Techniques to support diagnosis of muscular dystrophies based on image processing and reconfigurable systems

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Abstract— Prior to the diagnosis of muscular dystrophies, pathologists must perform a difficult and tedious procedure, including the extraction of cross-sectional samples of skeletal muscle, and the examination of the muscle fibres in the samples. Using optical microscopy, Type I and Type II fibres are manually quantified, and the lesser diameters of 100 random fibres of each type are manually measured. After this, other parameters of interest are calculated, in order to achieve a correct diagnosis. This procedure is not time-efficient and takes time that could be used in the actual diagnosis. In this work, a tool called MuCSA (Muscle Cross-Sectional Analyzer) is created to accelerate the process up to 20 times, and it also provides an automatic quantification and measurement of the lesser diameters of the fibres, besides the calculation of all the parameters of interest for the pathologists.

Keywords— Muscle, fibres, dystrophy, biopsy, digital image processing, segmentation, lesser diameter, graphical user interface.

I. INTRODUCTION

Muscular dystrophies are chronic diseases with a genetic origin. They cause progressively increasing damage, so it is important to make an early diagnosis in order to prevent deterioration of the muscle [1]. Skeletal muscle tissue is composed of fibres Type I and II, whose sizes are important for the diagnosis. The way to detect dystrophies is the study of fibres from cross-sectional muscle tissue, obtained after a biopsy. Optical microscopy images of the samples are taken to proceed with the study.

In the Department of Pathological Anatomy of the Hospital Insular – Materno Infantil de Las Palmas de Gran Canaria, the pathologists manually carry out the following main tasks, which usually spend more than 30 minutes to complete:

- Quantification of Type I and Type II fibres, as well as the total amount of them.
- Selection of 100 random fibres of each type, and the measurement of their lesser diameter, defined as the longest perpendicular to the longest diameter.
- Calculation of other parameters, like percentages of each type of fibre, mean lesser diameters, generation of histograms, standard deviation, Variability Coefficient and the Atrophy and Hypertrophy Coefficients [1].

Despite the need for an automated process, only few segmentation methods dedicated to these tasks have been presented [2,3,4,5,6]. This work aims to reduce the time spent in the analysis of muscle samples. To do it so, manual tasks have been automated by its implementation in MATLAB. Segmentation techniques were developed to detect the edges of the fibres. Then, a Graphical User Interface was created in order to give to the pathologists a custom easy to use tool.

II. METHODOLOGY

A. Muscle sample preparation and image acquisition

After the extraction of the cross-sectional muscle sample, the stain ATPase A with pH=9.4 technique must be applied to the sample for type distinction. Type I fibres will have light colour, and Type II, dark colour [1]. Both the histological cut during the biopsy and the staining are extremely important to prevent overlapping of fibres and to facilitate type distinction.

Once the samples are stained, the images must be taken with magnification x100. If the whole sample is not observed on the image, then several images must be taken to compose them into one. The Department of Pathological Anatomy supplied images from nine real cases. The four most representative images are shown in Fig. 1. The figure shows the importance of a correct histological cut and staining.

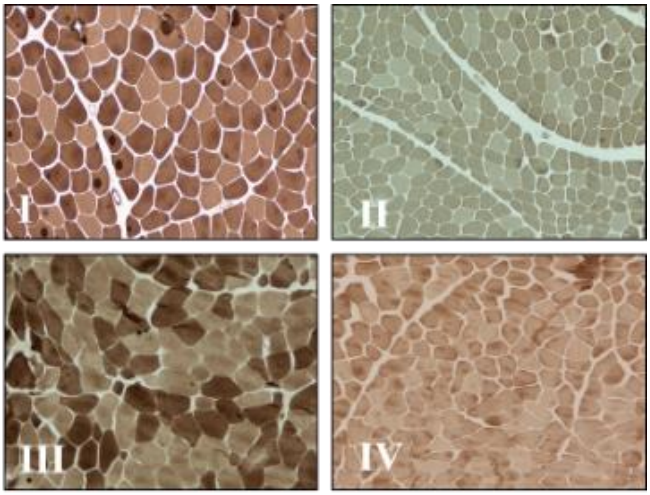

Figure 1: I) correct cut and staining; II) correct cut and deficient staining; III) cut with fibres overlapping and acceptable staining; IV) lots of overlaps and incorrect staining.

