

## Supplementary file

### MuscleJ2: a rebuilding of MuscleJ with new features for high content analysis of skeletal muscle immunofluorescence slides

Anne Danckaert<sup>1\*</sup>, Aurélie Trignol<sup>2</sup>, Guillaume Le Loher<sup>1,3</sup>, Sébastien Loubens<sup>4,5</sup>, Bart Staels<sup>4</sup>, Hélène Duez<sup>4</sup>, Spencer L. Shorte<sup>1</sup>, Alicia Mayeuf-Louchart<sup>4\*</sup>

1. Institut Pasteur, Université Paris Cité, UTechS Photonic BioImaging/C2RT, F-75015 Paris, France

2. French Armed Forces Biomedical Research Institute (IRBA), France; Université Paris Cité, VIFASOM (UPR 7330 Vigilance Fatigue, Sommeil et Santé Publique), France.

3. Present Address: École Centrale d'Electronique (ECE), Paris, France.

4. Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011- EGID, F-59000 Lille, France

5. CHU Lille, Service Neuropédiatrie, 59000 Lille, France

\*Corresponding authors: [anne.danckaert@pasteur.fr](mailto:anne.danckaert@pasteur.fr); [alicia.mayeuf-louchart@inserm.fr](mailto:alicia.mayeuf-louchart@inserm.fr)

### Figure Legends and Tables

**Fig. S1. Comparison of the *Homogeneous* and *Heterogeneous* options.** Comparison of the results and cartographies obtained by MuscleJ2 after fiber morphology analysis on laminin-stained *Tibialis anterior* sections after partial injury, with the *Healthy* option (left) and the *Damaged* option (right). Histograms at the bottom show the frequency of fibers with different cross-sectional areas. The results show that the number of included fibers is higher with the *Damaged* option than with the *Healthy* option where a number of fibers are excluded from the analysis (black fibers).

**Fig. S2. Analysis of the diaphragm with MuscleJ2.** (A) Cartography of the extracellular matrix (ECM) staining representing the diaphragm of the mouse rolled up on itself at the time of cutting. (B) Comparison of the results obtained by MuscleJ2 after ECM analysis on laminin-stained diaphragm sections, with the *Diaphragm* option and the *Limb* option. The results show that the total surface area is smaller with the *Diaphragm* section due to the removal of the fiberless parts (\*), which is not the case with the *Limb* option.

**Fig. S3. Comparison of the Data Acquisition panel between the macro and the plugin MuscleJ2.** Screenshots of the dialog boxes in the initial macro (A) and the plugin (B) showing changes in the Data acquisition section.

**Fig. S4. Perimyonuclei detection with MuscleJ2.** (A) Skeletal muscle section from injured mouse (21days post-injury) stained with Dapi, Pcm1 and laminin antibodies (SB=500µm) and representative cartography of perimyonuclei quantification results obtained with MuscleJ2. Only double Pcm1/Dapi-positive cells in the ROI<sup>MB</sup> are quantified and represented in the

cartography. (B) The grey table shows the results obtained from the global results file and (C) the green table a subset of the results obtained from the *PeriMyoNucleiDetails* file.

**Fig. S5. Fiber typing with MuscleJ2.** (A) Skeletal muscle section stained with Dapi, Myosin Heavy chain IIX, IIA, I and laminin antibodies (SB=600 $\mu$ m). (B) Representative cartographies (original image, Fiber morphology and Fiber Typing) of the results obtained with MuscleJ2 (SB=300 $\mu$ m). (C) Global file results and fiber detail results. All areas are given in  $\mu$ m<sup>2</sup>.

