

Fiber Manual

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Introduction

Given that currently, in many studies related to muscle fibers, the classification and counting of fibers is done manually by an expert, which leads to a high investment of time and effort, coupled with the availability of computational equipment with high processing capacities, arises the need for the creation of software that supports this task, which reduces the requirements of supervision by an expert.

Using artificial intelligence tools, this software was developed as a support tool for the classification, localization and counting of muscle fibers in histological images, as well as for storage in compatible formats that allow subsequent studies such as fractal dimension calculation or comparison of different specimens of the same muscle. In addition, when developed in the Java programming language, it offers cross-platform capabilities.

Minimum Requirements

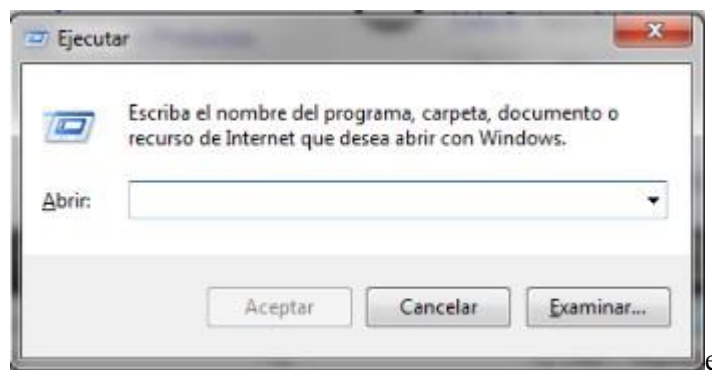
- Dual core CPU 2 GHz.
- Microsoft Windows 7 or Apple Mac OS.
- 4GB RAM
- Java

Running

For running the software we need to have a copy of the project and the libraries in the local storage, preferably in C:/, the root folder of the operative system.

Using command prompt:

Press Windows + R.



Type cmd and click ok button.

Finding program in storage

Go to the folder containing the program, command prompt starts at the directory:

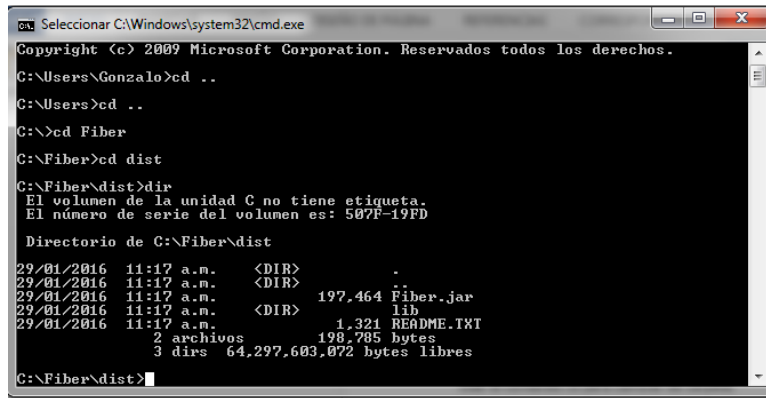
```
C:\Users\(\Nombre)>
```

Type the command dir to show the content of the folder.

Use the command cd to change folder.

```
C:\Users\(\Nombre)> cd (folder name).
```

Use cd .. to go back.



```
Selecionar C:\Windows\system32\cmd.exe
Copyright (c) 2009 Microsoft Corporation. Reservados todos los derechos.
C:\Users\Gonzalo>cd ..
C:\Users>cd ..
C:\>cd Fiber
C:\Fiber>cd dist
C:\Fiber\dist>dir
El volumen de la unidad C no tiene etiqueta.
El número de serie del volumen es: 507F-19FD

Directorio de C:\Fiber\dist
29/01/2016  11:17 a.m.    <DIR>          .
29/01/2016  11:17 a.m.    <DIR>          ..
29/01/2016  11:17 a.m.      197,464 Fiber.jar
29/01/2016  11:17 a.m.    <DIR>          lib
29/01/2016  11:17 a.m.      1,321 README.TXT
                2 archivos      198,785 bytes
                3 dirs    64.297.603.072 bytes libres
C:\Fiber\dist>
```

Optimum running parameters

In the folder dist type:

```
java -jar -Xms512m -Xmx1792m -XX:+UseG1GC Fiber.jar
```

The numbers depend on the available RAM on the system, the most the better, but sometimes the operative system has a maximum to this value, according to the used programs and other factors.