B. Edge detection algorithms

For edge segmentation purposes, three algorithms have been implemented. The first one was a Watershed Transform based algorithm [8], which fragments the image with divisory lines that depend on foreground and background markers. The second algorithm takes advantage of the unsupervised classification method, K-means [7], by modifying the result of the three colours classification with morphological operations.

To define the most accurate fibres edges, a new approach was developed in this work. The proposed algorithm combines, by using a "bitwise logical OR" operation, the resulted images of two edge detectors, that are based on the luminance gradient and Gaussian filters, in order to take advantage of them. A flow diagram of the proposed edge detection model is presented in Fig. 2. The final image has significantly better quality results in muscle fiber edge detection, compared to the resultant images before logical OR.

C. Quantification and classification of muscle fibres

To accomplish the classification and quantification of fibres over the segmented image, the mean luminance of each isolated area given by the segmentation process was calculated. Then by thresholding (Otsu Method), fibres are classified in Type I and Type II depending on their corresponding mean luminance. After classification, percentages of quantity of each fibre type are possible to calculate.

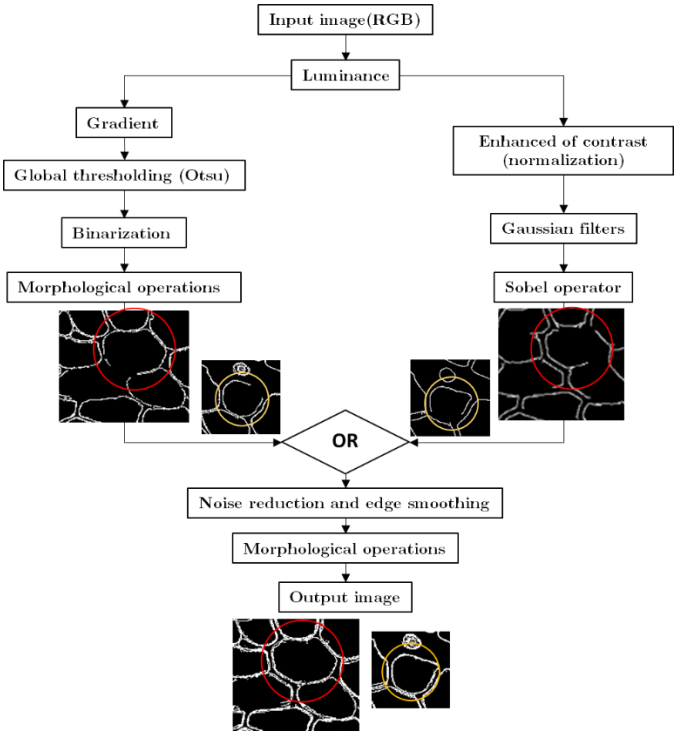

Figure 2: Flow diagram of the proposed algorithm.

D. *Calculation of lesser diameters*

To calculate the fibres lesser diameters, three algorithms have been developed. The first one is the brute-force search, which aims to find the longest euclidean distance in a fibre, so the lesser diameter can be found as its longest perpendicular diameter. The second one is based on the concept of Feret's diameter, which represents the distance between the two farthest tangential parallels of a fibre edge pixels. By rotating the fibre, it is possible to obtain the longest diameter, and the lesser, by finding its longest perpendicular. The degrees The degrees of rotation are defined by the programmer (in this case they are 5º). The third one, implies the adaptation of the principal stresses method, defining a stress as a covariance [9]. The principal stresses directions of a plane are defined as the eigenvalues from the original stresses matrix. In Fig. 3, the direction of the principal stress, σ_2, coincides with the lesser diameter direction, so then it is possible to calculate it.

