

Image J for analysis of biological image

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Scientific image

- Is different than a regular digital photograph of a beautiful scene you shot.
- Digital images are samples of information

What is Digital Image Analysis?

- Using the numbers in digital pictures to get useful information
- REMEMBER:
- Start with an image -- end with an answer

How can you download image J

•

https://www.embl.de/services/core_facilities/almf/serv ices/downloads/imageJ/imageJ_setup/

You just double click with <u>EMBL ImageJ setup v1</u> Of, and then file downloading will be start.

- You have to download Java using this link
- https://java.com/en/
- If you have any message concerning Javaw.exe just open the setuped version in program files and click that file then complete setup steps and open image J

When opening the program you will get this window just choose allow access



Some information about the program

- 1- Runs everywhere: it is written in Java, It to run on Linux, Mac OS X and Windows, in both 32-bit and 64-bit modes.
- 2- Open source: ImageJ and its Java source code are freely available and in the public domain. No license is required.
- 3- ImageJ has a large and knowledgeable worldwide user community.
- 4- Data types: 8-bit grayscale or indexed color, 16bit unsigned integer, 32-bit floating-point and RGB color

Some information about the program

- 5- File Formats: Open and save all supported data types as TIFF
- 6- All analysis and processing functions work at any magnification factor.
- 7-Selections:
- Create rectangular, elliptical or irregular area selections. Create line and point selections. Edit selections and automatically create them using the wand tool. Draw, fill, clear, filter or measure selections. Save selections and transfer them to other images.
- 8- Image Enhancement:
- Supports smoothing, sharpening, edge detection, median filtering and thresholding on both 8-bit grayscale and RGB color images. Interactively adjust brightness and contrast of 8, 16 and 32-bit images.

To know about the program after setup help then about image J



Different bars of the program



Common tools in image J



Adjusting image contrast and brightness



Important notes

- Adjusting contrast and brightness is just for pictures you take for demonstration and not in captured photo for analysis because when you capture it you adjust settings in all groups.
- It is not a tool to change anything about analyzed image.

Analysis done by image J

- Measure area, mean, standard deviation, min and max of selection or entire image.
- Measure lengths and angles. Use real world measurement units such as millimeters.

Topics that will be covered today

- 1- Analysis of gel and western blot images
- 2- Analysis of fluorescence and colocalization signal of immunofluorescence images
- 3-Analysis of nanoparticle size distribution
- 4- Analysis of stained liver tissue
- 5-Counting the cells

Analysis of DNA gel using image J

Steps you can follow as screen shot

• 1- open image J software from its setuped icon

Common Tasks ×	🛓 ImageJ	- 🗆 🗙
Bin Image RGB Merge	File Edit Image Process Analyze Plugins Window Help Image <	[™] ¶ (3) →
Subtract Background		
Enhance Contrast		
Measure		
Hot LUT		
Pale LUT		
Gamma		
Add Ramp		
Close Toolbar		

This is the image



Open the image of gel you want to analyze By clicking file menu then open



As image J can not read the white bands so invert colors in image by editinvert



Photo after color inversion.....bands will appear in black



Select the area of bands using rectangular selection



Analyzegelsselect the first lane



Analyze-gel-plot lanes





Use straight line tool and shift to detect bands



Another option to select area of each band separately



Use wand tool to detect areas of bands



Click analyze gel ----label peaks



You can save your results by clicking filesave as in results window



Then open in excel and analyze the results

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In this case which is semiquantitative analysis of DNA in gel image

- You can calculate the fold change by dividing over the area or percent of each group or band on that of control
- You can add the values on each band in the image that will be added in paper. Something like in this



PLOS ONE | www.plosone.org 1 January 2014 | Volume 9 | Issue 1 | e7979

Analysis of western blot image

Western blot overview



Harry Towbin, in Encyclopedia of Immunology (Second Edition), 1998

Image for western blot



Open the image in image J

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As there is too much free space crop the image


Rectangular selection then image crop

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Image became as in view



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Add Ramp			Re-plot Lanes
Close Toolbar			Reset
			Label Peaks Draw Lane Outlines Gel Analyzer Options

It will be selected with no. 1 in the middle



Move the selection to the last lane and analyze gel select next lane and no.2 will be added on this lane

	r and drag)
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Analyze ...gel ...plot lanes

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Close Toolbar	Reset Label Peaks Draw Lane Out Gel Analyzer O	ines ptions

Make selection like this using shift and line tool



Use wand tool to analyze area of each band



You just click wand tool and in the middle of area click you will get the area size in results file



Click analyze gels then label peaks you will get the percent of area

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Then file ---save as and save results for further analysis

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To get the fold change of each band first divide over that of each internal control then the resulted value on control value (non treated)

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Analysis of fluorescence signal you get from confocal microscopy



Immunofluorescence



Immunohistochemistry Schematic



Analyzing fluorescence in this image (lysotracker staining)



Open the image in image J



Split colors in this image as following

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You will get three images with different color layers



Note:

- The green layer is not included as you have only red fluorescence and blue for nucleus and we will use blue layer later in counting cells.
- So Just close the green layer window and other images will be analyzed separately one to get red fluorescence and the other for cell count.

For counting cells

Common Tasks	<u>₹</u>	Image	eJ	
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Editinvert



Remove the diameter of cell (as no.) by

eraser



Image -----adjust-----threshold



Move the down scroll of threshold window till adjusting nuclei view



Click apply and analyze---particles ...60infinity



Important

 You should fix the settings for analysis of images in different groups to get acceptable meaning.

