**IMAGEJ USER GUIDE**

ImageJ is a public domain Java image processing and analysis program. It can read many image formats including TIFF, GIF, JPEG, BMP. ImageJ can display multiple spatially or temporally related images in a single window. These image sets are called stacks. The images that make up a stack are called slices.

Fiji (Fiji Is Just ImageJ) is a distribution of ImageJ together with Java, Java 3D and several plugins, including the LOCI plugin required to open files generated with different microscopes. ( <http://wiki.imagej.net/Fiji> )

1. **Installation and maintenance of ImageJ/ Fiji**

ImageJ can be downloaded from <http://imagej.nih.gov/ij/download.html>

Details on how to install ImageJ are available at: <http://imagej.nih.gov/ij/docs/install/>

Fiji installation is described at <http://fiji.sc/wiki/index.php/Downloads>

The downloaded package may not contain the latest bug fixes so it is recommended to upgrade ImageJ right after a first installation. Updating IJ consists only of running Help -> Update ImageJ..., which will install the latest ij.jar in the ImageJ folder (on Linux and Windows) or inside the ImageJ.app (on Mac OSX).

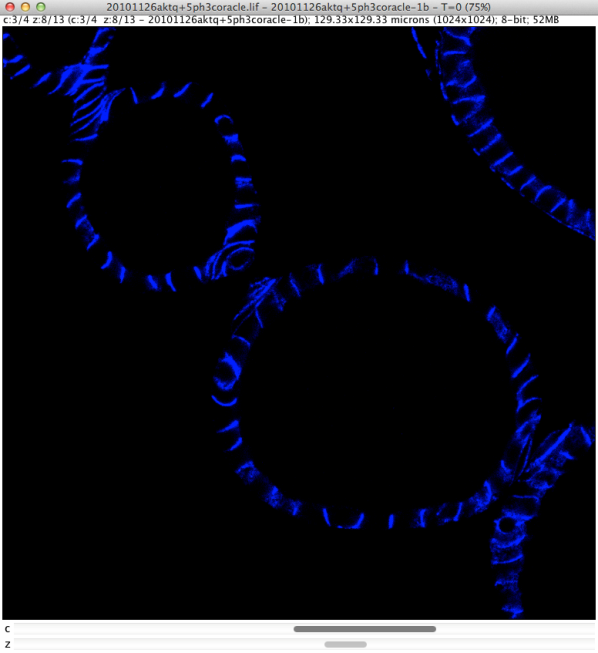
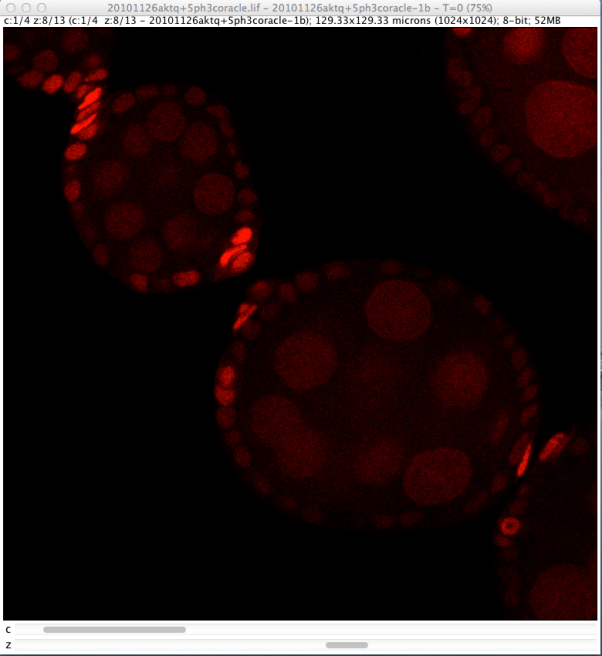
1. **Installation of plugins**

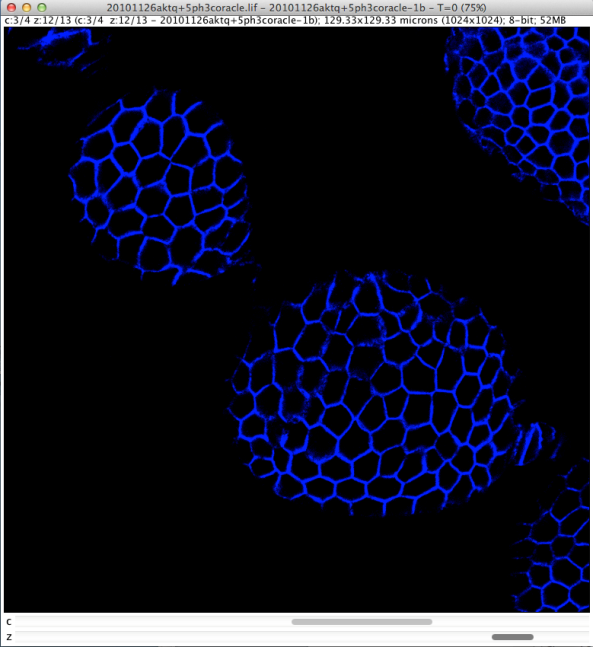
The real strength of ImageJ is the vast repertoire of Plugins that extend ImageJ’s functionality beyond its basic core. The plugins are available through the Image J web site: <http://imagej.nih.gov/ij/plugins/index.html>.