Thus it must be tested until the program runs successfully.

The number after -Xms indicates the minimum RAM to start the program, don't forget the m (Megabytes).

The number after -Xmx indicates the maximum RAM that the program can use.

In the tests the recommended values allow to process 8 Mpx images without trouble.

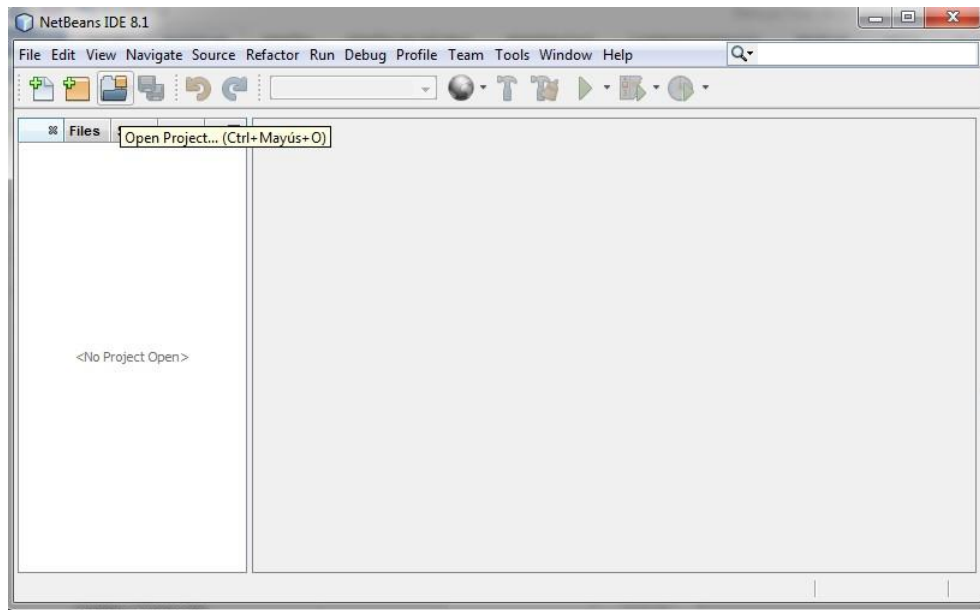
Using NetBeans

NetBeans is a integrated development environment for programming languages like HTML, PHP, JavaScript, C, C++ and of course Java. It features code completion, application organization in projects, visual design tools and a better environment to run applications.

Installation

It is needed to download and install the Java Development Kit (JDK). Also it is recommended to download and install the last version de NetBeans IDE.

The first time NetBeans is opened it will be shown a window like the following figure:



To add the project press the Open Project button and search the folder where is stored the project, it will be easily identified because the folder will change its icon.

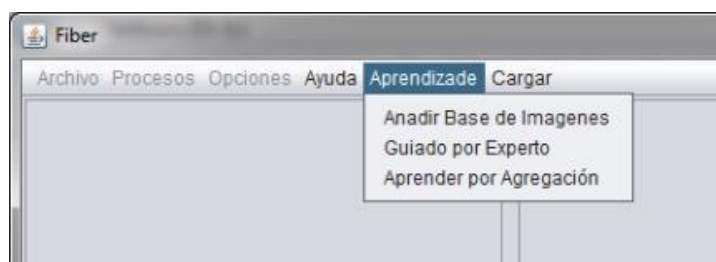
Once the project is open some button of NetBeans interface will be enabled, the most important is Run Project, with a green arrow icon.

In the program

The histochemical image analysis with this software is divided in 5 steps: Learning, Segmentation, Filtering, Counting and Correction.

