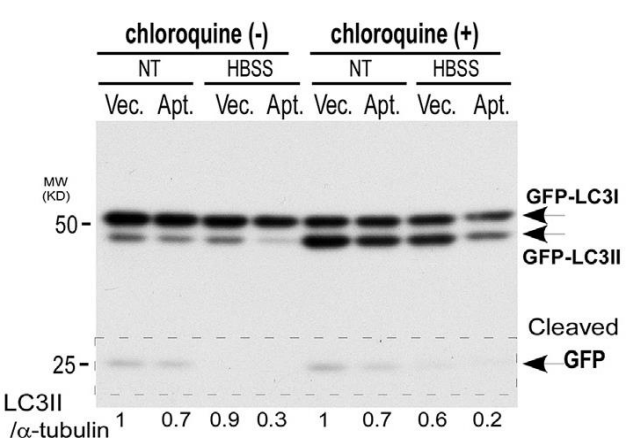


Image J for analysis of biological image

Dr. Thoria Donia

Associate Professor of Biochemistry,

Faculty of Science, Tanta University

Mail: thoria_donia@science.tanta.edu.eg

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?

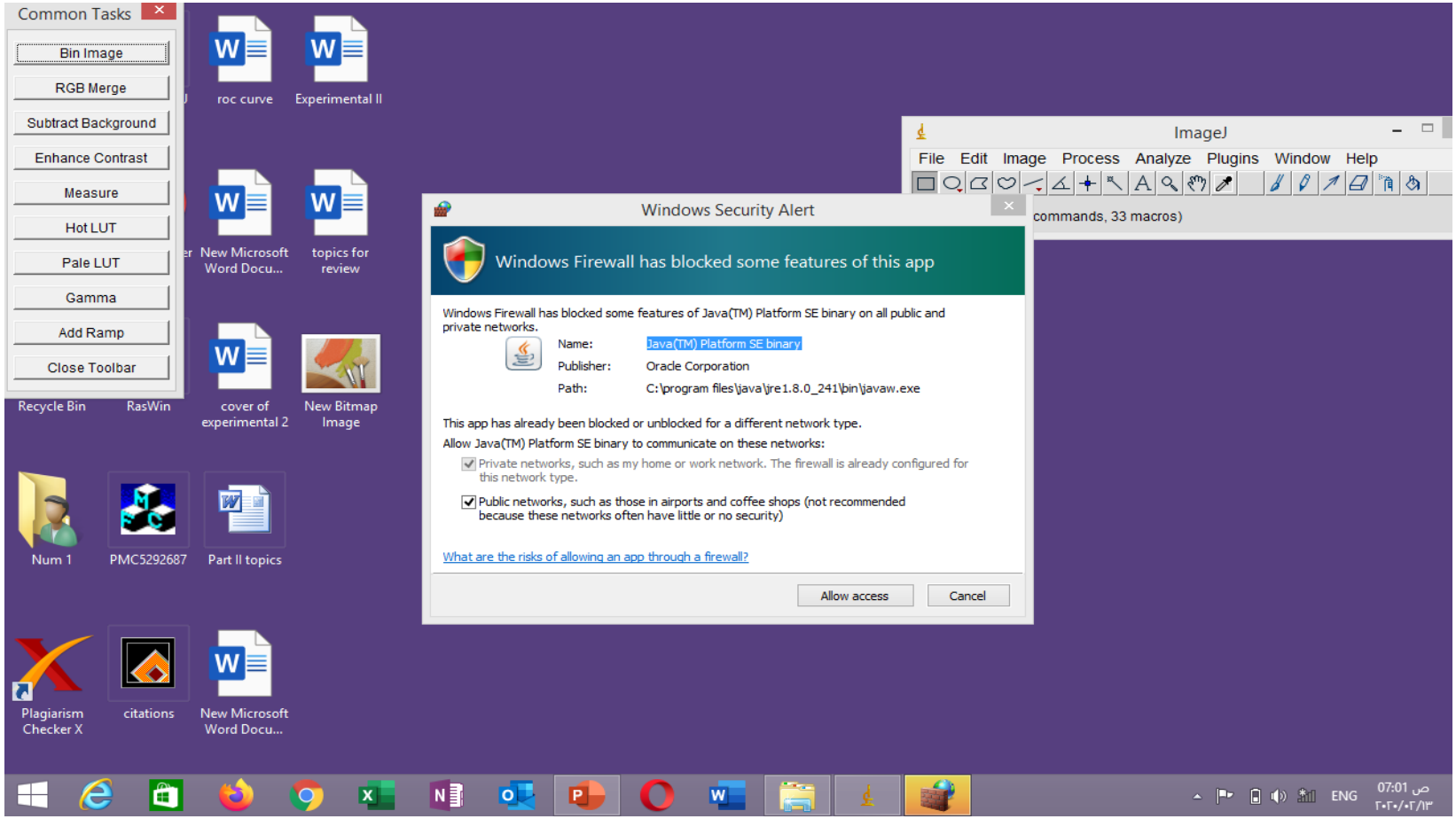
- Using the numbers in digital pictures to get useful information
- REMEMBER:
- Start with an image -- end with an answer

