robust algorithm which can be used for all sorts of applications, such as 3D surface reconstruction [6], navigation of spacecraft or autonomous robots [7], the visualization of weather satellite images [8], and, as described here, for the quantification of cytoskeletal deformation [9].

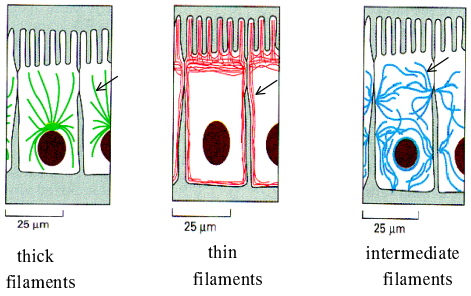

Figure 2: Constituents of the cytoskeleton.

Two equally sized images of the scenery are generated, and stored in a computer memory as two grids of pixels. Each pixel has a grey-level. To distinguish between the two images, one is called the *reference image* and the other the *search image*. A reference pixel is said to *correspond* with a search pixel if the reference pixel represents the same real-world location as the search pixel. However, we need to be careful with this definition. As we work with discrete grids of the images, the exact position that corresponds with a reference pixel may be *in between* several pixels in the search image.

Matching

Matching is performed to compute which reference pixels correspond with which search pixels. From the results, the *disparity map* is constructed. The disparity map contains the horizontal and vertical displacements of the search pixel with respect to the reference pixel. When a piece of the scenery is only visible in one image, for some pixels no match will be found. Most matching algorithms are based on the fact that two corresponding pixels have corresponding neighborhoods. One method frequently used is based on cross correlation between grey level values of pixels in the respective neighborhoods. Another matching technique which seems more appropriate for this application is based on Feature Vector Matching.

Feature Vector Matching

A *feature* of a pixel is a value which depends on the characteristics of the grey-levels of pixels within the neighborhood of the pixel. If we calculate the feature for all pixels of an image, we get a so-called *feature image*. We present a method to match pixels by comparing several features. Selected features are usually the local average, a number of horizontal and vertical edge operators and the local variance. Most of them are described as *convolutions* or can be approximated by means of convolutions. Applying a convolution on an image means that for each pixel a value is computed which depends on the grey-levels of the surrounding pixels and the convolution *kernel*. The result is another image containing the filtered values.

Suppose there are m features. If we denote all feature values of a certain pixel as a vector, we obtain the *feature vector* $\vec{f}$ for this pixel. From the contents of the feature images we can derive this vector for each pixel of the stereo image pair. Matching using feature vectors is called *Feature Vector Matching* or FVM. Finding a match for each pixel is performed by comparing its feature vector, the *reference vector*, to all feature vectors of the *search area*. For a pixel with coordinates (x, y), the search area is defined by the expected coordinates (i_x, j_y) and the extensions δ_i and δ_j. For all coordinates (k, l) in the search area we define the *search vector* $\vec{s}_{k,l}$ as the feature vector which belongs to (k, l) in the search image. The *search space* $\sigma_{x,y}$ is then defined as

$$\sigma_{x,y} = \{\vec{s}_{k,l} | k \in [i_x - \delta_i, i_x + \delta_i], \\ l \in [j_y - \delta_j, j_y + \delta_j]\}.$$

To compare the reference vector $\vec{r}$ to the search vectors $\vec{s}_{k,l}$ in the search space, we determine the *feature distance* $|\vec{r} - \vec{s}_{k,l}|$. The feature distance is defined such that each component of the vectors is weighted. If we denote the value of feature l of pixel p as $f_l(p)$ and the weight of feature l as w_l, then the feature distance between the vectors $\vec{r}$ and $\vec{s}$ is defined as

$$|\vec{r} - \vec{s}| = \sqrt{\frac{\sum_{l=1}^{m}((f_l(r) - f_l(s)) \cdot w_l)^2}{\sum_{l=1}^{m} {w_l}^2}}.$$

