Chapter 5

Conclusions

5.1 Summary

A novel experimental technique for measuring three-dimensional deformation fields in soft materials has been developed and its applicability to quantitatively investigate cell-matrix interactions has been demonstrated. This method utilizes the three-dimensional scanning ability of laser scanning confocal microscopy (LSCM) in conjunction with a digital volume correlation (DVC) algorithm as described in detail in Chapter 2. The utilization of a deconvolution algorithm to account for the effects of the point spread function along the optical imaging axis, is shown to improve the resolution of the LSCM images, leading to enhanced accuracy in the calculation of the displacement fields. The ability of this technique to determine full-field three-dimensional displacement information under large deformations was demonstrated in Chapter 2. One advantage of being able to measure large deformations accurately is being able to compile the cumulative evolution of the applied deformations. While incremental deformations can be added up to yield a cumulative representation of the deformation state, any perturbations or noise will not only affect the single measurement time point but rather the entire time evolution. Hence, a cumulative deformation evolution represents a more robust and potentially more accurate measurement.

The application of the LSCM-DVC technique to study the interaction of motile fibroblast cells with polyacrylamide substrates was discussed in Chapter 4. The results demonstrate the capability of this method to accurately map the cell-induced deformation fields during cell migration, and the

viability of determining the traction forces in three dimensions. While previous traction force methods provided significant insight into cell-matrix interactions in two dimensions, the newly developed technique demonstrates that these interactions can now be quantified in all three dimensions with high spatial resolution. Furthermore, all of the presented investigations in Chapter 4 were obtained dynamically with respect to the average speed of cell migration, allowing for an *in-situ* analysis of cell motility and the resulting mechanical interactions with the substrate. While previous studies focused primarily on quantifying traction forces and adhesion of spread cells [14, 41, 49], this study highlights the more dynamic substrate interactions of motile cells. While the main results of this study present quantitative experimental findings of cells interacting with soft biomaterials, in particular polyacrylamide gels of different moduli, Section 5.3 will provide an outlook on some of the experimental implications and future applications of these measurements and the LSCM-DVC technique.

5.2 Preliminary Cell Migration Studies on Artifical Extracellular Matrix Proteins (aECM)

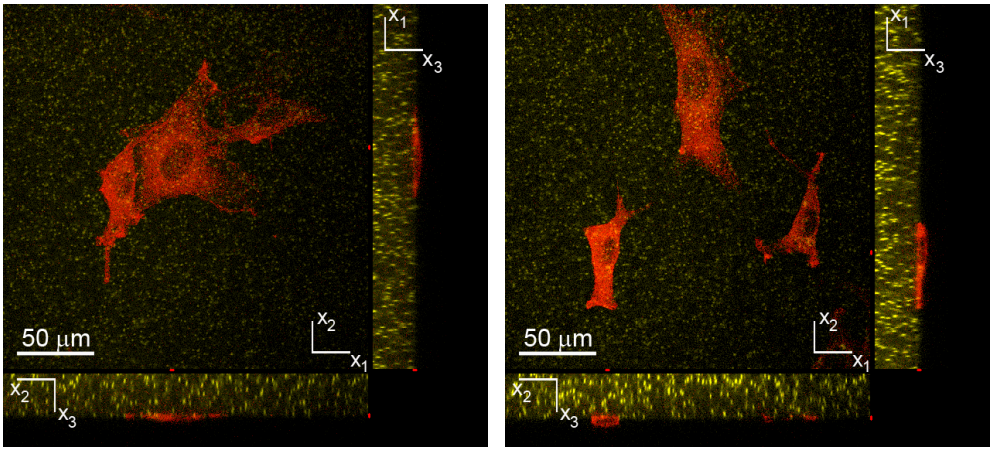
This section provides some preliminary results on 3T3 fibroblast cells migrating on artificial extracellular matrix proteins (aECM) developed by Tirrell and coworkers [28, 21]. This new class of biomaterials offers greater control and versatility than traditional biomaterials since their molecular structure can be precisely controlled through genetic engineering. These proteins are designed to be employed as implant materials mimicking the key features of the natural extracellular matrix [28, 21]. Since, a cell's response is most often comprised of a variety of biochemical and biomechanical stimuli at a particular point in time, decoupling these signals requires information on the spatial and temporal location of these cell-matrix exchanges. In other words, by controlling the exact composition of the extracellular matrix, cell-specific signals and cues can be analyzed and potentially controlled. In brief, these novel biomaterials incorporate certain amino repeat sequences to control both a specific cell binding domain, e.g., RGD, CS5, etc., as well as their mechanical