How can you download image J

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

  You just double click with EMBL ImageJ setup v1_0f, 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



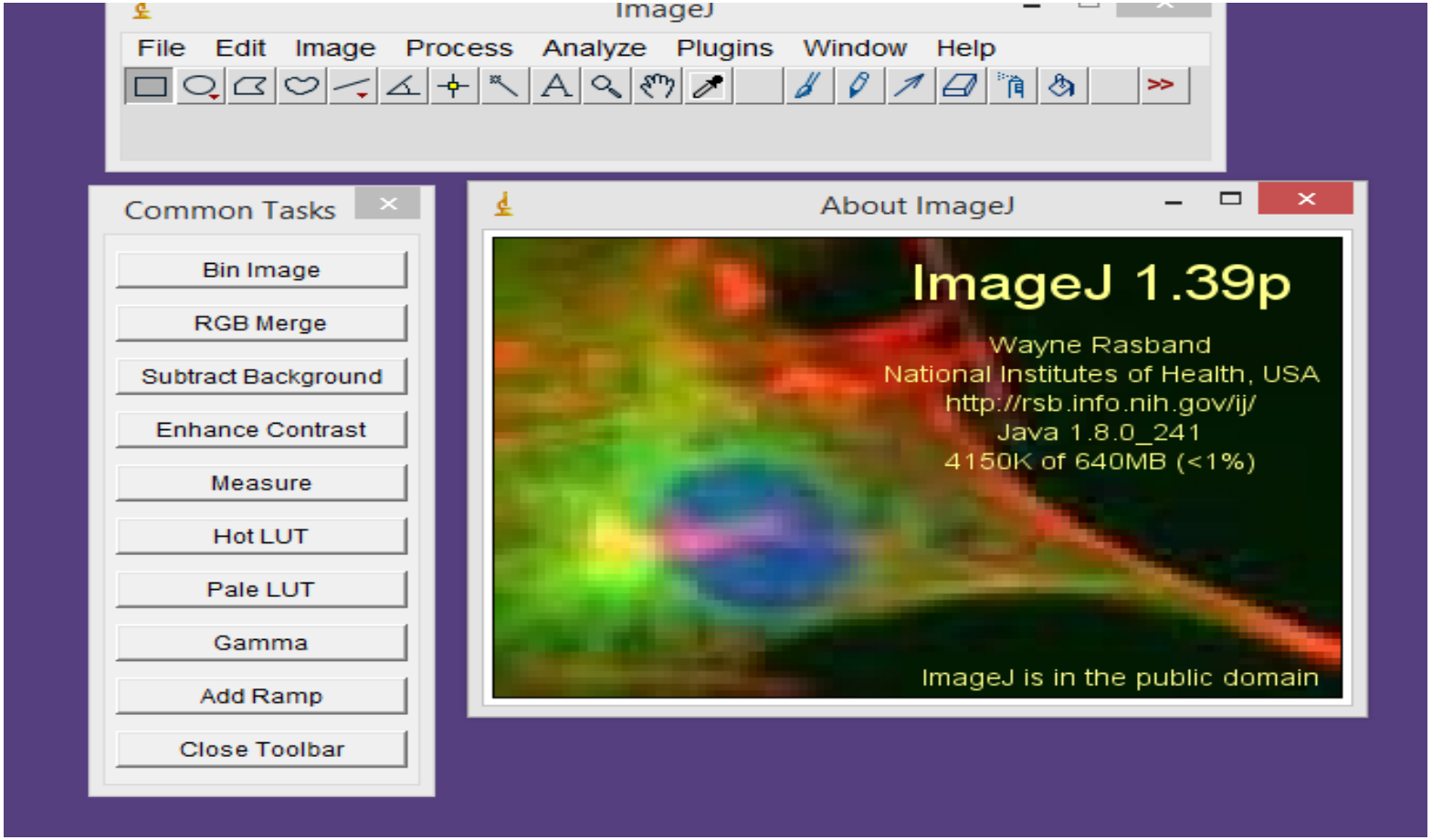
Some information about the program

- 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, 16-bit unsigned integer, 32-bit floating-point and RGB color

