

Brazilian Synchrotron Light Laboratory



ImageJ (Trainable Weka Segmentation)

FIJI

A quick start

X-Ray Imaging Beamline (IMX) July/2019

LNLS is part of the CNPEM an organization certified by the Ministry of Science, Technology, Innovation and Communication (MCTIC). Address: Rua Giuseppe Máximo Scolfaro, 10.000 - Polo II de Alta Tecnologia - Caixa Postal 6192 - 13083-970 Campinas/SP Telephone: +55.19.3512.1010 | www.lnls.cnpem.br

Documentation History

Date	Revision	Description	Author
10/07/2019	1	Version based on ImageJ 1.52	Paola R. R. Rosa

Summary

Documentation History	2
 Opening and modifying the data a. Opening ImageJ and loading data 	4 4
 2. ImageJ Window a. Menu Bar (blue) b. Toolbar (green + orange + yellow) 	5 5 5
3. File Menu: Opening Data	6
4. Edit Menu a. Selection Submenu b. Options Submenu	8 9 11
 5. Image Menu a. Type b. Adjust Submenu c. Cropping the image 	12 12 13 14
6. Process menu: Filters	15
 7. Segmentation a. Tools b. Training the classifier c. Other training buttons 	16 18 21 22
8. Saving and exporting	22
9. Batch Processing	23
10. Using Scripts for segmentation	26

ImageJ (FIJI)

1. Opening and modifying the data

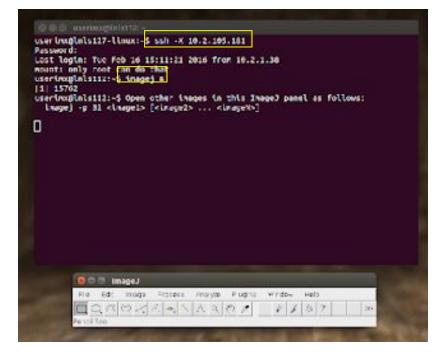
ImageJ has a image processing package called Fiji (Fiji Is Just ImageJ) and it is available online for free download and it has many plugins. If you are going to open the imageJ of CNPEM, get the following directions.

a. Opening ImageJ and loading data

From any terminal connected to the Recon computer, open ImageJ. To do this, follow the commands:

ssh –X 10.2.105.181 (Password: ask your local contact) imagej &

This will open the program and release the terminal for further use.



2. ImageJ Window

Ø					(Fiji	ls Ju	ust)	ImageJ						•		×
File	Edit	Image	Pi	roce	ss	Ana	alyze	Plugins	W	'indo	w			11. A)	He	elp
		♡ A,	Δ.	*** ** *		А	Q	8 ^m	Dev	Stk	Lur	Ø	b	\$		≫
Magnif	yin g g l	ass (<mark>or</mark> u	se "H	⊦" an	nd "-"	' key	rs)					Click	< her	e to s	sear	ch

a. Menu Bar (blue)

The menu is composed of: File: Open/Close/Save/Export/Quit Edit: Selection/Copy/Paste/Undo/ROI handling Image: Visualization/Adjust/Crop/Transform/stack manipulation/video editing Process: Image filters Analyze: Statistics/Measures Plugins Window Help

b. Toolbar (green + orange + yellow)

These features are used for selection, measurement and segmentation. When used for selection, information about the measures (angle, distance, height etc) will appear at the **Status Bar (Pink)**.

See the applications of area selection/line selection/angle/point/wand tools (green) for segmentation at <u>Section 7.a</u>.

Other tools **(orange)** are Text Tool, Magnifying glass (you can also zoom in and zoom out clicking – or + at the keyboard), Scrolling tool and Color picker.

Then, there is the *macro tools* (yellow).

The last button is the macro *toolset switcher* (red): select a tool to add to your window.

The search bar (purple) allows you to find tools based on keywords.

3. File Menu: Opening Data

Import the .b file, created during the reconstruction. Go to *File > Import > Raw*, then go to the *Sample* folder and open it.

