Region-Based Segmentation of Parasites for High-throughput Screening

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Abstract. This paper proposes a novel method for segmenting microscope images of schisotsomiasis. Schistosomiasis is a parasitic disease with a global impact second only to malaria. Automated analysis of the parasite's reaction to drug therapy enables high-throughput drug discovery. These reactions take the form of phenotypic changes that are currently evaluated manually via a researcher viewing the video and assigning phenotypes. The proposed method is capable of handling the unique challenges of this task including the complex set of morphological, appearance-based, motion-based, and behavioral changes of parasites caused by putative drug therapy. This approach adapts a region-based segmentation algorithm designed to quickly identify the background of an image. This modified implementation along with morphological post-processing provides accurate and efficient segmentation results. The results of this algorithm improve the correctness of automated phenotyping and provide promise for high-throughput drug screening.

1 Introduction

Schistosomiasis is one of the seven neglected tropical diseases as defined by the Centers for Disease Control [1]. Diseases in this category affect over one billion people and occur in developing nations. In particular, schistosomiasis is largely found in African countries where infection rates are reported above 50% [2] and in sections of East Asia and the subcontinent. The World Health Organization published in 1999 that all of the reported deaths and 99.8% of those disabled by the disease were considered low or middle income [3].

This disease is caused by several parasites of the genus Schistosoma. Freshwater snails are used as vector by the parasites. Once the larvae leave the snail they can infect anyone who comes in contact with the contaminated water. The parasites infect the host by penetrating the skin and traveling through the blood stream. Schistosomiasis targets the liver and bladder. If not treated over a period of time studies have shown an increased chance of bladder cancer [4].

Large pharmaceutical companies do not invest in finding cures for these diseases because there is little or no profit to be gained. One of the barriers to rapid drug

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discovery is the time it takes to analyze the complex reactions of the parasites to various drugs. High-throughput screening (HTS) is a recent technique in drug discovery that allows screening a large number of drugs in parallel against a target. In the given problem context HTS plates can contain up to 384 samples, thereby allowing the effect of a number of drug candidates to be tested in parallel. The reaction of the parasite is videotaped using a camera connected to the microscope. Researchers have described six different phenotypic responses to putative drugs resulting in changes in shape, color, texture, and movement [5]. It is highly probably that more phenotypes exist but have not been identified. Currently, researchers review these videos manually to assign phenotypes, and these assignments are used to determine the effectiveness of a specific treatment. Requiring a professional to analyze each individual video sample is a long and tedious process. Implementing a HTS process requires algorithms that can accurately and efficiently identify these changes. Segmentation is the first step in the process to achieving high accuracy.

With approximately 50 individuals in a well, parasites tend to touch, causing traditional segmentation methods to often merge them into a single segmented object. Parasites also exhibit complex shapes, textures, and movement. Finally, a proposed method also needs to allow for variability in imaging conditions.

We organize the paper as follows: Sec. 2 details the challenges faced when attempting to segment schistosomula in the context of the state of the art in biological image analysis methods. Sec. 3 provides an overview of recent research. This is followed by a brief discussion of the original Active Mask [6] segmentation method in Sec. 4, which is designed for segmentation of human cells, and how the region-based distributing function of this method is modified in the proposed method. Next is an explanation of the implementation of the proposed method. Finally, Sec. 5 contains the results of the implementation and a comparison to other morphology based methods.

2 Problem Characteristics and Challenges

Segmentation of parasites requires an understanding of their color, texture, shape, and movement. Each of these comes with possible challenges that need to be handled skillfully in the segmentation process to avoid inaccurate or unusable results. Among others, illumination conditions and image-capture technologies can vary. No assumptions can be made about the quality or composition of parasite data if the intention is to make the solution widely applicable. Traditional thresholding techniques are generally not effective in these situations. Additionally, due to illumination, crowding, or the nature of the substrate on which the parasite lives, the body boundaries can be obscure.

