Biases from model assumptions in texture sub-cellular image segmentation

Antonio Cardone, Julien Amelot, Ya-Shian Li-Baboud, Mary Brady, and Peter Bajcsy

A supervised segmentation technique classifies pixels in sub-cellular microscopy images into biologically meaningful regions with associated model bias quantification.

Actin is the most abundant protein in most multicellular animal cells. It forms a diverse array of structures, particularly filaments, that participate in important processes such as cell motility, division, and contraction. The location and structure of actin filaments used in cell motility has been studied at whole cell spatial resolution. But studies at the sub-cellular level are limited by the optical diffraction limits of light microscopes and by the destructive nature of imaging at resolutions higher than half of the wavelength of visible light. Previous studies at the sub-cellular level focused on actin interaction with myosin, another protein with which it often works in concert.\(^1\) Our work focuses specifically on actin structures, and analyzes sub-cellular regions using optical confocal fluorescent microscopy images at 200 nanometer resolution.

Segmentation techniques partition images (in this case, of sub-cellular regions) into segments or sets of pixels. The goal is to simplify the image and make it easier to analyze. Several sub-cellular segmentation techniques, many of which are based on the Gaussian mixture model (GMM) Bayes classifier, have been developed by researchers.\(^2-4\) GMM Bayes classifier assumes that cell features follow a Gaussian probability density function and are conditionally independent. Only a few studies have focused on how well cell features actually conform to Bayes classifier GMMs.\(^5\) Our objective is to analyze the model biases in these sub-cellular region segmentation techniques and so learn how the sub-cellular structures differ from these Gaussian assumptions.

The segmentation technique developed in this work is outlined in Figure 1. We used the gray-level co-occurrence matrix (GLCM) as a texture filter. Here, texture is the pattern of actin filaments in the sub-cellular regions. We partitioned images into hexagonal regions for spatial isotropy. Each hexagon was characterized by a vector of 15 features describing intensity, i.e., mean, mode, standard deviation, third moment, fourth moment, fifth moment, sixth moment, principal axes ratio, principal axes angle, and entropy; texture, i.e., directionality (angle), contrast, correlation, energy extracted from GLCM; and geometry, i.e., distance from known cell border. We estimated probabilities for GMM Bayes classification based on features from the training data. The region-based accuracy of classification was calculated on the remaining data by fourfold cross-validation.

In order to explore how cell features conformed to GMM assumptions, we evaluated feature normality and conditional independence. Our preliminary results showed that the majority of features do not follow normal distributions and many are conditionally dependent (data not reported). Both results

Figure 1. Overview of the segmentation algorithm for biologically meaningful image representation. GMM: Gaussian Mixture Model.

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confirm the importance of evaluating GMM outcome with respect to the conformity of features to GMM assumptions.

We also estimated classification accuracy as a function of the number of GMM components on two sub-cellular image sets containing, respectively, 88 and 70 fibroblast cells. We placed the cells on either soft or stiff extracellular matrix (ECM) and imaged one hour after seeding using confocal fluorescent microscopy imaging. Each cell is stained for actin, myosin, and focal adhesion channel. The IR channel was used to separate cell from background. The segmentation performance was evaluated against three-region reference segmentations obtained by expert visual inspection. The actin channel and segmentation images are shown in Figure 2. The GMM Bayes classifiers were applied only to actin images because of the biological focus on actin spatial distribution. For the ECMs, GMM classification accuracy was quantified with respect to the number of GMM components using hexagons with 127 pixel area: see Figure 3. The accuracy evaluation included a 10 pixel-wide background band around cell borders (region 4): see Figure 2.

Our segmentation results enabled quantification of some biases introduced in GMM Bayes-based segmentation and provided a better understanding of the role of actin at the sub-cellular level. In this case the variation of classification accuracy with the number of GMM components is reasonably low, probably due to careful feature specification and to image properties. However, we expect that significantly higher biases will be introduced by non-conformity to cellular model assumptions in general. We intend to explore new techniques to quantify how

Figure 2. Actin-stained cells and corresponding segmentations for (a) stiff and (b) soft extracellular matrix (ECM).

Table 1. Normality test for confidence level 90%. The values quantify the ratio of features used by the classifiers that satisfy the test.

<table>
<thead>
<tr>
<th>Location</th>
<th>T-test</th>
<th>Chi-square test</th>
<th>K-S test</th>
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<tbody>
<tr>
<td>Stiff ECM</td>
<td>0.013258</td>
<td>0.454545</td>
<td>0.551705</td>
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<tr>
<td>Soft ECM</td>
<td>0.005714</td>
<td>0.363571</td>
<td>0.485952</td>
</tr>
</tbody>
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Figure 3. Classification accuracy versus the number of GMM components. The variation in classification accuracy is within the interval range of 0.06/0.02 for stiff/soft ECM conditions over a large range of GMM component numbers, implying only a small bias with respect to number of components in the case.
much the accuracy of segmentation techniques suffers due to mismatches between the model assumptions and the real features of cells. This will in turn lead to improved feature design and selection techniques for image segmentation with applications to cell biology.

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References