RoiEditor 2.0

The goal of RoiEditor is to remove “bad ROIs” from microscope images and to generate the data to perform statistics on the basic ROI properties: area and the Feret-parameters.

RoiEditor 1.0 is a Jython plug-in for Fiji and was started from the LabelsToRois plug-in. RoiEditor 1.0 and 2.0 have extended editing capabilities, but lack the configurable erosion and multiple slice functionality of LabelsToRois. RoiEditor 2.0 is a standalone implementation of RoiEditor using regular Python aka CPython.

RoiEditor cannot segment ROIs in photographs. cellpose is used for that purpose.

This manual will guide you through the steps from the segmentation using cellpose to the generation of the data for further statistical analysis using RoiEditor 2.0.

An overview of the cellpose + RoiEditor workflow is shown in Figure 9 on page 11.

1 Cellpose basics

1.1 Installing Python

Cellpose and RoiEditor are Python applications. To run them, one needs to first install Python.

1. Option 1: Python installer in RoiEditor zip
    a. An experimental installer for Python can be found in the RoiEditor zip package.
    To use that installer, first execute step 2. until of section 2.1 on page 3.
    b. Double-click `install_python.bat`
2. Option 2: use the installer from the python.org website

1.2 Installing cellpose

1. Option 1: regular install using pip
    a. Open a Windows command prompt. 
    b. Type : `python -m pip install cellpose[gui]` .
    c. Cellpose will now be installed. If you freshly installed Python, this may take a while since next to cellpose quite some Python modules need to be installed.
    d. If things go wrong both the cellpose GitHub pages and cellpose website offer some help.
2. Option 2: cellpose installer in RoiEditor zip
    a. An experimental installer for Python can be found in the RoiEditor zip package.
    To use that installer, first execute step 2. until 5. of section 2.1 on page 3.
    b. Double-click `install_cellpose.bat`
    c. The script will install a desktop shortcut for cellpose too: 

1.3 Using cellpose

If you have a desktop icon for cellpose, just double click the icon: 

Alternatively start cellpose, following the steps below:

1. open a Windows command prompt.
2. Do not start Python, but type `python -m cellpose`
3. Now you need to open a photograph. Open the photograph using the menu File>Load ((A) in Figure 1).
4. Preferably do not change any settings, just make sure that in the lowest groupbox 'Image Restoration' cyto3 is selected.
5. Now click the 'run cyto3' button in the groupbox 'Segmentation'.
6. When the segmentation has finished, the photo will be overlayed with the identified ROIs.
7. Now you can save the results in several file formats. The three file formats RoiEditor can import are shown in Figure 1 and Table 1.

I.	*_seg.npy	This is a Python numpy specific file format. It contains more data than II and III, but does not offer extra info for RoiEditor
II.	PNG/tif	Although this is a regular picture format, most picture editors will not properly display this file. It is only used to transfer data to another application, not for visual inspection.
III.	.zip	The zip archive is a fileformat that is compatible with Fiji. It is the same fileformat used by RoiEditor to store the results after removing 'bad' ROIs