To install a plugin, you have to download it, copy it in the ImageJ/plugins/ folder, and restart Image J. All the plugins present in this folder are listed at the bottom of the Plugins menu.

1. **Open files from the microscope**

* **Plugins -> Bio-formats -> Bio-Formats importer ;** then select the file to open
* The window : **Bio-Formats import option** open, select « View stack with : Hyperstack » and the default parameters
* In a new window, you can now select the image(s) you want to open
* the selected images will open
* to change the channel, move the « c slider »
* to see the different z slices, move the « z slider »





1. **Duplicate an image / a stack**

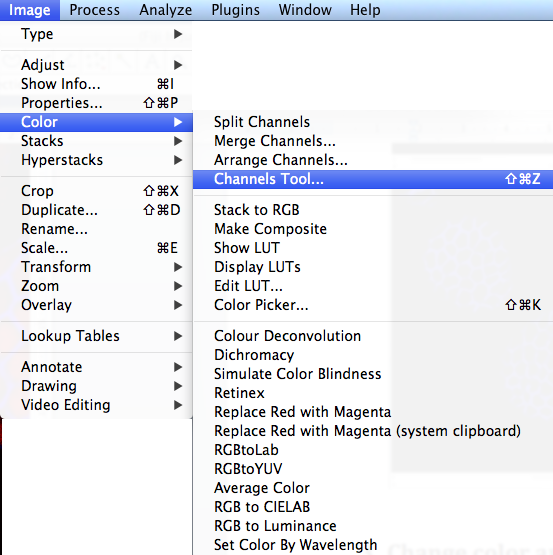
* Since the “undo” function does not work for stacks, it is recommended to duplicate your image before any operation:   
  **Image -> Duplicate…**
* A new window will open where you can change the name, and choose which channels, slices and frames to duplicate.

1. **Convert image to binary image (threshold)**

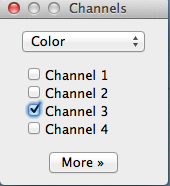
* To segment an image based on a threshold, you can use the function: Image -> Adjust -> Threshold…
* A new window will open, allowing you to manually define the thresholding values or choose an algorithm to automatically do the job.
* Clicking “Apply” will transform your image into a binary image (black and white) with values equal to 0 and 255.

1. **Change the colors and make an overlay**

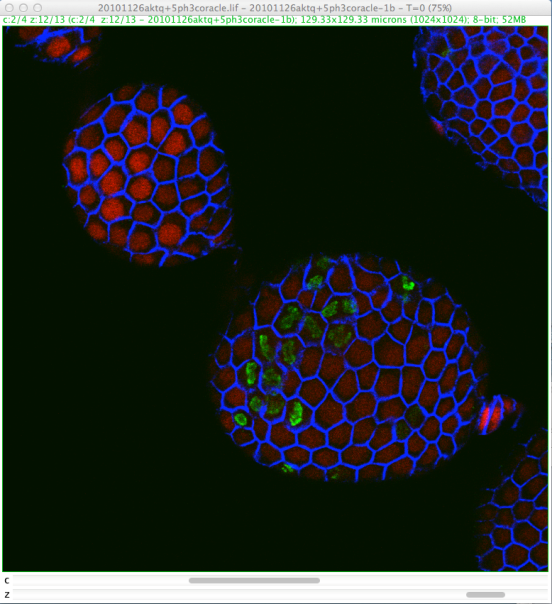
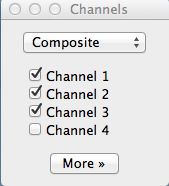
* **Image -> color -> channels tool**

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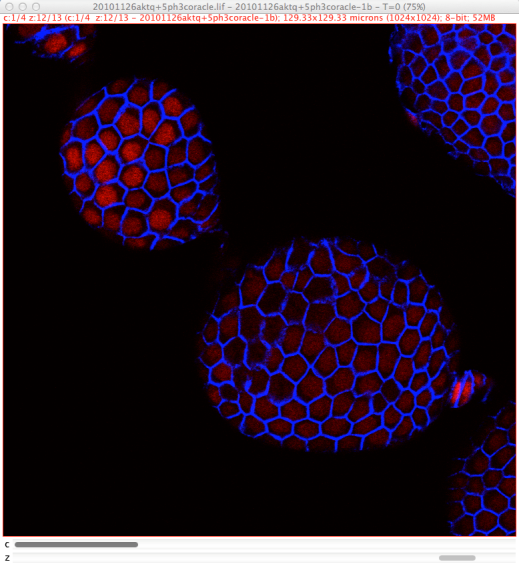
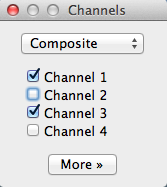
* the « channels » window opens