Several aspects of the parasite's movement and shape also cause difficulty in acquiring accurate segmentation results. Unlike in the original implementation of the Active Mask segmentation algorithm [6], a rounded shape cannot be assumed. The assumption of "roundedness" of shape is inherent in many methods developed for biological segmentation. Schistosomes movement are based on elongation and contraction of the musculature. This changes the shape of the body from narrow and straight edged on the sides to a more rounded shape within a single movement cycle. Additionally, parasites contain visible "inner" anatomical structures that complicate segmentation by creating edges that do not correspond to the boundaries of the body.

Lastly, it is important to note that these parasites tend to "stick" together. Thus, video data shows parasites that touch. Once two or more parasites touch, they tend to stay stuck to one another for a significant number of frames. This often results in poor splitting of the parasites where more than one parasite is considered a single region. The leftmost image in Fig. 1 shows an example of two parasites that might be segmented as a single region. All images in Fig. 1 display the various shapes and textures present in a single video.

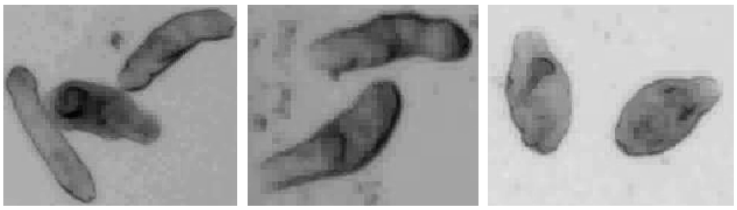

Fig. 1. Illustrations of the segmentation challenges: Touching parasites in the leftmost image cause merged regions. Note the "inner" dark structures that cause additional edges inside the body of the parasite. The center image displays the variance of the texture causing the left edge of the body to become unclear. Finally, the parasite's body is shown elongated in the leftmost image and rounded in the rightmost image.

3 Review of Recent Research

While segmentation of parasites has not been deeply investigated, there is a rich literature in segmentation of cells in biological images. An accurate automated segmentation algorithm as part of a high-throughput system capable of handling large datasets was shown in [7] to successfully measure 14 phenotypes of approximately 8.3 million human cells. Completing this type of analysis manually is unimaginable. In [8], a dataset generated using a Cellnomics ArrayScan VTi system (Cellnomics, Pittsburgh, PA) for high content screening (HCS) resulted in poor segmentation of over 50% of the SK-BR-3 (breast carcinoma) cells. Researchers found that the HCS system had difficulty handling the splitting and merging of cells which in turn significantly skewed the analysis of the dimension and shape of the cells. They noted the importance of discovering new techniques for image segmentation but were limited by the inability to integrate new software into the existing HCS system.

The watershed segmentation algorithm is known to over-segment images, which led the authors of [9] to implement a derivative called the marker-controlled watershed algorithm. The algorithm was seeded using a combination of fine and coarse filters to approximately identify the cell's center as a starting point for the watershed process. This approach was able to handle touching cells but relied on the elliptical shape of the cells and two defined thresholds based on testing to automate the process.

Leukocytes, or white blood cells, were effectively segmented using a shape and size constrained active contour approach in [10]. Researchers noted that the approach was only feasible due to *a priori* knowledge that leukocytes are approximately circular and their size is relatively stable.

In [6] researchers proposed a more sophisticated version of active-contour segmentation called active mask segmentation. This method was created to more accurately segment fluorescence microscope images of human cells. These experiments result in grayscale images with dark backgrounds and bright punctuate patterns that outline the cell and its components. The end result of segmentation is a collection of masks where each mask is the binary representation of a single region in the original image. The following section will briefly describe the overall method and go into more detail regarding the region-based distributing function which is the starting point of our method.

4 Proposed Method

4.1 Active Mask Overview

The Active Mask algorithm [6] incorporates multiscale and multiresolution blocks which encapsulate the region-based and voting-based distributing functions. The original image is padded, the scale is reduced, and the resolution decreased. This smaller low resolution image is used to apply weights to the collection of masks using a region-based distributing function to identify the background and then using the voting-based distributing function to split merged regions. The region-based function is applied to the first mask which is designated the background mask. Higher weights represent pixels identified as background pixels in the first mask. Next, the voting-based distributing function applies weights to each pixel based on the mask assignments of the neighborhood. As a result of region growing, each mask is propagated based on these weights. Subsequently, the weighting functions are iteratively applied at the current scale and resolution until convergence. The scale and resolution are increased and the process starts again until the image returns to its original scale and resolution. In [6], the authors were able to accurately split merged regions using the voting-based distributing function, given that human cells are rounded.