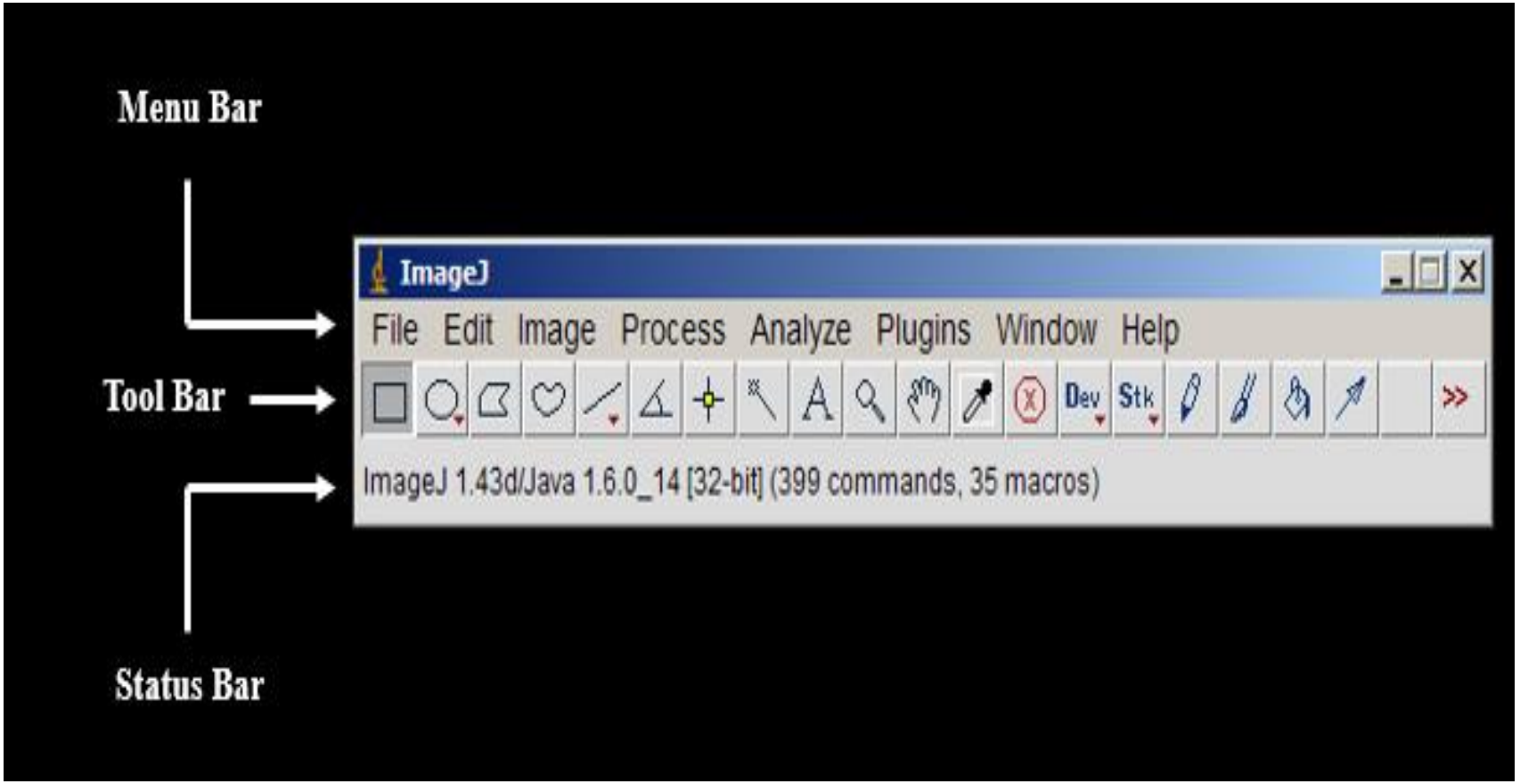
Some information about the program

- **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.

