Comparative Study of Histological and Histochemical Image Processing in Muscle Fibre Sections of Broiler Chicken

Abdelhamid Achouri (✉ abdelhamid.achouri@univ-soukahras.dz)

Institut des Sciences Agronomiques et Vétérinaire Souk Ahras <https://orcid.org/0000-0002-4298-1340>

Mohamed Melizi

Department of Veterinary, Veterinary Sciences and Agricultural Sciences institut, University of Batna

Hamid Belbedj

Department of Veterinary, Institute of Agriculture and Veterinary Sciences, Mohamed-Cherfi Mesaadia University-Soukahras

Abdenmour Azizi

Department of Veterinary, Veterinary Sciences and Agricultural Sciences, University of Batna

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Research

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Abstract

Image processing of muscular fibre has significantly improved over the last decades, from light microscopy acquisition to virtual microscopy and from manual segmentation and fibre typing to automatic. The study aims to discuss main tools used in different histoenzymological image processing phases of muscle fibre, which are consecutively, acquisition, segmentation and fibre typing and their efficacy in morphometric parameters' determination.

Firstly, the acquisition, optical microscopic image with different magnifications (x100, x200, x250 and x400) were compared with virtual slides digitized by Slide Scanner, in muscle fibre sections, for cell number counting. Secondly, the segmentation, three software (Fiji «Digitizing pen, Mouse», Image Pro Plus 10 ~~-semi-automatic-~~ and Cytoinformatics LLC ~~-automatic-~~) were compared for image segmentation quality and fibers number determination. Thirdly, manual fibre typing with Fiji of segmented images using three software cited previously were performed to calculate the accuracy of morphometric parameter (CSA, perimeter and DMF). Results of acquisition showed that scanner slide have a better resolution and detected a higher number of cells compared to optical microscopic with different magnifications. For this latter, the processing can be performed only with an acceptable resolution, where the number of cells is lesser, which requires unfortunately several repetitions and exhausting work. Our findings regarding segmentation indicate that Cytoinformatics LLC showed the best processing time and the highest quality followed by IP and Fiji. For the last step, morphometric parameter calculation showed the best accuracy using Fiji followed by Cytoinformatics and finally by IP. The findings of this study suggest that Fiji (semi-automatic) showed the best quality/price ratio (open access software) for segmentation and fibre typing, but time consuming compared to Image Pro Plus 10 (semi-automatic) and Cytoinformatics LLC (automatic) which are a paid service.

Introduction

Research on skeletal muscle is a broad and complicated field, structural and functional evaluation (quantitative and qualitative) related to this tissue, remain challenging. As a result, many muscle diseases still not fully understood, and continue worrying scientists and suffering patients. An essential approach to study these latter in muscle biology are: physiological (metabolic or contractile type) and morphological determinism (number, Cross sectional Area «CSA», Minimal Feret's Diameter (MFD) and perimeter); similarly, these parameters used for quality determination of farm animal meat.

In recent decades, muscle histological image processing has been considerably developed. It is a vast field that encompasses all the methods and techniques operating to make the processing simpler and faster with image visual aspect improved and relevant data extracted. Currently, histoenzymological images processing (histological and histochemical) of the muscle is successfully advanced in muscle fibre typing, as well as their morphometric parameters determination (number, CSA, MFD and perimeter). The mastery of histoenzymological image processing requires three main determinants: excellent image acquisition, perfect segmentation, and adequate fibre typology.

For the microscopic acquisition of the histological image of the muscle, different magnifications have been used by scientists, but variably, where several have been used in the same research, aiming to reach an acceptable level of studied fibres, which influences the accuracy of the results. Magnification of x200 was commonly used [1–9] compared to x4 [1], x45 [11], x50 [12, 13], x90 [11], x100 [14]; [5, 6, 15, 16], x400 [17]. However, researchers were unable to capture the entire surface of a stained section, which considered the whole area of a muscle, into a single image, as a consequence some of them were forced to perform several images with low magnification. Garton *et al.* [10] and Wen *et al.* [8] were used respectively, objectives of x20 and x40 to cover the entire surface of the muscle in order to join

them after, into a single complete image using Photoshop. Moreover, there is considerable regional variability in fibre size in some muscles; therefore, measuring or sampling only a few small areas will not necessarily provide representative measures [4]. In recent years, bioinformatics researchers have successfully developed scanners that digitize slides sections coloured by histoenzymological reactions [9, 18–23].