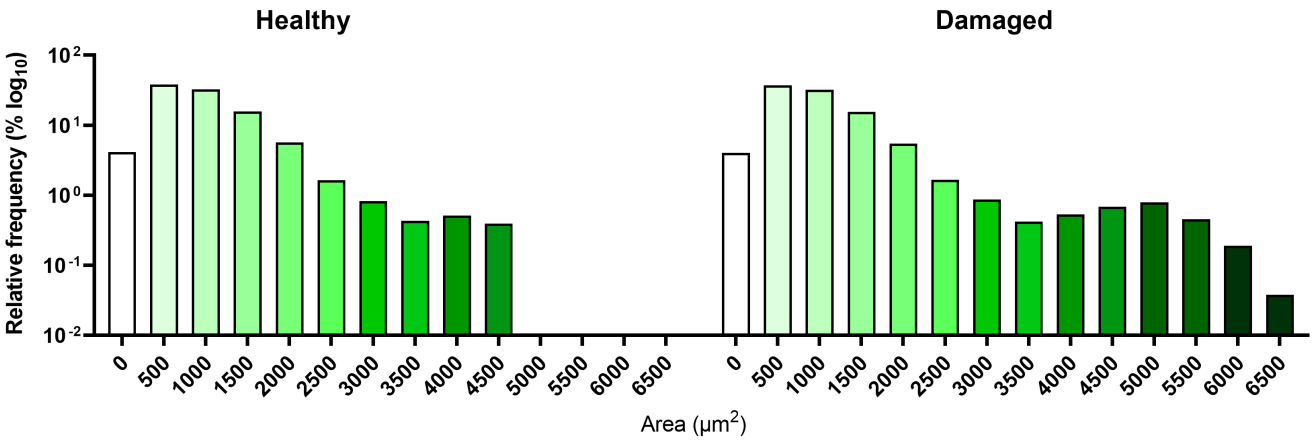
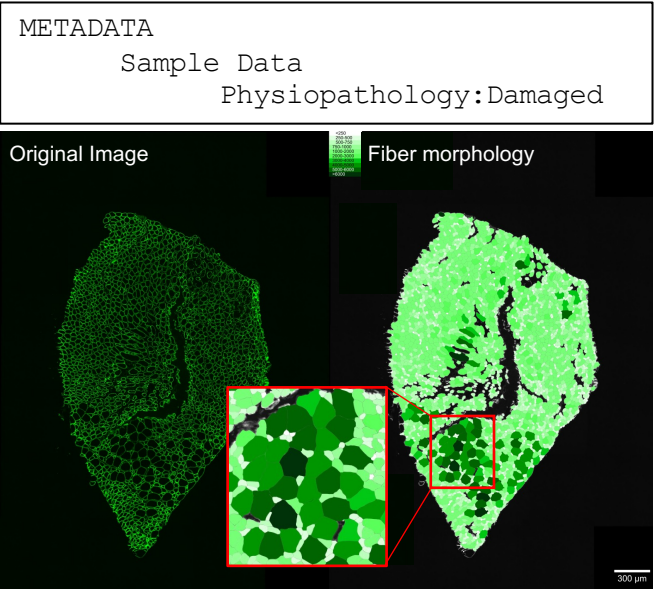
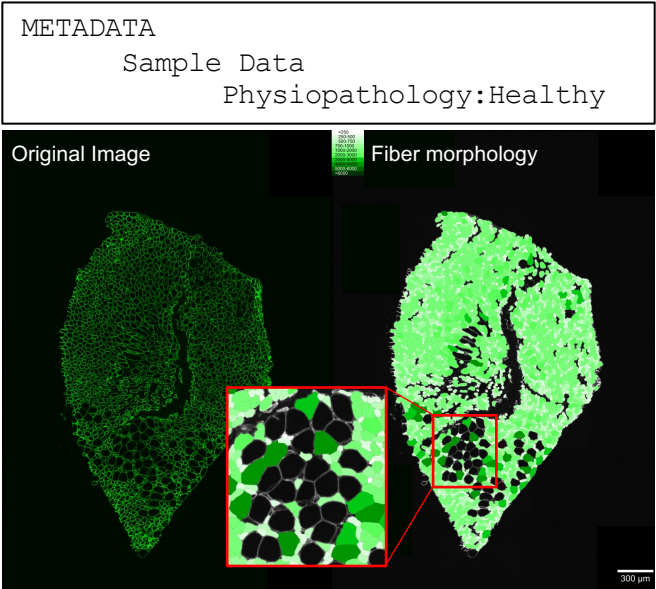
**Fig. S6. Global information associated with a MuscleJ2 batch run.** Screenshots of the BATCH\_LOG text file corresponding to the meta data and the analysis performed during the batch run as well as channel attributions, to keep a printout of options and global information.

**Sup Table 1: Main features of tools and software available to perform a fully or semi-automatic analysis on skeletal muscle immunofluorescence images.**

List of abbreviations used: AI: artificial intelligence; DPI: days post injury; EBD: Evans blue dye; IF: immunofluorescence; IHC: immunohistochemistry; MYH: myosin heavy chain (gene); NCAM: neural cell adhesion molecule; PFA: paraformaldehyde; ref: reference.

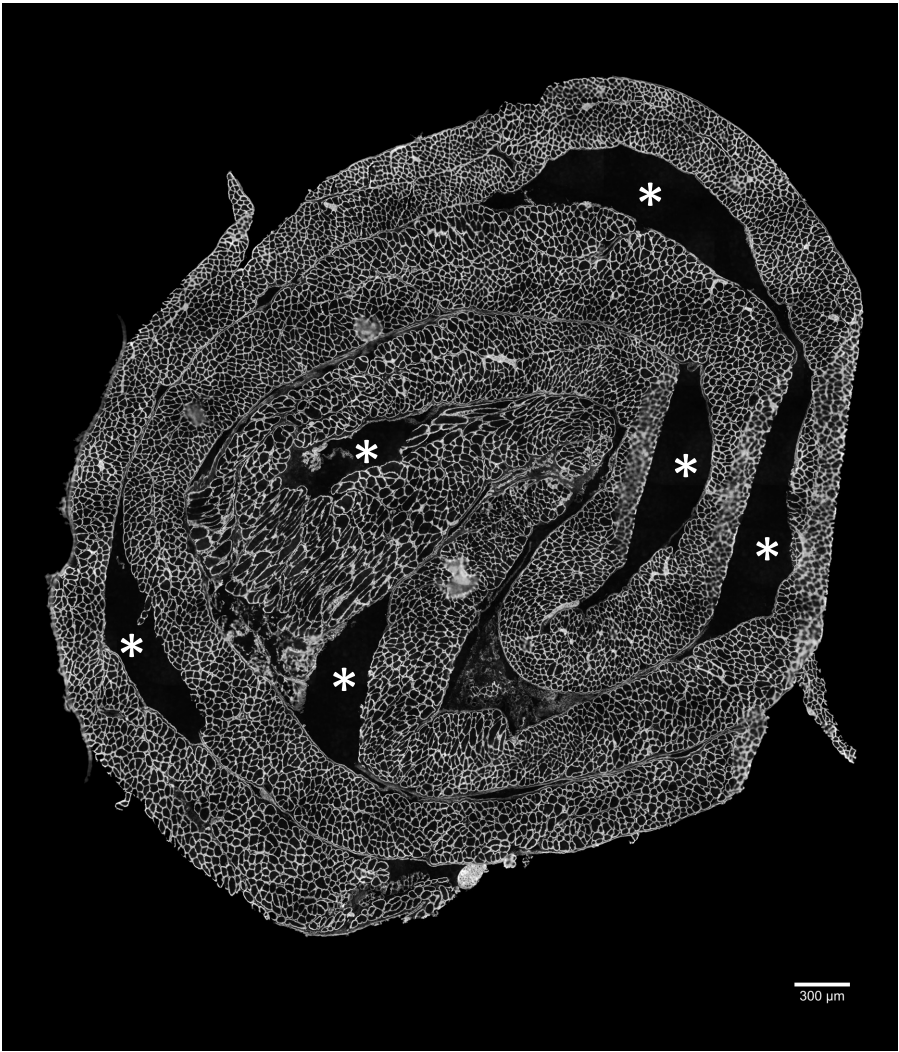
**Sup Table 2: List of antibodies and specific protocols used**

FigS1.