Best correspondence is found on position (k_0, l_0) in the search area, where the feature distance between the reference vector and the search vector is minimal, i.e.

$$|\vec{r} - \vec{s}_{k_0,l_0}| = (\textbf{min}\ \vec{s} : \vec{s} \in \sigma : |\vec{r} - \vec{s}|).$$

Recapitulating, Feature Vector Matching can be described as:

1. Create feature images for both images of the stereo image pair.
2. Compare each reference vector to all search vectors of the search space. Best correspondence is found where the feature distance has a minimal value. If the minimum feature distance exceeds a given threshold, the correspondence is invalid and the reference pixel is not matchable. As a result, the disparity remains undefined.
3. Remove errors and interpolate undefined disparities.

In Figure 3 Feature Vector Matching is depicted. Applying this algorithm results in a disparity map.

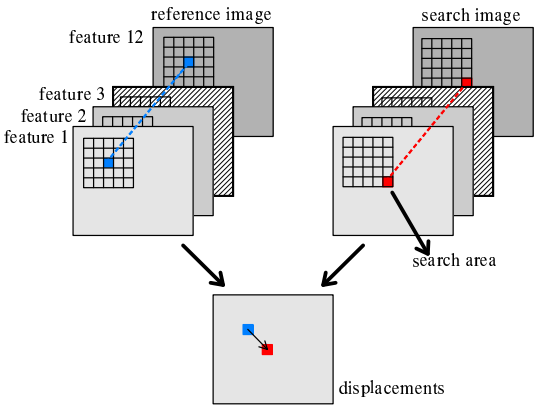

Figure 3: Feature Vector Matching.

Pyramids

Hierarchical Feature Vector Matching is an extension of Feature Vector Matching. The computation time of matching algorithms can be reduced by limiting the search areas. To construct smaller search areas, the disparity map of the same stereo image pair, but with a resolution which is half the resolution of the original image pair, is calculated. This disparity map is used as initial disparity map in order to reduce the size of the search areas in the original image. For a stereo image pair of size 512×512, 4 or 5 iterations will do. These images together are called the *pyramid*. The smallest image is the top level of the pyramid and the original image is level 0. Building the pyramid starts with the original image. To create the next level of the pyramid first a *Gaussian* convolution is performed and all pixels in

the even rows and columns are retained (*subsampling*). Applying a Gaussian convolution on an image results in an image for which each pixel is a weighted average of the original pixel and its surroundings. In Figure 4, the creation of the pyramid is visualized.

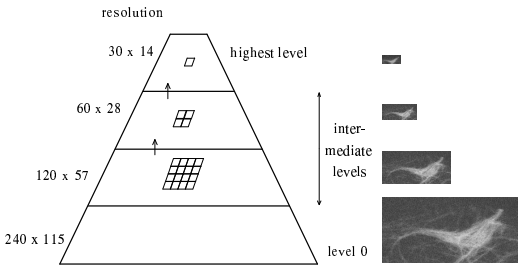

Figure 4: Pyramid of images, with level 0 the original input image.

Matching starts at the top level of the pyramid. The result of the matching algorithm is a disparity map. This map has to be filtered and the undefined disparities have to be interpolated, before it can be used as initial disparity map for matching the next, lower, level of the pyramid.

In order to assure that the disparities from the reference to the search image are of good quality, matching is performed in two directions. Consistency checking is done by *backmatching*. For each pixel r of the reference image, the matching pixel in the search image s is obtained by applying the disparity map. Next, for pixel s the matching pixel in the reference image r' is obtained by applying the second disparity map. If the distance between r and r' exceeds a specified distance, say 1 pixel, the match will be defined as invalid.

Hierarchical Feature Vector Matching can be described as follows: for both images of the stereo image pair the following steps have to be taken:

1. Build the pyramid.
2. Compute the feature images for each pyramid level.
3. Match the top level of the pyramid.
4. Filter the resulting disparity map.
5. Check matching consistency by backmatching.
6. Interpolate the undefined disparities.
7. Use the resulting disparity map as initial disparity map to match the next, lower, pyramid level.