Similar to acquisition, segmentation of histological muscle image into distinct fibres in order to calculate their morphometric parameters is difficult, and simple thresholding or morphology technics have a big difficulty to separate precisely tightly affixed fibres [4, 10, 24]. To overcome these limitations, researchers use universal semi-automatic methods for image processing of different types of tissue (Fiji [25], Image Pro Plus « Media Cybernetics », Metamorph « Universel Imaging Corporation »), and specialized ones for processing histoenzymological images of muscle tissue (Racine [26–29]. In parallel, the development of completely automated methods for the histoenzymological image processing of the muscle has gradually taking place [2–4, 20, 21, 23, 24, 30, 31,]. These technics are more effective (i.e. the boundaries between muscle fibres are well-defined and more discriminating information is provided).

The methods of typing muscle fibers are highly advanced and developed, starting by manual typing technics [32], then by interactive tools, which measure the optical density of the fibres using spectro-photo-microscopy [27, 33], and finally, skeletonization approaches of the inter-fibre network in different serial sections, which classify fibres according to the gray levels of muscle cells [26, 34]. Recently, there was an emergence of automated tools that classify the fibres according to their densitometry by superimposing various serial sections coloured differently on a reference image [6, 14].

To our knowledge, no study has compared muscle image acquisition methods between light microscopy and slide scanner, with a focus on image quality and number of fibres studied per image. Also, few studies have compared between image analysis tools regarding their applicability to micrography of muscle cells and various segmentation and analysis tasks. Therefore, choosing a suitable tool for the analysis of muscle fibre images remains a challenge.

In order to perform a suitable processing of the histological image of skeletal muscle tissue and bridging the chasm between image processing methods and muscle scientists, we have chosen three muscles of chicken broiler, on which we carried out this work. The study aims, in a first part, to compare the processing of classical histoenzymological images of muscle tissue in terms of image acquisition by light microscopy and slide scanner. In the second part, to compare three software in terms of segmentation, where two are semi-automatic (Fiji and Image Pro Plus 10 «IP») and one automatic which belongs to the Cytoinformatics LLC group. In the third part, we try to explain our method of muscle fibres typing in order to calculate their morphometric characteristics.

Material And Methods

STUDY DESIGN AND METHODS

Serial images used in this study are from an unpublished research conducted on morphometry and histoenzymology of three different types of muscles (Pectoralis superficialis, Sartorius mixed part, Sartorius rapid part and Anterior Latissimus Dorsi (ALD)) in broiler.

This study was carried out on chickens at different post hatching ages, respectively: D0, D7, D14, D21, D28, D35, D42, D49 and D56, with 10 replicates for each age. For each muscle, we performed three serials sections mounted on three different slides and coloured differently. The first one was stained with red azorubin (considered as a reference slide), the second was intended to reveal the metabolic activity of the enzyme Succinate Dehydrogenase and the third was preincubated in an ATPase solution with a pH of 4.10, to reveal the contractile activity. The three slides were fully

scanned using Leica Slide Scanner (Leica) at 40x magnification by the Cytoinformatics LLC group (Lexington, Kuntacky, USA) and recorded in Aperio-SVS format.

For visualization of scanned slides, Image Scope software (v12.3.2.8013) was used. Serial images were recorded in JPEG format from image scope, where a single serial image was selected for each muscle and age studied. All red azorubin stained images were segmented by Cytoinformatics (CytoF).

Only ALD muscle was selected for our comparative study, because its a slow type, and contain mush conjunctive tissue and less overlapping fibres which make image processing easy to perform.

Image acquisition

From 90 images segmented by the Cytoinformatics group, 63 images of ALD muscle stained with red azorubin were chosen, approximately 7 images for each category of age. Same areas selected from the 63 images, were also captured with different magnifications (x10, x20, x25, x40) for red azorubin images using light microscopy (Optika B-293) equipped with an integrated camera (18MP of resolution). Muscle cells number determination in each images was performed by Cell Counter application in Fiji software, and the results were compared with their contemporaries segmented using CytoF (Fig. 1).

Segmentation

For each category of age, one image of red azorubin was selected randomly from the 90 images of the ALD muscle. A total of 9 images were segmented again in order to calculate the time required for each image using two universal semi-automatic software, which are, IP (Media Cybernetics (USA), trial version « 10.0.2 build 6912 ») and Fiji [25], a new free release of ImageJ). Fiji segmentation was conducted by two methods, one by computer mouse (FM) and the other by a digitizing pen (FDP).

For these two software the segmentation was conducted in two steps:

Muscle fibre individualization

we separate only the overlaying fibres.