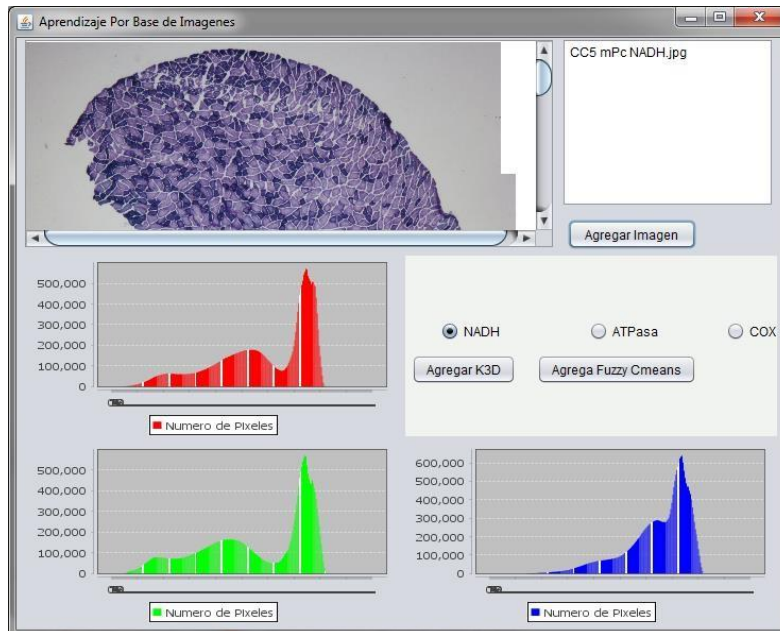
Training

In this step are determined the colors of each one of the present fibers in an histochemical image, i.e. the fibers are classified by their color. There are 3 supported types of learning: through Data Mining in a images' database, Expert Guided and Aggregation, that it is a combination of the previous.



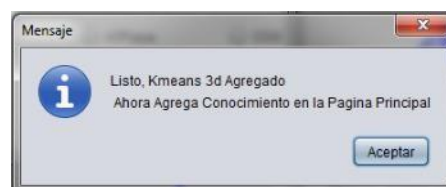
Images database

A set of images of the same histochemical staining is used to determine the colors of each one of the present fiber types. To add images we press the Agregar Imagen (Add Image) button, a file explorer is opened, then press the Abrir (Open) button and a preview of the image will be shown and also histograms of colors in the image splitted in its RGB components, as shown in the following figure.



When all images are added choose the histochemical staining type and press the button according to the desired algorithm, in can be K-means, Fuzzy C-means, or Kohonen map learning.

When an algorithm is done the following message will be shown:

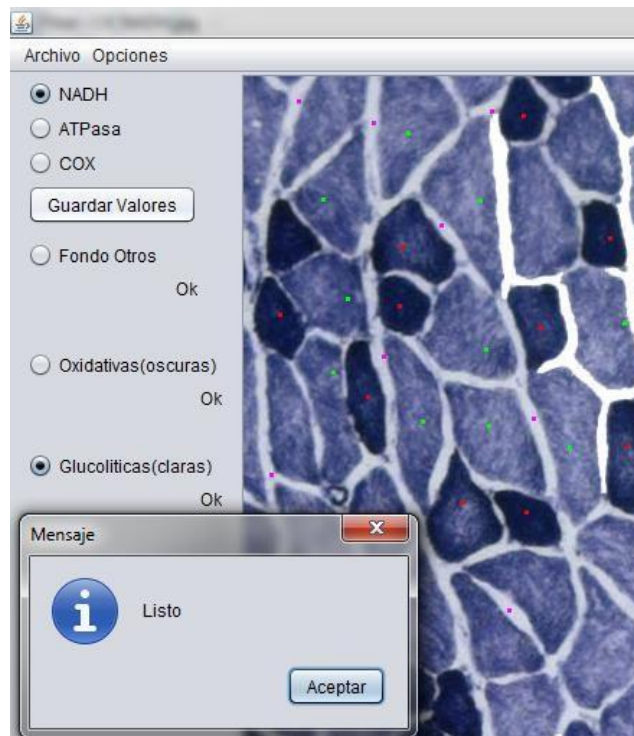


As it is indicated, after adding knowledge in training window, it must be added in the main window at the menu Cargar -> Carga Conocimiento, this will enable the file choosing to analyze in the next step of the process: Segmentation.