properties. These materials can be crosslinked into thin films or three-dimensional matrices through the incorporation of the photosensitive non-canonical amino acid *p*-azidophenylalanine (pN$_3$Phe). Exposure of pN$_3$Phe to ultraviolet (UV) light results in the photodecomposition of the aryl azide, which mediates non-specific crosslinking, either by electrophilic trapping via ring expansion or by the diradical behavior of the triplet nitrene. Thus, this technique is well suited for fabrication of particular thin film geometric patterns [8]. Furthermore, the mechanical properties, such as the Young's modulus, can be tuned by altering the incorporation level of pN$_3$Phe with a typical modulus range of 0.3 - 1.0 MPa [33].

The experimental setup utilized is similar to the one described in Chapter 3 and hence, only the differences are discussed here. aECM proteins incorporating photosensitive pN$_3$Phe residues with elastin-based repeats for mechanical integrity and RGD cell binding domains are expressed and purified, dissolved, and deposited onto glass coverslips yielding typical thicknesses of 50 - 80 μm. As the protein is dissolved in dimethylsiloxane (DMSO), 0.5 μm fluorescent red microspheres are mixed with the protein solution similar in fashion to the preparation protocol described in Chapter 3. The final step consists of crosslinking the protein, which is achieved through UV irradiation for several minutes. Next, GFP-actin expressing cells are seeded on the top surface of the aECM matrices and imaged in 45 min time increments over time periods up to 24 hours. The imaging setup and the imaging conditions are the same as described in Chapter 3.

Figure 5.1 shows that the cells appear well adhered to the aECM substrate and remain motile and alive after several hours of imaging. Comparing the general cell shape in Fig. 5.1, the fibroblast seeded on top of the aECM protein substrates are spread over a larger area and are thinner in cross-section when compared to the fibroblast seeded on top of the presented polyacrylamide gels in Chapter 4. A very similar cell morphology is observed when fibroblasts are placed on glass substrates indicating a potential connection between cell shape and substrate Young's modulus.

Figure 5.2 shows preliminary cell displacement measurements during fibroblast migration on the aECM substrate shown in Fig. 5.1. It is important to note that the generally observed displacements in Figs. 5.2(a) - 5.2(d) are indiscernible from measurement noise, whose threshold was previously

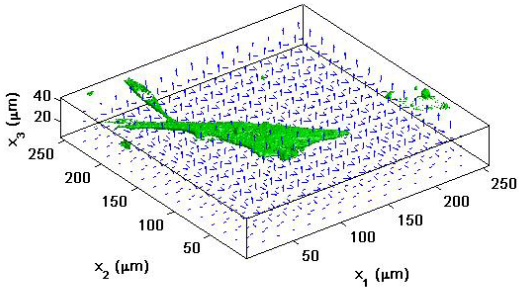


(a) LSCM composite image at t_0 (beginning of imaging)

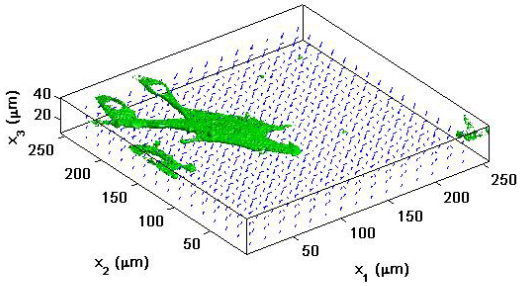
(b) LSCM composite image at $t_{10} = 450$ min

Figure 5.1: LSCM image depicting three arbitrary planar slices of the entire volumetric image stack at two different imaging times. Fibroblast cells displaying GFP-actin are shown in red, whereas the 0.5 µm fluorescent microspheres are shown in yellow.

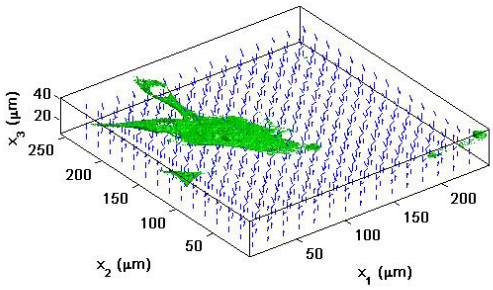
established ~ 0.12 µm. One possible explanation is that cells have a finite amount of internal force they can generate and transmit to the substrate. Hence, the resulting surface deformations due to the higher substrate stiffness cannot be detected by the optical methods employed here. It is also worth noting that most traction force studies typically report Young's moduli in the range of $\sim$ 0.1 - 25 kPa [14, 49, 41]. New proteins are currently being designed to increase the compliance of the aECM substrates by increasing the molecular weight between individual crosslinks, such that the LSCM-DVC can be successfully employed to study cell-induced surface deformations and their connection to specific biochemical stimuli. These studies have great potential in providing deeper insight into the biomechanochemical coupling during cell-matrix interactions.