For IP, segmentation takes place in two steps (Fig. 2). Firstly, automatic segmentation realized with a variance filter in the green channel in order to improve muscle fibres' boundaries and obtain image with areas of segmented and unsegmented fibres (black arrows in image A). Secondly, manual segmentation by digitizing pen leaves behind a purple striation around the unsegmented fibres (black arrows in image B), which require a second one to obtain a better quality of segmentation (image C). The tool "Mask all frams" in option of the IP software was applied on image C to obtain the final image D.

Similarly, FDP and FM require two steps to achieve the segmentation (Fig. 3). The thresholding tool on red azorubin image gives us an image (A) with a large number of muscle fibres' which are not segmented. Manual segmentation conducted on image (B) with two techniques, digitizing pen (C) and computer mouse (D).

The thresholding with the two software Fiji «FDP, FM» and IP merge the closely overlaying muscle cells, which requires some concentration of the manipulator at the time of segmentation to separate them.

Post processing of segmented images

After segmentation, post-processing step with the Fiji software aimed to remove the artefacts, irrelevant tissues and separate any overlaying muscle cells from the segmented images (by Fiji «FDP, FM», CytoF and IP), and also to calculate the time required for this operation for each software (Fig. 4).

Typology of muscle fibres

Same areas from the nine previously segmented red azorubin images were also selected for images revealing ATPase activity (Fig. 5). Slides showing SDH activity were unconsidered because all the fibres of this muscle shows a positive reaction.

Red azorubin images segmented by the three software (Fiji «FDP, FM», CytoF and IP) were considered later as reference images in Fiji for fibres' typing and their morphometric parameters (Number, CSA, MFD and Perimeter); this selection was done on segmented image using the Fiji "Add to Manager" tool based on the image that reveals the ATPase activity (Fig. 5).

STATISTICAL ANALYSIS

Multiple comparisons between images recorded from Scanner Slide and light microscopy at different magnifications regarding the number muscle fibres (image acquisition) at different ages using ANOVA followed by Bonferroni correction multiple comparison tests.

Multiples descriptive comparisons regarding the segmentation methods were conducted using Excel between:

- Two different segmentation methods of Fiji software «FDP, FM» and IP concerning the time required for the segmentation of the 9 images.
- Three software packages Fiji «FDP, FM», CytoF and IP regarding the time required for image post processing and fibres number in the same image.

Comparison between the three software regarding fibres' morphometric parameters (CSA, MFD and Perimeter) were conducted were conducted using ANOVA followed by Tukey's multiple comparison tests. All statistical analysis were performed using SPSS 22 and data were presented as Mean $\pm$ SD and differences in means were considered statistically significant at $p < 0.05$.

Results

Overall results (Mean $\pm$ SE) are presented in Tables 1,2 and 3 for different comparison between automatic (CytoF) and semi-automatic (Fiji «FDP, FM » and IP) software regarding fibres' number and morphometric parameters.

Image acquisition

There is a significative difference between Slide Scanner and different magnifications of light microscopy regarding the number of fibres ($P < 0.000$, Table 1). Slide Scanned showed the highest detection of fibres' Mean number (765 ± 33.5) compared with different microscopic Mean magnifications consecutively, x10 (468 ± 32.8), x20 (201 ± 32.2), x25 (187 ± 30.6) and x40 (50 ± 8.6). Multiple comparisons were also significative ($P < 0.000$, Table 1) between Slide Scanner and different magnifications of light microscopy at different stage of age. As highlighted in Table 1, the

number of fibres detected in muscle section image decrease gradually in function of age using different magnifications of light microscopy; however, there is a slight increase of fibres from 7 to 42 days of age detected by Slide Scanner.

As shown in Table 1, x20 magnification (950 ± 70.5) showed the greater number of fibres detected at age of hatching (day 0), followed by x25 (902 ± 61.0), then by Slide Scanner (796 ± 65.0). Moreover, the magnification x10 have more fibres' number detection compared with Slide Scanner at D7 (837 ± 56.4 vs 626 ± 44.8) and D14 (839 ± 112.0 vs 623 ± 65.7), and for this later it was impossible to identify the number of fibres at age of hatching. There was no significant difference between magnification x20 and x25 at different stages of age. It can be seen that x40 magnification showed the lowest fibres' number detection at different category of age.

Time required for segmentation comparison between the three software (CytoF, Fiji «FDP, FM» and IP)

Time needed for segmentation of the 9 selected images was not provided by CytoF group because they segmented all images of our project. Time was expressed in hours, minutes and seconds (hour: minute: second). To achieve a complete segmentation, two steps were conducted to enhance the quality of the finale image by cell separation and artefacts deletion.