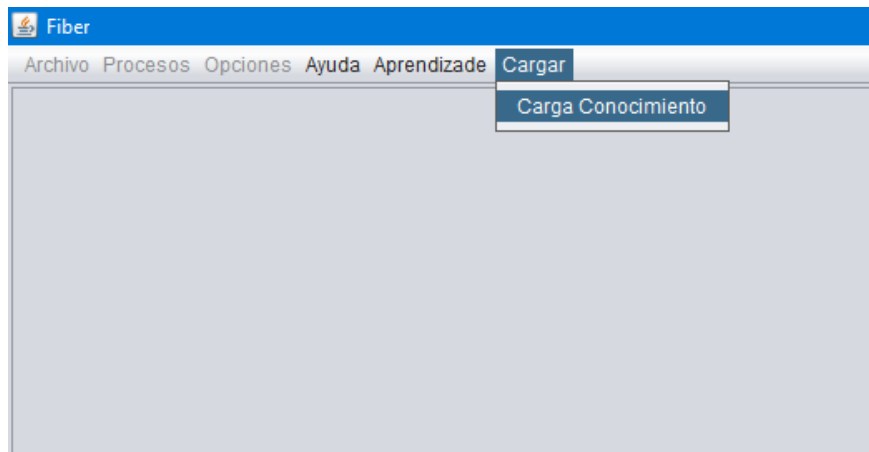
Expert guided

It should be noted that given the characteristics of the colors in ATPasa staining images, the data mining algorithms most of the time are not able to do a correct classification, thus its proposed a Expert guided learning, i.e. the color of each fiber type must be indicated manually.

In the Expert guided window select the histochemical staining (NADH, ATPasa or COX) of the image to be used, then open it from the Archivo (Archive) menu (JPG, GIF, BMP, TIFF or PNG extension). Select 10 places whit the background color (and connective tissue) and 10 fibers of each type (slow, intermediate and fast for ATPasa or glycolytic and oxidative for NADH and COX), finally click the Guardar Valores (Save Values) button and a confirmation message will be shown.



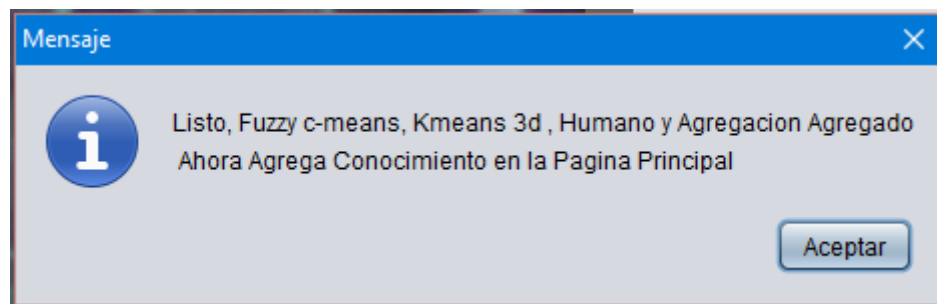
After accept the last message go to the main window and click on menu Cargar (Load) and select the option Cargar Conocimiento (Load Knowledge).



Aggregation

It is a combination of the previous methods: while the expert is indicating manually the colors of each fiber type the algorithms are running in background and generate their results that are saved for the next steps of the process and additionally a proposed data combination is saved. With this method we get information from all the sources, and in addition the information used to the segmentation step can be easily changed among the added on this step.

The procedure is the same of the Expert Guided approach, only the user must wait until all the algorithms ends their execution.

