Segmentation of Parasites for High-Content Screening Using Phase Congruency and Grayscale Morphology

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Abstract. Schistosomiasis is a parasitic disease with a global health impact second only to malaria. The World Health Organization has determined new therapies for schistosomiasis are urgently needed, however the causative parasite is refractory to high-throughput drug screening due to the need for a human expert to analyze the effects of putative drugs. Currently, there is no vision system capable of relieving this bottleneck with sufficient accuracy for the automated analysis of parasite phenotypes. We presented a region-based method with performance limited primarily by poor edge detection caused by body irregularities, groups of touching parasites and unpredictable effects of drug exposure. Towards ameliorating this difficulty, we propose an edge detector utilizing phase congruency and grayscale thinning. The detector can be used to impose the correct topology on a segmented image – an essential step towards accurate segmentation of parasites.

1 Introduction

1.1 Background

Schistosomiasis is a parasitic disease considered to have global health and socio-economic impacts second only to malaria. Although incidence of the disease in developed countries is extremely low, more than 200 million people are infected worldwide, with an additional 800 million at risk. The chronic illness is caused by infection with one of several species of trematodes, chiefly *Schistosoma mansoni*, *Schistosoma haematobium* and *Schistosoma japonicum*, which are carried to humans through water contaminated with their larvae. Early on, infection is characterized by an inflammatory response to the parasites' eggs, eventually leading to fibrotic granulomas that can occlude the hepatic portal vein and cause hydronephrosis (kidney swelling from urine buildup) and squamous cell bladder cancer. Other effects of schistosomiasis include diarrhea, lesions in the central nervous system and genital sores which enhance the transmission of HIV. The World Health Organization (WHO) has classified schistosomiasis as one of 17 neglected tropical diseases, a set of illnesses grouped together

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because they (1) are proxies for poverty, (2) affect politically disadvantaged populations, (3) do not travel out of the third world, (4) lead to discrimination, especially of women, (5) have serious, widespread health effects, (6) are neglected by research and (7) might be controlled through currently feasible means [1].

Whole-organism drug screens against schistosomiasis have recently been adapted to automated, high-throughput data collection, but the need for an expert observer remains a significant bottleneck. We therefore proposed an image segmentation algorithm [2] for bright-field microscopy images of the juvenile schistosomula, towards the development of a fully automated screen against schistosomiasis. Unfortunately, the separation of touching parasites obtained by this method was often insufficient for precise phenotypic measurements [3].

1.2 Problem Formulation

In order to support the precise measurement of parasite attributes from a video, segmentation must be accurate, robust and able to separate individual parasites. Segmentation of schistosomula in particular raises challenges which are distinct from the segmentation of cells. These include:

- The parasites are all unique individuals and exhibit marked variation in size and shape. In addition, elongation and contraction of the musculature can result in drastic alterations in proportion, shape and orientation. These facts preclude the assumption of an a priori geometric model of shape.
- The proclivity of schistosomula to touch or overlap slightly. This leads to the formation of large groups of parasites in physical contact, which are segmented as a single object due to weak or nonexistent edges.
- The presence of visible anatomical structures within the parasites. These structures create internal edges which do not correspond to parasite boundaries.
- Alterations in each of the above due to the effects of drug exposure, which again differs between individual parasites.

A proposed method must successfully address these difficulties, which are illustrated in Figure 1. Detection of those edges which are most salient to the perceptual separation of individual parasites is essential if the challenges enumerated above are to be overcome.

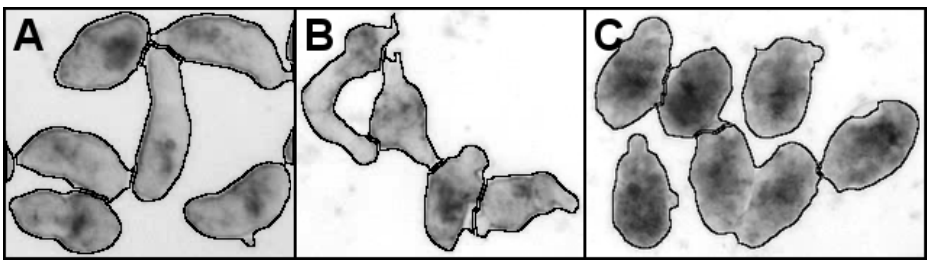

Fig. 1. Phenotypic diversity of schistosomula. (A) Control and exposed to the drugs Praziquantel (B) and Simvastatin (C). Black lines indicate edges found by proposed method.

