Utilizing phase retardation features for segmenting cells in phase contrast microscopy images

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Abstract

Label free imaging, especially, phase contrast microscopy, plays an important role in high-content microscopy, in particular live cell screening. We aim to develop a fast and robust segmentation algorithm that enables the extraction of accurate morphometric measurements at a single cell level. Here we make use of a recently introduced image restoration method, which captures intrinsic features of phase contrast images. The resulting features are aggregated into superpixels and then grouped into objects using standard clustering methods.

The resulting method is computationally effective and only requires a minimal amount of user annotation. Proposed method is tested on a set of 10 phase contrast images of cervical cancer cell colonies of the HeLa cells. In order to compare our results with the literature, we report a Tanimoto coefficient of 0.94 for the set of images that were used in this study. The reported results indicate that our method does compare with the current state of the art.

1 Introduction

High-content microscopy plays an increasingly important role in drug development and basic life science research. Here we are particularly interested in enabling live cell imaging [6] experiments that reveal more detailed information about cellular function than standard end point assays. Given the volume and complexity of these image data sets there is a need for analysis tools that measure parameters such as overall cell morphology, measure parameters associated with cell division, motility, mechanisms of death and population statistics. Label free imaging, in particular, phase contrast microscopy, plays an important role since it eliminates the need for potentially cytotoxic fluorescent markers.

One fundamental task in quantitative biological imaging is cell segmentation. While a number of sophisticated and robust segmentation techniques have been developed for fluorescent microscopy, cell segmentation in phase contrast image sequences is still challenging. Artefacts that are introduced by the specific optics, observed as halo and shade off effects, as

well as the lack of clear boundaries among cluttered cells, poor contrast between cells boundaries need to be addressed. Ali *et al.* [2] uses local phase analysis and level set techniques to automatically extract cells from background. Wang *et al.* [8] apply machine learning to learn the specific edge profiles observed in phase contrast data whereas Arteta *et al.*[3] employs a structured learning scheme with dot annotations over non-overlapping extremal region features. We are particularly interested in a recently developed image restoration method [7, 9] that produces phase retardation features, which are intrinsic to phase contrast microscopy.

Here we are presenting a weakly supervised method that makes direct use of the phase retardation features introduced in [7]. After extracting the phase retardation features [9], the pixel features are aggregated into SLIC superpixels [1]. Subsequently, we utilize standard clustering and watershed segmentation to find an accurate delineation of the cell boundaries. Weak *a priori* knowledge about the location of the individual cells is being used to achieve robustness of the overall method. Details of our approach are presented in Section 2.

Results from our experimental evaluation are presented in Section 3. Our experiments show that the proposed method gives a good segmentation of cells, achieving an average Tanimoto coefficient (TC) of 0.94. Common failure modes, as for example the risk of over-segmentation are discussed in more detail. In the conclusion section, Section 4 we will outline why the proposed method is well suited for tracking cells from frame-to-frame in a time lapse sequence.

2 Method

Extracting accurate morphometric information for each individual cell requires reliable detection of object boundaries. Given the nature of phase contrast microscopy, the appearance of such object boundaries depends on the specimen itself as well as the local imaging conditions. This motivates why we utilize the phase retardation features, which are then aggregated into superpixels. Clustering is used to label the superpixels, which results in an over-segmentation of the image. The identification of the individual cell bodies is explained in Section 2.3.

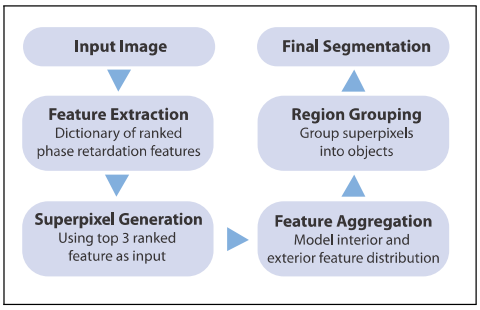
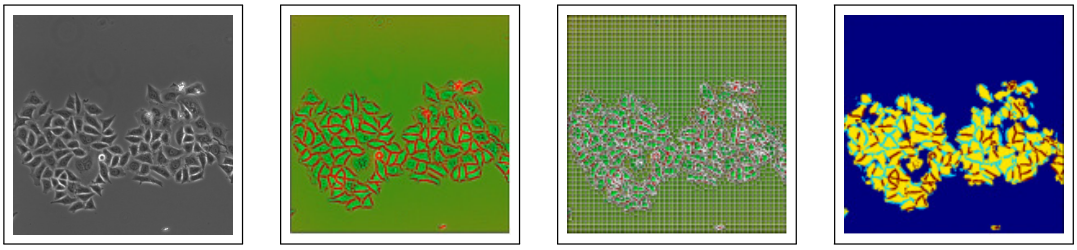


