Fully-automated Analysis of Muscle Fiber Images with Combined Region and Edge-based Active Contours

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Abstract. This paper presents a new approach to automated muscle fiber analysis based on segmenting myofibers with combined region and edge-based active contours. It provides reliable and fully-automated processing, thus, enabling time-saving batch processing of the entire biopsy sample stemming from routinely HE-stained cryostat sections. The method combines color, texture, and edge cues in a level set based active contour model succeeded by a refinement with morphological filters. False-positive segmentations as compared to former methods are minimized. A quantitative comparison between manual and automated analysis of muscle fibers images did not reveal any significant differences.
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1 Introduction

The size and shape of muscle fibers is an important cue to diagnose neuromuscular diseases, since it has been shown that morphometric data can discern very early changes in the distribution pattern of fiber size in muscle biopsy samples [1]. While fiber size estimation by simple inspection, as yet performed in routine diagnostics, is inaccurate and subjective, accurate measurements by means of manual segmentation of fibers is time-consuming and tedious, particularly since a few hundreds of fibers per specimen have to be analyzed in order to obtain statistical significance. This brings up the need for an automated analysis routine of muscle fiber images.

Despite this need for an automated process, until today, only few segmentation methods dedicated to this task have been presented [2, 3, 4, 5]. The reported methods are mostly based on border shape enhancement routines, followed by application of user-defined or histogram based thresholds, and interactive manual editing. Due to inaccurate delineation of fibers as well as the need of special histochemical stains and partially extensive user interaction, the usability of these methods in practice is limited.

For a fully-automated yet reliable automated analysis, a solid and accurate separation of single myofibers from the remaining parts in the image (i.e., connective tissue, nuclear clumps, and blood vessels) is essential. To succeed in this task, the presented method makes use of recent advances in level set based segmentation, where classical edge-based active contours [6, 7, 8] are extended by region based cues, such as the color and texture. Region based active contour models that partition the image into two classes have been introduced in [9, 10]. In the present application, the two classes are the class of the myofibers and the class of the background.

This extension allows for exploiting three sources of information to separate the classes: color, texture, and edges. They are combined in a sound and transparent way by means of a cost functional. The separating contour is thereby modelled by an implicit level set function that allows for topological changes and can, hence, handle the area of myofibers as one class, though the separate fibers are not connected. The functional and its minimization are detailed in Section 3.

With the single fibers at hand, a multitude of morphometric parameters can be assessed for all detected fibers in a single pass. The whole method works without any user interaction and with a fixed set of parameters. Despite this full automation, the empirical evaluation in Section 4 shows a high reliability of the method. Section 5 concludes the paper by discussing the impact of this outcome.

2 Muscle Sample Preparation and Image Acquisition

Biopsy samples were trimmed, mounted, and frozen in isopentane-cooled liquid nitrogen, before storage at $-70°$C. Transverse sections (10μm) were cut with a cryotome at $-20°$C and attached to slides by thawing. After keeping the slides at room temperature for at least 30 min., the sections were stained with hematoxylin and eosin.

Microscopic images were then taken in artifact-free areas in muscle cross sections with a $20\times$ objective (Nikon Eclipse E600 microscope, Nikon DN100 CCD-camera) and stored as 640×480 pixel ($571 \times 428\mu$m) RGB color images.

3 Segmentation of Myofibers

Given the fiber image $I : \Omega \to \mathbb{R}^3$, the decisive task is to determine the outline of the myofibers. To this end, we suggest a level set based active contour model that combines multiple cues to separate the fibers from the remaining parts of the image.

In level set based segmentation methods, the contour is represented by the zero-level line of a so-called embedding function $\Phi : \Omega \to \mathbb{R}$. This implicit representation of the contour has several advantages, among others that parts of the two regions need not necessarily be connected and may split and merge. Since the region of the muscle fibers itself consists of numerous connected components, this flexibility in the topology of the regions is an important property of the method.

The active contour model is described by a cost functional, in which undesirable properties of a possible solution are penalized by high costs:

$$E(\Phi) = -\int_\Omega \Big(\underbrace{H(\Phi)\log p_1 + (1-H(\Phi))\log p_2}_{\text{region based}} - \underbrace{\nu\, g(|\nabla I|^2)|\nabla H(\Phi)|}_{\text{edge based}} \Big)\, dx. \quad (1)$$

The region based part is a probabilistic model, that maximizes the total a-posteriori probability of pixels assigned to the best of two regions. The membership of a pixel to one of the regions is determined by the sought level set function Φ evaluated by means of the step function H, which is smoothed in order to ensure differentiability [9]. The regions are modelled by the probability densities p_1 and p_2 that are estimated within the two regions, respectively. For the density estimation we employ the Parzen method that comes down to smoothed region histograms; see [11]. The region based part exploits that all fibers have a similar color that can generally be distinguished from the color of the intermyofibrillar connective tissue. Since color alone may not always be sufficient to distinguish the two regions, it is supported by texture information represented by the feature space provided in [12]. It captures the fact that the muscle fibers are mainly homogeneous without much textural variations, whereas the endomysial connective tissue contains collagenous fibers, blood vessels, fibrocytes, and other cellular components. The joint densities p_1 and p_2 are approximated as the product of the densities of the single feature channels.