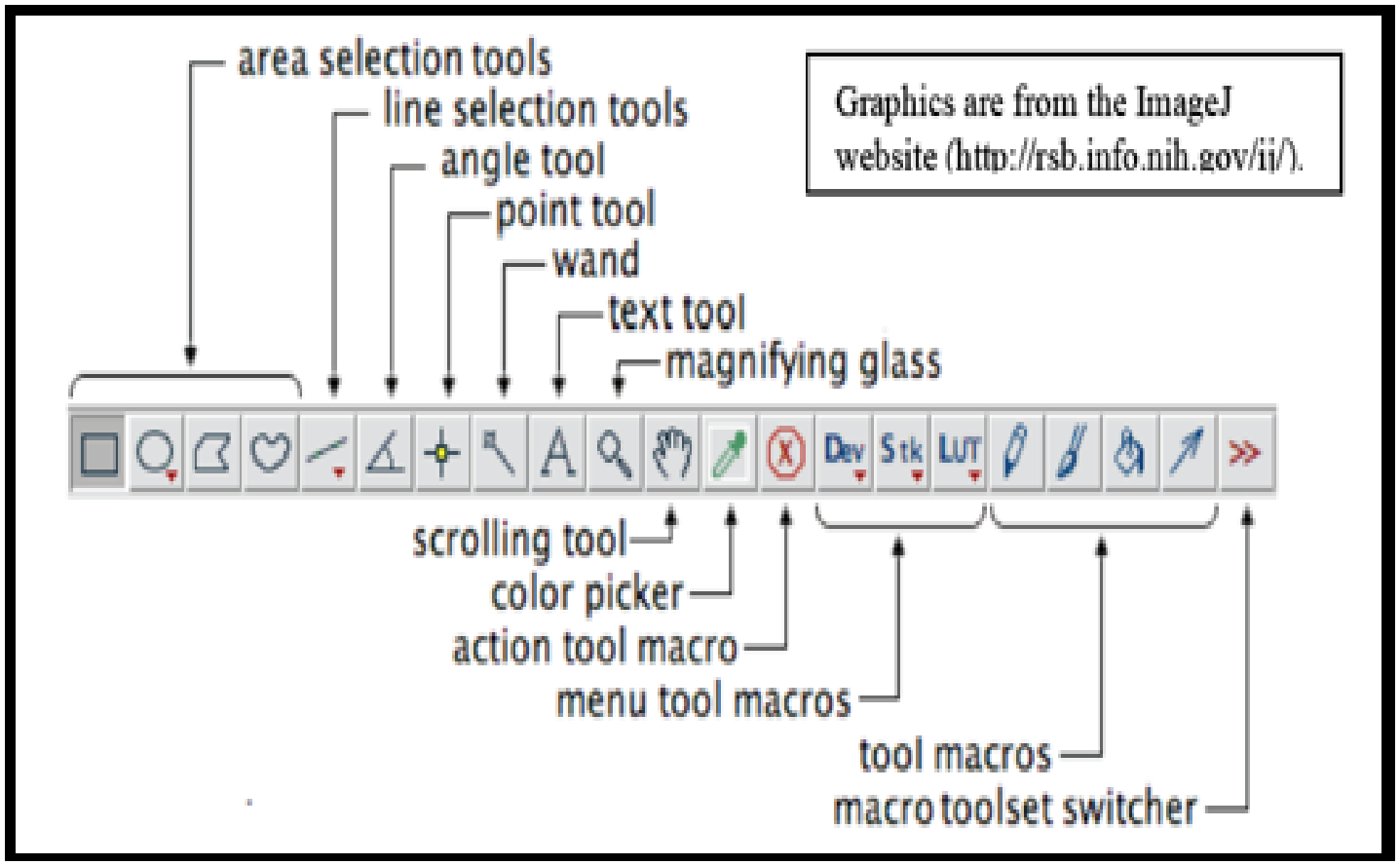
To know about the program after setup help then about image J