First step of segmentation (fibre individualization)

According to Fig. 6, IP (Mean time, 1:18:06) showed the lowest time of segmentation at consecutive stages of age, followed by FM (Mean time, 2:39:05) and finally the longest time required was observed in FDP (Mean time, 3:02:46). Also, we can see that the time required for fibre individualization decrease with age until 28 days, where there is a slight increase from 35 to 42 days.

Second step of segmentation (Post processing of segmented images)

The nine images segmented by IP were rechecked by Fiji to remove irrelevant tissue, artefacts and unsegmented cells which took more time. From the Fig. 7, we can note that the time required for post processing of segmented image decrease with age until 28 days, where there is an increase from 35 to 42 days. Moreover, there is a slight difference in time estimated for post processing between FM (Mean time, 0:25:36) and FDP (Mean time, 0:26:13), with difference not exceeding 1 minute. CytoF has the lowest time required for post processing (Mean time, 0:10:00) and IP the highest (Mean time, 0:40:31).

Comparison of muscle fibre parameters obtained after segmentation with the three software

Total number of fibres in muscle

Total number of muscle fibres calculated after segmentation was approximately equal at different category of age using the three software. Mean number of fibres in all stages of age was respectively, 612, 614, 613 and 623 for FM, FDP, IP and CytoF (difference did not exceed 11 fibres). (Table 2)

CSA

There is a significant difference between the three software in CSA at different ages ($P < 0.000$, Table 3), only at 42 days of age, where no effect of software type was observed ($P < 0.349$, Table 2). Moreover, IP has more significant difference at different category of age and the lowest value of Mean CSA compared to other software (1743.16 ± 17.82 , Table 3). The results obtained from Table 3 analysis demonstrate that FM and FDP showed the highest values

of Mean CSA (1863.61 ± 19.44 and 1869.47 ± 18.57 , respectively) followed by CytoF (1833.77 ± 18.92). These later showed no difference in comparison.

Perimeter

Results showed a significant difference between the three software in perimeter at different stages of age ($P < 0.000$, Table 3). Irrespective of age, closer inspection of the Table 3 shows that significant differences were mostly observed between FM and FDP, and IP. As Table 3 shows, there is no significant difference between FM and FDP and they detected the highest Mean values of perimeter (171.83 ± 1.04 and 171.84 ± 0.99 , respectively) followed by CytoF (166.01 ± 0.99). Also, IP showed the lowest Mean value of perimeter with more difference compared to other software (159.45 ± 0.93).

MFD

Results showed a significant difference in MFD between the three software in function of age ($P < 0.000$, Table 3). The results obtained from Table 3 analysis demonstrate that FM and FDP showed the highest Mean values of MFD with no difference in comparison (38.91 ± 0.24 and 38.98 ± 0.22 , respectively). Moreover, IP and CytoF showed the lowest values of MFD with no difference in comparison (37.20 ± 0.22 and 37.24 ± 0.23 , respectively).

Discussion

Image acquisition

It may be appropriate for good research on muscle to work with a number greater than 400 fibres [10]. In the current study, this number is reached using x200 magnification with at least two fields for each type of coloration. The number of fields increase with age, this latter was respectively, 2 at the post hatching, D7 and D14, 3 fields at D21, 4 fields at D28, more than 6 fields at D35, J42, J49 and J56, which inevitably require more time for taking the serial images. This problem forced researchers to minimize the number of fields as well as the number of fibres to be analysed [3, 4]; or to use different magnifications in the same study with high magnifications for muscles of early ages and low magnifications for muscles of adults [11]. This last author conducted a study on muscles of developing chicken from D0 post hatching until the age of 55 weeks and analysed an average of 400 fibres in two microscopic fields with different magnifications (x45, x90, and x215). However, most researches have not specified the magnification used and / or the number of fibres analysed [24, 26, 28, 29, 31, 35–38]. In our study, the average number of fibres by image at the early ages, D0, D7 and D14 post hatching is 950, 227 and 253, respectively, which considered acceptable. However, the segmentation of these images was very difficult as result of fibres' small size despite the acceptable resolution of the camera (18 MP). Similarly, same observations were produced using the magnification x250. The images of x100 magnification are more adequate regarding the average number of fibres / image, which exceeds 400 fibres at D7, J14, J21 and J28, and 200 fibres at D35, J42, J49 and J56; although the segmentation was very difficult especially at the early ages D7, D 14 and D21, where the images become blurred when the zoom was applied to recognize boundaries of the cells. In addition, it is impossible to work on images using this magnification at D0 post hatching.

