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An image processing approach to analyze morphological features of microscopic images of muscle fibers

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ABSTRACT

We present an image processing approach to automatically analyze duo-channel microscopic images of muscular fiber nuclei and cytoplasm. Nuclei and cytoplasm play a critical role in determining the health and functioning of muscular fibers as changes of nuclei and cytoplasm manifest in many diseases such as muscular dystrophy and hypertrophy. Quantitative evaluation of muscle fiber nuclei and cytoplasm thus is of great importance to researchers in musculoskeletal studies. The proposed computational approach consists of steps of image processing to segment and delineate cytoplasm and identify nuclei in two-channel images. Morphological operations like skeletonization is applied to extract the length of cytoplasm for quantification. We tested the approach on real images and found that it can achieve high accuracy, objectivity, and robustness.

1. Introduction

Skeletal muscle is the most abundant tissue of the human body, occupying about half of the body weight in healthy adults [1]. Skeletal muscle is principally composed of individual muscle fibers (or muscle cells) that are arranged in a structured manner. Muscle fibers are formed in the development process from the fusion of myoblasts (primitive muscle cells that can potentially develop into a muscle fiber) in a process known as myogenesis. The long, cylindrical, multinucleated muscle fibers is the biggest cell in the body, generally of tens of micrometers in diameter and hundreds of micrometer in length. The cytoplasm of skeletal muscle fibers mainly consists of regularly arranged myofibrils, in which two components, actin and myosin filaments, are responsible for the striated appearance of skeletal muscles. Functionally, skeletal muscles not only control bodily movement through their contraction and relax but also constitute the largest metabolic pool of the body. Aberration of skeletal muscle is the cause of many muscular diseases, including cardiomyopathy [2–4], muscular hypertrophy [5,6], inflammatory muscle damage [7–9], muscle development [10,11], muscular dystrophies [12–14], amyotrophic lateral sclerosis (ALS) [15], and myasthenia gravis [16]. Many of these diseases have no effective treatment and some of them like muscular dystrophies have a high fatality rate [17,18].

In studying skeletal muscles there are multiple criteria in evaluating the healthy status of muscles, including functional improvement of muscles, biochemical changes in muscle mass, and morphology of muscle fibers observed under microscopes. In this paper we focus on developing an image processing approach to automatically analyze and quantify morphology of muscle fibers because many muscular diseases manifest themselves as abnormality in terms of changes in nuclei and cytoplasm of muscle fibers. For example, skeletal muscles are capable of undergoing hypertrophy (increase in their sizes) in response to increased loading, while it can become atrophy upon unloading or immobilization. Skeletal muscle fibers have been shown to gain newly formed nuclei via the fusion of myogenic cells with the adult fibers in muscular hypertrophy. In muscular atrophy, muscle fibers are shown to usually lose nuclei through apoptosis. As such, one important criterion in evaluating the efficacy of treatment under development is to assess whether normality in muscle fiber morphology can be restored, in combination with dynamic evaluation like muscle functions and *in vivo* imaging [19,20]. The large number of microscopic images of muscle fibers generated in a typical experiment can be on the

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order of hundreds, making it is time prohibitive and error prone for manual analysis. Computerized analysis can provide objective and quantitative measurements in a high-throughput manner and assist researchers to assess many direct features and derived characteristics of muscle fibers. For example, after finding nuclei and cytoplasm in images, computerized analysis can drive the nuclei-to-cytoplasm ratio of muscle fibers, a criterion widely used to assess the health of muscle fibers [21,22].

In this work we developed an image processing pipeline consisting of image segmentation, quantification, and morphological operations to analyze muscle fiber images in a high-throughput manner. We tested the method on muscle fiber images acquired from tissue samples stained for both nuclei and cytoplasm and found the method can achieve high objectivity and accuracy.

2. Materials and methods

2.1. Cell culture, immunostaining, and image acquisition

We harvested primary myoblasts from hind limb muscles of 4-week old C57BL/10 male mice as described in Rando et al. [23]. The myoblasts were expanded in Ham's F10 medium supplemented with 20% fetal calf serum and 5 ng/ml basic fibroblast growth factor on collagen-coated plates. After clone culture, the myoblasts were identified with anti-desmin antibody through immunocytochemistry. To induce myogenic differentiation of the cultured myoblasts, the growth medium was replaced with differentiation medium (DMEM with 2% horse serum) after the percentage of coverage reached over 70%. For immunostaining, the differentiated cells were fixed with 4% paraformaldehyde for 30 min at 4 °C, washed and treated with 0.5% Triton-X 100 in PBS for 5 min at room temperature. Then the cells were incubated with primary antibody Myosin Heavy Chain (MHC) diluted in 1:50 (MEDCLA66, Accurate Chemical & Scientific Corp, NY) followed by incubation with a CyTm3-conjugated secondary antibody (Jackson Lab) diluted in 1:500 to observe the cytoplasm. The nuclei were counter-stained with 4,6-diamidino-2-phenylindole (DAPI). Pictures were taken using a laser microscope (Nikon Eclipse E600) and saved as TIFF images with a pixel size of 0.76 μm. Fig. 1 shows a typical image after merging the DAPI and MHC channels.