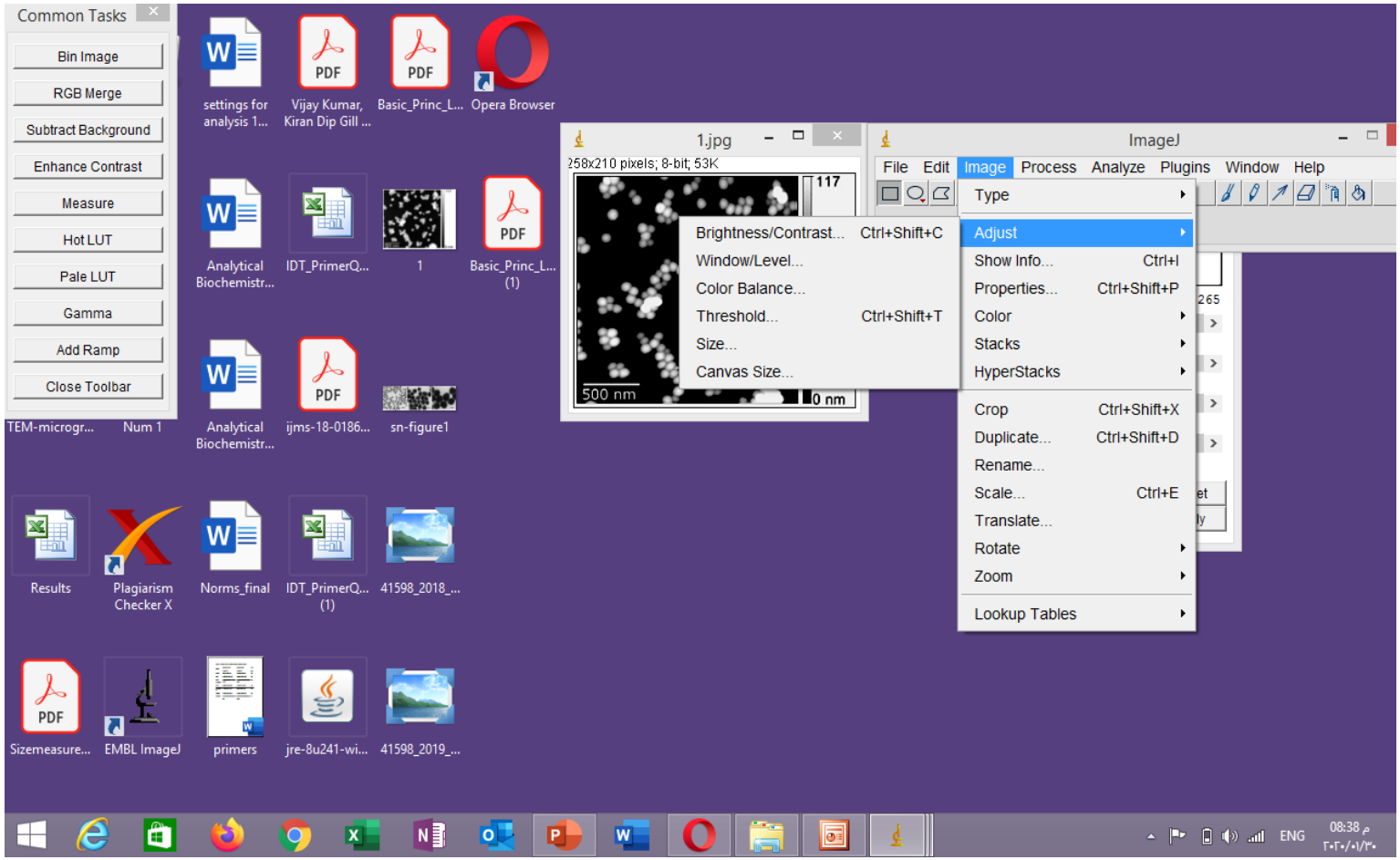
Different bars of the program



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.

