How to Automate ImageJ Analysis with Macros and Batch Processing

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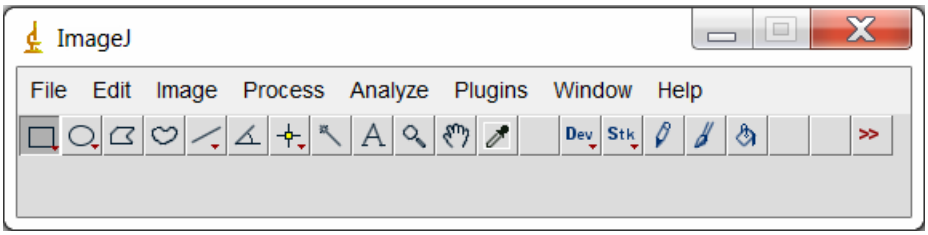
This tutorial covers how to automate ImageJ analysis using **macros** and **batch processing**. This technique can save you a lot of time by turning what normally takes hours by hand into a 60 second process. Automation of the "threshold" tool will be used as an example, but the same technique can be applied to all commands under the "Image", "Process", or "Analyze" tabs from the ImageJ menu. ImageJ version 1.45s was used for this tutorial, and can be downloaded at http://rsbweb.nih.gov/ij/index.html .



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What is an ImageJ macro?

An ImageJ macro is a program that automatically performs a sequence of commands that would normally be performed manually. If you have multiple images that need to undergo the same analysis process, manually repeating the same task over and over again is very time consuming. For example, when analyzing images of fluorescently stained nuclei, I usually click Image->Adjust->Threshold. Then I select the threshold constraints (Hue, Saturation, Brightness), the threshold color. Lastly, I click Analyze->Analyze Particles, modify the options, and click "Ok." Doing that for each image takes too long!

That's where an ImageJ macro comes in: it lets you record a sequence of steps (like what I described above), and then it performs the entire sequence automatically. The upcoming section covers how to create a macro, and section after that demonstrates how to run the same ImageJ macro on multiple images.

How to create an ImageJ Macro

1. Open one of the files you want to analyze inside of ImageJ.
2. Click **Plugins->Macros->Record**. A macro Recorder window will pop up. Ignore it for now.
3. On the ImageJ menu, click the command you wish to execute. For example, click Image->Adjust->Threshold.
4. Modify that command's options (if applicable). For example, I set the Hue, Saturation, and Brightness ranges of the threshold, and then set "Threshold color" to "B&W."
5. If there is a **"Macro" button** at the bottom of the window (**Figure 1**), click it. This will tell the macro to use the current settings for the command. If the "Macro" button does not appear, simply click "Ok" or "Apply" etc. to execute the command. The sequence of steps will automatically be added to the macro window.

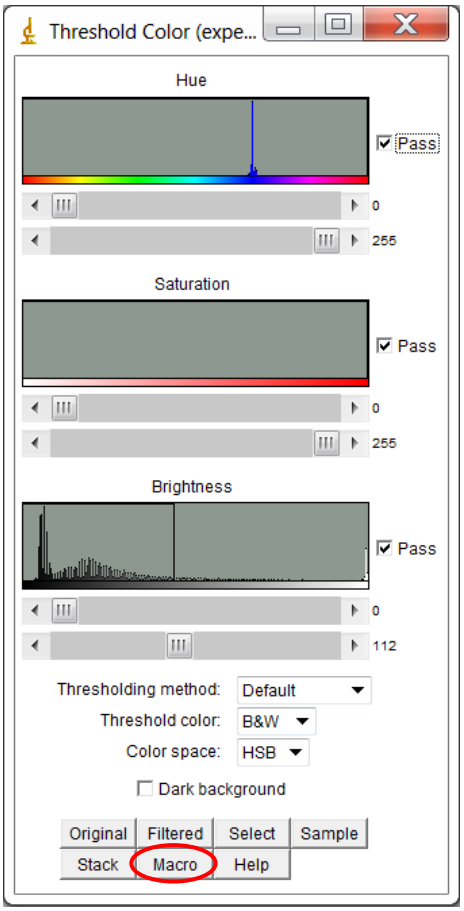
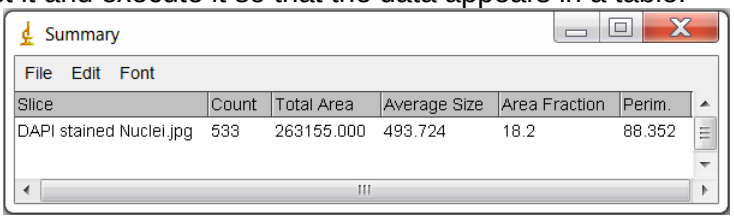