Steps 4 through 7 are repeated till a disparity map of level 0 is computed. The HFVM method is depicted in Figure 5.

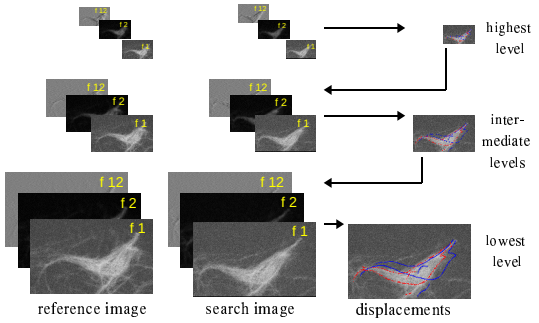

Figure 5: Hierarchical Feature Vector Matching.

Results

After optimalisation of the parameters and the feature set, the HFVM program was applied on images of the spontaneous movement of the cytoskeleton of living cells [3]. To this end, seven images (each taken every five minutes) of a time series of the spontaneous movement of the cytoskeleton were matched. In the first image, points belonging to the cytoskeleton were selected. Of the selected points, 98% could be traced until the last image. However, due to discretisation errors which occur when matching each intermediate image with the previous image in the time series, the structures found after matching were not smooth and did not always coincide with the fibers. If the changes in the local characteristics between successive images were too large, the program was not able to find enough matches with high reliability.

The results of matching the first and last image of the time series are depicted in Figures 6 and 7.

The total displacement in both horizontal and vertical directions is depicted in Figure 8. Maximum displacement measured is 42 pixels, which is about 2.1 μm.

In Figures 9 and 10 details are shown of the matching results with and without intermediate matching.

Notice for instance the different matching results at the intersection of two fibers in the lower right corner of the two images.

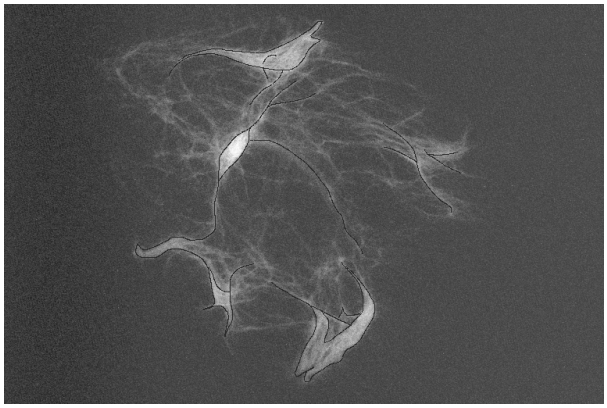

Figure 6: First image of time series with pixels belonging to the cytoskeleton selected (selected pixels are black).

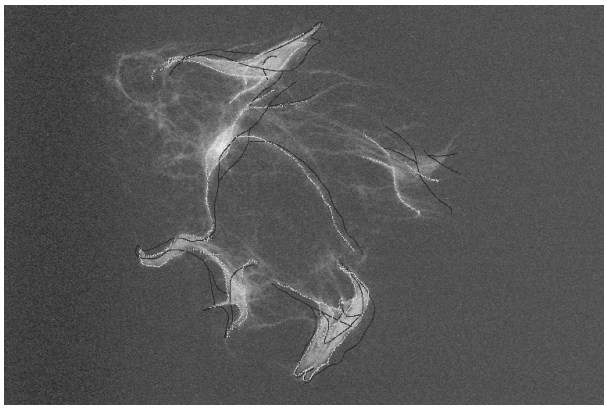

Figure 7: Matching results after applying HFVM on first and last image of time series (matches for selected pixels are white).