The magnification of x400 is more laborious due to the number of fields that exceeds 6 for each muscle studied from the day 7 of post hatching.

The average number of fibres / image in all the ages studied varies between 600 and 1000 using CytoF, which considered very acceptable. This related to the software used by this group, which can work on images in SVS format.

In addition, the capacity of segmentation of this later arrive the whole virtual section but more expensive.

Time required for segmentation comparison between the three software (CytoF, Fiji «FDP, FM» and IP)

Image analysis algorithms are widely used by biomedical researchers and software engineers. To carry out this part of the work, firstly, we selected two semi-automatic software which are ImageJ (Fiji) from NIH and IP, from Media Cybernetics since these two software are the most popular currently according to Kostraminova et al. [6] and their popularity in research field associated to several factors such as, accuracy of results, accessibility (price and availability). Secondly, we worked with the group Cytoinformatics LLC for segmentation with automatic software. The comparison between the three software was conducted based on:

The time required for segmentation and the number of fibres obtained

The observation of the time of segmentation of the nine images by the three software reveals that this time decreases with age but it is fluctuating from one image to another and that could be explained

Firstly, by the degree of automatism the tool used for segmentation:

The total time required for segmentation, in hours: minutes: seconds, of the nine images by Fiji/M using the computer mouse is 26: 23:37 and their average segmentation time is 02: 56: 25; so in our unpublished work which was carried out on four muscles with 10 repetitions for nine ages studied, we needed 1061.6 hours to complete this segmentation which is the equivalent of 44.33 days of non-stop work or almost 353.23 days with 8 hours of work per day (Fig. 6).

To segment these images by the same software with the use of FDP this time is the highest compared to the other segmentation methods which is 27: 24: 56, therefore an average segmentation time of 3: 05: 00, but it remains the closest to measuring the fibre parameters.

The total time required for the segmentation of these same nine images by IP is 10:21:35 and their average segmentation time is 1:13:06; so for our work it took 438.6 hours which is the equivalent of 18.27 days of non-stop work or almost 146.2 days with 8 hours of work (Fig. 6).

On the other hand, the new image processing algorithms can manage an automatic segmentation of the image in less than a minute [4, 23] even if these images have a large scale which can reach up to 9000×9000 [23] which considerably reduces the average image processing time.

Secondarily, this time is also influenced by the number of fibres / image which linked to several factors such as:

Age: the number of fibres / image decreases progressively with age due to the radial growth of the fibre and therefore the segmentation time is negatively correlated with age.

The shape and size of the fibre: the irregular and overlapping shape as well as the small size of the fibre increase the segmentation time; these same findings are observed by Liu et al. [4], and Wang [29].

The percentage of connective tissue: when the connective tissue is abundant in the image, the number of fibres and overlapped ones decrease, which reduce the time of segmentation. The percentage of connective tissue varies within

the same muscle and from one muscle to another; it has decreasing value of the slow $\diamond$ intermediate $\diamond$ rapid, according to our unpublished work. As an example, the images chosen at ages D21 and D28 which contain a lot of connective tissue and the number did not exceed 420 fibres, presented a segmentation time lower than the other images (Fig. 6 and Table 2).

Finally, by the image quality: the well-coloured image with good resolution and less debris and artefacts has a low segmentation time.

The time required for post processing of segmented images

The performance of image processing software for muscle tissue is linked to its ability to remove artefacts, irrelevant tissue and to separate any contact between the fibres. The average time (in hour: minute: second) of the post processing of the nine images segmented by FDP and FM was around 00: 25: 00 (Fig. 7) and therefore a total time for the post processing of all the images of our work which is around 112: 50: 00.

The average time of post processing of the nine images segmented by CytoF which was carried out by Fiji is 0: 10: 16 h (Fig. 7) and therefore a total average time for the post-processing of all the images of our work which is estimated at 45: 72: 00 h. The post-processing time of the segmented images by CytoF is very low because these images do not contain artefacts and irrelevant tissues and this time is intended only for the segmentation of the touching fibres, in addition the average time of the post-processing of these same images by Image Pro Plus 10 (IP) is the highest compared to the previous software with a time of 00: 32: 06 h (Fig. 7) and therefore a total time which is by means of 144: 45: 00 h, this is due the presence of many artefacts and irrelevant tissue in the images segmented with this software (Fig. 8).