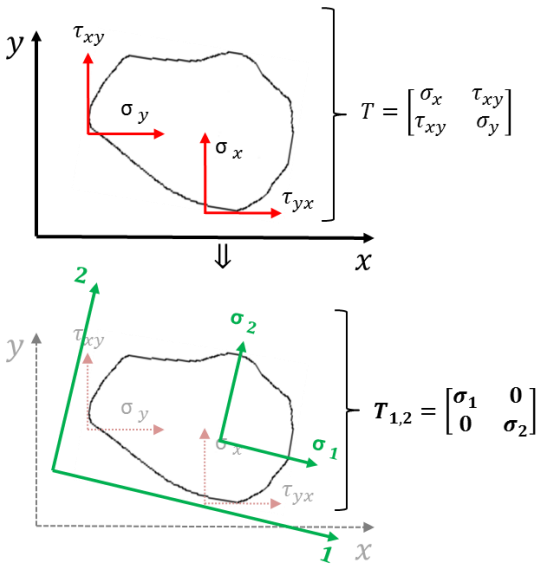

Figure 3: Calculation of fibre lesser diameter with the principal stresses method. Symbols σ_x, σ_y, τ_{yx} and τ_{xy} show the original stresses. σ_1 and σ_2 show the principal stress of the plane (fibre). σ_2 corresponds with the direction of the lesser diameter.

III. RESULTS

A. *Results of the edge detection methods*

To express the quality of the methods, Table 1 shows the results obtained after applying the methods to the four representative images. The last column, a Figure Of Merit (FOM) is introduced:

$$\mathrm{FOM}_{\mathrm{Segmentation}} = \frac{2 \cdot (\text{Correspondance with real fibres})}{\text{Isolated areas} + \text{Real fibres}} \cdot 100 \quad (1)$$

where "Isolated areas" expresses the number of isolated areas over the segmented image, "Correspondance with real fibres" indicates the number of real fibres among the isolated areas, and "Real fibres" expresses the quantity of fibres manually quantified over the original image. FOM shows how the best algorithm is the one proposed here, because it presents the FOM values that are closest to 100 (the ideal value).

	Method	Isolated areas	Correspondence with real fibres	$\mathrm{FOM_s}$
Image I	**Watershed**	195	97	55.8
	K-means	87	58	48.3
	Proposed	155	151	**98.0**
	Real fibres = 153			
Image II	**Method**	**Isolated areas**	**Correspondence with real fibres**	**$\mathrm{FOM_s}$**
	Watershed	258	222	82.5
	K-means	199	156	65.1
	Proposed	290	278	**97.5**
	Real fibres = 280			
Image III	**Method**	**Isolated areas**	**Correspondence with real fibres**	**$\mathrm{FOM_s}$**
	Watershed	75	45	38.9
	K-means	15	10	11.7
	Proposed	135	120	**82.4**
	Real fibres = 156			
Image IV	**Method**	**Isolated areas**	**Correspondence with real fibres**	**$\mathrm{FOM_s}$**
	Watershed	194	109	53.7
	K-means	56	25	18.7
	Proposed	246	195	**82.6**
	Real fibres = 226			

Table 1: Results of edge detection methods.

B. *Results of the quantification and classification*

Table 2 shows the results obtained after the classification of fibres. To evaluate the results, the quantity of fibres of each type was manually calculated over the original image.

Image	Type I (manual)	Type I (software)	% of accuracy	Type II (manual)	Type II (software)	% of accuracy
I	36	34	94.4 %	117	122	95.9 %
II	49	51	96.1 %	231	239	96.7 %
III	70	55	78.5 %	86	80	93 %
IV	56	50	89.3 %	170	192	88.5 %

Table 2: Results of the quantification and classification of fibres.

After classification, percentages of quantity of each fibre type are now possible to calculate.