4.2 Region-Based Distributing Function

The region-based distributing function constitutes the first step of the proposed solution for segmenting schistosomula. The purpose of this function is to rapidly identify the background by assigning a higher weight to background pixels. Initially, a low-pass filter is applied to the original image to remove noise and smooth the image. Next the average border intensity γ is subtracted and the image is multiplied by the harshness of the threshold β. β is inversely proportional to the difference of the average background and foreground intensities and is assigned a higher weight the smaller the difference. The result is asymptotically bounded using a sigmoid function and finally a skewing factor α is applied. This results in pixel values below the average border intensity γ being skewed towards the background because they have a higher weight. Following [6] we define the *region-based distributing function* R_1 as:

$$R_1(n) \coloneqq \alpha \, sig\left(\beta\left((f * h)(n) - \gamma\right)\right). \tag{1}$$

Where $\alpha \in (-1, 0)$ is the weight of the region-based distributing function, $\beta = 4/(\text{high}-\text{low})$ is the harshness of the threshold, and $\gamma = (\text{high}+\text{low})/2$ is the average border

intensity. High and low are set to the average region and background intensity values respectively. The lowpass filter h and the sigmoid function as defined in [6] as:

$$h(n) = e^{-|n|^2/\alpha^2} .$$

$$sig(x) = \text{erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x e^{-t^2/2} dt . \tag{2}$$

The lowpass filter removes high frequencies from the frequency domain that correspond to details and noise. This results in a blurred or smoothed image. The sigmoid function defines horizontal asymptotes that restrict the possible value range to between ±1.

4.3 Region-Based Distributing Function Adaptation

The region-based distributing function from [6] displayed the ability to not only roughly identify the background but also to accurately locate each parasite in frame. In studies carried out by us, even though it surpassed all previous methods at locating every parasite it did not provide accurate segmentation using the suggested parameter values based on the average background and foreground intensities. Tests were conducted by converting the image to grayscale, inverting it to create a dark background and supplying high and low intensity values based on the converted image. Various values were tested for the initial number of masks, the scale parameter, and function weights. See [6] for more details regarding these parameters.

While the region-based distributing function is not intended to fully segment an image, it is able to efficiently determine the background by weighting the image in the frequency domain. In our approach it is used to over-segment the frame and is followed by post-processing to remove unnecessary regions. This method has been shown to provide efficient and accurate results through tests reviewed in Sec. 5. We propose a modified version of equation (1):

$$R_1(n) \coloneqq -1 * sig\big((f * h)(n) - \gamma\big) . \tag{3}$$

Where we remove α and β whose weights are not necessary without the voting-based distributing function. Additionally, γ becomes our threshold representing the distance in intensity between the background and foreground. By adjusting this threshold it is possible to adjust the quality of the results as can be seen in Fig. 2.

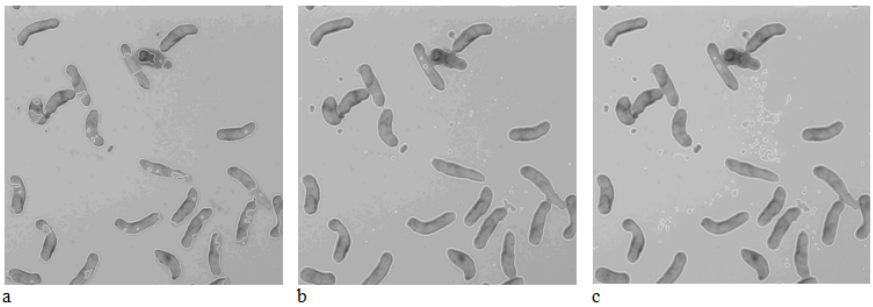

Fig. 2. The results of the region-based distributing function with the boundaries shown in white at the following threshold values: (a) 50; (b) 15; (c) 11