Figure 1: Click the "Macro" button to add the threshold settings to the macro sequence.

Figure 2: After clicking the "Macro" button, the sequence of steps gets added to the macro recorder window.

6. If you want to analyze the images, such as with the "Measure" or "Analyze Particles" command, select it and execute it so that the data appears in a table.



7. Once you have completed all the tasks you would like the macro to perform, find the macro Recorder window, and click **"Create."** A second window will pop up (shown to the right in **Figure 3**). Click **File->Save As** to save it somewhere on your computer. Make sure the file name has the extension ".ijm" at the end. This signifies it is an ImageJ Macro file.

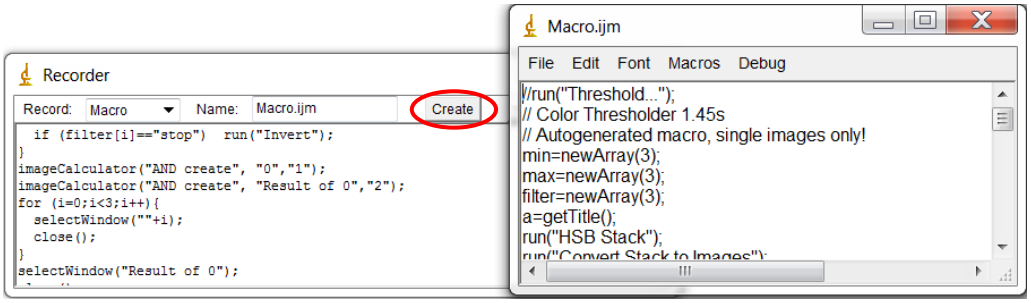


Figure 3: The macro Recorder window is shown to the left. After clicking **"Create"** the macro will appear to the right. Click **File->Save As** to save it somewhere on your computer.

How to use an ImageJ Macro

1. Open one of the files you want to analyze inside of ImageJ.
2. Click **Plugins->Macros->Run**.
3. A window will pop up, allowing you to browse your computer. Select the macro file you just created (the file must have a ".ijm" extension at the end).
4. Click **"Open"** to open the macro, and it should automatically run the sequence of steps you performed, as well as output the final analyzed data.

How to install an ImageJ macro for repeated use

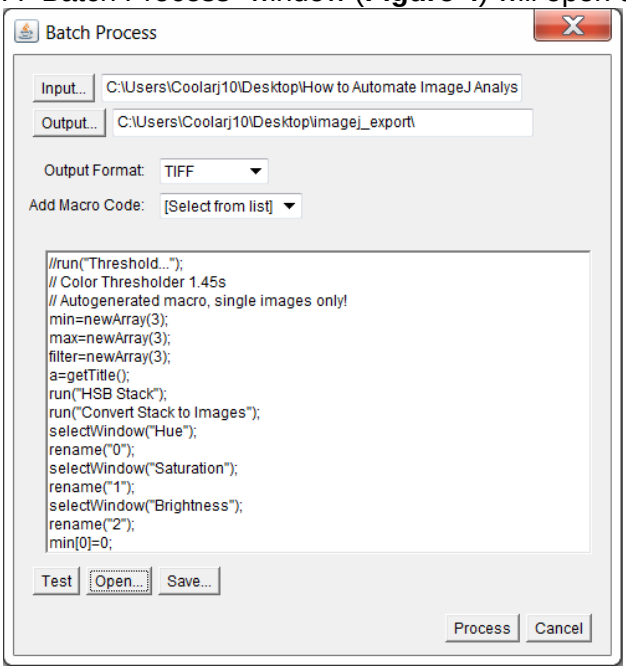
If you don't want to constantly click Plugins->Macros->Run every time you want to run a macro, you can install it instead. This way, the macro will be listed under "Plugins->Macros".