		Image Sequence	
		Raw	
		LUT	
μ.	(Fiji I	Is Text Image	↑ □
File Edit Ima	age Process	م Results	Hel
Open	Ctrl+0	Table	0 8 3
Open Next	Ctrl+Shift+O	URL	Click here to searc
Open Samples Open Recent	-	Stack From List TIFF Virtual Stack	
Import		AVI XY Coordinates	
Show Folder	-	 Av coordinates 	
Close Close All Save Save As	Ctrl+W Ctrl+Shift+W Ctrl+S	HDF5 Analyze MHD/MHA Koala Binary DF3	
Revert	Ctrl+R	FIB-SEM	
Page Setup Print	Ctrl+P	_ FIB-SEM MRC Leginon PDF	
Export	5	Extract Images From PDF	
Quit		DM3 Reader	
Fix Funny Filename Make Screencast	98	TorstenRaw GZ Reader Nrrd – ICO	
4		lcns	

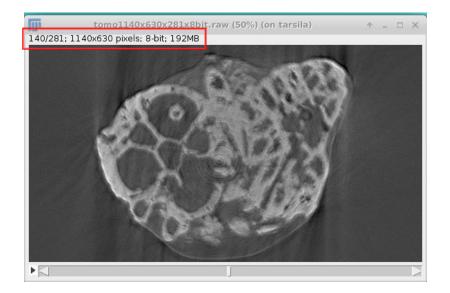
Fill the *Import > Raw* window with image information:

Image type: e.g. 8-*bit* Width and Height: e.g. *1140* Number of images: e.g. 281 because this data is already cropped. It is typically *2048*, but it can be less if not all slices are reconstructed. Be sure that *little-endian byte order* and *virtual stack* is enabled (depending on the operation in ImageJ, you can't load with virtual stack).

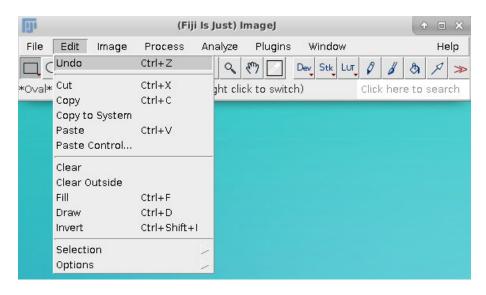
(j)	(Fiji Is Just) ImageJ	Ϋ́
File Edit Image	Process Analyze Plugins Window	Help
	L *** × A Q () Dev Stk Lut &	1 8 8 >
Importing: tomo1140×6	30×281×8bit.raw Click	here to search
Import>Ra	w + • ×	
Image type:	8-bit	
Width:	1140 pixels	
Height:	630 pixels	
Offset to first image:	0 bytes	
Number of images:	281	
Gap between images:	0 bytes	
▼Little-endian byte	order	
	older	
□Use virtual stack		
He	lp <u>Cancel OK</u>	
٤	Console	* - ¤ ×

This is an example of a carbonate sample. First, check all slices to ensure the reconstruction is as expected. If strong rings are seen or the image contrast is not very good, (i.e. it is hard to separate the phases of interest), there are some parameters on the reconstruction process that can be changed. Talk to your local contact, so he/she could help you.

From the header (red rectangle), you get information regarding the image: the first information (e.g. 140/281) is the slice number shown (e.g. 140), out of the total (e.g. 281); the three following numbers are the size (in pixels) in X and Y directions, respectively. The next information is the bit depth (8-bit), followed by the image size (192 MB).

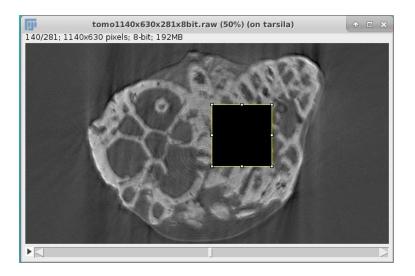


4. Edit Menu



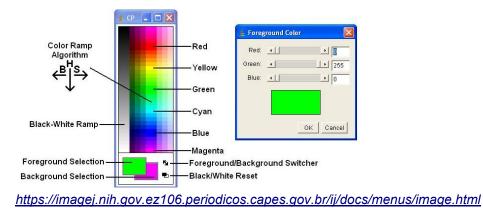
Undo: Reverse the last action (only 1 reversion is possible – if you click more then one time, the tool will redo the action). Operations on entire stacks (e.g. filtering) won't be reversed.

Cut: Removes the area selected (and fills with the background color) and copies to the transference area.



Copy/Paste: Copies and pastes the content – If the area is not selected, the program copies or pastes the entire image.