2.2. Image processing pipeline

As the original images were collected in two channels, one for cytoplasm and the other for nuclei, our image processing pipeline features two paths to analyze each channel separately. There are

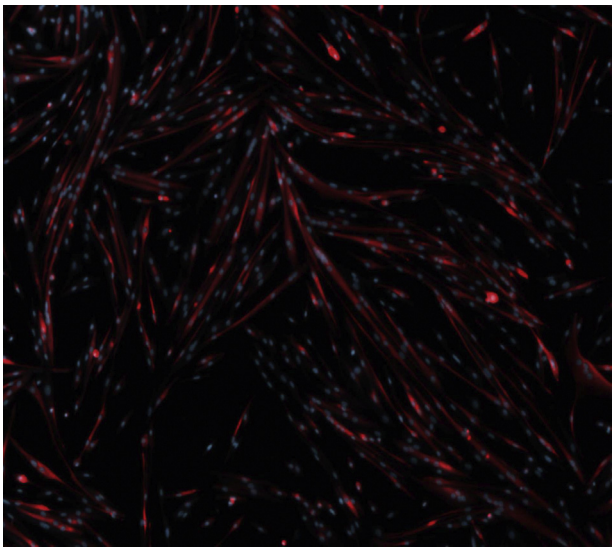

Fig. 1. An original image showing both cytoplasm and nuclei of harvested TA muscle from a mouse model of muscular dystrophy. We can observe that cytoplasm generally has an elongated ellipsoidal shape. Due to the presence of nuclei and other factors in imaging, cytoplasm has an uneven signal intensity, making them a challenge for automatic detection.

two challenges in extracting objects from this type of two-channel images. The first challenge is that the images may have an uneven background and objects have close adjacency among them. The second challenge is that due to limitations in staining and imaging, there are residual signals from the MHC channel in the DAPI channel and vice versa. For example, Fig. 2(a) shows the DAPI channel image of Fig. 1 and we can observe some weak components of cytoplasm in the image. Fig. 2(b) shows the MHC-stained cytoplasm of Fig. 1 and because nuclei are not stained by MHC, they appear as dark holes on the MHC channel image, which may affect the accuracy of segmenting cytoplasm if the dark holes are not taken into consideration.

From Fig. 1 we note that cytoplasm generally has an elongated ellipsoidal shape with varying lengths. We also note that cytoplasm tends to have an approximately straight profile, a fact that we will explore in our algorithm design to detect them. Our image processing pipeline is shown in Fig. 3, which consists of two paths, with one to process the cytoplasm channel and the other to process the nuclei channel. Each branch has two main steps, binarization and morphological analysis. At first, we give an overview of

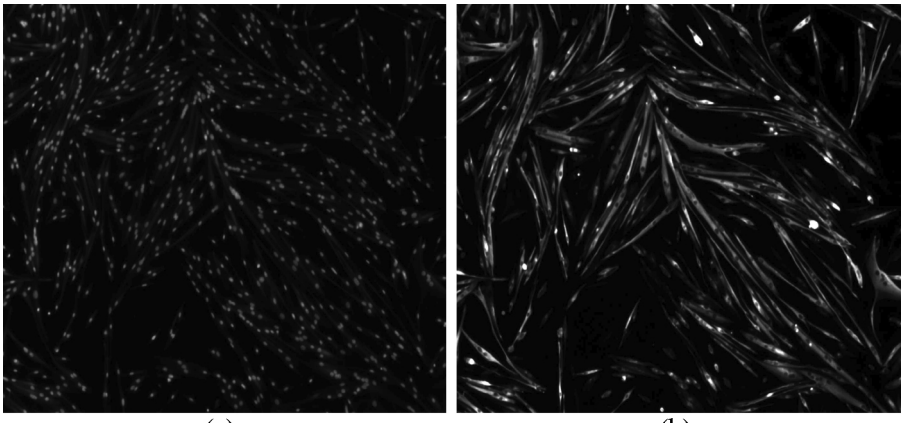

Fig. 2. (a) The DAPI channel of Fig. 1 showing nuclei, from which we can observe some residual signals from the MHC channel. (b) The MHC channel of cytoplasm, from which we can observe that as nuclei are not stained by MHC they constitute dark holes in cytoplasm.