The second part of (1) represents the classical geodesic active contour model that seeks a contour of minimal weighted length. A decreasing function $g(s^2) = 1/\sqrt{s^2+1}$ of the image gradient magnitude $s^2 = \sum_{k=1}^{3} |\nabla I_k|^2$ serves as weighting function in order to reduce the cost of the contour length in the presence of edges. This part of the model exploits the fact that the muscle fibers are often separated from the connective tissue by more or less strong edges. Hence, it supplements a third cue for the partitioning besides the color and texture information. Moreover, the penalty on the contour length avoids single noise pixels or small artifacts to be separated from the surrounding region. The relative influence of the edge-based term is determined by the parameter $\nu = 2$.

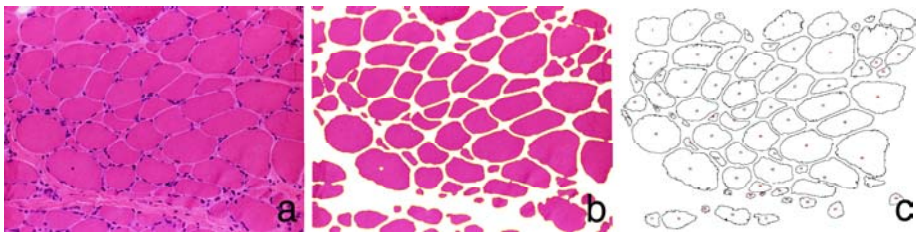
Starting from an initial partitioning obtained by a histogram based thresholding, a minimum of (1) is obtained by steepest descent. This yields the update

$$\Phi^{k+1} = \Phi^k + \frac{\tau}{\nu} H'(\Phi^k) \left(\log \frac{p_1}{p_2} + \nu \operatorname{div} \left(g(|\nabla I|^2) \frac{\nabla \Phi^k}{|\nabla \Phi^k|} \right) \right) \quad (2)$$

with iteration index k, time step size $\tau = 0.5$, and H' being the derivative of H with respect to its argument. In each iteration, also the densities p_1 and p_2 are updated. After 200 iterations one obtains the sought contour separating the muscle fibers from the connective tissue; see Fig. 1b.

Although the active contour model yields accurate boundaries in large parts of the image, some myofibers may not be completely separated. In order to refine the result, it is merged with the outcome of an edge detector, binarized by automated thresholding, and enhanced by filters from mathematical morphology [13]. The final result of this postprocessing is shown in Fig. 1c.

Fig. 1. (a) Original input RGB image of a dystrophic muscle biopsy sample. (b) Resulting segmentation with the active contour model. Non-fiber structures are removed. (c) Final result: each analyzed fiber is outlined and numbered. Fibers touching the image boundaries are not considered.



4 Experiments

A total of 30 digital images containing 679 fibers on five human muscle specimens were segmented. Parameters were kept fixed for all images to ensure a fully automated processing. The segmentation results were evaluated independently by two neuropathologists yielding a misclassification rate of only 2%.

Additionally, the accuracy of the morphometric analysis was assessed by comparison between human and machine measurements in 10 out of the 30 digital images containing a total of 191 fibers on five human muscle specimens. Three experts manually traced fiber outlines with a computer mouse using the "free-hand" ROI selection tool. Calculation of cross sectional area, perimeter, circularity, and Feret diameter were then analyzed automatically by the computer for each outlined fiber. The manually collected data were compared to the machine measurement of the same images. For each fiber, the three human measurements and the results obtained by the automated system were averaged. This was used as standard of comparison. The mean error was defined as the mean of deviations from the standard of comparison assessed for each fiber. The results shown in Tab. 1 do not reveal any significant differences between human and machine analysis.

5 Discussion

The experiments show that, despite the full automation, the method works very reliably with a mixed set of muscle specimen. This is in contrast to previous techniques that either yield many inaccurate segmentations, or need a significant amount of manual user interaction. The robustness of the presented approach can be explained by the sound combination of three types of information. This ensures that in case of one cue not being available, or reliable, the others can promote the solution. Moreover, the method is easy to use, and it neither requires special knowledge in image processing nor special histochemical stains. As a consequence, the method satisfies all premises for being valuable in high-content image analysis of muscle samples for research purposes as well as in clinical diagnostics. Since the method enables full automation, convenient batch

Table 1. Average morphometrical values and mean error from standard of comparison as assessed for 191 fibers obtained manually by three investigators and by machine.

	human 1	human 2	human 3	machine
fiber area	$5707\mu m^2$	$5639\mu m^2$	$5617\mu m^2$	$5625\mu m^2$
	7.6%	7%	6.7%	7%
perimeter	$292.5\mu m$	$301.1\mu m$	$301.1\mu m$	$302.7\mu m$
	5.6%	4.1%	4.3%	4%
circularity	0.74	0.70	0.69	0.68
	6.1%	9.7%	9.4%	10.5%
diameter	$111.3\mu m$	$111.4\mu m$	$110.9\mu m$	$111\mu m$
	3.7%	4.1%	3.5%	3.6%

processing of the entire biopsy sample may be possible and selection bias can be avoided.

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