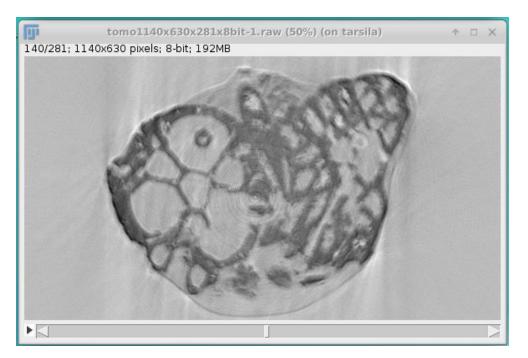
Clear/Clear Outside - Erases the area selected and fills with the background color. If you activate the clear outside, the other part of the image will be erased. If you want to change the background color, select: *Image>Colors>Color Picker.*



Fill: Fills the area selected with a picked color. To pick the color, click on *Image>Colors>Color Picker* and select a color of your choice. Then, select an area and click on Fill. A dialog is displayed offering the option to fill the selection in all stack images.

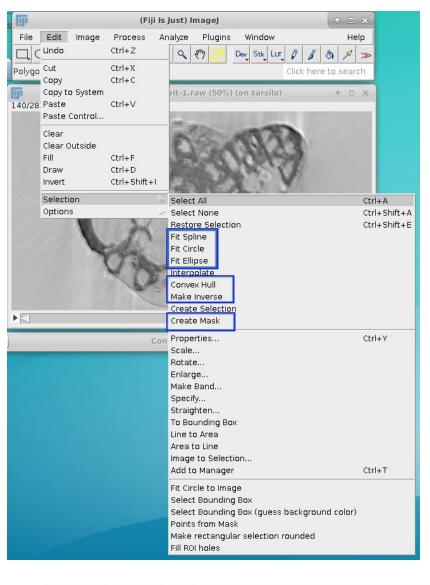
Draw: Outlines the selected perimeter/line.

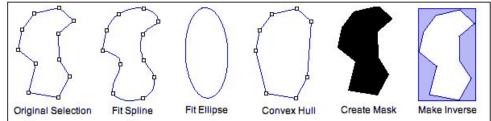
Invert: Reverts the image (e.g. black areas becomes white).



a. Selection Submenu

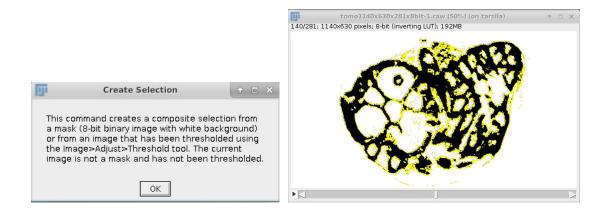
Some results of this features (blue) are resumed in the picture down bellow.



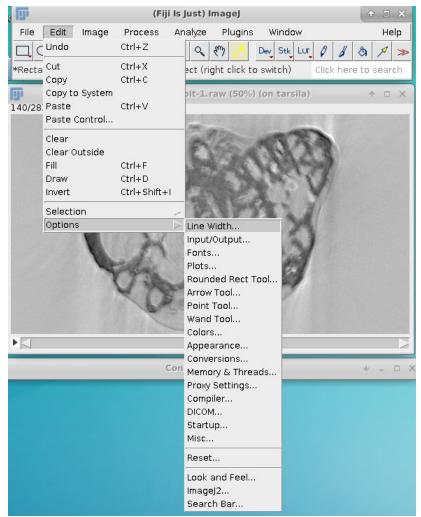


https://imagej.nih.gov.ez106.periodicos.capes.gov.br/ij/docs/menus/edit.html

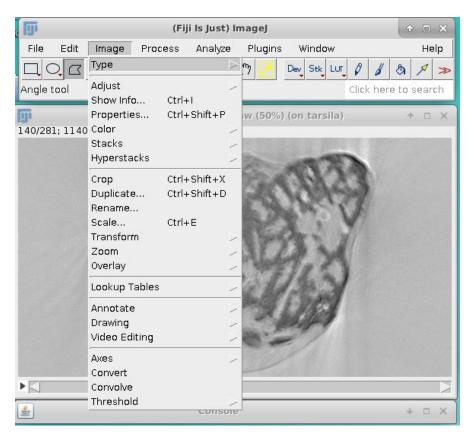
Select all: It makes a rectangular selection of all the image.
Select none: Clears the selection of the image.
Restore selection: Redo the previous selection.
Interpolate: Interpolation of pre-existing selections.
Create selection: Based on a threshold image or binary mask.



b. Options Submenu

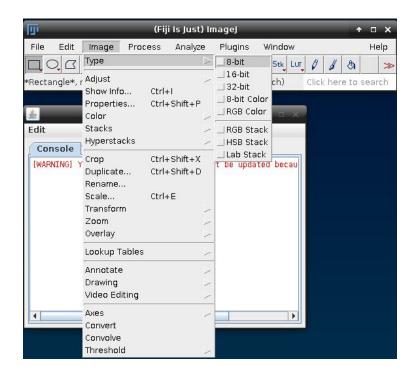


5. Image Menu



а. Туре

In case you have a 32-bit image type, you can reduce the bit depth to 8-bit or 16-bit. It is desirable as Avizo uses RAM memory for most of the processes.