FigS2.

A



B

|                    | Option Diaphragm | Option Limb |
|--------------------|------------------|-------------|
| Whole Section Area | 7785545.0        | 1.5096652E7 |
| ECM Area Tot       | 1684250.4        | 1684294.8   |
| % ECMArea          | 21.633043        | 11.156744   |



FigS3.

A Macro Muscle J 1.0.1 (2018)

Data Acquisition

=====

Microscopy

☒ Apotome/WideField

☐ Confocal/Spinning Disk

Volume

☒ Single Z

☐ Z stack

Scanned muscle area

☒ Entire

☐ Crop

Data Format

☒ Original File Format

☐ TIFF (16bits) by channel

B Plugin Muscle J 2.0 (2022)

Volume :

☒ Single Z

☐ Z stack

Scanned Area :

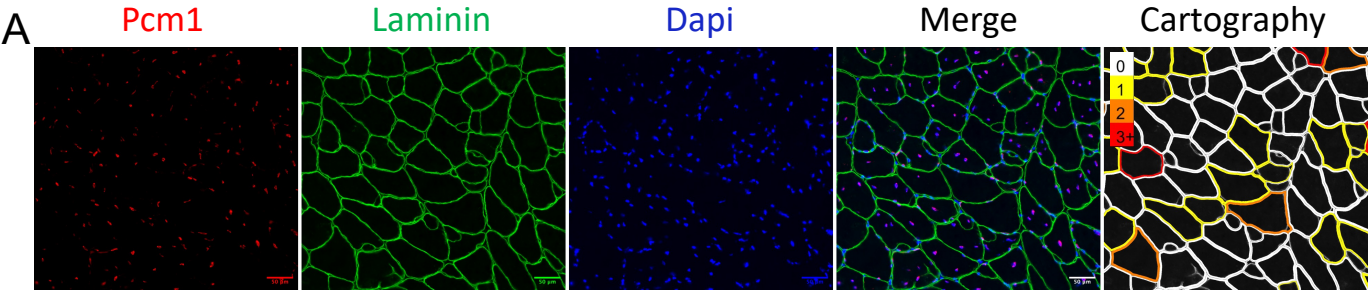
☒ Entire section

☐ Crop

Artefact Detection (%area min):

40 %

FigS4.



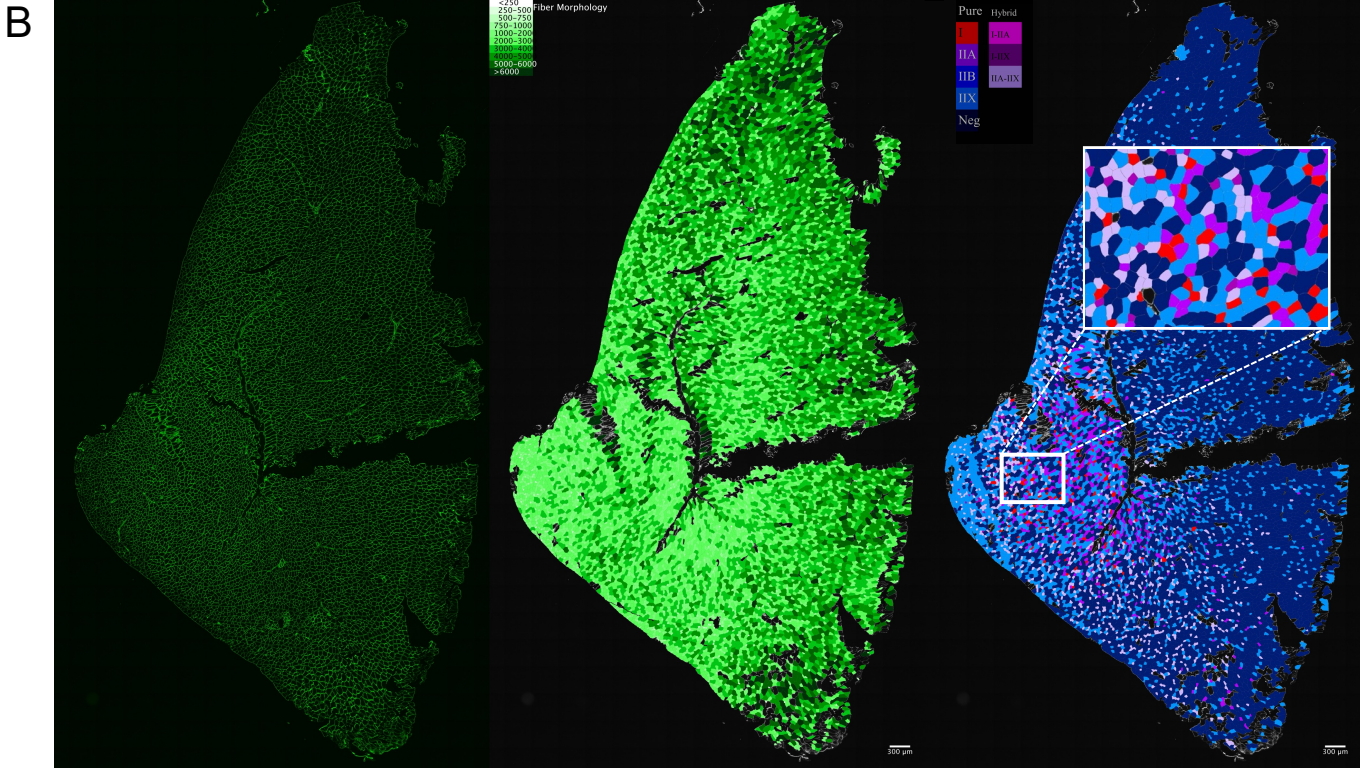
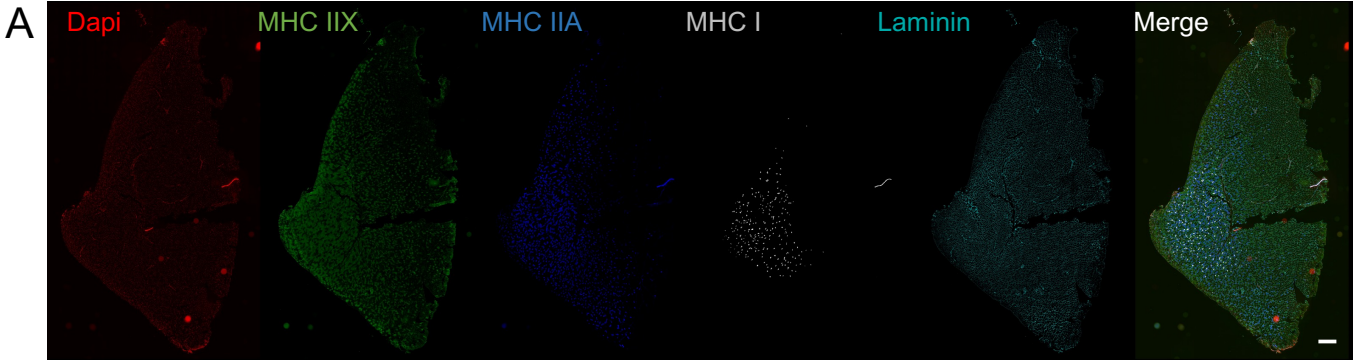
B