Table 1: file formats supported by RoiEditor 2.0

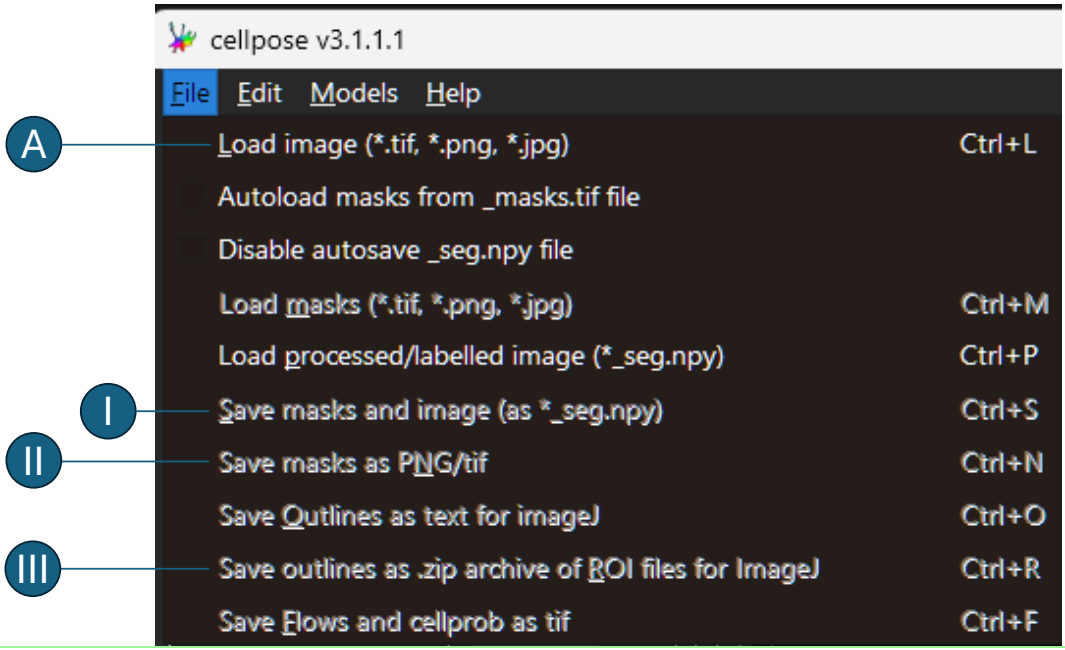

Figure 1: Reading and writing files

2 RoiEditor

2.1 Installation on Windows

1. It is assumed that you have Python already installed on your machine. If not, follow the steps in 1.1 on page 1 to install Python.
2. Change folder to a folder where you are allowed to install software
3. Copy the RoiEditor.zip into that folder
4. Extract/Unzip the zip-file
5. This will create an `RoiEditor` folder
6. Inside the folder there is an `install.bat` batch file
7. Double-click the batch file
8. When your Python environment lacks some required packages, they will be installed now
9. After finishing the installation, you should be able to start RoiEditor as described in "2.2.1 Start RoiEditor" below.
10. An icon should appear on the desktop: 

2.2 Use

2.2.1 Start RoiEditor

There are a few options to start the RoiEditor:

- If the installation has run smoothly, an icon should have appeared on the desktop. Double-clicking the icon will start the RoiEditor. 

Alternatively:

- Start a windows command prompt: 
- Try:
  - `Python -m RoiEditor`
  - `RoiEditor`
- If the above do not work, change the folder to the RoiEditor folder and type:
  - `Python -m RoiEditor`

2.2.2 Select the files

The files generated by cellpose can now be imported in RoiEditor. As indicated in the columns 'Usable combination', one always needs to specify at least the original file and a label file.

For the label file either a .png or a _seg.npy, numpy file format, can be specified. If no zip/ROI file is specified, RoiEditor derives the information from original & label file.

File	Figure 1	Usable combination	Usable combination
Original	A	√	√
Label	I or II	√	√
Zip / ROI	III		√

Table 2: Selecting files

2.2.2.1 Autocompletion

When the user first selects the Original file, RoiEditor will search the current folder for the 'most probable' candidates to be used as Label file and Zip file and select them. If this does not correspond to the intended choice of the user, the names can be wiped by clicking the trash can button.

2.2.2.2 Two in one go

When the Original file is being selected, one can select two files. The one with shortest name will be used for the Original file and the longest name for the Label file

2.2.2.3 The checkboxes & radio buttons

2.2.2.3.1 Process Labels

Table 3 explains the function of the checkboxes in the "Choose paths and settings">"Process labels" group box. The settings are only applied when the Next button is clicked to process the data.

While the Image Window is shown, the checkboxes have no effect.

Remove at edge	When checked, incomplete ROIs at the edges of the photograph will be removed automatically. The deleted ROIs are tagged with 'edge.image'
Remove small	When checked, the ROIs smaller than the size specified in the textbox 'Min size' pixels will be removed automatically. The deleted ROIs are tagged with 'small'

Table 3: Proces labels checkboxes

2.2.2.3.2 Unit & Scale

Table 4 explains the function of the radio buttons in the "Choose paths and settings">"Units & Scale" group box. The settings are only applied when the Next button is clicked to process the data.