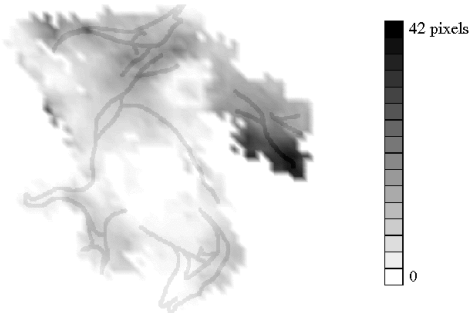

Figure 8: Total displacement field.

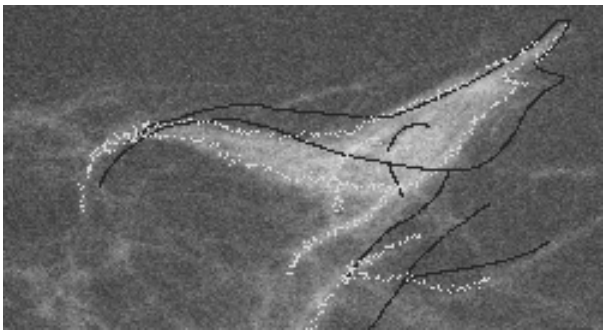

Figure 9: Detail of matching results with intermediate matching.

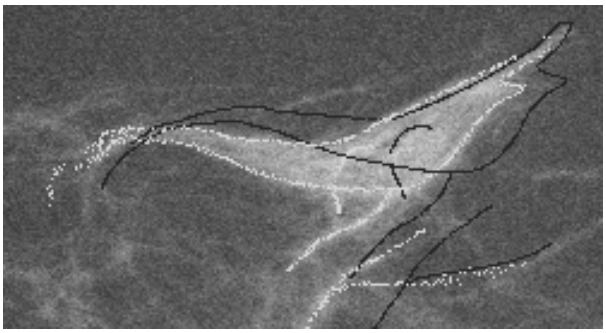

Figure 10: Detail of matching results without intermediate matching.

Conclusions and Future Directions

The aim of this study was to quantify cytoskeletal deformation. The requirements for the applied image analysis were: quantify deformations of the entire cytoskeleton with the size of the deformations up to maximal physiological loading, the analysis must not be hampered by rotation or translation of the cell during the image acquisition, and determine deformations in three dimensions, if possible from multiple image stacks.

The matching results of HFVM are very good when matching two images of natural deformations of the cytoskeleton. Approximately 98% of the points in the image belonging to the cytoskeleton could be matched with points in the last image of a time series of images of the spontaneous movement of the cytoskeleton, taken thirty minutes after the first image.

When matching the complete time series of images, the percentage of points that could be traced is still approximately the same. The global displacement field of the cytoskeleton is correct, but locally the

matched points start to let go of the filaments. This is due to the accumulation of discretisation errors. After seven image matching steps the matching results do not coincide with the filaments.

A more structure-based matching algorithm, that allows tracing of corresponding structures, such as complete cytoskeletal filaments, in a series of images, can be a solution to this problem. The ultimate goal of this research project will be to determine cytosckeletal deformations inside the cell in three dimensions. By describing the structures by means of graphs, the image matching transforms into a graph matching problem. If the graphs can be obtained from a stack of images, the graph matching can also be applied to solve the matching problem in three dimensions. We refer to [9] for more details on this idea of structure matching.

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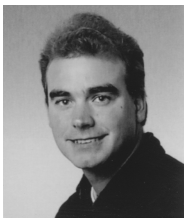
About the authors

Saskia van Engeland graduated in Applied Physics at the Eindhoven University of Technology (TUE) in August 2000. Her graduation project, which is covered by this paper, concerned the application of image matching algorithms on confocal microscopy images of the cytoskeleton. Momentarily she is working as PhD. student on the computer aided detection and interpretation of abnormalities in mammograms at the Department of Radiology of the University Medical Centre (UMC) St. Radboud in Nijmegen.



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