2 Prior Work

Few computer vision methods targeting parasitic organisms exist in the literature. One image based assay against *T. cruzi*, responsible for Chagas' disease, was designed utilizing the IN Cell Analyzer 1000 (GE Healthcare), a dedicated, high-throughput experimentation and imaging apparatus [4]. The only measurement made is the quantity of parasite in the foreground and no attempt is made to uniquely identify individuals. While not a parasite, *C. elegans* is related to a number of parasite species and is visually similar in some respects to vermiform macroparasites such as Schistosomatidae. Some computer vision studies of *C. elegans* have undertaken to segment multiple touching or overlapping individuals. The approaches taken include articulated models [5] and path searching on probabilistic shape models [6].

In contrast to parasites, segmentation and tracking of cells has been an active research area for some time. Zimmer et al. [7] use a parametric active contour with a repulsive term between regions which enforces the separation of closely touching cells, while Srinivasa et al. [8] devised an "active mask" algorithm in which region masks are iteratively evolved and/or discarded in order to produce a correct labeling of each pixel.

3 Methods

3.1 Overview

In broad terms, the algorithm presented in [2] consists in an initial segmentation using a region-based distributing function adapted from [8], in which touching parasites are typically merged into a single object, followed by edge-based region splitting in which edge information extracted from the image is used to correct border placement and separate erroneously merged parasites. Edges that are irrelevant to the separation of merged parasites are eliminated, giving a pruned set of edges for splitting merged regions. The edge detection component, which is the focus of this paper, originally employed the Canny edge detector. However, the Canny operator proved very susceptible to noise. Furthermore, gradient-based detectors in general are sensitive only to features with a very limited range of phase angles of the spatial frequencies. The remainder of this section further describes the shortcomings of gradient methods and then presents a new edge detector, suitable for localizing perceptual edges between schistosomes – even if they are not well represented by the intensity gradient.

3.2 Edge Detection

Traditional edge detection methods rely on the image gradient. The best known examples of such methods are the Prewitt-Sobel-Roberts family of derivative approximations and the Canny operator [9]. The latter is of particular interest, as it is optimal among the gradient-based approaches and is widely recognized for its efficacy.

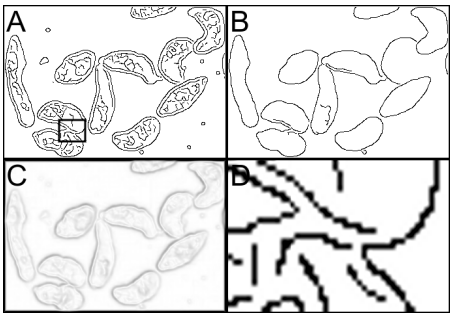

Fig. 2. Canny edges with (A) permissive thresholds, (B) with conservative thresholds, (C) gradient magnitude and (D) close up of problematic result from black box in (A)

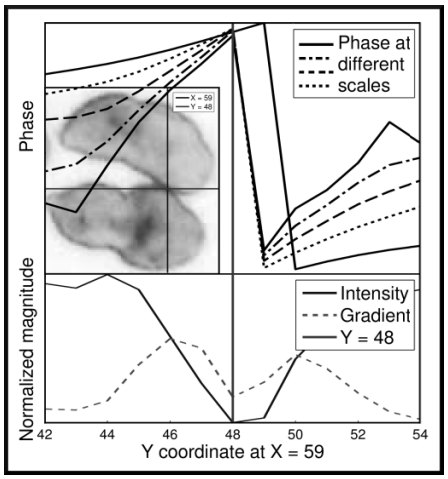

Fig. 3. Motion for phase congruency; graphs show that phases (top) are maximally congruent just at the perceptual edge indicated by the vertical line, while the gradient (bottom, dashed line) has peaks to either side. Inset shows origin of intensity profile. Note the steep change in phase after the edge is just a 180 degree shift.