Pixel is unit	When checked, all shown lengths and areas are expressed in pixels.
From file	tif(f) image files may contain metadata about the microscope used. When the Original file has been selected and it contains the necessary data, it is shown here. When checked, the scaler read from file will be used.
Specified	The user can manually enter a scaler $\left(\frac{\mu m}{pixel}\right)$ converting pixel-width and height into microns. When checked the user-specified scaler will be used.

Table 4: Unit & Scale radio buttons

2.2.2.4 Next

Figure 2 shows that when the 'Next' button (B) is clicked after the files have been selected (A), the files will be processed taking into account the choices in the checkboxes. The result will be shown in an Image Window as shown in Figure 3. At the same time a Histogram window will appear.

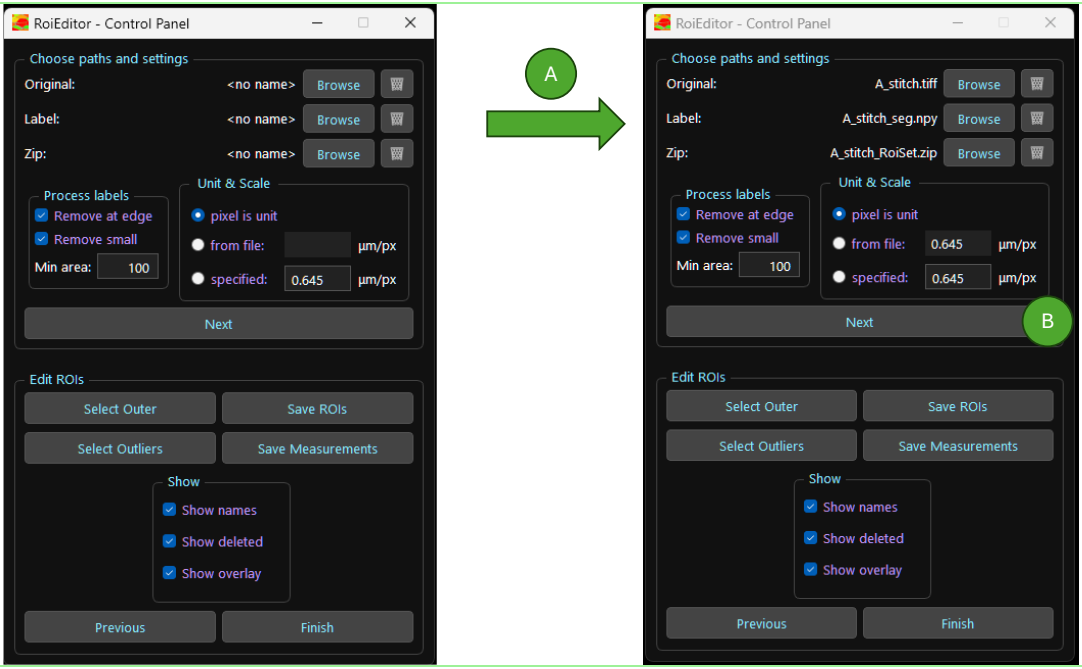

Figure 2 Clicking Next after selecting files

2.3 Editing ROIs

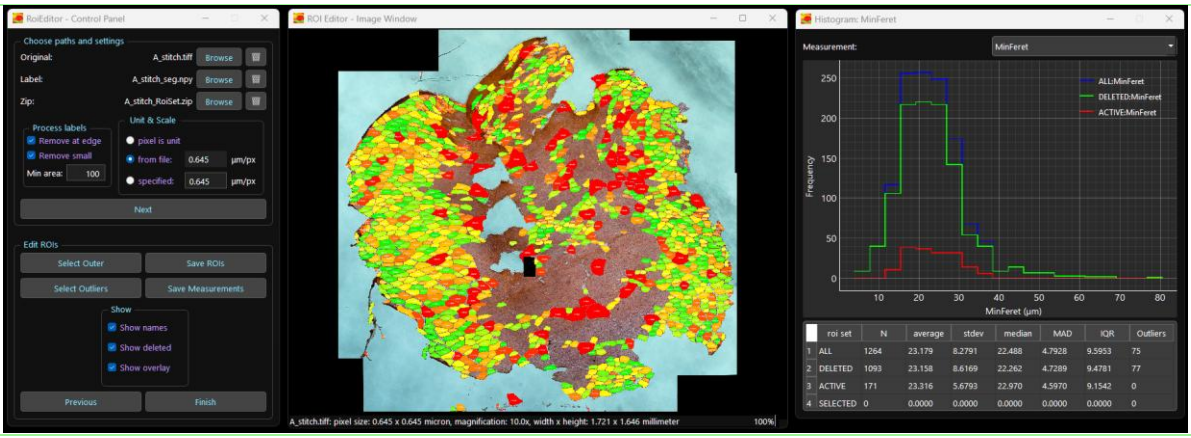

Figure 3: the context for editing ROIs

ROIs have three possible states in the RoiEditor: ACTIVE, DELETED, or SELECTED. ROIs are never removed from the output generated by RoiEditor. When the status of the work is saved by 'Save ROIs' or 'Save Measurements', the data of all ROIs is kept, and their state is stored too.

2.3.1 The Image Window

2.3.1.1 Basic Actions

Action	Effect
single right click	Toggles the selection of the clicked ROI. The selection of other ROIs is not changed