C. *Results of the lesser diameter calculation*

The calculation of the lesser diameter has been implemented for three fibres with the three methods described before. The lesser diameters manually measured of each fibre were, in pixels, 162.8, 143.5 and 159. The runtime has been expressed as a quality result. The Figure Of Merit shown in the last column of Table 3, is defined as:

$$\mathrm{FOM}_{\mathrm{diameters}} = \frac{\%\ \text{accuracy longest diameter} + \%\ \text{accuracy lesser diameter}}{100 \cdot \text{Runtime}} \quad (2)$$

where "% accuracy longest diameter" and "% accuracy lesser diameter" indicate the percentage of accuracy achieved with the algorithms regarding the measured values. "Runtime" expresses the time spent in the calculation. The larger is the FOM value, the better is the method.

Fibre I	% accuracy		Runtime	Estimated time for 200 fibres	FOM_d
	Longest	Lesser			
Brute-Force	99.7%	99.9%	19.9 s	66.6 min	0.10
Feret (5º)	99.5%	99.3%	3.2 s	10.7 min	0.62
Stresses	99.5%	99.4%	0.48 s	1.6 min	**4.14**
Fibre II	**% accuracy**		**Runtime**	**Estimated time for 200 fibres**	**FOM_d**
	Longest	Lesser			
Brute-Force	99.9%	99.7%	19.1 s	63.7 min	0.11
Feret (5º)	99.8%	98.3%	3.1 s	10.3 min	0.32
Stresses	99.6%	98.8%	0.45 s	1.5 min	**4.41**
Fibre III	**% accuracy**		**Runtime**	**Estimated time f or 200 fibres**	**FOM_d**
	Longest	Lesser			
Brute-Force	99.7%	99.9%	19.9 s	66.6 min	0.10
Feret (5º)	99.5%	99.3%	3.2 s	10.7 min	0.62
Stresses	99.5%	99.4%	0.48 s	1.6 min	**4.14**

Table 3: Results of the lesser diameter calculation.

D. Graphical User Interface: MuCSA v1.0 (Muscle Cross-Sectional Analyzer)

A GUI has been developed to give the pathologists a custom tool easy to use and that allows them to make decisions, like the manual selection of the fibres for the calculation of the lesser diameter after the fibre random selection by the software.

Since they yielded the best FOM values, MuCSA includes:

- The proposed algorithm for segmentation.
- The principal stresses method for the lesser diameters calculation.

Other parameters of interest are calculated from the results of the algorithms, like percentages of each type of fibre, mean lesser diameters, generation of histograms, standard deviation, Variability, Atrophy and Hypertrophy Coefficients.

E. Profiling of algorithms

Runtime of each algorithm has been measured by making a profiling of them. It has been determined that the code parts with the longest runtime are those executed for the quantification and classification of the fibres and for the calculation of the lesser diameters.

	Image I	Image II	Image III	Image IV
Classification and quantification	49.4 s	84.1 s	12.3 s	56.1 s
Diameters calculation	37.2 s	45.1 s	14.9 s	49.7 s
Number of diameters	97	138	107	162
Average time per fibre	0.39 s	0.33 s	0.14 s	0.31 s

Table 4: Profiling of the algorithms with largest runtime.

These algorithms have parts that could be independently executed. As a consequence, they are potentially parallelizable.

IV. CONCLUSIONS

This paper presents algorithms, which have been unified in MuCSA v1.0, that accelerate the manual procedure for the diagnosis of muscular dystrophies. In particular, the time spent during the procedure has been significantly reduced, from 30 minutes to 2 minutes. The last algorithm proposed for segmentation provides more accuracy for edge detection. The principal stresses method for the calculation of lesser diameters provides insignificant error and shorter runtime than the others.

Other parameters are calculated from the results of the algorithms, like percentages of each type of fibre, mean lesser diameters, generation of histograms, standard deviation, Variability Coefficient and the Atrophy and Hypertrophy Coefficients.

This work is the first step for the development of a commercial tool, that automatically solves the tasks required and that can be used in any hospital or clinical laboratory.

Furthermore, the algorithms with the larger runtime have been analyzed, and they resulted potentially parallelizable, since they do not need to be executed sequentially.

Once detected, these routines can be accelerated, with the use of reconfigurable systems such as FPGAs, GPUs or multi-core CPUs, being this study a part of a future research.

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