| FileName | Whole Section Area | Nb Segmented Fiber | Nb Segmented Fiber | Nb Real PeriMyoNuclei |
|----------|--------------------|--------------------|--------------------|-----------------------|
| Image 1  | 7351347.5          | 3314               | 3314               | 9053                  |

C

| <i>Perimynonuclei details</i> | Area      | Max Feret | Min Feret | Nuclei GC X | Nuclei GC Y | Intensity |
|-------------------------------|-----------|-----------|-----------|-------------|-------------|-----------|
| 1                             | 47.32     | 9.825541  | 7.8       | 2253        | 537         | 3201.2812 |
| 2                             | 71.4025   | 11.830178 | 8.953805  | 2367        | 550         | 4157.487  |
| 3                             | 32.638126 | 9.475297  | 5.285623  | 2304        | 567         | 3148.1326 |
| 4                             | 15.21     | 8.604832  | 3.71222   | 2297        | 574         | 2688.9307 |
| 5                             | 26.82875  | 7.914267  | 4.665732  | 2115        | 575         | 3261.9211 |

FigS5.



C

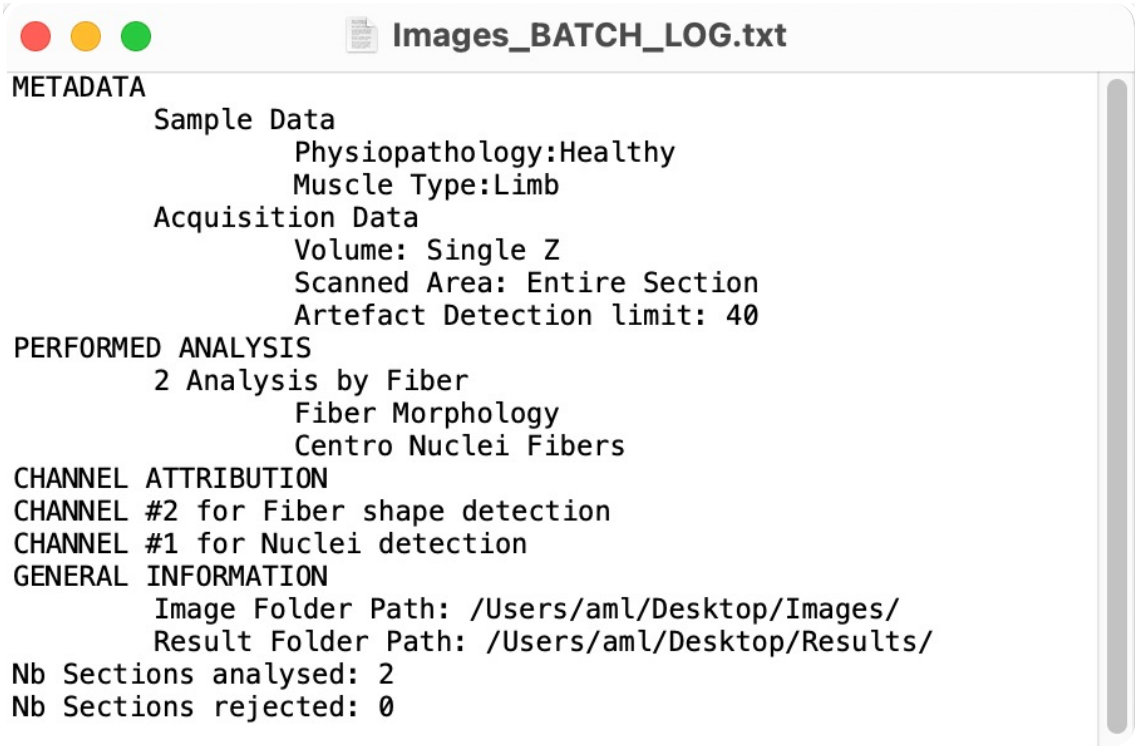
**GlobalResults file**

| FileName | Whole Section Area | Nb Segmented Fiber | Nb Type I | Thres. I  | Nb Type IIA | Thres. IIA | Nb Type IIX | Thres. IIX | Nb Type Neg | Nb Type I-IIA | Nb Type I-IIX | Nb Type IIA-IIX |
|----------|--------------------|--------------------|-----------|-----------|-------------|------------|-------------|------------|-------------|---------------|---------------|-----------------|
| Image    | 3.6394256E7        | 11501              | 161       | 3.1257656 | 567         | 9.799161   | 2848        | 1.3097754  | 6553        | 5             | 90            | 1303            |