Alt +single right click	Immediately marks the ROI as deleted
right-click hold & drag release	Selects all ROIs that are completely inside the indicated rectangle
Delete key	Marks all selected ROIs as deleted
Esc(ape) key	Deselects all ROIs

Table 5: Basic actions

2.3.1.2 Tagged delete

The goal of the tagged delete is to facilitate keeping a record of why specific ROIs have been marked as deleted.

When one or more ROIs have been selected and one of the function keys listed in Table 6 is pressed, the selected ROIs are deleted and marked with the text shown in the 'Tag' column.

Key pressed	Tag	Artifact
F5	freeze	The deleted ROIs shows a freeze artifact
F6	fold	The deleted ROIs shows a folding artifact
F7	vessel	The deleted is a vessel
F9	section.tear	The deleted ROIs were torn during section
F10	section.stretch	The deleted ROIs were stretched during section

Table 6: Tagging artifacts

2.3.1.3 Select outer edge

ROIs at the edge of the photographed object are often damaged or deformed. They can be selected with a single button click on 'Select outer'. When they are deleted after selection, they are marked with the tag 'edge.outer'. When one or more ROIs are manually removed from the selection by right-clicking them, the tag is 'forgotten' for these ROIs.

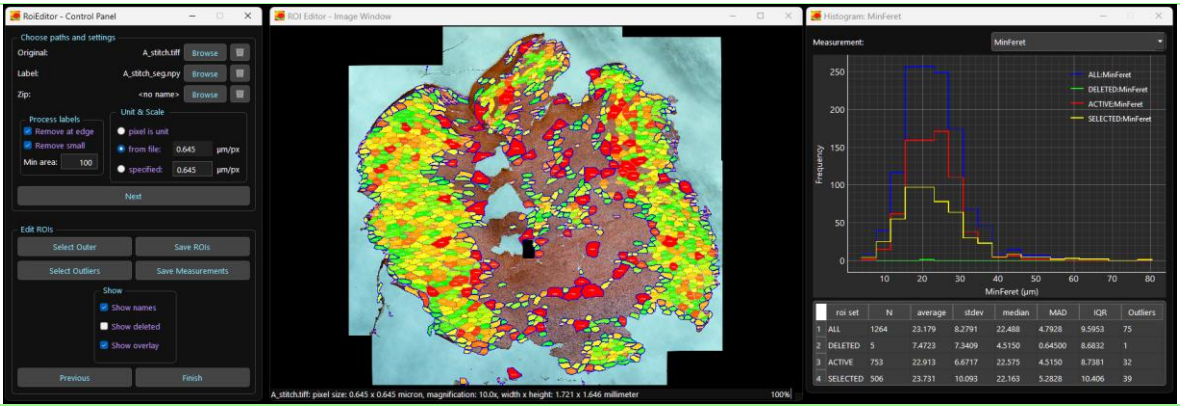

Figure 4: Select outer

2.3.2 The Histogram window

The Histogram window shows the statistics of the 4 subsets of ROIs in real-time.