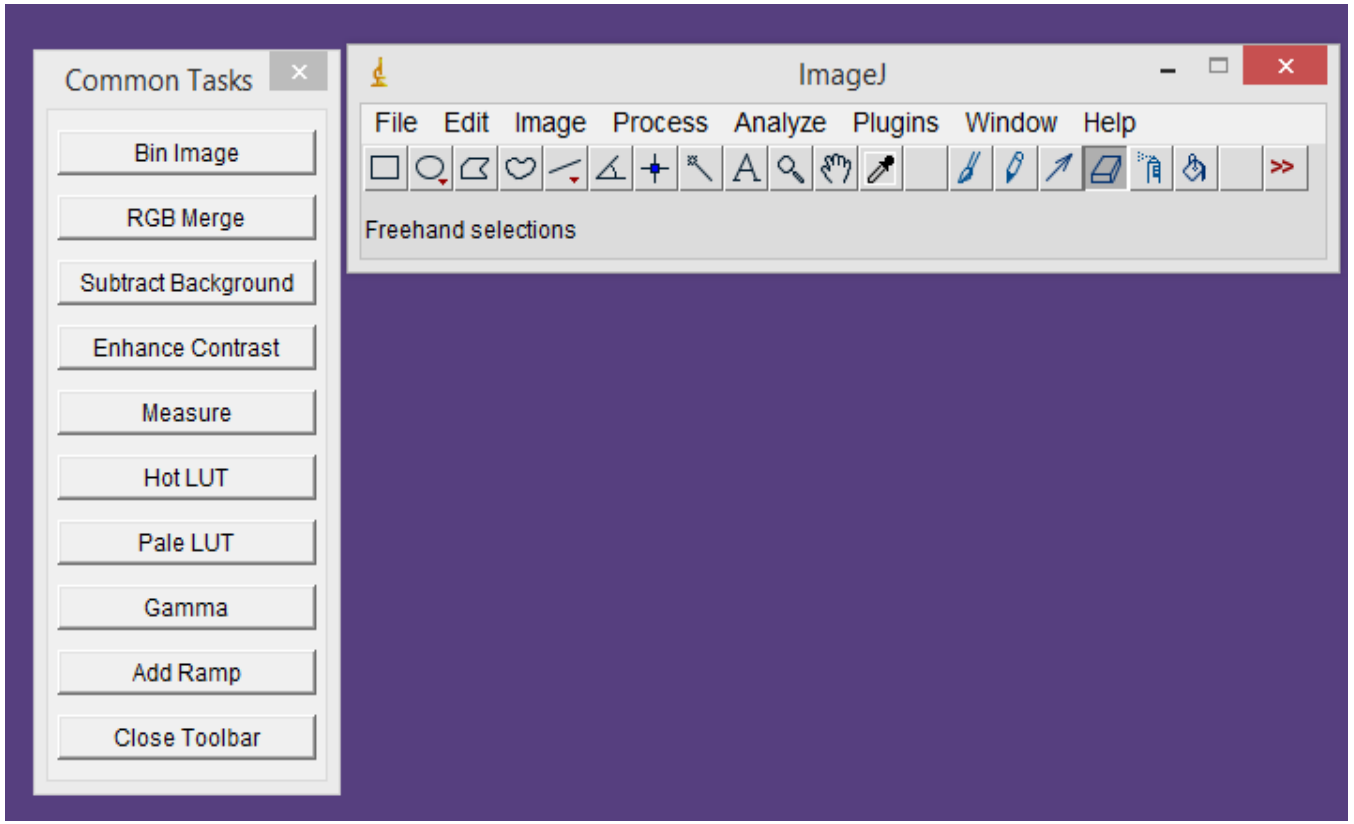
Topics that will be covered today

- **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