Figure 1: **Algorithm Overview.**

2.1 Feature extraction

Phase contrast microscopy is essentially a contrast-enhancing technique that employs an optical mechanism to translate small variations in phase (diffraction pattern) into corresponding changes in amplitude, which can then be visualized as differences in image intensity. This image formation process can therefore be exploited in an image reconstruction method to obtain features for each pixel based on the diffraction and hence the phase retardation experienced by each pixel element. We adopt the feature extraction method outlined in [7, 9] which proposes a dictionary-based imaging model for an N-pixel grayscale image as:

$$g = \sum_{m=0}^{M-1} H_m \Psi_m, \quad s.t.\ \Psi_m \geq 0, \tag{1}$$



(a) (b) (c) (d)

Figure 2: Initial processes resulting in cluster regions: (a) Original phase contrast image; (b) The top three(3) principal features for each pixel mapped onto RGB of a color image; (c) SLIC superpixel over-segmentation; (d) Result of GMM clustering

where g is the vectorized image intensities ($N \times 1$) expressed as a linear combination of a dictionary of M bases $\{H_m\}_{m=0}^{M-1}$. The vectorized coefficients, Ψ_m for each pixel corresponds to a diffraction pattern with phase retardation $\theta_m = \frac{2\pi m}{M}$. Hence, the n^{th} row of an H_m ($N \times N$) matrix corresponds to the vectorized kernel function obtained from a point spread function around pixel location x_n. The vectorized coefficients for each grayscale pixel are then utilized as the M-dimensional feature vector for that pixel.

The principal features are obtained from the top 3 bases that produce the least residual errors when used in the reconstruction of the original image. These features are observed to preserve the structure of the original image and therefore can be mapped onto the RGB of a colour image as shown in Figure 2

2.2 Generating Superpixels

Aggregating the pixel features into superpixels eliminates the local neighbourhood redundancy in the feature space and reduces the computational cost of partitioning the image into the required regions. We perform an oversegmentation of the phase contrast image using simple linear iterative clustering (SLIC) superpixels [1]. For an image with N pixels, we obtain K superpixels which consist of small regions with an approximate size N/K that stay within the object boundaries in the original image as shown in Figure 2. Hence, for roughly equally-sized superpixels there would be a superpixel center at every grid interval $Q = \sqrt{N/K}$.

For an image with phase retardation features $\{\Psi_x, \Psi_y\}$ for arbitrary pixel locations $\{x, y\}$, the similarity distance measure for aggregating the pixels, d_s is defined as:

$$d_s(x,y) := (\Psi_x - \Psi_y) + \frac{c}{Q}||x-y||_2 \tag{2}$$

where d_s accounts for both feature variation and distance in pixel location. The compactness, c determines the extent of emphasis on spatial proximity. The higher its value, the more compact the superpixels.

2.3 Multiclass Clustering and Region Extraction

We perform multi-class clustering on the features associated with each superpixel to group the segments into regions that correspond to the main region partitions expected in a typical phase contrast image. These include dark cells, bright cells, and halos.

Each superpixel is initially characterized by the mean and covariance of the multi dimensional pixel phase feature vector within it. It is observed that bright cells and halos are both represented by similar phase retardation features (shown in red in Figure 2(b)). Hence bright cells and halos cannot be distinguished by using only the features within each superpixel. As these are usually surrounded by different structures, we augment the feature vector of each superpixel by incorporating neighbourhood information. To include neighbourhood information, we construct the adjacency graph, $G(V,E)$, of the superpixels $s_i \in S$, where E is the set of edges formed between pairs of adjacent superpixels (s_i, s_j) in the image.

By estimating the dissimilarity between the feature distribution, P_s, in a given superpixel, s, and the feature distribution, $P_{N(s)}$, in its neighbourhood, $N(s)$, we capture some basic context information. We use the Jensen-Shannon divergence (JSD) [4], which is a symmetrized and smoothed version of the Kullback-Leibler divergence, KL, can be written as:

$$JSD(P_s||P_{N(s)}) = \frac{1}{2}KL(P_s||M) + \frac{1}{2}KL(M||P_{N(s)}) \quad \text{where} \quad M = \frac{1}{2}(P_s + P_{N(s)}) \,. \tag{3}$$

The divergence between P_s and $P_{N(s)}$ is used to select the most distinctive neighbour feature which is used as an additional feature for each superpixel s. As a result, we obtain a feature vector for each superpixel that consists of its mean and covariance, and that of its most distinctive neighbour. This ensures that neighbourhood information that is included is the one that shows the most deviation from the distribution of a given superpixel.

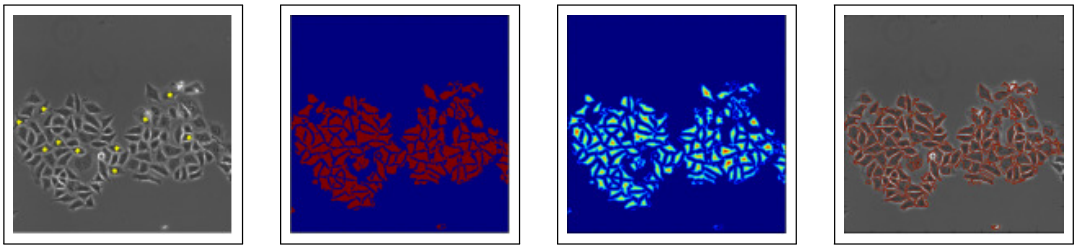
Considering every superpixel is modelled by the mean, μ and covariance, σ of a multivariate Gaussian, we proceed to make the assumption that groups of superpixel features that share similar mean and covariance could be modelled as the components of a Gaussian Mixture Model (GMM). We then proceed to fit the superfeatures to q components of a GMM via expectation maximization (EM). This amounts to clustering the superpixels in a given image into q clusters. Figure 2(d) shows the output of the clustering.