1. Click **Plugins->Macros->Install**.
2. A window will pop up, allowing you to browse your computer for the macro. Open that macro.
3. The macro will now be listed at the bottom of the Plugins->Macros tab, and can be quickly executed from there.

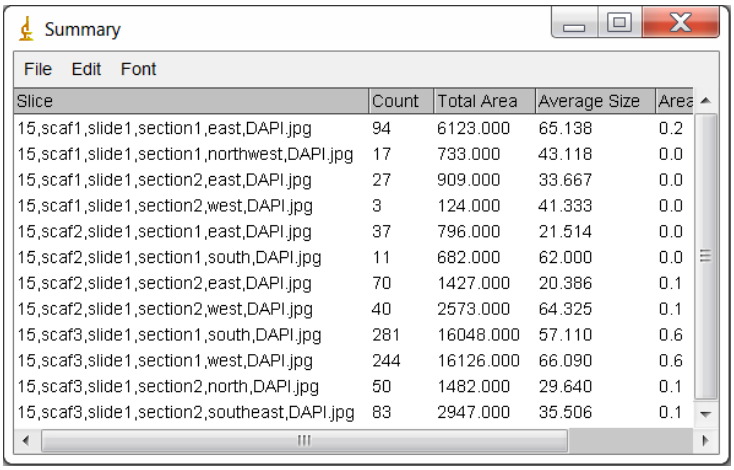
Analyzing multiple images with batch processing

This is the holy grail of ImageJ automation. If you have many images and want to run the exact same macro on each one, batch processing is for you. After analyzing each image, batch processing will generate a table that lists the analyzed data from each image, allowing you to easily export it to a spreadsheet such as Microsoft Excel.

1. Put all images that need to be analyzed in the same folder on your computer.
2. In ImageJ, click **Process->Batch->Macro**
3. A "Batch Process" window (**Figure 4**) will open up.



4. Click **"Input..."** and select the folder containing all of your images.
5. Click **"Output..."** and select or create a folder in which ImageJ should place all of the analyzed images. (You need to create this folder even if you don't need the images. Just delete the folder when you are done).
6. Click **"Open..."** towards the bottom of the Batch Process window to open a macro file located on your computer. This is the macro that will be executed on each image.
7. Once you are ready, click **"Process"**, and watch the magic happen. ImageJ will automatically open each image from the folder you chose, apply the macro to that image, and export the analyzed data into a nice summary table (**Figure 5**).



Slice	Count	Total Area	Average Size	Area
15,scaf1,slide1,section1,east,DAPI.jpg	94	6123.000	65.138	0.2
15,scaf1,slide1,section1,northwest,DAPI.jpg	17	733.000	43.118	0.0
15,scaf1,slide1,section2,east,DAPI.jpg	27	909.000	33.667	0.0
15,scaf1,slide1,section2,west,DAPI.jpg	3	124.000	41.333	0.0
15,scaf2,slide1,section1,east,DAPI.jpg	37	796.000	21.514	0.0
15,scaf2,slide1,section1,south,DAPI.jpg	11	682.000	62.000	0.0
15,scaf2,slide1,section2,east,DAPI.jpg	70	1427.000	20.386	0.1
15,scaf2,slide1,section2,west,DAPI.jpg	40	2573.000	64.325	0.1
15,scaf3,slide1,section1,south,DAPI.jpg	281	16048.000	57.110	0.6
15,scaf3,slide1,section1,west,DAPI.jpg	244	16126.000	66.090	0.6
15,scaf3,slide1,section2,north,DAPI.jpg	50	1482.000	29.640	0.1
15,scaf3,slide1,section2,southeast,DAPI.jpg	83	2947.000	35.506	0.1

Figure 5: A list of data from all the analyzed images. To set what measurements appear in the summary table, click **Analyze->Set Measurements** before running the batch process.