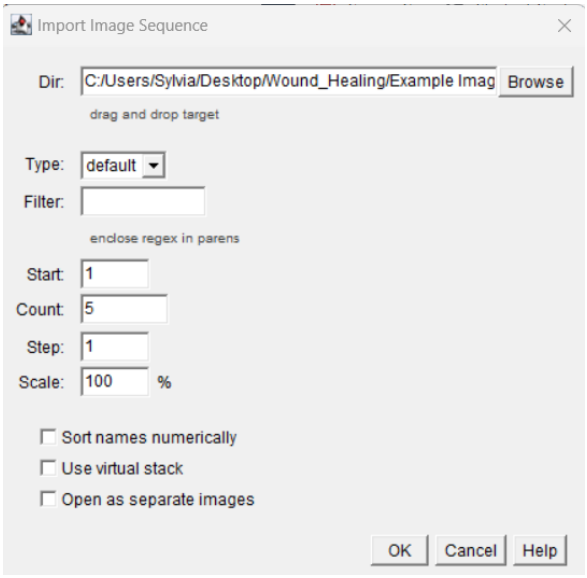
Wound Healing ImageJ Tool Nowak Lab Guide

1. Go to https://github.com/Nowak-Lab/ImageJ_ScratchAssay and download wound-healing-sjh.ijm (wound-healing-sjh.ijm).

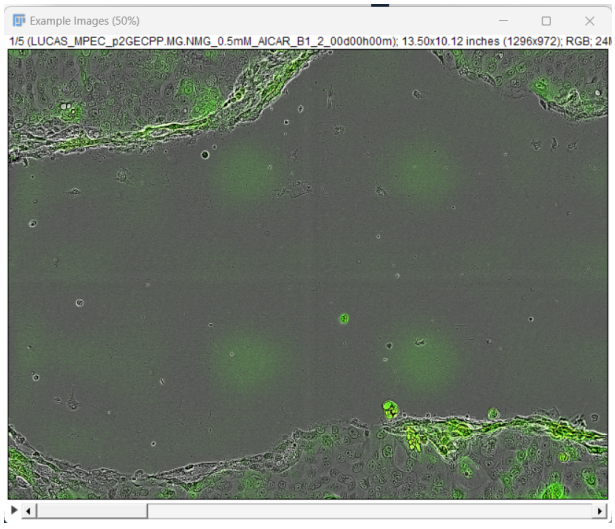
2. Open ImageJ.

3. To open a folder of images go to $File \rightarrow Import \rightarrow Image\ Sequence$. The popup below should appear. Click the *Browse* button to select the folder with your images.



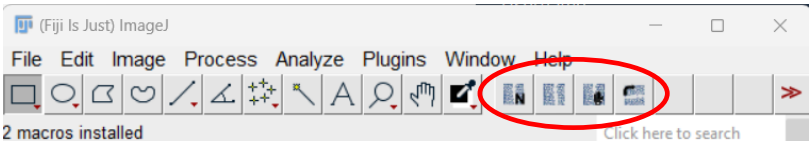
Important: make sure to uncheck *Sort names numerically*. It may be checked by default. Make sure it is unchecked. (Your screen should look like the one above before you click *OK*. The only values that may be different from the image above are the ones for *Dir*, the folder location, and *Count*, the number of images in the folder).

4. After your images finish loading, they should appear.



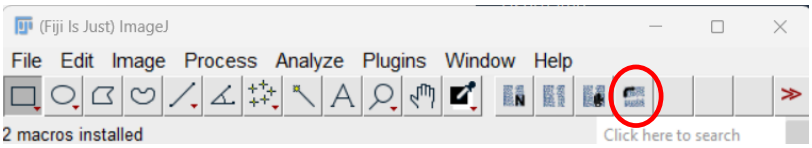
5. On ImageJ, click $Plugins \rightarrow Macros \rightarrow Install$ and select the file wound-healing-sjh.ijm

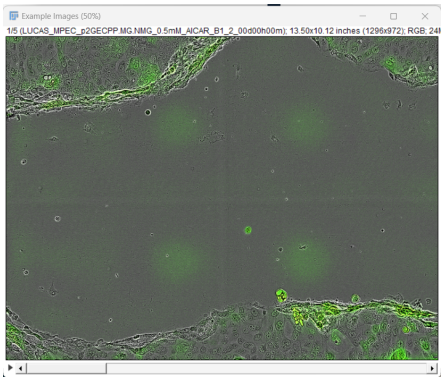
6. The plugin should appear like so:



7. To use the plugin, click one of the four icons and then click on the wound images.

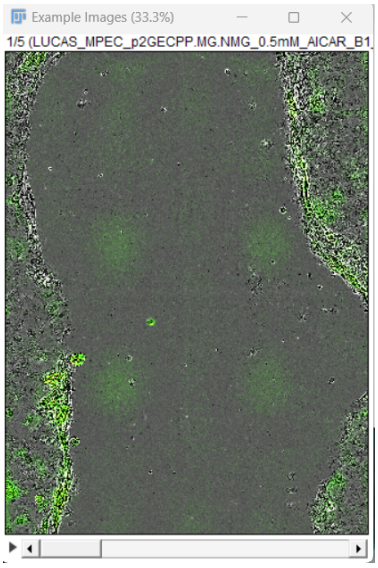
   a. If the scratch wounds are horizontal, click on the  icon to rotate them.