Comparison of muscle fibre parameters obtained after segmentation with the three software

The three segmentation methods FDP, FM and CytoF gave more consistent results for CSA with non-significant differences for the average surface area which is less than 3.34% for CytoF compared to FDP (Table 3), this parameter (the Area) which is the main criterion for studying muscle fibre confirms that the measurements obtained by CytoF concerning CSA are closer to the measurements obtained by manual segmentation performed by FDP and FM. No significant difference between FDP and FM.

However, statistically, there are highly significant differences for the Perimeter and DMF between CytoF and FDP but the differences are not large (Table 3) with fairly encouraging percentages for these two parameters calculated by the CytoF (less than 5.06% and 5.01% for the Perimeter and DMF respectively) compared to those calculated by FDP.

According to Kostraminova et al. [6], Image J (Fiji) and IP are widely accepted for precise measurements of CSA. The highly significant difference between FDP and IP regarding the average area of the fibre is related to the thresholding step of IP, which leaves in some cases a small margin (Fig. 9). This influence the calculation of average value which is less than 5.02% compared to that calculated by FDP and which remains very satisfactory.

The highly significant differences between FDP and IP concerning the perimeter and MFD are associated to fibre Area calculation (less Area = less perimeter and less MFD). The MFD is very robust against experimental errors such as the orientation of the angle of section [4], and it is recommended by Markus et al. [16]. However, it is influenced by the segmentation technic because it measures the minor diameter in the muscle cell [38]. Despite this, the average values

calculated by IP of these two parameters (Perimeter and MFD) are also acceptable, they are less than 7.19% and 3.96% respectively compared to those found by FDP.

Semi-automatic tools (Fiji and IP), always require prior processing, where the operator must interact manually with the computer, using a digitizing pen or a computer mouse, to remove artefacts and irrelevant tissue and trace the contours of unsegmented muscle fibres. This interaction makes the analysis of a large number of images more laborious and impractical, these observations are confirmed by some authors [23, 30, 39]; despite that, this software remain used by a large community of researchers.

For automatic tools and despite their expensive prices and availability, in addition to the critics addressed to many of these programs such as the use of shortcuts (assuming for example that the fibres are circles or ellipses) which can make the measurements of the morphological parameters of the fibres inaccurate [39]; the software of the CytoF remain far from these criticisms and gives satisfactory results.

Fibre typology

We adopted the manual method for typing muscle fibres for two reasons; the first is the additional costs and the second is the difficulties of classifying all muscle cells, especially in sections where the muscle fibres are not parallel to each other or they change shape through a large series of sections, or if certain fibres disappear from the section or divide; these cases are well presented in our work and proved by Karen et al. [28].

Conclusion

The main goal of the current study was to compare tools (automatic and semi-automatic) used in muscle histological image processing steps, where the main are: acquisition, segmentation and fibre typing and determine morphometric parameters of each tool.

This study has identified that Slide Scanner allows a large-scale selection of image with a good resolution and a very satisfactory number of muscle cells per image compared to images captured with different magnifications commonly used in research; therefore, a reliable work in muscle tissue field, this type of image will gradually replace the conventional images of the microscope. Automation progress in image acquisition and processing, help researchers to reduce the amount of time required for image processing from hours to minutes or seconds and save more time for other steps of their research.

The research has also shown that CytoF in the field of muscle cell segmentation provide a very satisfactory and more precise service, it remains a small concern for the segmentation which not performed by the researcher himself and this require a post-image processing step to remove any contact between cells using another tool.

Free access advantage of Fiji makes it sanctuary for researchers and small laboratories with limited resources. However, this latter is universal software, in which, the menu contains a lot of functions with a complex graphical interface that requires a good training. It will be better to create a special version for the treatment of muscular images with only specific tools for this field and a complete documentation for this type of picture. Similarly, IP is simpler and easier and make faster the processing of muscle tissue images compared with Fiji, but it requires a good mastery of its tools for very satisfactory results.

Abbreviations

CSA: Cross Section Area; **MFD:** Minimal Feret's Diameter; **FDP:** Fiji Digitizing Pen; **FM:** Fiji Mouse; **IP:** Image Pro Plus 10; **CytoF:** Cytoinformatics LLC

Declarations

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Availability of data and materials

N/A.

Authors' contributions

H.B. and A.Ac participated in image analysis. A.Az. analysed data. A.Ac. and A.Az. wrote the manuscript. M.M. supervised the findings of this work. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Ethics approval and consent to participate

N/A

Consent for publication

N/A

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1

Multiple comparisons at different ages of chicken broiler for muscle fibres numbers determination between different magnifications of light microscopy and Slide Scanner.