The Histogram window displays statistics for area and the Feret numbers as shown in Figure 6. The typical shape of a Feret Angle distribution has a bathtub shape due to the cyclical nature of angles. To facilitate the interpretation of the Feret angle an extra measurement has been created, AngleShifted. AngleShifted shows the distribution of the Feret Angle shifted by 90° typically creating a bell-shaped distribution that is easier to interpret. This is shown in Figure 7.

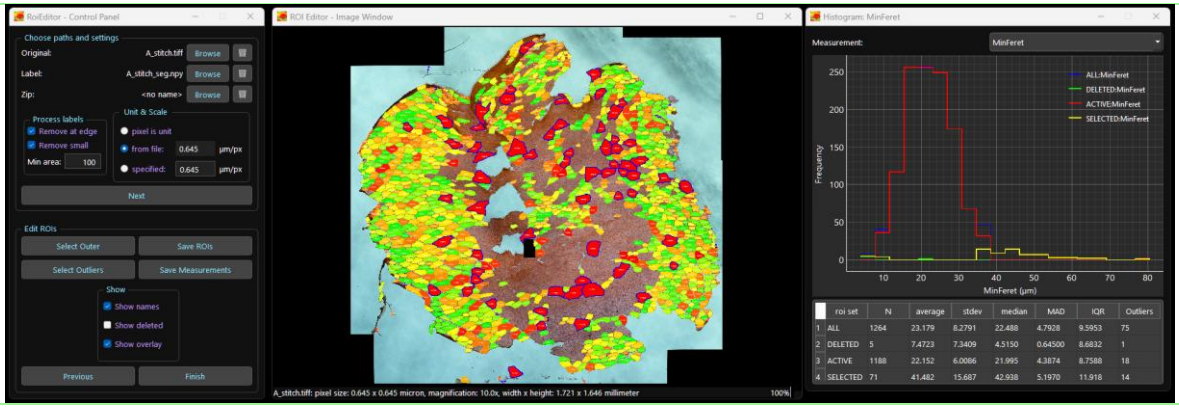

Figure 5: Select outliers

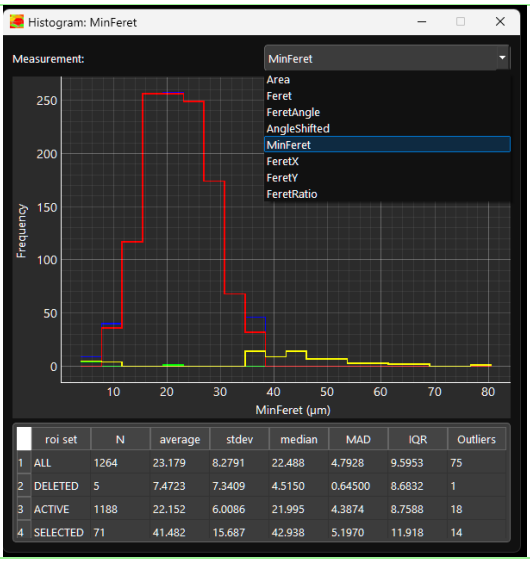

Figure 6: Selecting a measurement

Figure 7: FeretAngle and AngleShifted

2.3.3 Selecting Outliers

When the checkbox 'Show overlay' is checked, the ROIs are filled with a color ranging from green to red. Green ROIs have a value for the selected measurement that is close to the median, red ROIs have a value close to or beyond $median \pm 1.5 \times IQR$.

When the button 'Select Outliers' is clicked, all ROIs having a measurement value outside of the range $median \pm 1.5 \times IQR$ are selected.