**FiberDetails file**

| Fiber details | Area      | Intensity Type I | Intensity Type IIA | Intensity Type IIX | Fiber Type |
|---------------|-----------|------------------|--------------------|--------------------|------------|
| 3286          | 1385.0057 | 1.1461766        | 16.572737          | 1.5782285          | IIA-IIX    |
| 3287          | 1162.2657 | 1.0533646        | 9.317839           | 2.2394698          | IIX        |
| 3288          | 1499.1063 | 0.840229         | 15.722873          | 2.899948           | IIA-IIX    |
| 3289          | 1632.9064 | 0.75740564       | 1.4910415          | 2.4351408          | IIX        |
| 3290          | 1040.9486 | 18.883455        | 4.2055464          | 1.8933858          | I-IIX      |
| 3291          | 1340.5358 | 1.0152771        | 19.135895          | 2.5192783          | IIA-IIX    |
| 3292          | 1786.2108 | 0.8130596        | 8.119895           | 2.3356628          | IIX        |
| 3293          | 2545.321  | 0.8429119        | 2.162912           | 2.2293487          | IIX        |
| 3294          | 3860.5012 | 0.50881624       | 1.1416663          | 0.669277           | Neg        |
| 3295          | 1527.5828 | 0.5188968        | 2.1446629          | 2.5491574          | IIX        |
| 3296          | 1876.126  | 0.7485186        | 1.5382056          | 1.3699969          | IIX        |
| 3297          | 2460.282  | 0.9463295        | 2.7606628          | 3.2271285          | IIX        |

FigS6.



Sup Table 1.