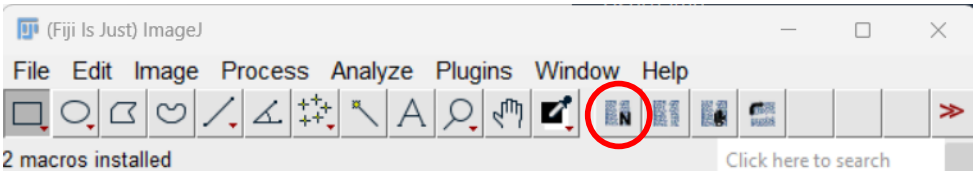


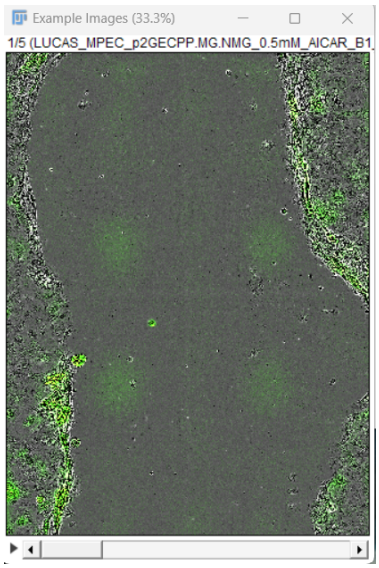
(click anywhere on the wound images after selecting the plugin)

The wound images should now be displayed vertically.



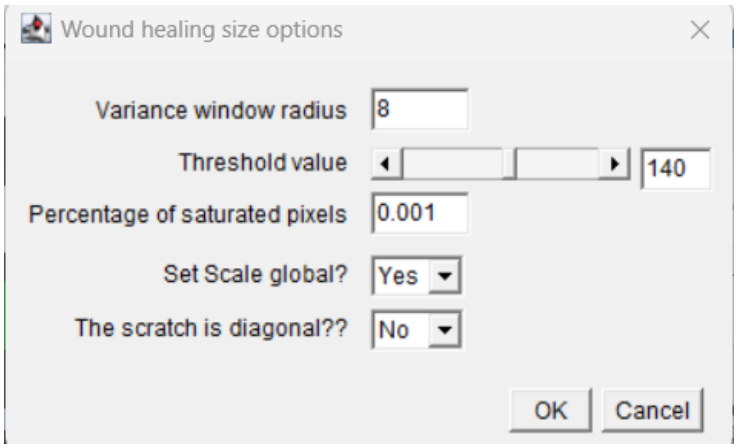
b. To process the images, click on the  icon.





(click anywhere on the wound images after selecting the plugin)

The following popup should appear:



(The parameters that Sylvia was using previously are shown above. Feel free to change any of the fields if needed. Descriptions of what each field represents are explained below:

o Variance window radius: Represent the radius of variance filter which is established to determine the empty or the occupied zones. The radius must be big enough, so the noise variance has no impact on tissue variance.

o Threshold: The image resulting from the variance filter is converted to a mask by applying the given threshold.

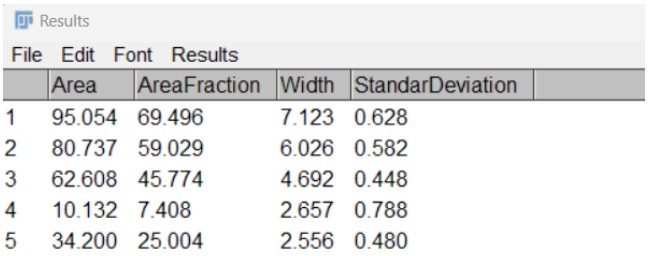
o Percentages of saturated pixels: Enhance the contrast of the image by determining the number of pixels in the image that can become saturated. Increasing this value increases contrast. This value should be greater than zero.

o Set Scale global? Select "Yes" if the scale calibration applies to all the images. Select "No" if the scale calibration only applies to the select image.

o The scratch is diagonal? Select "Yes" if you can observe that the scratch has an inclination differently of 0°. Select "No" if the scratch has not an inclination.

After clicking *OK* you should see various animations on the wound images, such as their borders being traced out in blue. There will also be many popup boxes that appear.
Important: Do not click on the wound images OR any of the popup boxes that appear while this process is happening or else the program might bug out. If you have a lot of images to process you can leave this plugin running in the background as you do other things on your computer.

When the plugin is done running, a table should appear


Results

File Edit Font Results

	Area	AreaFraction	Width	StandarDeviation
1	95.054	69.496	7.123	0.628
2	80.737	59.029	6.026	0.582
3	62.608	45.774	4.692	0.448
4	10.132	7.408	2.657	0.788
5	34.200	25.004	2.556	0.480

Ctrl+a and *Ctrl+v* to select all the values in the table and paste the values into an excel file.

c. For reference, the  icon is used to process individual images (instead of a folder of images). To do this, open an image by clicking $File \rightarrow Open$. Rotate the image if needed using the  icon and process the image using the  icon.

d. For reference, the  icon is used if you want to manually draw a border on the wound instead of having the plugin draw one for you.