When ROIs are deleted after being selected by clicking 'Select Outliers', the ROIs are tagged with '<measurement name>.outlier', e.g., 'Area.outlier'.

2.4 Output files

2.4.1 ROIs

When RoiEditor writes the zip file it adds a file containing the states and tags to the zip-file. This extra file called 'tags.json' may confuse some "Fiji compatible ROI readers/writers". When this occurs and it is necessary to open the zip in another application: just remove the 'tags.json' file.

Contrary to other applications using Fiji ROI format, RoiEditor never removes ROIs from the zip-file: it stores the state (ACTIVE,DELETED,SELECTED) in the 'tags.json' file.

2.4.2 Measurement data

The .csv file contains the area and other metrics for all ROIs expressed in pixels, so no unit or scaling is applied.

The .xlsx file contains multiple sheets as explained in Table 7.

Sheet	Content	Unit (*)	This worksheet contains…
pixels_ALL	numbers	pixels	the same data as the .csv file
pixels_ACT	numbers	pixels	the data of the ROIs in ACTIVE state
scalers	numbers	$\left(\frac{\mu m}{pixel}\right)$ and $\left(\frac{\mu m^2}{pixel}\right)$	the scalers to convert pixels into the correct unit
scaled_ACT	formulas	μm and μm^2	the result of applying the 'scalers' to the data of 'pixels_ACT'
summary_ACT	formulas	μm and μm^2	the stats for all metrics of sheet 'scaled_ACT'

Table 7: xlsx workbook worksheets

(*) some metrics are expressed in degrees (FeretAngle, AngleShifted) or have no unit (FeretRatio) the user may have chosen not to apply a scaling then the unit is pixels in all rows

2.5 Erosion

Unless the ROIs are very clearly separated, cellpose creates neigboring ROIs without any gap in between. When RoiEditor reads the labels from either an .npy or a .png file the algorithms that identify the labels create an open area of 3 pixels between the ROIs. No statistics have been collected to estimate the possible error when ROIs have already been separated by cellpose and RoiEditor widens that gap. Visually the error seems to average out.