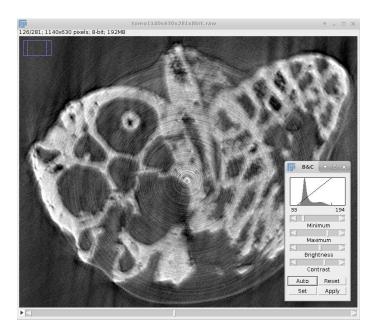


b. Adjust Submenu

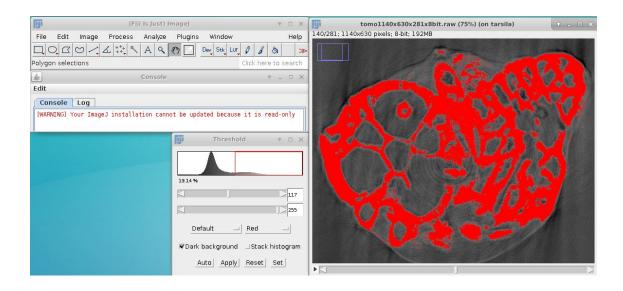
Before proceeding to segmentation, some adjust might be necessary. Select Image > Adjust > **Brightness/Contrast**

(in				(Fij	i Is Just) In	nageJ				•	• ×	III
File	Edit	Image	Proc	ess	Analyze	e	Plugins	Window				Help	126/2
		Туре				2	m	Dev Stk Lu	0	8	\$	≫	
Edit Con	sole [Adjust Show Inf Propertie Color Stacks Hypersta Crop Duplicate Rename. Scale Transfor Zoorn Overlay Lookup T Annotate Drawing Video Ec Axes Convert Convolve Threshol	es e m Fables e	Ctrl+ Ctrl+ Ctrl+	- I - Shift + P - Shift + X - Shift + D - E	/ /	Window/ Color Ba Thresho Color Th Size Canvas - Line Wid Coordina Auto Loo Auto Thi Bleach C Auto Cro	ess/Contrast. Level Iance Id reshold Size th ates cal Threshold correction op op (guess ba	1	bund		Ctrl+S	hift+C hift+T

A table will appear. Click on Auto and the image will get a good contrast. That is mostly necessary when the histogram of your data is too narrow and you can barely see the sample when you upload.



Other option is to adjust the threshold to make the segmentation easier. Select Image > Adjust > **Threshold**. Select apply and then ok.





c. Cropping the image

Use the square tool to choose the area of interest, i.e. the area to crop. Be sure that the whole image is inside the square. Check all slices by moving the bar. Then click on Image > Crop. Save the cropped image as a raw data (File > Save As > Raw Data...).

As a suggestion, use the following termination: tomoXxYxZsizes_nbit.vol. An example of name is tomo980x1132x2048_8bit.vol. Image dimension and type are required information to load the data in Avizo.

Observation: A more complete way of cropping is available. Search for Plugins > Stacks > Crop (3D). It allows you to crop in any direction (x, y, z).

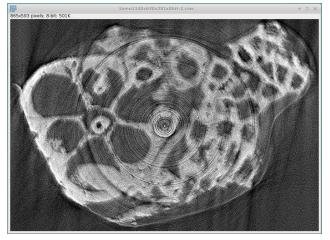
6. Process menu: Filters

The options are: Smooth, Sharpen, Find edges, Enhance contrast. Once the hole image is filtered there is no way to reverse it. So duplicate the image to test the result. Here are some examples:

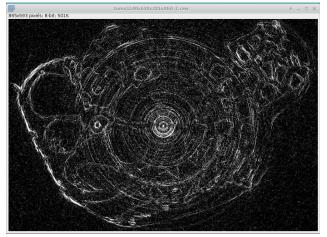
Smooth:



Sharpen:



Find edges:



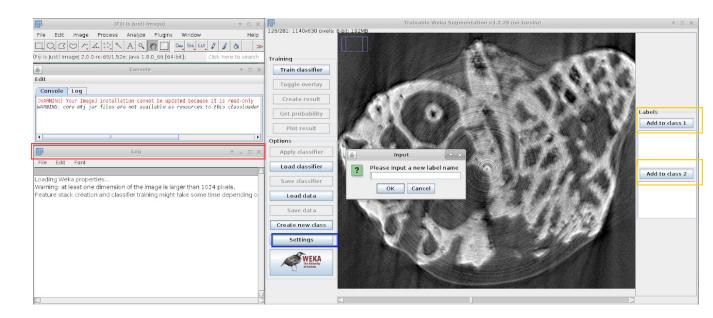
Observation: We emphasize that is not always good to filter your image if you plan to use it for segmentation via Trainable Weka Segmentation (the following section).