Days		Acquisition Type					<i>P</i> < 0.05
		M x10	M x20	M x25	M x40	SC	
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	
Fibers Number	0	.1	950.00±70.56 ^a	901.67±60.99 ^a	233.33±39.40 ^b	795.67±64.78 ^a	.000
	07	837.00 ± 56.40 ^a	227.00 ± 20.03 ^b	202.40 ± 10.94 ^b	64.20 ± 9.39 ^c	626.20 ± 44.83 ^a	.000
	14	839.00 ± 112.07 ^a	253.00 ± 23.77 ^b	246.17 ± 20.13 ^b	66.83 ± 10.19 ^c	623.33 ± 65.70 ^a	.000
	21	559.50 ± 50.19 ^a	161.50 ± 21.05 ^b	142.38 ± 18.33 ^b	40.88 ± 3.89 ^c	612.88 ± 70.88 ^a	.000
	28	538.11 ± 59.37 ^a	122.56 ± 9.41 ^b	118.22 ± 8.21 ^b	29.56 ± 2.58 ^c	653.67 ± 131.42 ^a	.000
	35	295.43 ± 20.54 ^a	77.57 ± 7.33 ^b	69.43 ± 6.32 ^b	17.57 ± 1.93 ^c	867.29 ± 131.84 ^d	.000
	42	329.75 ± 16.68 ^a	79.25 ± 3.99 ^b	76.63 ± 6.43 ^b	18.00 ± 1.66 ^c	988.25 ± 46.34 ^d	.000
	49	290.63 ± 19.01 ^a	69.25 ± 4.03 ^b	58.00 ± 6.12 ^b	14.63 ± 1.87 ^c	888.00 ± 90.99 ^d	.000
	56	222.57 ± 17.15 ^a	57.86 ± 4.46 ^b	45.57 ± 3.10 ^b	11.86 ± 0.76 ^c	780.14 ± 82.66 ^d	.000
	Mean	467.71±32.8 ^a	201.31±32.2 ^b	187.25±30.6 ^b	49.72±8.6 ^c	765.20±33.5 ^d	.000
<i>P</i> < 0.05 M: Magnification of light microscopy							

Table 2
Total number of muscle fibres calculated after segmentation in function of age using three software (Fiji « FDP, FM », IP and CytoF).

	D0	D07	D14	D21	D28	D35	D42	D49	D56	Mean number
FM	737	534	736	401	334	714	1017	422	620	612,77
FDP	732	528	735	409	338	703	1015	435	635	614,44
IP	717	530	746	401	343	726	1026	408	622	613,22
CytoF	706	517	751	416	348	734	1048	448	645	623,66
D: days										

Table 3

Multiple comparisons at different stages of age for Fibre morphometries (CSA, perimeter, MFD) between FDP, FM, CytoF and IP segmented images.