|                                           |      |            |                         |                   |                | Software features |                                                     |                |                 |             |            |                    |                                            |     |                |             |                                                                   |                                                                                                                                  |
|-------------------------------------------|------|------------|-------------------------|-------------------|----------------|-------------------|-----------------------------------------------------|----------------|-----------------|-------------|------------|--------------------|--------------------------------------------|-----|----------------|-------------|-------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Software (ref)                            | Year | Image/Fiji | Sample preparation      | Staining protocol | Human biopsies | Fiber morphology  | Myonuclei                                           | Central nuclei | Satellite cells | Capillaries | Fiber type | Myofiber staining  | Sarcolemmal staining                       | ECM | Specific cells | Cartography | Conditions tested                                                 | Comments                                                                                                                         |
| MyoScan (1)                               | 2013 |            | Frozen                  | IF/IHC            |                | X                 |                                                     | X              |                 |             |            |                    |                                            |     |                |             | Healthy and mdx mice samples                                      | Using web-based image analysis system S.CORE                                                                                     |
| Skeletal Muscle Algorithm (2)             | 2013 |            | Frozen                  | IF staining       |                | X                 |                                                     |                |                 |             | X          | NCAM+              |                                            |     |                |             | Mice (healthy and injured), rat, swine and monkey samples         | Using CyteSeer                                                                                                                   |
| Algorithm by Beekman et al. (3)           | 2014 |            | Frozen                  | IF staining       | X              | X                 |                                                     |                |                 |             |            |                    | Dystrophin+, Spectrin+                     |     |                |             | Healthy human samples and patients with a myopathy                | Using Definiens, providing dystrophin and sarcolemmal intensities                                                                |
| SMASH (4)                                 | 2014 |            | Frozen                  | IF staining       |                | X                 |                                                     | X              |                 | X           | X          |                    |                                            |     |                |             | Healthy and mdx mice                                              | Using Matlab, providing capillaries density                                                                                      |
| MyoVision (5)                             | 2018 |            | Frozen                  | IF staining       |                | X                 | Dapi staining                                       |                |                 |             | X          |                    |                                            |     |                |             | Mice muscle samples                                               |                                                                                                                                  |
| By Sardone et al. (6)                     | 2018 |            | Frozen                  | IF staining       | X              | X                 |                                                     |                |                 |             |            |                    | Dystrophin+, Spectrin+, Laminin+           |     |                |             | Healthy human samples and patients with a myopathy                | Using Definiens, providing dystrophin and sarcolemmal intensities                                                                |
| MuscleJ (7)                               | 2018 | X          | Frozen                  | IF staining       |                | X                 | Dapi staining                                       | X              | X               | X           | X          |                    |                                            |     |                | X           | Healthy, notexin-injected (21 DPI) and mdx mice                   |                                                                                                                                  |
| MuscleAnalyzer (8)                        | 2018 |            | Frozen                  | IF staining       |                | X                 |                                                     | X              |                 |             |            |                    |                                            |     |                |             | Healthy and mdx mice samples                                      | Using CellProfiler                                                                                                               |
| Open-CSAM (9)                             | 2019 | X          | Frozen                  | IF staining       |                | X                 |                                                     |                |                 |             |            |                    |                                            |     |                |             | Healthy, cardiotoxin-injured (8, 14 and 28 DPI), mdx and old mice |                                                                                                                                  |
| MuscleMap (10)                            | 2019 |            | Frozen                  | IF staining       | X              |                   |                                                     |                |                 |             |            |                    | Dystrophin+                                |     |                |             | Healthy human samples and patients with a myopathy                | By Flagship Biosciences, providing also a positive-fiber classification and quantify the number of myofibers                     |
| Macro by Reyes-Fernandez et al.(11)       | 2019 | X          | Frozen                  | IF staining       | X              | X                 |                                                     |                |                 |             | X          |                    |                                            |     |                | X           | Healthy human samples (young, old, obese) or with a myopathy      |                                                                                                                                  |
| QuantiMus (12)                            | 2019 |            | Frozen                  | IF staining       | X              | X                 |                                                     | X              |                 |             | X          | NCAM+, MYH3+, EBD+ |                                            |     |                |             | Healthy and mdx mice; patients with a suspected myopathy          | Using Filika (Python), AI algorithm to improve efficiency and speed                                                              |
| SLCV (13)                                 | 2019 |            | Frozen                  | IF staining       |                | X                 |                                                     |                |                 |             |            |                    |                                            |     |                |             | Healthy mice samples                                              | AI algorithm for various immunostaining qualities                                                                                |
| Muscle2View (14)                          | 2019 |            | Frozen                  | IF staining       | X              | X                 | Dapi staining                                       | X              |                 | X           | X          |                    |                                            |     |                |             | Healthy human samples                                             | Using CellProfiler, providing also capillarization indices                                                                       |
| Myosoft (15)                              | 2020 | X          | Frozen                  | IF staining       |                | X                 |                                                     |                |                 |             | X          |                    |                                            |     |                | X           | Healthy and mdx mice samples                                      | Could be used with suboptimal stains                                                                                             |
| By Scaglioni et al. (16)                  | 2020 |            | Frozen                  | IF staining       | X              | X                 |                                                     |                |                 |             |            | MYH3+, MYH8+       | Dystrophin+, αSarcoglycan+, βDystroglycan+ |     |                | X           | Healthy human samples and patients with a myopathy                | Using Definiens, providing also a positive-fiber classification and a % of sarcolemmal circumference coverage                    |
| MyoSight (17)                             | 2020 | X          | Frozen                  | IF staining       |                | X                 | Dapi staining                                       | X              |                 |             | X          |                    |                                            |     |                |             | Healthy and mdx mice samples                                      | Single software with multiple analyses, using channels                                                                           |
| MuscleJ modified by Bonilla et al.(18)    | 2020 | X          | Frozen                  | IF staining       | X              | X                 |                                                     |                |                 |             | X          |                    |                                            |     |                |             | Human samples (older adults to study muscle aging)                |                                                                                                                                  |
| MyoSAT (19)                               | 2020 | X          | Frozen                  | IF staining       |                | X                 |                                                     |                |                 |             |            |                    |                                            |     |                | X           | Healthy and after nerve-injured mice samples; healthy dogs        | For weak or non-uniform staining                                                                                                 |
| MuscleJ modified by Bindellini et al.(20) | 2021 | X          | Frozen                  | IF staining       |                | X                 |                                                     |                |                 |             | X          |                    |                                            |     |                |             | Healthy mice samples                                              |                                                                                                                                  |
| LabelsToRois (21)                         | 2021 | X          | Frozen (+/- PFA before) | IF staining       |                | X                 |                                                     |                |                 |             |            |                    |                                            |     |                | X           | Mdx and injury models (cardiotoxin or glycerol at 21 DPI) mice    | Coupled to Cellpose (AI algorithm) for suboptimal stainings                                                                      |
| MyoView (22)                              | 2021 |            | Frozen                  | IF staining       |                | X                 | Dapi staining                                       |                | X               |             | X          |                    |                                            |     |                |             | Mdx and healthy mice (exercise-induced injury protocol)           | Using Matlab                                                                                                                     |
| By Vetter et al. (23)                     | 2021 |            | Frozen                  | IF staining       | X              | X                 |                                                     |                |                 |             |            |                    | Dystrophin+, Spectrin+, Laminin+           |     |                | X           | Healthy human samples and patients with a myopathy                | Using NIS-Elements (Nikon), providing also a positive-fiber classification                                                       |
| MyoProfiler/SiriusProfiler (24)           | 2022 |            | Frozen                  | IF/Sirius/Masson  | X              | X                 | Dapi staining                                       | X              | In theory       |             |            |                    |                                            | X   | F4/80+         |             | Healthy and mdx mice samples                                      | Using CellProfiler                                                                                                               |
| MyoVision 2.0 (25)                        | 2022 |            | Frozen                  | IF staining       |                | X                 | Dapi staining +/- second staining (co-localization) |                |                 |             | X          |                    |                                            |     |                |             | Healthy rat (electrical stimulation and atrophy protocols)        | Using U-net (segmentation) and neural networks (fiber detection and fiber-type classification), providing myonuclear domain size |