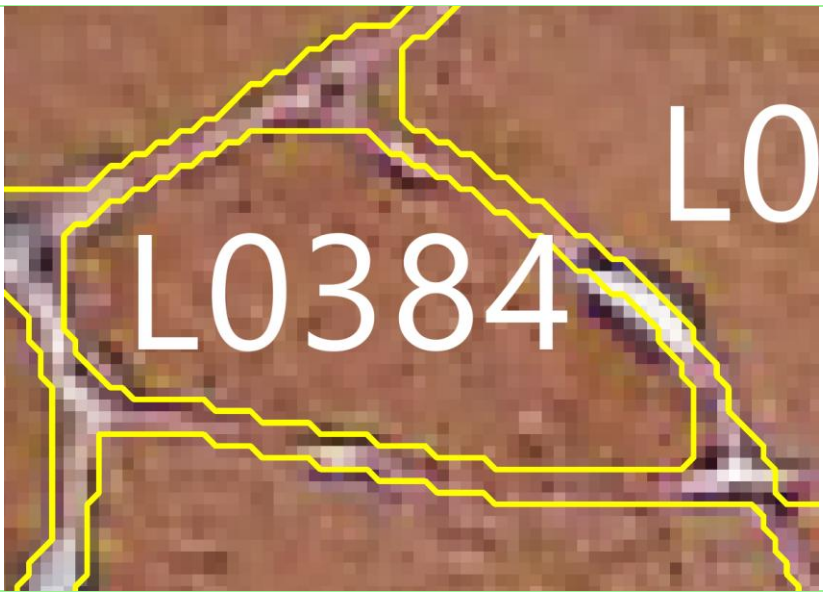

Figure 8: FeretAngle and AngleShifted

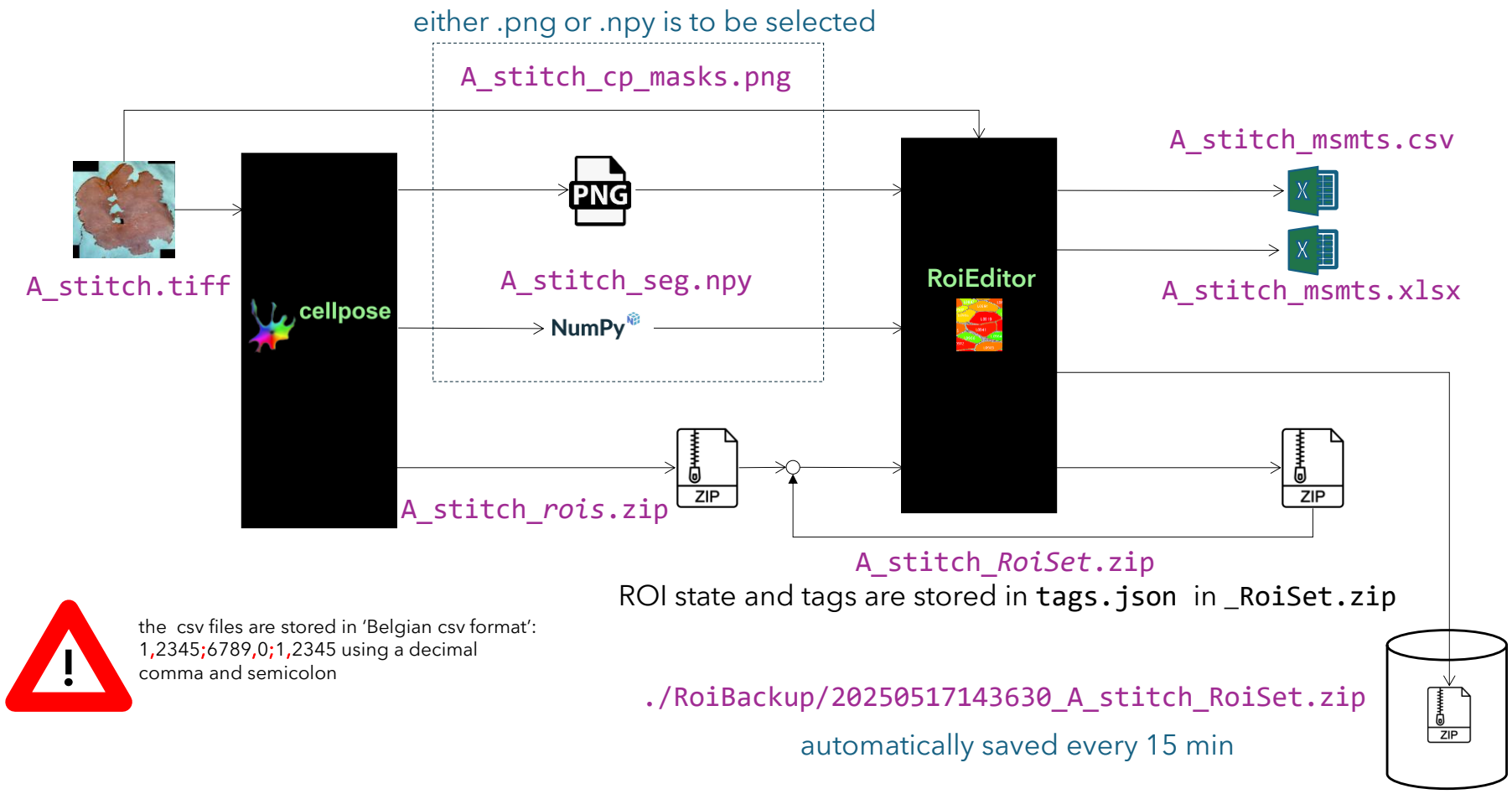

Figure 9: cellpose and RoiEditor Workflow

RoiEditor 2.0

Contents

The original article about LabelsToRois

https://www.researchgate.net/publication/348672590_Automatic_and_unbiased_segmentation_and_quantification_of_myofibers_in_skeletal_muscle

The github site of LabelsToRois

https://labelstorois.github.io/