Comparison of Pre-Processing Techniques for Fluorescence Microscopy Images of Cells Labeled for Actin
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Abstract
Automated analysis of fluorescence microscopy images of endothelial cells labeled for actin is important for quantifying changes in the size, directionality, and density of actin stress fibers. The current manual approach is laborious and inefficient. The goal of our work is to develop automated image analysis methods, thereby increasing cell analysis throughput.

Background
The formation of a well-developed actin-based cytoskeleton is important for endothelial cell integrity. The ability to define various "cellular states and phenotypes" through automated image processing techniques is of clinical interest in determining endothelial cell behavior during wound healing and metastasis processes.

• The size, directionality and density of actin stress fibers in a variety of cell types has been indicative of the cellular mechanical interaction with its environment through its adhesions. Mechanotransduction influences a variety of intracellular signaling processes and influences cell motility.

• One approach to systematically modulate the force transmitted through adhesions is to investigate the influence of the adhesion size on the cell in the range of adhesion sizes from about 100 nm to 1000 nm. A strong influence on the adhesion size on the cell in the range of adhesion sizes from about 100 nm to 1000 nm is to determine the dominant orientation of the actin fibers.

• Reduction of the homogeneous background can be seen as a problem of improving the signal to noise ratio and we achieved this via the use of Radon transform and enhancement of linear structures by linear filtering in the Radon domain.

• The changes in the actin cytoskeleton of cells on nanopatterned adhesion sites are associated with changes in other phenotypical parameters of the cells, and quantification of the actin stress fiber arrangement is required to firmly establish these relationships.

Materials and Methods

Materials
• Figure 1 shows an image of an endothelial cell at 50x zoom after acquisition from an oil immersion Carl Zeiss ApoTome inverted microscope. Ten such images were used for this analysis.

Methods
• To obtain an estimate of the cell spread area, a key step is to automatically outline the cell on the image, for which we used an approach based on geometric active contours proposed by Li et al [1].

• To obtain an estimate of the actin fiber density, a key step is to minimize the homogeneous background seen in figure 1 without compromising the integrity of the actin fiber.

• Once the homogeneous background is reduced, the next step is to determine the dominant orientation of the actin fibers for deciding the cross section angle and we used the widely employed steerable filters for orientation estimation [2].

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Results

• Figures 2 and 3 illustrate a couple of examples of good cell outline detection obtained using the method described above, while Figure 4 illustrates poor cell outline detection.

• Figures (5, 6) and (7, 8) illustrate examples of homogeneous background reduction and the orientation response determined using steerable filters. The orientation response gives a good estimate of the dominant orientation of the actin fibers.

• Reduction of the homogeneous background can be seen as a problem of improving the signal to noise ratio and we achieved this via the use of Radon transform and enhancement of linear structures by linear filtering in the Radon domain.

• The number of iterations required for the active contour to converge on the cell outline is currently empirical and we plan to develop a stopping criteria to completely automate cell outline detection.

Future Work
• Once the cell outline is detected, we will then estimate the cell spread area.

• We plan to use the dominant orientation estimate to obtain a line of cross section along which the actin fiber density will be automatically computed.

References

Figure 1. Endothelial cell image acquired at 50x zoom using an oil immersion Carl Zeiss ApoTome inverted microscope.