Fibre Parameters	Age (Days)	Software				P < 0.05
		FDP	FM	Cyto	IP	
		Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE	
CSA	1	162.28 $\pm$ 1.78 ^b	145.59 $\pm$ 1.73 ^c	155.52 $\pm$ 1.59 ^a	156.00 $\pm$ 1.88 ^{a,b}	.000
	7	999.96 $\pm$ 12.73 ^a	993.99 $\pm$ 16.08 ^{a,b}	970.56 $\pm$ 11.45 ^{a,b}	949.16 $\pm$ 12.26 ^b	.017
	14	778.65 $\pm$ 7.86 ^b	743.45 $\pm$ 7.69 ^a	740.91 $\pm$ 7.32 ^a	718.89 $\pm$ 7.66 ^a	.000
	21	1350.73 $\pm$ 16.43 ^{a,b}	1390.82 $\pm$ 16.91 ^b	1326.78 $\pm$ 16.76 ^a	1319.65 $\pm$ 18.02 ^a	.015
	28	1560.76 $\pm$ 26.46 ^a	1565.06 $\pm$ 26.69 ^a	1470.06 $\pm$ 25.15 ^a	1487.48 $\pm$ 25.26 ^a	.013
	35	3013.37 $\pm$ 37.61 ^a	2934.64 $\pm$ 38.69 ^a	2914.07 $\pm$ 37.40 ^a	2774.29 $\pm$ 36.66 ^b	.000
	42	2072.81 $\pm$ 25.10 ^a	2060.46 $\pm$ 24.68 ^a	2063.37 $\pm$ 24.14 ^a	2014.60 $\pm$ 25.27 ^a	.349
	49	2443.28 $\pm$ 23.81 ^b	2411.41 $\pm$ 26.67 ^{a,b}	2323.36 $\pm$ 29.80 ^{a,c}	2234.13 $\pm$ 29.23 ^c	.000
	56	4280.97 $\pm$ 48.05 ^a	4292.66 $\pm$ 47.02 ^a	4216.67 $\pm$ 48.80 ^a	3966.59 $\pm$ 44.63 ^b	.000
	Mean	1869.47 $\pm$ 18.57 ^a	1863.61 $\pm$ 19.44 ^a	1833.77 $\pm$ 18.92 ^a	1743.16 $\pm$ 17.82 ^b	.000
Perimeter	1	53.14 $\pm$ 0.32 ^b	49.35 $\pm$ 0.33 ^a	49.57 $\pm$ 0.28 ^a	51.15 $\pm$ 0.34 ^c	.000
	7	130.77 $\pm$ 0.99 ^b	131.17 $\pm$ 1.24 ^b	125.14 $\pm$ 0.85 ^a	123.45 $\pm$ 0.93 ^a	.000
	14	121.21 $\pm$ 0.74 ^b	118.12 $\pm$ 0.71 ^c	110.40 $\pm$ 0.60 ^a	108.94 $\pm$ 0.65 ^a	.000
	21	161.78 $\pm$ 1.18 ^b	160.96 $\pm$ 1.08 ^b	151.54 $\pm$ 1.19 ^a	150.11 $\pm$ 1.24 ^a	.000
	28	174.02 $\pm$ 1.72 ^b	171.16 $\pm$ 1.61 ^b	159.64 $\pm$ 1.49 ^a	158.33 $\pm$ 1.43 ^a	.000
	35	233.43 $\pm$ 1.77 ^b	230.90 $\pm$ 1.83 ^b	223.82 $\pm$ 1.88 ^a	217.71 $\pm$ 1.78 ^a	.000
<i>P</i> < 0.05						

	42	192.18 ± 1.34 ^a	193.46 ± 1.30 ^a	191.33 ± 1.31 ^a	184.88 ± 1.39 ^b	.000
	49	218.97 ± 1.37 ^b	216.72 ± 1.52 ^b	205.29 ± 1.81 ^a	196.80 ± 1.67 ^c	.000
	56	273.44 ± 1.94 ^a	276.59 ± 1.84 ^a	269.62 ± 2.08 ^a	251.23 ± 1.71 ^b	.000
	Mean	171.84 ± 0.99 ^b	171.83 ± 1.04 ^b	166.01 ± 0.99 ^a	159.45 ± 0.93 ^c	.000
MFD	1	12.45 ± 0.08 ^b	11.87 ± 0.09 ^a	12.09 ± 0.07 ^a	12.43 ± 0.09 ^b	.000
	7	31.56 ± 0.24 ^b	31.60 ± 0.30 ^{b,c}	30.48 ± 0.22 ^a	30.66 ± 0.23 ^{a, c}	.001
	14	26.97 ± 0.17 ^b	26.34 ± 0.17 ^b	24.98 ± 0.16 ^a	25.41 ± 0.16 ^a	.000
	21	35.47 ± 0.29 ^b	35.57 ± 0.29 ^b	33.29 ± 0.31 ^a	34.00 ± 0.30 ^a	.000
	28	38.10 ± 0.42 ^{b,c}	38.21 ± 0.42 ^b	35.69 ± 0.43 ^a	36.53 ± 0.40 ^{a, c}	.000
	35	52.66 ± 0.42 ^b	52.19 ± 0.43 ^b	49.79 ± 0.44 ^a	50.12 ± 0.41 ^a	.000
	42	42.74 ± 0.33 ^b	43.20 ± 0.32 ^b	41.13 ± 0.32 ^a	42.10 ± 0.32 ^{a, b}	.000
	49	48.44 ± 0.36 ^b	47.95 ± 0.38 ^b	45.57 ± 0.44 ^a	44.85 ± 0.44 ^a	.000
	56	64.05 ± 0.48 ^b	64.54 ± 0.46 ^b	61.49 ± 0.516 ^a	60.46 ± 0.45 ^a	.000
	Mean	38.98±0.22 ^b	38.91±0.24 ^b	37.24±0.23 ^a	37.20±0.22 ^a	.000
<i>P</i> < 0.05						

Figures

Figure 1

Figure 1

Figure 2

Figure 2

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Figure 4

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