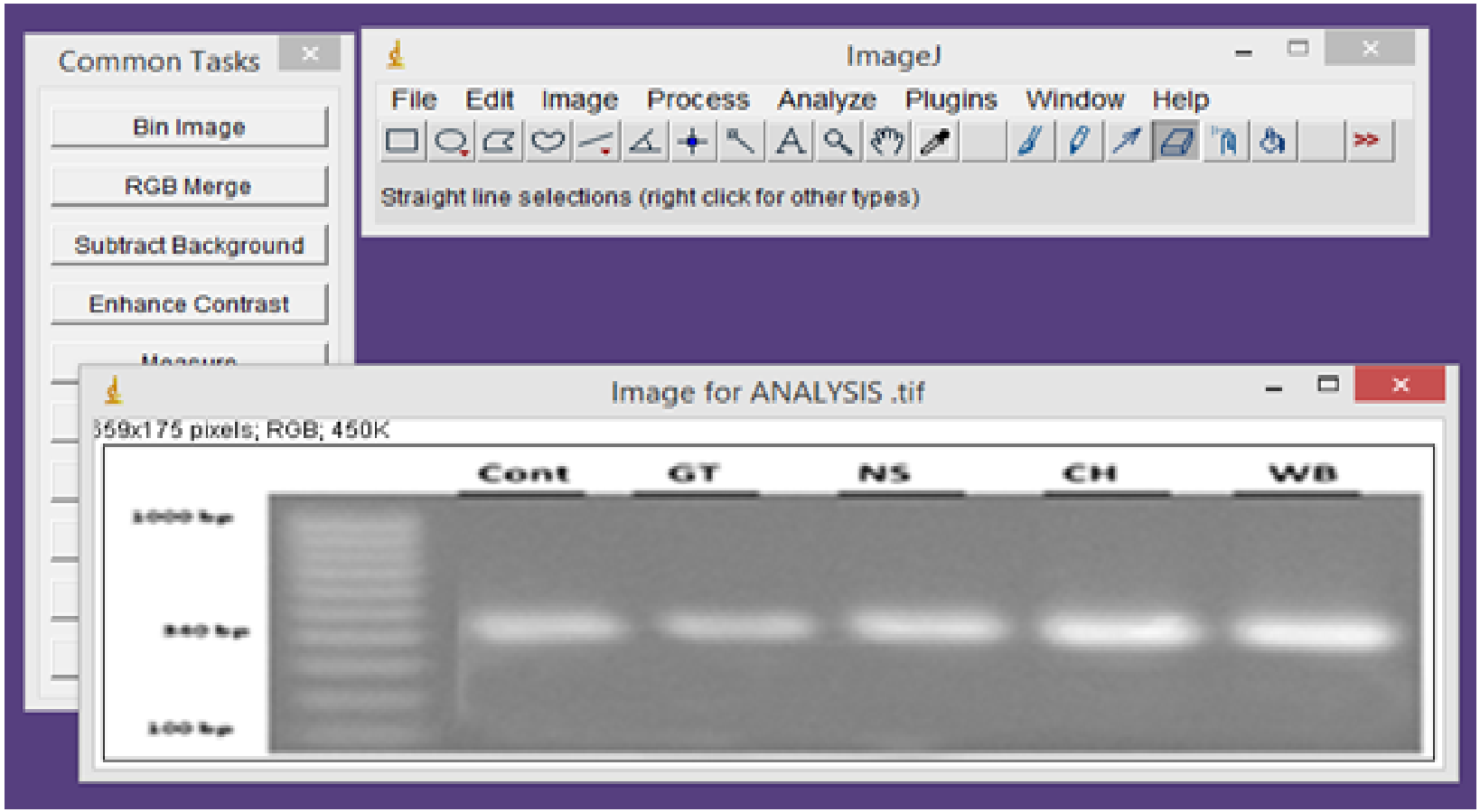
Steps you can follow as screen shot

- 1- open image J software from its setuped icon