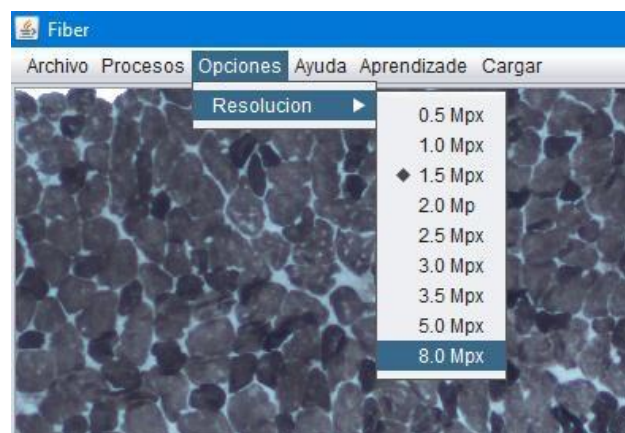


Segmentation

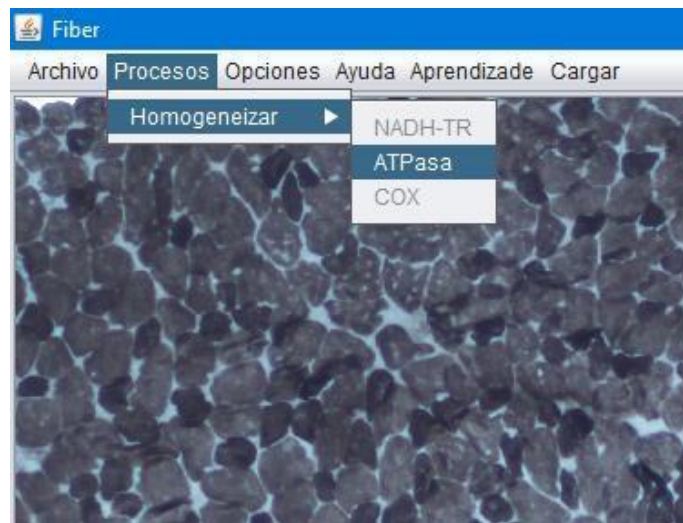
In this step each image is splitted in images containing only one fiber type.

When any of the Learning algorithms is added in the main window the menus Archivo (Archive) and Procesos (Processes) will be enabled. In the Archivo menu select the option Abrir imagen (Open Image) and select the image to work in the file explorer (the same image used in the Learning step).

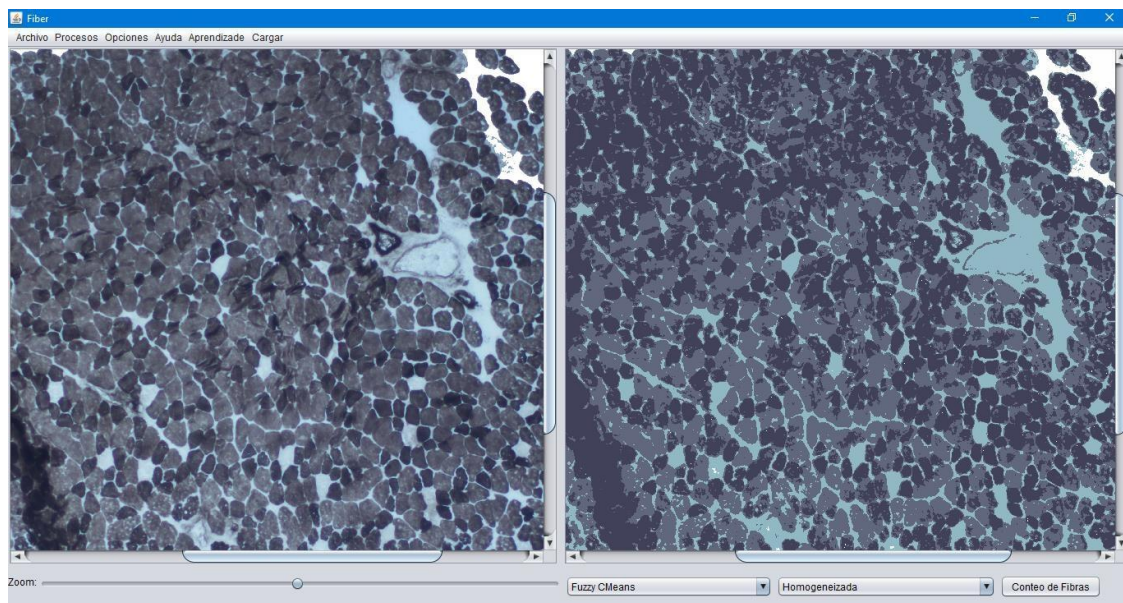
It is possible to select the resolution to process the image, because when a image is processed using its original resolution the RAM memory was insufficient, moreover it is necessary a lot of computing power. There are options from 0.5 Mpx up to 8 Mpx, with 1.5 Mpx as default. It can be selected in the menu Opciones (Options) → Resolución (Resolution).