# Sup Table 2.

| Specific labelling                      | Primary antibody                                                                       | Secondary antibody and others (45min RT)                                                        | Muscle sample             |
|-----------------------------------------|----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|---------------------------|
| ECM                                     |                                                                                        |                                                                                                 |                           |
| Oligosaccharide residues                | /                                                                                      | Wheat Germ Agglutinin Alexa Fluor 555 (#W32464, Invitrogen) 1:1000                              | Rat samples               |
| Vascularization/Capillaries             |                                                                                        |                                                                                                 |                           |
| Endothelial cells                       | Mouse IgG1 monoclonal Anti CD31 (#ab64543, Abcam) 1:200 ON 4°C                         | Donkey anti Mouse IgG (H+L) Alexa Fluor 594 (#A21203, Thermo Fisher Scientific) 1:500           | Rat samples               |
| Laminin                                 | Rabbit IgG polyclonal Anti Laminin (#ab11575, Abcam) 1:200 ON 4°C                      | Goat anti Rabbit IgG (H+L) Alexa Fluor 488 (#A21206, Thermo Fisher Scientific) 1:500            |                           |
| Specific cells                          |                                                                                        |                                                                                                 |                           |
| Macrophages                             | Rat IgG2b monoclonal Anti F4/80 (#MCA497G, Bio-Rad) 1:100 ON 4°C                       | Donkey Anti-Rat IgG DyLight 550 (#NBP1-75652, NOVUSBIO) 1:200                                   | Mice samples              |
| Laminin                                 | Rabbit IgG polyclonal Anti Laminin (#L9393, Sigma-Aldrich) 1:200 ON 4°C                | Donkey Anti-Rabbit IgG DyLight 488 (#NBP1-75285, NOVUSBIO) 1:200                                |                           |
| Nuclei                                  | /                                                                                      | Hoechst H33342 (#B2261, Sigma-Aldrich) 1:1000                                                   |                           |
| Fiber Type                              |                                                                                        |                                                                                                 |                           |
| Fiber type I (MYH7)                     | Mouse IgG2b BA-D5-s (Developmental Studies Hybridoma Bank) 1:200 45min 37°C            | Goat anti Mouse IgG2b Alexa Fluor 647 (#A21242, Thermo Fisher Scientific) 1:500                 | Rat samples (not use PFA) |
| Fiber type IIA (MYH2)                   | Mouse IgG1 SC-71-s (Developmental Studies Hybridoma Bank) 1:200 45min 37°C             | Goat anti Mouse IgG1 Alexa Fluor 568 (#A21124, Thermo Fisher Scientific) 1:500                  |                           |
| Fiber type IIB (MYH4)                   | Mouse IgM BF-F3-s (Developmental Studies Hybridoma Bank) 1:50 45min 37°C               | Goat anti Mouse IgM Alexa Fluor 488 (#A21042, Thermo Fisher Scientific) 1:500                   |                           |
| Laminin                                 | Rabbit IgG polyclonal Anti Laminin (#ab11575, Abcam) 1:200 45min 37°C                  | Goat anti Rabbit IgG (H+L) Alexa Fluor 750 (#A21039, Thermo Fisher Scientific) 1:500            |                           |
| Nuclei                                  | /                                                                                      | Dapi in mouting medium (#ab104139, Abcam)                                                       |                           |
| Fiber Intensity by ROI MB               |                                                                                        |                                                                                                 |                           |
| Dystrophin                              | Mouse IgG2a MANDYS1(3B7)-s (Developmental Studies Hybridoma Bank) 1:250 ON 4°C         | F(ab')2-Goat anti Mouse IgG (H+L) Alexa Fluor 647 (#A21237, Thermo Fisher Scientific) 1:500     | Mice samples              |
| Laminin                                 | Rat IgG1 monoclonal Anti Laminin a2 (4H8-2) (#sc-59854, SantaCruz) 1:250 ON 4°C        | Donkey anti Rat IgG (H+L) Alexa Fluor 488 (#A21208, Thermo Fisher Scientific) 1:500             |                           |
| Dystrophin                              | Mouse IgG1 monoclonal Anti Dystrophin (#ab7164, Abcam) 1:200 ON 4°C                    | Goat anti Mouse IgG1 Alexa Fluor 568 (#A21124, Thermo Fisher Scientific) 1:500                  | Rat samples               |
| Laminin                                 | Rabbit IgG polyclonal Anti Laminin (#ab11575, Abcam) 1:200 ON 4°C                      | Goat anti Rabbit IgG (H+L) Alexa Fluor 488 (#A21206, Thermo Fisher Scientific) 1:500            |                           |
| Fiber Intensity by ROI F                |                                                                                        |                                                                                                 |                           |
| Embryonic fiber type (MYH3)             | Mouse IgG1 F1.652-s (Developmental Studies Hybridoma Bank) 1:250 ON 4°C                | Donkey anti Mouse IgG (H+L) Alexa Fluor 488 (#A21202, Thermo Fisher Scientific) 1:500           | Mice samples              |
| Laminin                                 | Rat IgG1 monoclonal Anti Laminin a2 (4H8-2) (#sc-59854, SantaCruz) 1:250 ON 4°C        | Goat anti Rat IgG (H+L) Alexa Fluor 647 (#A21247, Thermo Fisher Scientific) 1:500               |                           |
| Capillaries/Fiber Type/Fiber morphology |                                                                                        |                                                                                                 |                           |
| Endothelial cells                       | Mouse IgG1 monoclonal Anti CD31 (#ab64543, Abcam) 1:200 1h 37°C (1st step)             | Goat anti Mouse IgG1 Alexa Fluor 568 (#A21124, Thermo Fisher Scientific) 1:500 (1st step)       | Rat samples (not use PFA) |
| Laminin                                 | Rabbit IgG polyclonal Anti Laminin (#ab11575, Abcam) 1:200 1h 37°C (1st step)          | Goat anti Rabbit IgG (H+L) Alexa Fluor 405 (#A31556, Thermo Fisher Scientific) 1:500 (1st step) |                           |
| Fiber type I (MYH7)                     | Mouse IgG2b BA-D5-s (Developmental Studies Hybridoma Bank) 1:200 45min 37°C (2nd step) | Goat anti Mouse IgG2b Alexa Fluor 647 (#A21242, Thermo Fisher Scientific) 1:500 (2nd step)      |                           |
| Fiber type IIX (MYH1)                   | Mouse IgM 6H1-s (Developmental Studies Hybridoma Bank) 1:50 45min 37°C (2nd step)      | Goat anti Mouse IgM Alexa Fluor 488 (#A21042, Thermo Fisher Scientific) 1:500 (2nd step)        |                           |



