

## Quantitative comparison of several basic automated segmentation algorithms for identifying the cell periphery

John Elliott ( <a href="mailto:jelliott@nist.gov">jelliott@nist.gov</a> )
Alden Dima ( <a href="mailto:alden.dima@nist.gov">alden.dima@nist.gov</a> )
James Filliben ( <a href="mailto:james.filliben@nist.gov">james.filliben@nist.gov</a> )
Michael Halter ( <a href="mailto:michael.halter@nist.gov">michael.halter@nist.gov</a> )
Anne Plant ( <a href="mailto:anne.plant@nist.gov">anne.plant@nist.gov</a> )

High quality and robust image analysis routines are essential for automated analysis of large image datasets such as high-content cell-based assay data. Because automated algorithms require little human intervention, the data generated are considered to be less biased than those from image analysis methods that required manual thresholding. We tested the effect of eleven different basic automated image segmentation routines on a set of microscopy images of cells treated with high contrast fluorescent cell body and nuclear stains. Ground truth data were generated by manually outlining cells. Variations in the image collection conditions for replicate fields of view, and the use of images from two different cell types, provided the opportunity to evaluate the sensitivity of each image analysis routine to cellular features and data acquisition parameters. Several metrics for comparison of algorithms are considered. While accuracy relative to ground truth is important, robustness to different image and object characteristics such as size and shape of the object and the ratio of signal to background may also be critical for application to large and diverse datasets. Our results indicate that the higher order clustering algorithms such as k-means ( $n=4,5$ ) performed well on the analysis of this dataset. We are currently evaluating improvements in measurement quality that results from using automated image analysis routines compared with manual thresholding techniques.