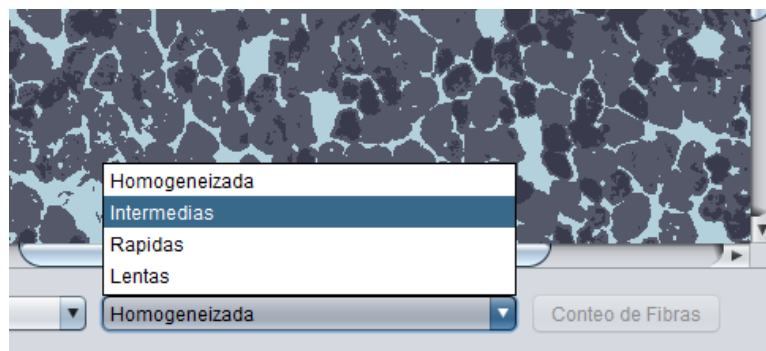
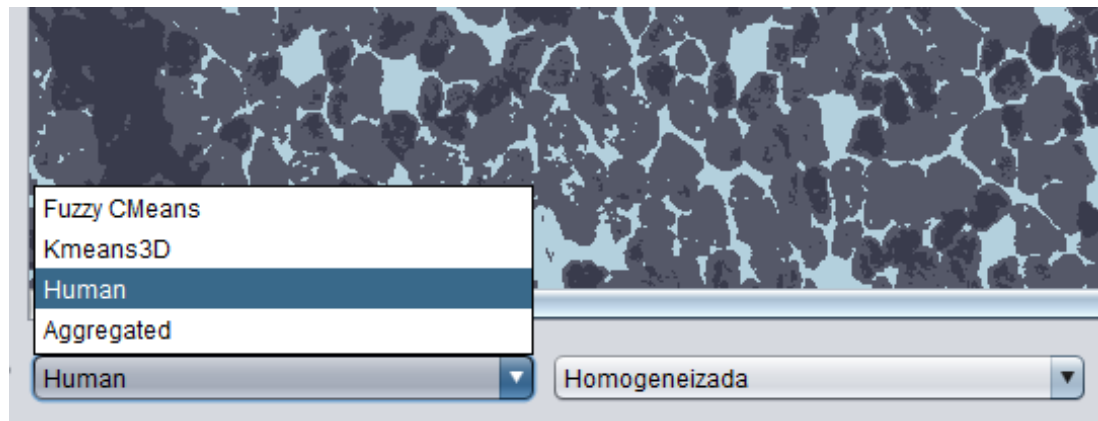
When the desired resolution is selected go to menu Procesos (Processes) -> Homogeneizar (Homogenize) and choose the histochemical staining.



Once the process is done the result will be shown in the right panel, i.e. the homogenized image.



There are two dropdown menus at the bottom of the main window, the first one to choose the learning algorithm data to be used to the segmentation, here will be shown only these that was added in the learning step. The second dropdown menu is used to change the image shown in the right panel, it can be the homogenized image or the images with only one fiber type.

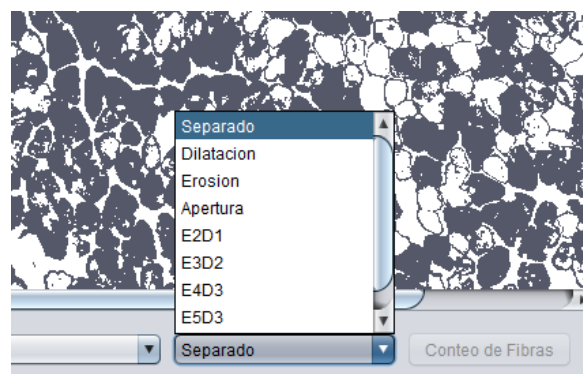


When a showing a image with only one fiber type a third dropdown menu will appear that corresponds to the next step: Filtering.

Filtering

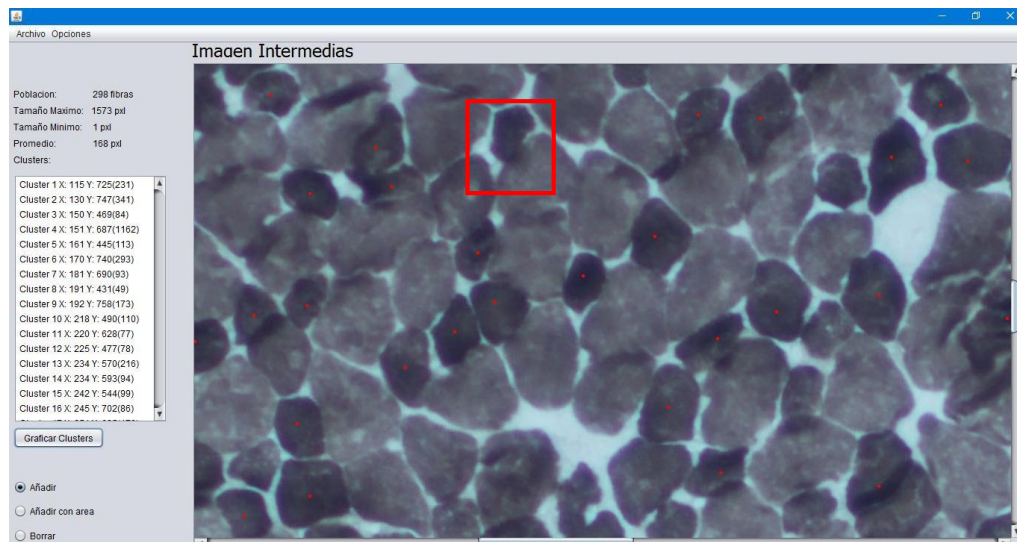
At this step it is pretended to prepare a image with only one fiber type to apply the fiber counting algorithm because in initial tests the counting was wrong due to noisy or homogeneous colors in images.