Although there is oversegmentation, the results also show that most of the parasites are accurately segmented. Additionally, any small erroneous regions can be easily identified. For instance by the fact that their area will be significantly less that the average area of the parasites. As the threshold decreases the frames are over-segmented because segmentation starts to include regions with intensity values closer to the background intensity. The increase in the number of regions becomes steep and the maximum increase is identified to be used as the threshold. For the results shown in Fig. 2, the threshold that maximized the slope of the graph was equal to 11 and is noted with an asterisk in Fig. 3, which plots the number of regions in relation to the threshold.

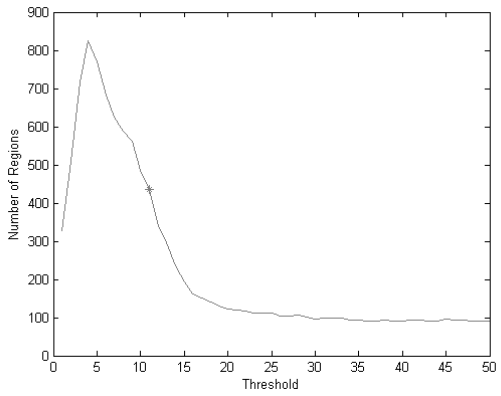

Fig. 3. As the threshold value decreases the number of regions segmented increases significantly due to over-segmentation

A starting threshold is established by taking the minimum of the average of the difference between the minimum and maximum intensities present in the grayscale image and a cutoff value of 50. The cutoff value was established during testing and is based on the grayscale color range of [0, 255] where 100, or twice the cutoff, is an acceptable starting difference between foreground and background. The region-based distributing function is iteratively applied with a decreasing threshold. The optimal threshold is identified, the region-based distributing function is applied, and the results are refined using the following morphological techniques.

4.4 Morphological Analysis and Processing

The proposed approach takes advantage of the quick identification of the background by the region-based distributing function and then further refines those results through morphological analysis and processing. Our implementation uses morphological techniques to remove noise and improve edge detection based on our prior research [11].

The image is cleaned using closing, filling holes inside of regions, eroding the edges and removing inconsistent regions. Edges that were missed during application of the region-based distributing function create cavities. Closing is performed to aid in filling interior holes by creating the missing edge of the parasite that closes in the

cavity allowing us to identify and fill holes inside the parasite. Edges are then eroded to compensate for the over estimation of the boundary by the region-based distributing function as shown in Fig. 2. The last step in the cleaning process is removing inconsistent regions by comparing each region's average intensity value to the overall average. Regions with values outside of one and half times the standard deviation are considered background and removed.

At this point the image still contains regions where multiple touching parasites are considered a single region. The Canny edge detection algorithm is applied to the original image as the first step in identifying edges to separate parasites. The edge image is analyzed to identify edge pixels surrounded by more than one region. The resulting relevant edges image is subtracted from the black and white segmented image as shown below in Fig. 4(e). This new image, called the final labels image, is processed to remove small regions and fill in any holes created in the edge removal process to produce the final segmentation result. Examples of this process are shown in Fig. 4.