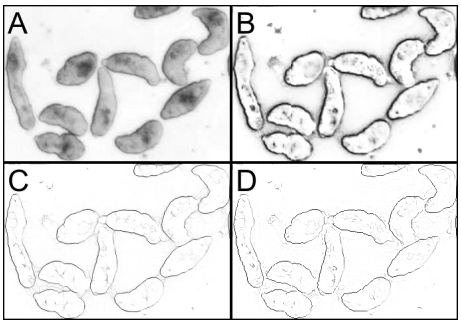

Fig. 4. Original image, (B) phase congruency and ridge detection by (C) grayscale thinning and (D) non-maxima suppression.

Application of the classical formulation of the Canny operator to schistosomula images produces edges which are insufficiently accurate to effectively separate merged parasites without falsely splitting individuals. When permissive thresholds are used, the results are characterized by a high degree of noise and artifacting, while conservative thresholds fail to detect the weak edges which often occur between touching parasites (Figure 2A-B). Furthermore, gradient based edge detection suffers from a "double edge" artifact: edges wide enough to have a small gradient in the center are not identified correctly and in many cases are incapable of separating touching objects regardless of the thresholds used (Figure 2C-D, Figure 3). In order to address these issues, we replace the Canny detector with a novel edge operator aimed at producing accurate edge contours with maximum perceptual salience.

Calculation of valid edge weights is crucial to the performance of the edge detector. Rather than the intensity gradient, we propose using the phase congruency of the grayscale image. Phase congruency (PC) is an approach to feature detection based on the Local Energy Model [10], which holds that perceptually salient features occur where an image's Fourier components are maximally in phase with one another.

PC has a number of qualities which are advantageous in comparison to image gradients. First, it is a dimensionless quantity restricted to the interval [0,1]. Second this notion is illumination and contrast invariant, and can detect and correctly place perceptual elements which do not coincide with steps in the image gradient (such as thick edges). Finally, PC is naturally extensible to include noise cancellation and multiscale analysis and additionally may be implemented efficiently using fast wavelet transforms [11]. That PC is particularly suitable for images of schistosomula, is demonstrated by Figure 3, which illustrates that congruency of phase coincides with perceptual edges even where the gradient intensity does not.

PC is defined as the ratio between the local energy, or absolute magnitude in frequency space, and the total path length of all frequency component vectors. If F(x) denotes the real components of the spatial frequencies and H(x) the imaginary component, then the local energy is defined by Eq. (1), and the phase congruency itself by Eq. (2). ε is a small constant representing numerical precision.

$$E(x) = \sqrt{F(x)^2 + H(x)^2} \tag{1}$$

$$PC(x) = \frac{E(x)}{\sum_n A_n(x) + \varepsilon} \tag{2}$$

Calculating phase congruency requires a local, phase-preserving frequency analysis. An appropriate method is the wavelet transform, using quadrature pairs of matched even and odd filters. Energy *E* may then be easily extracted by comparing responses to the symmetric filter *f* and anti-symmetric filter *h*, representing real and imaginary parts of Eq. (1). Phase angle φ is computed via Eq. (3).

$$\varphi(x) = atan2(f(x), h(x)) \tag{3}$$

Log-Gabor filters, characterized by a Gaussian transfer function on a logarithmically scaled frequency axis, are chosen because they are psychophysically justified [12], and because they possess zero mean at arbitrary bandwidths. In the Fourier domain, the log-Gabor transfer function with center frequency ω is given by:

$$\mathcal{G}(\omega) = e^{\frac{-\ln\left(\omega/\omega_0\right)^2}{2\ln\left(\kappa/\omega_0\right)}} \tag{4}$$

Using Eq. (4), a log-Gabor filter bank comprised of filters of at different frequency scales is constructed. The implied sense of scale derives not from the spatial extent of a feature itself, but from the spatial extent of its constituent frequency components. Once the filter bank is constructed, analysis at multiple scales can be performed by summing the filter response at each scale. The reader is referred to [11] for a detailed treatment of this approach.