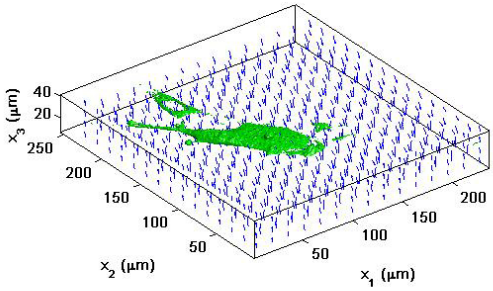
(a) Measured cell displacement fields at $t_1 = 45$ min



(b) Measured cell displacement fields at $t_3 = 135$ min



(c) Measured cell displacement fields at $t_5 = 225$ min



(d) Measured cell displacement fields at $t_7 = 315$ min

Figure 5.2: Time series of the LSCM-DVC measured three-dimensional displacement vector fields. The average vector length in each plot is between 0.06 - 0.18 µm. The fibroblasts cells are superimposed in green (GFP-actin).

5.3 Recommendation for Future Work

As demonstrated, the LSCM-DVC technique is capable of determining full-field three-dimensional displacement and strain information inside transparent materials with subpixel or submicron resolution. Also, the addition of a stretch-correction algorithm was shown to improve the overall precision of the methodology, especially under large deformation applications (strain $> 5\%$). However, in the large deformation formulation presented, only the three principal stretches were considered excluding both large shear deformations and rotations. In the future, the inclusion of the full stretch and rotation tensors should provide an even more accurate and precise full-field displacement measurement. While one study has addressed the issue of finite rotations [4], there has been no study to date that accounted for all 12 degrees of freedom. While this implementation, namely of the entire deformation gradient, is straightforward in the two-dimensional case of digital image correlation (DIC), it is non-trivial in three dimensions due to the high computational cost. The development of time-effective algorithms to incorporate both finite rotations and stretches in three-dimensional should alleviate this issue in the future. In particular, one of the potential next steps in the continued evolution of the DVC algorithm presented here is the incorporation of the already existing finite rotation algorithm into the stretch-correlation correction. Since DVC is not dependent on any particular image capturing method, its application might find further application in other imaging techniques, such as magnetic resonance imaging (MRI) and computer tomography (CT) scanning, where the only necessity is the generation of volumetric images entailing some sort of speckle or trackable pattern.

The combination of LSCM and DVC can lead to numerous quantitative applications in particular, but not exclusive, to cell mechanics. In particular, the method can be used to quantitatively study local force transmission by monitoring certain focal adhesion proteins and their force transmission locally using a smaller field of view. This can be achieved by using submicron tracker particles in conjunction with fluorescently labeled focal adhesion proteins, such as vinculin, talin, etc. Furthermore, interactions between multiple cells, including cell clusters and sheets, can be investigated quantitatively using the LSCM-DVC technique. In addition the development of novel biomaterials

relies on the capability of actively controlling or stimulating a particular cell response with the goal of reproducing the natural cell-cell or cell-matrix behavior. This technique has the ability to assist in the quantitative correlation between biomechanical and biochemical events. Furthermore, fundamental questions between biochemical signaling and mechanical stimulation for fully encapsulated cells, such as environmentally induced cell transformations from benign to malignant cancer cells can be addressed in a quantitative manner.

While these applications highlight the versatility of the technique to cell mechanics research, there are also many opportunities to study mechanics-based problems using the combination of LSCM and DVC. Some of these problems include contact studies, such as indentation and adhesion problems, where the contact area might be changing or non-linearities in the employed geometries make the observations difficult. For instance, some of the difficulties in calculating the mechanical properties from indentation data stems from the fact, that the contact area of the indenter and the surface interactions between the indenter and the sample surface are either unknown or poorly understood. The LSCM-DVC method could provide the deformation information needed to better understand these interactions for transparent materials. In conclusion, the quantitative three-dimensional full-field imaging technique presented here offers a new way to investigate cell-mediated mechanical interactions and three-dimensional mechanics problems with high spatial resolution.