We observe that the clusters partition the image into various regions. Considering this partitioning is completely unsupervised, we proceed to to extract the label associated with cell regions by incorporating prior knowledge of some pixel coordinates indicating cell location. A single user mouse click in any part of a cell region corresponding to a coordinate per cell is required for only a few cells in the image (See Figure 3(a)). We obtain a binary mask, as shown in Figure 3, of cell regions and background for each image by labelling all regions that have the same label as the known cell pixel points to 1 (foreground) and all other regions to 0 (background).

We further observe that some cell regions (especially for cluttered cells) in the mask merge along some length of their shared boundary. To split such regions, we compute the Euclidean distance transform on the mask such that the value of each pixel is replaced by its distance to the nearest background pixel (See Figure 3). We then find the local peaks in the distance transform and label them as markers. We the proceed to run a marker-controlled watershed routine to find the *watershed line* that separates adjoining regions.

3 Experiments and Results

10 phase contrast images of cervical cancer cell colonies of the HeLa cells were used to evaluate the proposed algorithm. The cells contained in the data set vary significantly in size and shape. Each image is 400×400 pixels in size and contains roughly 100 cells each imaged at a magnification of 10X.



(a) (b) (c) (d)

Figure 3: Final processing leading to final segmentation output: (a) *a priori* knowledge of cell location as dots (b) Cell region mask; (c) Euclidean distance transform map; (d) Segmentation contours overlaid on image

3.1 Parameter Selection

We detail the various parameter settings we adopted for the experiments run on our data here. In computing the phase retardation features for each pixel, we set the length of the dictionary of diffraction patterns, $M = 20$ resulting in a 20-dimensional feature vector for each pixel. Also, we set the number of superpixels, $K = 2500$ for each image with a corresponding high compactness, $c = 20$ to emphasize spatial compactness that ensures that the superpixels do not spread over boundaries in the image. For a 400×400 image with $N = 160000$ pixels, we obtain an average superpixel size, $N/K = 64$ pixels and grid size $Q = 8$. For multiclass clustering using GMM, we set $q = 4$ components representing dark cell regions, bright cell regions, halos and background.

3.2 Evaluation

We perform a quantitative assessment of our method on our data by computing the pixel-wise Tanimoto coefficient (TC) introduced in [5] as:

$$TC = \frac{L \cap L_o}{L \cup L_o} \tag{4}$$

where L is the predicted label obtained from the final segmentation mask and L_o is the ground truth label obtained by manually segmenting all cells in all images used.

We achieve an average TC of 0.94 ± 0.03. Table 1 compares our result to that of similar work. We achieve similar performance to Su *et al.*[7] yet provide only weak cell location annotation with dots for only about 5% of cells in an image compared to 20% of complete cell segmentation in [7].

Our approach however is dependent on majority of the labelled positions being correctly grouped into the cluster that corresponds to cells. In the event of poor clustering, there is a risk of poor delineation of boundaries when the majority of extracted labels from a priori locations in the multiclass clustering do not correspond to that cells regions. This is however unlikely to occur in our case unless the features are poorly extracted and the clustering fails completely.

Method	Tanimoto Coefficient
Our proposal	0.94 ± 0.03
Su *et al.* [7]	0.94 ± 0.02
Yin *et al.* [9]	0.83 ± 0.06

Table 1: Quantitative comparison with similar work

4 Conclusions and Further Work

We have demonstrated a method for cell segmentation by utilizing phase retardation features that compares in performance to the state-of-the-art. It should be considered that in comparison to Su *et al.* [7] we make use of weaker and fewer annotations. While annotations are typically a very good way for improving algorithm performance, it is typically hard to obtain these in practice. Further work is needed to limit the number of parameter adjustments that are necessary to account for different imaging conditions, especially in case intrinsic values of the microscope are not known.

We aim to utilize the proposed segmentation algorithm for tracking cells in time-lapse imagery. The only annotation we will require is to obtain the cell location at the time of initialization. But it would also be possible to obtain this information using some type of purpose build object detector. For tracking cells in any consecutive frame, the intrinsic location information from the tracking process could be exploited to increase the amount of prior location knowledge and hence improve the robustness of the overall segmentation. Cell state parameters based on the cellular morphology from such segmentation and tracking in time-lapse could then be obtained efficiently.

Furthermore, we are interested in extracting additional parameters that characterize each cell. Here it is, for example, possible to extract features on the nuclei regions and treat these as features. Additional studies are necessary to analyse if changes in nuclear texture can be correlated with functional fluorescent markers.

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