* then click on « More » to select the color you want to attribute to the selected channel
* to make an overlay select «  **composite** »

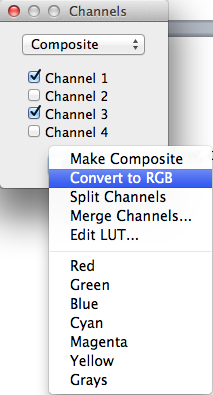


* you can select or deselect a channel to make it visible or hide it

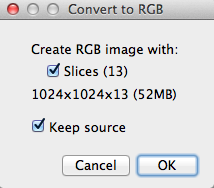


1. **Convert to RGB (required before saving in tiff or jpeg to obtain a file with the overlay)**

* First select the channels , then in the « channels » window, click on **More >>** **convert to RGB**



* If you have several z slices, the following window appears :

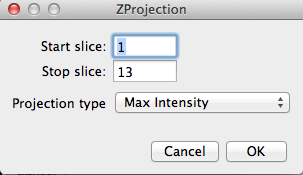


a. **if “slices” is selected:** you get a file with all the slices converted in RGB

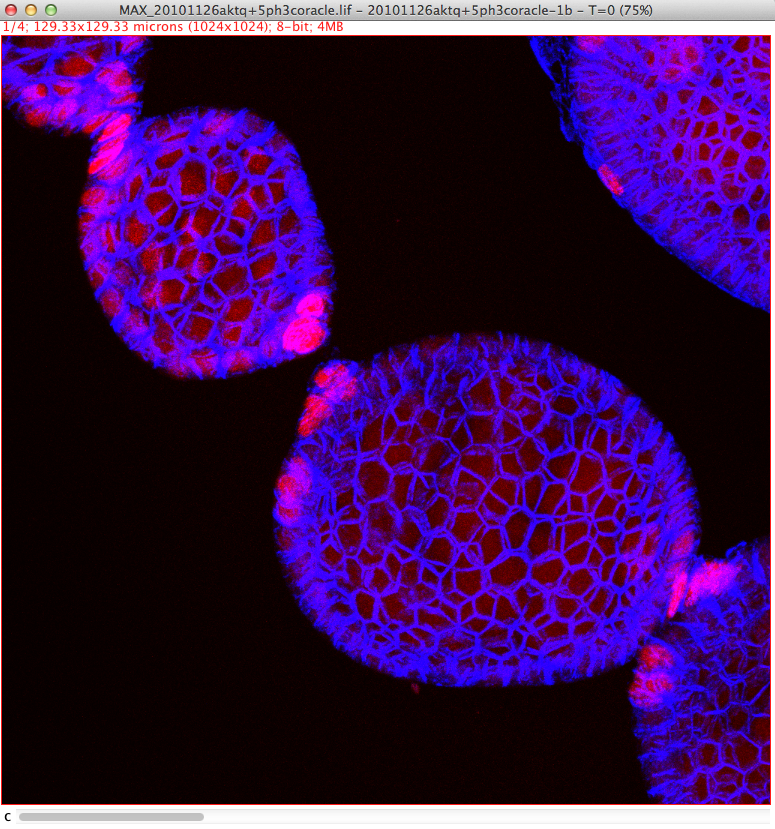
b. **if “slices" is not selected**: you obtain a file with only the « current » slice converted in RGB

1. **Make a projection**

**- Image -> stack -> z project:**

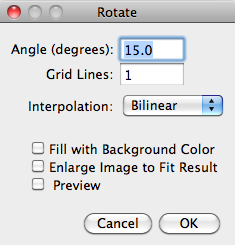


* Select the first and last slices you want to include in your projection and select the type of projection



1. **Rotation**

* Image -> transform -> rotate

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* then enter the angle of rotation

1. **Crop an image**



* Select the rectangle tools in the toolbar
* Design a rectangle around your region of interest
* Image -> crop

1. **Adjust brightness/contrast, levels…**

- Image -> adjust ->brightness/contrast or window level…

1. **Insert a scale bar**

- Analyze -> Tools -> Scale Bar

1. **Save in different formats**

- File -> Save as (select your favorite format)

1. **Record a macro**

* Open an image
* **Plugins -> Macros -> Record…**; a new window will appear
* Proceed with the operations you want to record: for each one of them, a new line will be displayed in the recorder.
* Once you are done, you can click “create”: this will open the script editor, which will allow you to edit and save your macro.
* However, if your process involves hard-coded values which need to be changed for each image (e.g. the image name), you will have to modify the generated code yourself by replacing those values with variables.