7. Segmentation

To start the segmentation, select Plugins > Segmentation > Trainable Weka Segmentation 3D. This is a machine learning tool that produces a segmented result based on the training of classifier. It works for 3D image. To use in 2D image, select Trainable Weka Segmentation.

(Fiji Is Just) Ir	nagej	↑ □ >	
File Edit Image Process Analyze	Plugins Window	Help	
	Macros		
	Shortcuts	2	
Wand (tracing) tool	Utilities	2	
	New		
🝰 Console	Compile and Run		
Edit	Install	Ctrl+Shift+M	
Console Log	Install PlugIn		
[WARNING] Your ImageJ installation cannot	3D Viewer		
	Analyze	>	
	BigDataViewer	>	
	Bio-Formats	2	
	Cluster	/	
	Color Inspector 3D		
	Examples	/	
	Feature Extraction	>	
	HDF5	~	
	lmage5D	\geq	
	Integral Image Filters	~	
	Janelia H265 Reader		
•	LOCI	~	
	LSM Toolbox	<	
	Landmarks	<	
	Multiview Reconstruction	-	
	Optic Flow	<	
	Process		
	Registration	_	
	SPIM Registration Segmentation		Compared black in OD Manuar
	Skeleton		Segment blob in 3D Viewer
	Stacks		Balloon Graph Cut
	Stitching		Graph Cut Blow/Lasso Tool
	Time Lapse		Robust Automatic Threshold Selection
	Tracking		Simple Neurite Tracer
	Transform		SIOX: Simple Interactive Object Extraction
	Utilities		Apply saved SIOX segmentator
	Volume Viewer		Statistical Region Merging
			Trainable Weka Segmentation
	Debug		Trainable Weka Segmentation 3D
	Sandbox		deprecated
	AutoRun	-	Color Clustering
	Kymograph Seripting	-	Segmentation Editor
	Scripting	/	Level Sets

Once selected, a log table will appear (red rectangle) and there will be two default classifiers (yellow rectangle). To add another classifier select **create new class** (blue rectangle).



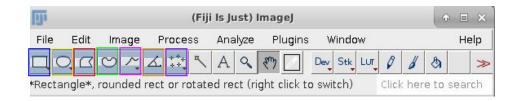
To modify the names of the other labels, select settings and then edit the class names (e.g. Class 1 - Rock; Class 2 - Background). At settings you can also able and disable training features. You can also change the type of classifier (select choose).

Other feature options are: **Minimum sigma** (minimum radius of the isotropic filters – by default 1 pixel). **Maximum sigma** (maximum radius of the isotropic filters - by default 16 pixels in 2D and 8 pixels in 3D).

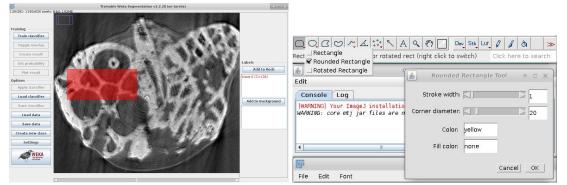
Ĩø	Trainable Weka Segmentati	ion v3.2.28 (on tarsila)	* 🗆 X
126/281: 1140x630 pixels: 8-bit: 192MB			
	Segmentation settings	O ■ X	
Training			
Train classifier	Training features:	A 4 4	
Toggle overlay	☐Gaussian blur ☐Hessian □Derivatives ☐Laplacia		
Create result	_ Structure _ Edges	1 9 L 1 1	
Get probability	⊔Difference of Gaussian	n	Labels Add to class 1
Plot result	Median ♥Variance	e	Add to class 1
Options	Minimum sigma: 1.0		
Apply classifier	Maximum sigma: 8.0		
Load classifier	Classifier options:		Add to class 2
Save classifier	Choose FastRandomForest -	200 -K 2 -S 196	
Load data	Class names:		
Save data	Class 1 class 1		
Create new class	Class 2 class 2	A BEING	
Settings	Advanced options:		
	⊒Balance classes		
WEKA The Delevant	Save feature stack		
	Result overlay opacity	33	
	Help	Cancel OK	
	1		

a. Tools

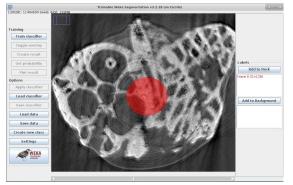
Once the classifiers are settled, use the best segmentation tool for your objective.