Analysis of red fluorescence



Edit -----invert



You will get white background and black fluorescent signal



Remove diameter by eraser

Common Tasks ×	₫ ImageJ	- 🗆 🛛
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1	•	

Image----adjust----threshold



Click apply or move the down scroll to clarify the fluorescence then analyzeanalyze particles



Keep all settings in default version with just changing size to be 0-infinity



Important note:



0 (infinitely elongated polygon) to 1 (perfect circle).

Image J guide book

Results will appear in summary


To calculate the signal per cell

• Just divide the red signal results on number of cells and the signal will be per cell as this example



T. Donia et 150 al. / Biochemical and Biophysical Research Communications 517 (2019) 146e154

Important note:

 Thresholding tool setting in a positive control specimen should then be duplicated in every image to be compared

Colocalization Analysis

Colocalizationobservation of the spatial overlap between two (or more) different fluorescent labels, each having a separate emission wavelength, to see if the different "targets" are located in the same area of the cell or very near to one another. "



Images for analysis of colocalization



Open the image in image J



Split image colors

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Delete the blue color image for DAPI



Click plugins ----choose colocalization analysis then colocalization highlighter



Record the settings you used to apply the same for all studied images

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You will get two images

- One for colocalized points in 8 bit and the other in RGB
- You can save them and the image for analysis will be 8 bit



Colocalized point (8 bit)



Editinvert



Erase the diameter



Analyze -----analyze particleskeep default settings....ok

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We just focus on count column which expresses colocalization



Count the number of cells by splitting the image colors and keeping blue one.



Edit ...invert



Then remove the diameter by eraser



Adjustthreshold



Adjust the down scroll to right till you get the clear nuclei



Apply to get such view



Analyzeanalyze particles



Keep all settings in default form with changing size to be 60- infinity

	🛓 ImageJ – 🗆 🗡
	File Edit Image Process Analyze Plugins Window Help
	Color picker (0,0,0)
 ▲ Ana Size (pixel^2): Circularity: Show: □ Display Result □ Clear Result □ Clear Result □ Summarize □ Add to Manage 	Color picker (0,0,0)

Save analysis or continue analyzing other images and all results will appear in summary



Final result should be like that colocalized pixel/cell by dividing the colocalized area (total area column) over the no. of cells



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Nanoparticle size distribution analysis by image J

Published paper

nature > scientific reports >	articles > article	our products. <u>Sign up to take part.</u>	a natureresearch journal					
SCIENTIFIC RE	PORTS		Search 1	E-alert				
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		nature						
Abstract								
In the light of promising p	rticles in	communications						
biomedical applications, t	his is the first study to repo	rt the synergistic						

Picture containing SEM analysis



I separate the picture in paint and saved as TIFF file



In image J file -----open ----choose SEM image from specific partition on your computer



Click straight line tool with shift and select the scale at the end of picture

• In this SEM picture, it is 200 nm

Analyze then set scale



Add 200 nm in the known distance and in unit of length add nm then ok



Choose Rectangular selection and select the area you want to analyze then image crop



Image then 8 bit then image and adjust threshold and change the upper scroll then apply

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You will get such view



Analyze -----analyze particles and in show choose masks and you can save the new image

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File -----save as

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Select specific partition and analyze your results using excel

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Edit ----distribution to see distribution of particle size in the image and you will get distribution window keep all information in default version then click ok



You will get area distribution graph as following by clicking on list you will get the next window

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Distribution histogram

• Size rangesplit into small size classes or bins and the no. of particles contained in each size bin are counted.

You can save data for area distribution for further analysis

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Calculate the Diameter of a Circle, from Its Area

- 1. Divide the **area** (in square units) by Pi (approximately 3.14159). Example: 303,000/3.14159 = 96447.98.
- 2. Take the square root of the result (Example: 310.56). This is the radius.
- 3. Now double the radius to get the **diameter** (Example: 621.12 meters).

Quantifying Stained Liver Tissue



Open the image in image J

• Fileopenchoose its location.....open

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shift with straight line selection on 200um scale at the end of image



Enter the scale bar length (200 µm) as the "Known Distance" and "um" as the "Unit of Length".

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Dimensions changed to um scale



Image ----type ----RGB stack to split the image into red, green and blue channels.



You will get such view



Move the slider to view each of the channels. Notice that the green channel has the best separation.



Image>Stacks>Make Montage to view all three channels at the same time.



Click ok in make montage box



All three channels will appear at the same time appear



Select the RGB stack (with the Green channel selected) and press shift-t (*Image>Adjust>Threshold*).



Set threshold level in the upper threshold level



Click apply



Analyze>Set Measurements dialog and checking "Area", "Area Fraction", "Limit to Threshold" and "Display Label"



(Analyse>Measure)



The area and percent area will be displayed in the "Results" window

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Counting cells in this image first open this image in image J



To remove noise click process....subtract background



Add any number according to intensity of noise for example 12 and then ok



You will get such view



Image ...adjust threshold and adjust only black and white



If nothing happen just make your image 8 bit and then change the upper scroll till you get clear cells



Click apply



To fill holes in cells choose process then binary then fill holes



For much processing process...binaryconvert to mask


To separate the connected cells process ----binarywatershed



You will get a line between connected cells



Thoria Donia

Analyze.....analyze particles add size to be 120-infinity and show outlines and keep other settings in default format

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You will get summary and detailed results and all noise was deleted