In general, the edge weights obtained via phase congruency (or gradient methods) are, for a given image feature, diffused over a width greater than that needed or desired for edge based region splitting (Figure 4C). In order to accurately localize edges,

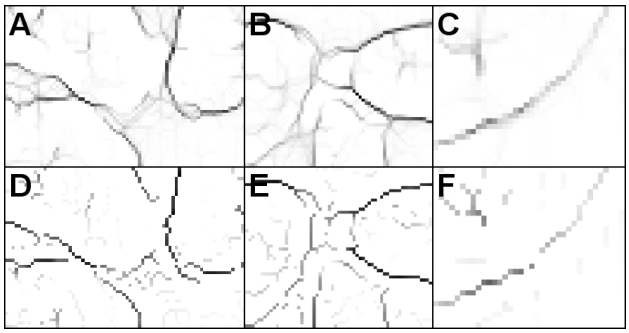

Fig. 5. Comparison of (A)-(C) grayscale thinning versus (D)-(F) non-maxima suppression, demonstrating that thinning is significantly more conservative

as well as reduce the number of pixels under consideration, the intensities of pixels which are not along the center-lines or ridges of edge features must be damped and/or eliminated. Anisotropic non-maxima suppression, using feature orientation estimates, is a well-known method which aligns with topographical sense, in that a ridge point ought to be a peak in the projection along the edge normal. On the other hand, orientation estimates may be subject to noise, and the exact pixel location of a numerical maximum may deviate from the center of the perceptual edge, leading to broken contours. Although the phase angles computed as intermediates in the phase congruency procedure may be used as estimates of feature orientation, in our method an approach to ridge detection is taken which does not rely on any such estimates.

Ridge detection is related to "thinning" of image features, in that the ridge is defined as the high-intensity center of a wider perceptual structure. The operation is therefore analogous to (infinite) morphological thinning of binary images, in which the set theoretic approach of mathematical morphology is used to eliminate pixels whose neighborhoods conform to constraints on their structure. Unlike fundamental morphological operations such as erosion and dilation, the hit-or-miss transform (from which most thinning algorithms are derived) is not well defined for intensity images. Nevertheless, any binary image operation can be extended to grayscale via linear superposition of global thresholding at all intensity values [13]. To carry out a morphological operation by threshold superposition, the intensities of a grayscale image are first quantized by restricting them to N bins. The center of each bin is used as a global threshold to convert the quantized image to binary. A morphological operation is performed on the thresholded images (until convergence if desired), which are then summed to yield a new image under the same quantization. This technique is practically limited to relatively small values of N, due to the exponential proliferation of possible thresholds, and down-sampling may be needed to obtain acceptable performance on available hardware.

Threshold superposition can be used to take advantage of the relationship between ridge detection and skeletonization [14]. In our work, grayscale thinning by threshold superposition is used to thin the phase congruency edge weights. A mathematical formulation of threshold superposition requires the global threshold operator T_t defined over a set of pixels $X=\{x_i\}$ with intensities I_x as given in Eq. (5).

$$T_i = \{x_i \in X | I(x_i) > t \tag{5}$$

Grayscale thinning by superposition is given by the expression in Eq. (6). The notation thin∞ indicates an infinite (binary) morphological thinning operation. Note that the phase congruency PCi is normalized by definition.

$$W_i = \sum_{t=0}^{N-1} thin_\infty \left(T_t \left(round(PC_i \cdot N) \right) \right) \tag{6}$$

The parallel thinning algorithm of Guo and Hall [15], which is simple to implement and obtains good results, is reapplied until convergence at each threshold independently. As shown by Figures 4 and 5, grayscale thinning proves significantly more conservative than non-maxima suppression, preserving the complete ridges that are necessary for effective region splitting.

Once the thinned edge weights are available, a binary edge image is determined using hysteresis thresholding. Hysteresis consists in locating pixels which are above a high threshold, or which are above a low threshold and are 8-connected to a pixel above the high threshold. In terms of binary sub-images given by high and low global threshold operations T_H and T_L, the hysteresis edges may be written as the components of T_L which are supersets of TH. The notation $\prod_i(X)$ denotes the partitioning of a binary image into its connected components.

$$E_i = \prod\nolimits_i T_L(W_i) \supseteq \prod\nolimits_i T_H(W_i) \tag{7}$$

The high threshold is taken to be that determined for the (thinned) PC image using Otsu's method; the low is taken as that value times ¼. The hysteresis thresholds are thus reflective of the intensity distribution within each sub-image. The product of hysteresis is finally subjected to infinite (binary) morphological thinning, in order to ensure that edges are one pixel wide in all cases.