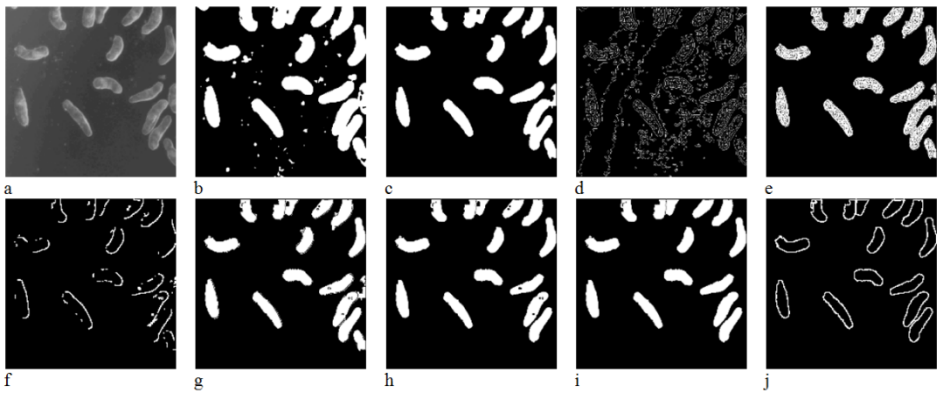

Fig. 4. (a) The inverted image; (b) the results of the region-based distributing function; (c) region-based function results after removing noise; (d) Canny edge detection results; (e) edges subtracted from c; (f) relevant edges; (g) subtraction of relevant edges from c; (h) g after removal of small regions; (i) h after filling holes in regions; (j) the outlines of segmented regions

5 Results and Comparison

To establish the effectiveness of the proposed method it was compared against our previous implementation using EDISON [12] mean-shift and morphological segmentation [11]. It was also compared to JSEG [13], a color quantization and spatial segmentation technique. The Codebook model [14] provided a comparison against a motion based segmentation approach. The Codebook model establishes a background codebook using a training set. This codebook is then used to segment an entire video based on motion. Lastly, the original Active Mask implementation was applied and compared. An image was selected and each method's result compared and runtime measured. The results are shown in Fig. 5.

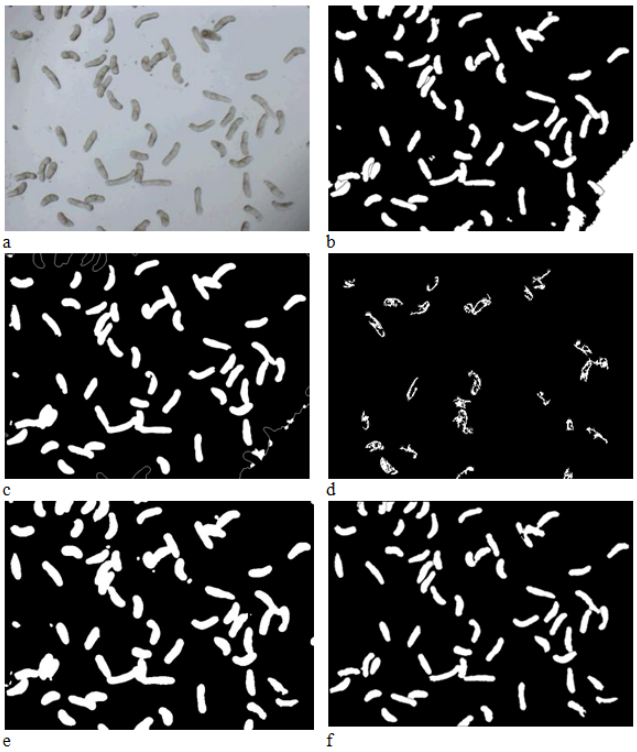

Fig. 5. (a) The original 1384 x 1024 image prior to segmentation and the following segmentation methods: (b) mean-shift and morphology-based segmentation, (c) JSEG segmentation, (d) Codebook model segmentation at 25% scale of original image, (e) Active Mask segmentation at 50% scale, and (f) the proposed region-based morphological segmentation

The mean-shift and morphological segmentation provides decent results with a reasonable runtime of approximately 26.28 seconds. It has difficulties with the uneven illumination in the lower right-hand corner and tends to merge touching parasites. The JSEG segmentation suffers similar problems in addition to missing several parasites with a slower runtime at 104.80 seconds. The Codebook model produces poor results due to the limited movement captured in the image with a runtime after codebook creation of approximately 91.16 seconds (processing the image at 50% of the original scale). The Active Mask implementation provides reasonable results but looking at the identified regions in Fig. 6 shows the algorithm did not successfully split and merge regions.

In addition, the image had to be reduced in size by 50% to avoid running out of memory during processing and the segmentation runtime was slowest at 888.86 seconds. Finally, the results of the proposed method show improved identification and splitting of regions with a reasonable runtime of approximately 15.96 seconds.