## Supplementary references

1. Pertl C, Eblenkamp M, Pertl A, Pfeifer S, Wintermantel E, Lochmüller H, et al. A new web-based method for automated analysis of muscle histology. *BMC Musculoskelet Disord*. 2013; 14:26.
2. Kostrominova TY, Reiner DS, Haas RH, Ingemannson R, McDonough PM. Chapter Seven - Automated Methods for the Analysis of Skeletal Muscle Fiber Size and Metabolic Type. In: Jeon KW, éditeur. *International Review of Cell and Molecular Biology* [Internet]. Academic Press; 2013 [cité 18 oct 2022]. p. 275-332. (*International Review of Cell and Molecular Biology*; vol.306).
3. Beekman C, Sipkens JA, Testerink J, Giannakopoulos S, Kreuger D, van Deutekom JC, et al. A sensitive, reproducible and objective immunofluorescence analysis method of dystrophin in individual fibers in samples from patients with duchenne muscular dystrophy. *PloS One*. 2014;9(9):e107494.
4. Smith LR, Barton ER. SMASH - semi-automatic muscle analysis using segmentation of histology: a MATLAB application. *Skelet Muscle*. 2014;4:21.
5. Wen Y, Murach KA, Vechetti IJ, Fry CS, Vickery C, Peterson CA, et al. MyoVision: software for automated high-content analysis of skeletal muscle immunohistochemistry. *J Appl Physiol Bethesda Md 1985*. 2018;124(1):40-51.
6. Sardone V, Ellis M, Torelli S, Feng L, Chambers D, Eastwood D, et al. A novel high-throughput immunofluorescence analysis method for quantifying dystrophin intensity in entire transverse sections of Duchenne muscular dystrophy muscle biopsy samples. *PloS One*. 2018;13(3):e0194540.
7. Mayeuf-Louchart A, Hardy D, Thorel Q, Roux P, Gueniot L, Briand D, et al. MuscleJ: a high-content analysis method to study skeletal muscle with a new Fiji tool. *Skelet Muscle*. 2018;8(1):25.
8. Lau YS, Xu L, Gao Y, Han R. Automated muscle histopathology analysis using CellProfiler. *Skelet Muscle*. 2018;8(1):32.
9. Desgeorges T, Liot S, Lyon S, Bouvière J, Kemmel A, Trignol A, et al. Open-CSAM, a new tool for semi-automated analysis of myofiber cross-sectional area in regenerating adult skeletal muscle. *Skelet Muscle*. 2019;9(1):2.
10. Aeffner F, Faelan C, Moore SA, Moody A, Black JC, Charleston JS, et al. Validation of a Muscle-Specific Tissue Image Analysis Tool for Quantitative Assessment of Dystrophin Staining in Frozen Muscle Biopsies. *Arch Pathol Lab Med*. 2019;143(2):197-205.
11. Reyes-Fernandez PC, Periou B, Decrouy X, Relaix F, Authier FJ. Automated image-analysis method for the quantification of fiber morphometry and fiber type population in human skeletal muscle. *Skelet Muscle*. 2019;9(1):15.

12. Kastenschmidt JM, Ellefsen KL, Mannaa AH, Giebel JJ, Yahia R, Ayer RE, et al. QuantiMus: A Machine Learning-Based Approach for High Precision Analysis of Skeletal Muscle Morphology. *Front Physiol.* 2019; 10:1416.
13. Rettig A, Haase T, Pletnyov A, Kohl B, Ertel W, von Kleist M, et al. SLCV-a supervised learning-computer vision combined strategy for automated muscle fibre detection in cross-sectional images. *PeerJ.* 2019;7:e7053.
14. Sanz G, Martínez-Aranda LM, Tesch PA, Fernandez-Gonzalo R, Lundberg TR. Muscle2View, a CellProfiler pipeline for detection of the capillary-to-muscle fiber interface and high-content quantification of fiber type-specific histology. *J Appl Physiol Bethesda Md* 1985. 2019;127(6):1698-709.
15. Encarnacion-Rivera L, Foltz S, Hartzell HC, Choo H. Myosoft: An automated muscle histology analysis tool using machine learning algorithm utilizing FIJI/ImageJ software. *PloS One.* 2020; 15(3):e0229041.
16. Scaglioni D, Ellis M, Catapano F, Torelli S, Chambers D, Feng L, et al. A high-throughput digital script for multiplexed immunofluorescent analysis and quantification of sarcolemmal and sarcomeric proteins in muscular dystrophies. *Acta Neuropathol Commun.* 2020;8(1):53.
17. Babcock LW, Hanna AD, Agha NH, Hamilton SL. MyoSight-semi-automated image analysis of skeletal muscle cross sections. *Skelet Muscle.* 2020;10(1):33.
18. Bonilla HJ, Messi ML, Sadieva KA, Hamilton CA, Buchman AS, Delbono O. Semiautomatic morphometric analysis of skeletal muscle obtained by needle biopsy in older adults. *GeroScience.* 2020;42(6):1431-43.
19. Stevens CR, Berenson J, Sledziona M, Moore TP, Dong L, Cheetham J. Approach for semi-automated measurement of fiber diameter in murine and canine skeletal muscle. *PloS One.* 2020;15(12):e0243163.
20. Bindellini D, Voortman LM, Olie CS, van Putten M, van den Akker E, Raz V. Discovering fiber type architecture over the entire muscle using data-driven analysis. *Cytometry A.* 2021;99(12):1240-9.
21. Waisman A, Norris AM, Elías Costa M, Kopinke D. Automatic and unbiased segmentation and quantification of myofibers in skeletal muscle. *Sci Rep.* 2021;11(1):11793.
22. Rahmati M, Rashno A. Automated image segmentation method to analyse skeletal muscle cross section in exercise-induced regenerating myofibers. *Sci Rep.* 2021;11(1):21327.
23. Vetter TA, Nicolau S, Bradley AJ, Frair EC, Flanigan KM. Automated immunofluorescence analysis for sensitive and precise dystrophin quantification in muscle biopsies. *Neuropathol Appl Neurobiol.* 2022;48(3):e12785.



24. Laghi V, Ricci V, De Santa F, Torcinaro A. A User-Friendly Approach for Routine Histopathological and Morphometric Analysis of Skeletal Muscle Using CellProfiler Software. *Diagnostics*. 2022;12(3):561.
25. Viggars MR, Wen Y, Peterson CA, Jarvis JC. Automated cross-sectional analysis of trained, severely atrophied, and recovering rat skeletal muscles using MyoVision 2.0. *J Appl Physiol Bethesda Md 1985*. 2022;132(3):593-610.