Having arrived at a binary edge set, we wish to use it to split connected components found in the initial segmentation. However, the edges will include some which are not relevant to this task; only edges which form closed contours or are connected to the background are capable of internally disconnecting a region. These irrelevant edges are located and eliminated as follows. A marker image is generated by direct subtraction of the edge set from the initial segmentation. Then, each edge pixel is marked as relevant if and only if its 8-neighborhood contains more than one marker region. The presence of multiple markers within the 8-neighborhood is determined by labeling the marker regions and determining if the cardinality of the unique labels within the neighborhood is greater than one. The labeling operation is denoted as $label_8$ and the set of unique elements about a pixel x is denoted as $unique_8$.

$$M_i = label_8(C_i - E_i) \tag{8}$$

$$F_i = \left\{ x \in M_i \middle| \left| unique_8(x) \right| > 1 \right\} \tag{9}$$

The relevant edges Fi constitute one pixel wide, 8-connected edge contours, which will alter the topology of segmentation upon subtraction (i.e. by splitting regions). Finally, the minimum subset of the relevant edges which reproduces the same segmentation topology is computed using the watershed transform.

4 Experiments

The algorithm we describe is designed to satisfy the criteria for accuracy laid out above, and to mitigate the specific challenges presented by data from HTS of Schistosoma. As discussed previously, it is especially important that accuracy be maintained across the diverse phenotypes exhibited by individual schistosomes, even with this diversity is exacerbated by drug insult. It is therefore necessary to evaluate results for a variety of experimental conditions:

- Control conditions. Parasites exhibiting their natural phenotypes provide an essential baseline for detecting drug induced phenotypic changes. Healthy parasites typically have stronger edges and more regular shapes than those exposed to drugs.
- Extreme phenotypes which occur because of the presence of different compounds. In particular, parasites exposed to the drugs Simvastatin (Sim), Chlorpromazine (Chl) and PZQ provide a diverse phenotypic set.

Volunteers hand-segmented 8 images from these conditions to serve as ground truth for quantitatively evaluating the proposed method versus Canny edge detection. Table 1 summarizes the accuracy of the two methods using two measures: the mean deviation of object boundaries, estimated using a Euclidean distance transform, and correct detection of individual parasites, represented by an excess or deficiency of connected components. The tests show the proposed method is accurate over a wide range of experimental conditions, supplementing results shown in Figure 1. While both methods place edge pixels relatively close to the ground truth, use of Canny results in the loss of 15% of parasites compared to just 3% with the proposed method. As expected, the highest accuracy is obtained for control images. In comparison to the controls, long term exposure to Sim or Chl causes edges between touching parasites to become very weak. For this reason, parasites in these conditions are more difficult to separate when they are in physical contact and are not correctly segmented in all frames. The presence of PZQ evokes a peculiar phenotype in which the parasites tend to "shrivel," adopting very irregular shapes which often bear narrow protrusions from the body. Here, changes in the appearance of anatomical features within the parasites contribute to false edges with the potential to induce the false splitting of single parasites. Success of the proposed method in spite of these difficulties testifies to the high sensitivity of phase congruency to perceptual edge features.

5 Conclusion

One essential component of a fully automated, high-throughput assay for drug discovery against neglected diseases is a computer vision system capable of ameliorating

Table 1. Quantitative Evaluation of the Proposed Method

Experimental conditions	Mean boundary deviation		Number of regions (ratio)	
	Proposed	Canny	Proposed	Canny
Control	0.003	0.002	0.005	-0.067
Sim	4.15	3.20	-0.067	-0.194
Chl	0.596	2.37	-0.077	-0.151
Pzq	2.60	1.08	0.005	-0.199
Overall	1.84	1.92	-0.033	-0.153

the bottleneck created when human experts must be relied upon for data analysis. However, extant methods do not address the distinct challenges found in the segmentation of schistosomes. We presented a segmentation algorithm designed specifically towards the segmentation of standard bright-field microscopy images of schistosomes, which achieved some success without any dependence on proprietary HTS systems. We now improve our method with the development of a novel, high-sensitivity edge operator – the first to combine phase congruency with ridge-detection by grayscale thinning. Quantitative and qualitative analysis demonstrates the power of the algorithm, and paves the way for an effective, high-throughput, phenotypic screen against schistosomiasis. It is hoped that such a system will lead to new drugs which will ameliorate the global health impacts of schistosomiasis.

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