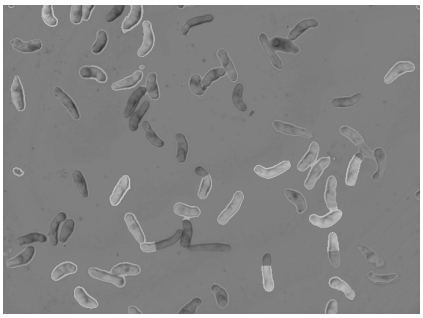

Fig. 6. The results of the Active Mask method highlighting merged and split regions using varying grayscale to identify unique regions

Table 1 provides a breakdown of the runtimes and accuracy for each method. Recall measures the ratio of pixels correctly identified as foreground to the total number of hand segmented foreground pixels. Precision measures the ratio of pixels correctly identified as foreground to the total number of pixels identified as foreground during the segmentation process.

Table 1. Each method was used to segment a set of 5 images (1384 x 1024) and the accuracy and runtime recorded. The processing times were evaluated on an Intel(R) Core(TM)2 Duo CPU T9300 @ 2.50GHz with 4.00GB RAM. Codebook generation is the training period for the Codebook method. Threshold Est. is the time taken to establish the threshold from the first image in our proposed method. The threshold and Codebook are used to segment subsequent images with the approximate segmentation runtime as shown under Segmentation.

Method	Image Process	Scale	Avg. Runtime	Recall	Precision
Mean-Shift Based	Segmentation	100%	26.28s	92.25%	69.98%
JSEG	Segmentation	100%	104.80s	85.04%	87.51%
Codebook	Codebook Generation	50%	6246.35s	N/A	N/A
Codebook	Segmentation	50%	91.16s	31.83%	88.13%
Active Mask	Segmentation	50%	888.86s	98.85%	67.98%
Proposed Method	Threshold Est.	100%	128.19s	N/A	N/A
Proposed Method	Segmentation	100%	15.96s	89.54%	97.70%

A set of five images were hand segmented by us to compare against the results of the proposed method. These images serve as the ground truth with which we establish the accuracy of our method. The comparison against the ground truth is visualized in Fig. 7.

Fig. 7 shows the background in black and the parasites in their grayscale representation. False positives and false negatives are captured using white and dark gray respectively. Over the set of five images each pixel was evaluated to establish percentages of false negatives and false positives. The region-based morphological segmentation resulted in 0.28% false positives and 1.39% false negatives over five images. The segmentation results correctly identified 98.33% of the pixels as either background or foreground.

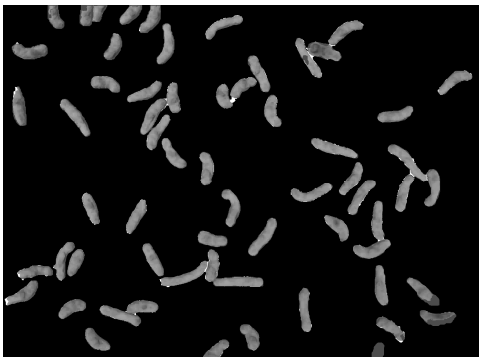

Fig. 7. First image taken from a control sample illustrates the accuracy of the proposed segmentation algorithm. Areas in black represent the background, white regions represent false positives (areas designated foreground that are actually background), dark gray areas represent false negatives (areas designated background that are actually foreground), and the correctly segmented parasite bodies are shown in the original grayscale

6 Conclusion

The promise of the region-based morphological algorithm for segmentation of parasite microscope images is evident in the results shown here. The algorithm is able to efficiently and accurately segment while handling complex shapes and textures. The algorithm outperformed our original mean-shift based method, JSEG, the Codebook model, and the original Active Mask method in both segmentation accuracy and runtime. In addition to performance our algorithm makes no assumptions about the data itself, making it more amenable to different data sets. Providing quality segmentation as part of a system that automates drug discovery for schistosomiasis has a worldwide impact. A freely available system can be used by researchers all over the world, which speeds drug discovery and in turn promotes better health.

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