Rectangle (blue) - Click and drag at the image. Then click on the Rock classifier. It makes a rectangle. If you right click, two other options are available: rounded rectangle and rotated rectangle. If you double click, appears a tool to adjust the parameters.



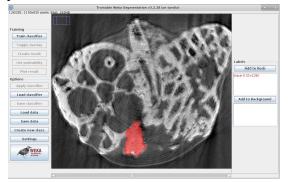
Circle (yellow) - Click and drag at the image. Then click on the Rock classifier. It makes a circle. If you right click, two other options are available: elliptical selections and selection brush tool. If you double click, appears a tool to adjust the parameters.



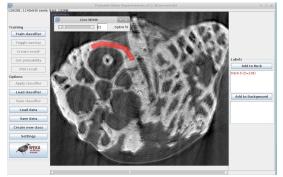
Polygon (red) – It makes straight lines connected to each other to form a polygon. Then click on the Rock classifier.



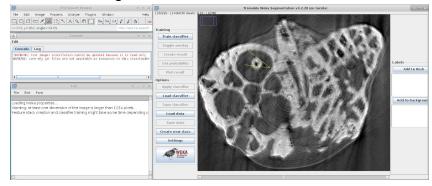
Freehand (green) – Make a form of your choice and it will fill automatically after you Add to Rock.



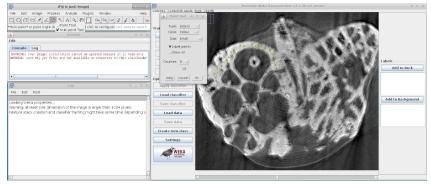
Straight (pink) – Freehand, but for lines. Right click to other options (straight line/ segmented line/ freehand line/ arrow tool, and double click to adjust the parameters.



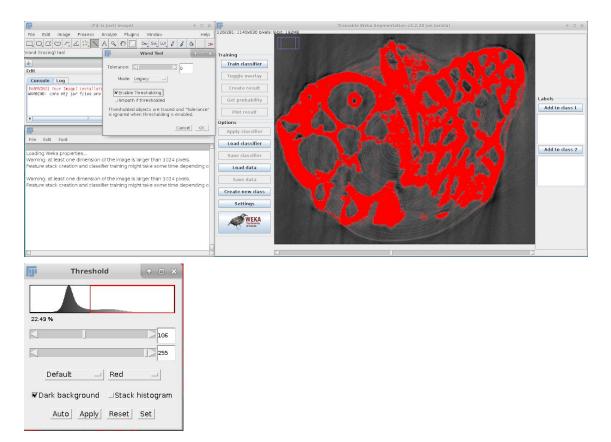
Angle (orange) – Gives the angle between two points. If you add to rock, it will mark as a triangle.



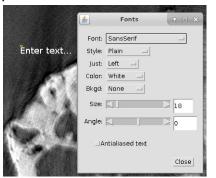
Multi-point tool (purple) - Right click to alter to Point tool and double click to adjust the parameters.



Wand (tracing) (black) – It selects parts of the image connected to each other with the same gray or color level. That tool also allows the use of thresholding. Right click and select Enable Thresholding and an auto threshold will be applied by default. Adjust the maximum and minimum at the threshold table that appears. Once you apply the Threshold, the image will become black and white and this can't be undone. It is worth to duplicate your image before testing (Ctrl + Shift + d).

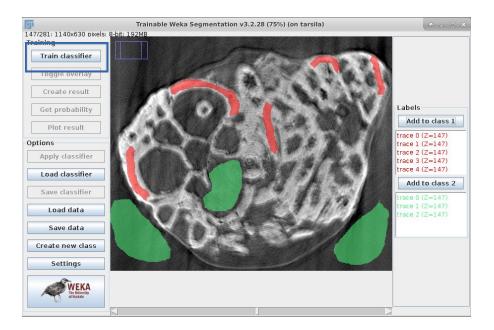


Text tool – Click on the region you want to write. Double click to adjust the parameters.