In the third dropdown menu can be chosen the best personalized filters used in while testing the software, however the best one must be selected by the expert according to some factors like the histochemical staining, the size of the muscle, the objective's zoom, the image resolution to process, noise while acquisition etc.

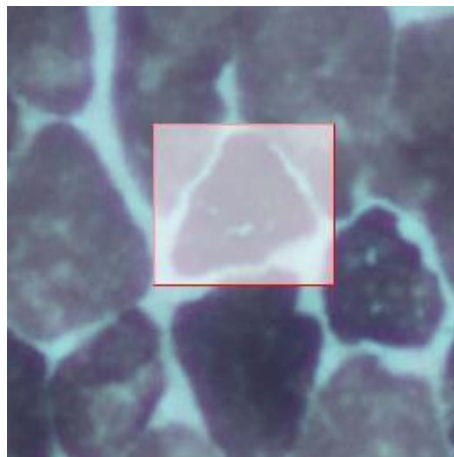


Counting and Correction

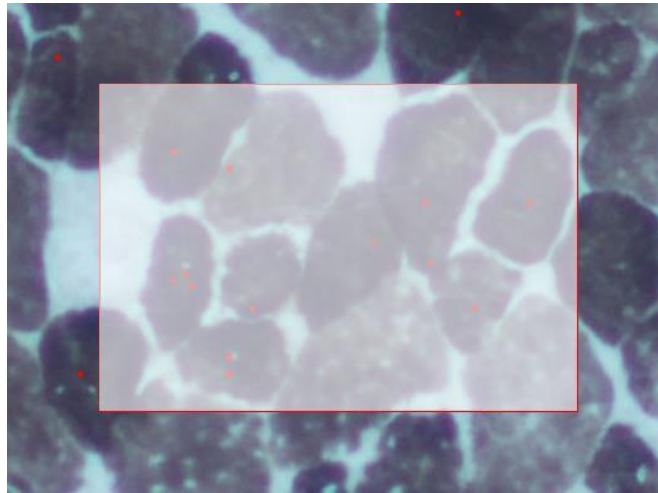
Once the filter is selected click the button Conteo de Fibras (Fibers counting), a window will be opened showing the amount of fibers as well as an image with the centroids of each identified fiber drawn.



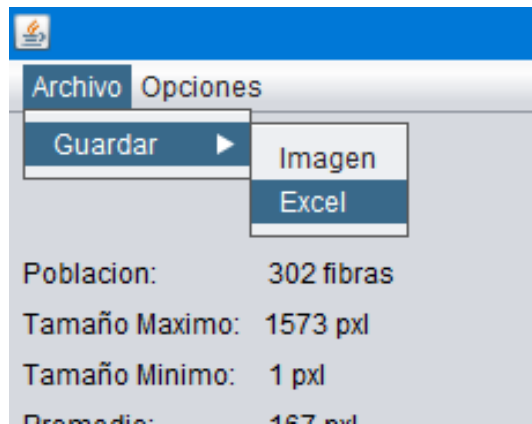
When the software does not identify a fiber (as shown in the previous figure) it can be used the option Añadir (Add) (bottom left), it will add a fiber (and its coordinates) to the total when clicking over the image. Also the option Añadir con área (Add with area), can estimate the area of each fiber in pixels by drawing a rectangle over the whole fiber.



If a fiber is wrongly added to the counting the option Borrar (Delete) can be used, only by clicking over the drawn point. Additionally this option allows deleting an area only drawing a rectangle over the undesired points.



Finally go to menu Archivo → Guardar (Save) choosing Excel option and selecting the location to save the file.



To count another fiber type image close the Counting window and go to the main window and change the fiber type on the dropdown menu and repeat from the Filtering step.