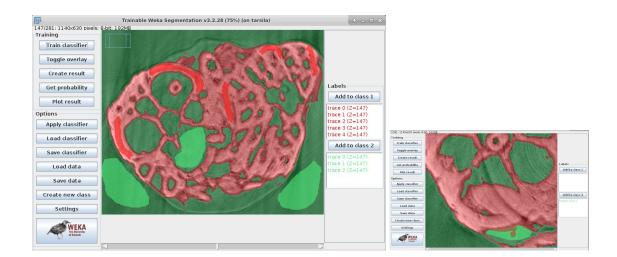
b. Training the classifier

Segment the image adding some seeds/markers in both of the classes. Then click on Train Classifier. Depending on the size of the image, the process can take minutes to hours. The first training usually takes longer. While training, you will see "STOP" instead of Train classifier. By clicking on it, the whole training process will be interrupted.



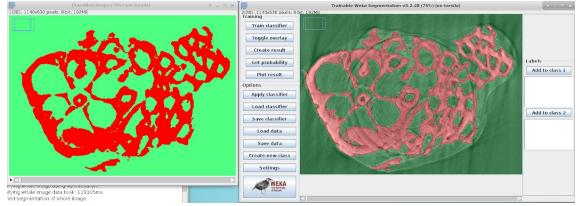
This is the first training. To see the black and white image, press Toggle Overlay. Make more seeds and apply train classifier until you get the result you expect.

Observation: For a better training, you might need to do the annotation in the beginning, in the middle (preferably) and at the end (but pay attention if the images at the top and bottom of your sample are in a good quality, if not, crop them).

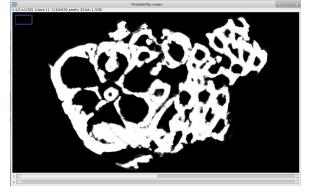


c. Other training buttons

Create result: It displays the resulting labeled image. It is a 8-bit Color image.



Get probability: It is related to the current classifier. This gives you the probability that each pixel belongs to each class.



8. Saving and exporting

To save what you have:

Click Save Classifier, than Save Data. If you already have your final segmented image (create result), click on this image and then File > Save as > Choose the type of file you prefer. **We suggest raw data or tiff.**

To open saved classifier and data:

After Opening the data and the Trainable Weka Segmentation Plugin, click on Load Classifier and then load data. If your classifier have different class names, you need to rename them before applying the data.

Then, click on train classifier. If you need, segment more and then save everything again.

9. Batch Processing

That is a feature that allows the application of scripts and macros in order to apply them for more than one image. A complete explanation about Macro Programming is available at:

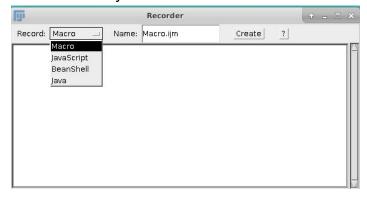
https://imagej.net/Introduction_into_Macro_Programming#The_recorder

Basically it is a scripting language that is used to do repetitive tasks automatically. It applies a "recipe" to several images.

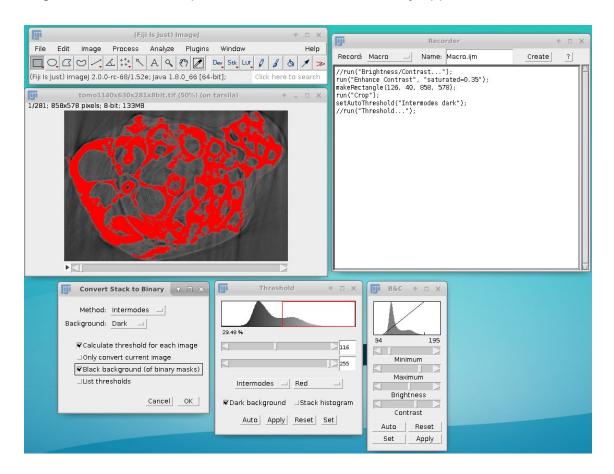
First, open the data (**it can't be a raw type**; we suggest tiff). To start your script select: Plugins > Macro > Record

	Macros		Install	YouTube - M
	Shortcuts Utilities New Compile and Run	1 / /	Provide State Stat	
(Fiji Is Just) ImageJ le Edit Image Process Analyze Plugins	Install Install Plugin 3D IO - 3D Toolkit	Ctrl+Shift+M	Pencil Tool Options Paintbrush Tool Options Flood Fill Tool Options Set Drawing Color	Rec Name: Macr
LOCOLOS A CONTRACTOR C	3D Viewer Analyze BigDataViewer Bio-Formats Cluster		About Startup Macros Save As JPEG [j] Save Inverted FITS	

The following table will appear. Make sure the Macro option is selected at Record. And alter the name if you want.



Then, proceed with the steps that you want to be registered: e.g. Brightness/contrast, crop, threshold. It will automatically appear at the table.



If something that you don't want to appear is scripted (e.g. zoom out, run ("close"), just select it with your mouse and then delete it.

(j)	Recorder						
Record:	ord: Macro 💷 Name: Macro.ijm Cre						?
makeRect run("Cro setAutoT //run("T setOptio	anče Con angle(12 p"); hreshold hreshold n("Black vert to e black"	trast" 6, 40, ("Inte "); Backgr Mask",	, "satur 858, 57 rmodes d ound", 1	rated=0.35"); 78); dark");	oackground=	Dark	

Once your done, click on Create. Then select File > Save as.

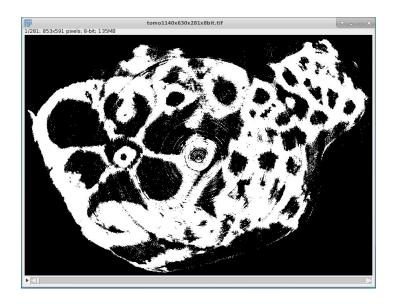
🔬 *Macro.ijm.ijm (on tarsila)	+ _ = ×
<u>F</u> ile <u>E</u> dit <u>L</u> anguage <u>T</u> emplates <u>R</u> un T <u>o</u> ols T <u>a</u> bs	
/ *Macro.ijm.ijm	
<pre>1 //run("Brightness/Contrast"); 2 run("Enhance Contrast", "saturated=0.35"); 3 makeRectangle(126, 40, 858, 578); 4 run("Crop"); 5 setAutoThreshold("Intermodes dark"); 6 //run("Threshold"); 7 setOption("BlackBackground", false); 8 run("Convert to Mask", "method=Intermodes background=Dark calculate black 9</pre>	
Run Batch Kill Show E	rrors Clear

Close this table, the record table and the result image. To apply your scrip to other images select: Process > batch > Macro

This window will appear. Select Open and then the scrip created. Then Process.

Batch Process + • ×
Input /ddn/IMX/_usersdata_/20180561/datasets/AvizoGuide/Este/
Output /ddn/IMX/_usersdata_/20180561/datasets/AvizoGuide/Outpu
Output format: TIFF Add macro code: [Select from list] File name contains:
<pre>//run("Brightness/Contrast"); run("Enhance Contrast", "saturated=0.35"); makeRectangle(126, 34, 853, 591); run("Crop"); setAutoThreshold("Intermodes dark"); //run("Threshold"); setOption("BlackBackground", false); run("Convert to Mask", "method=Intermodes background=Dark calculate black");</pre>
Test Open] Save
Cancel Process

Open the Output image:

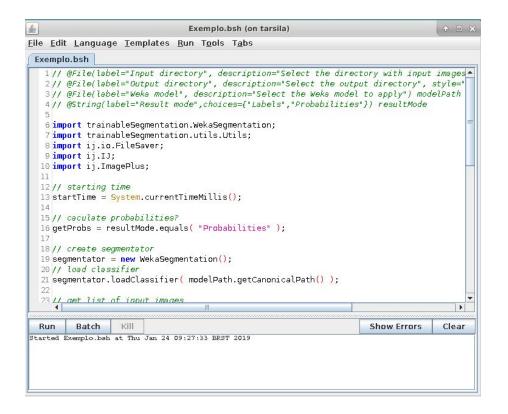


10. Using Scripts for segmentation

At this link you can access plenty of scripts related to the Trainable weka segmentation: https://imagej.net/Scripting_the_Trainable_Weka_Segmentation

In this tutorial we'll use *"Example: apply classifier to all images in folder".* First, you need to have already a .model file (after training a image, save the classifier). Then, copy the script and save it in a .bsh file (use a text editor, e.g. Gedit).

Open the ImageJ and select File > Open > .bsh file A table like this should appear:



Click Run and then define the folder where the image you want to be segmented is situated, where you want the output to be saved and the .model file.

Input directory	/ddn/IMX/_usersdata_/20180561	Browse
Output directory	/ddn/IMX/_usersdata_/20180561	Browse
Weka model	/ddn/IMX/_usersdata_/20180561	Browse
Result mode	Labels	

The result image will be at your output folder.