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 edit ….invert

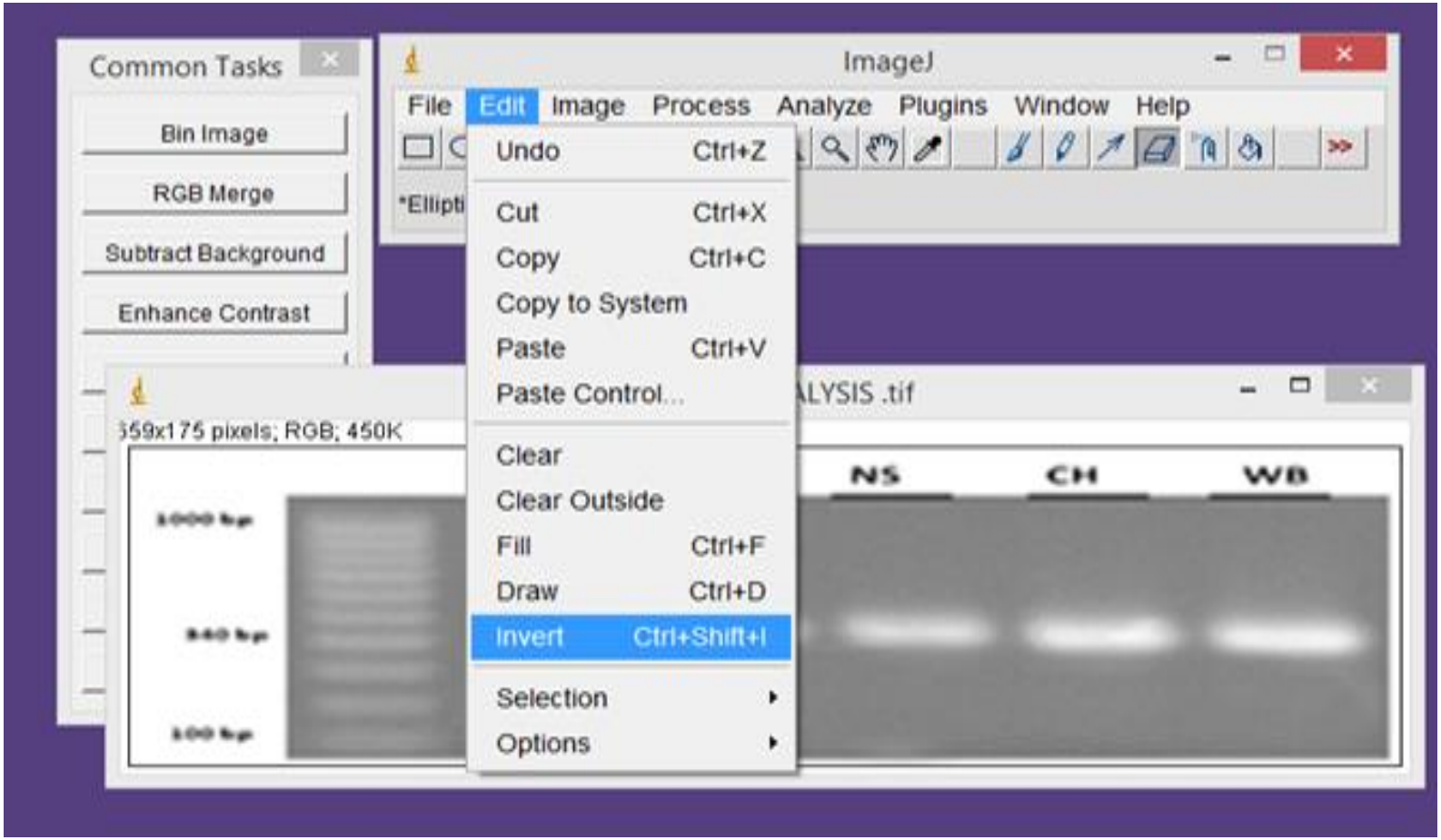
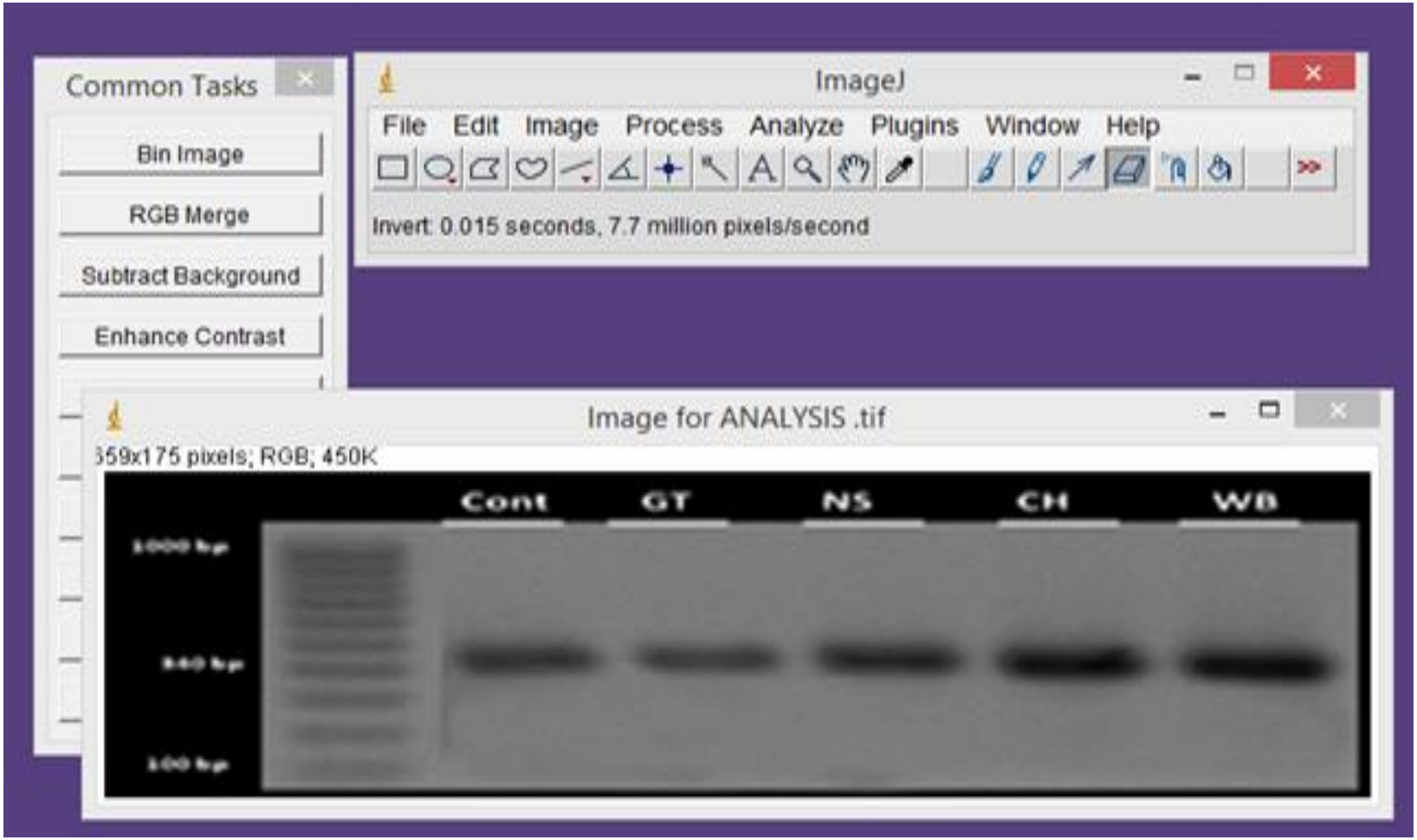
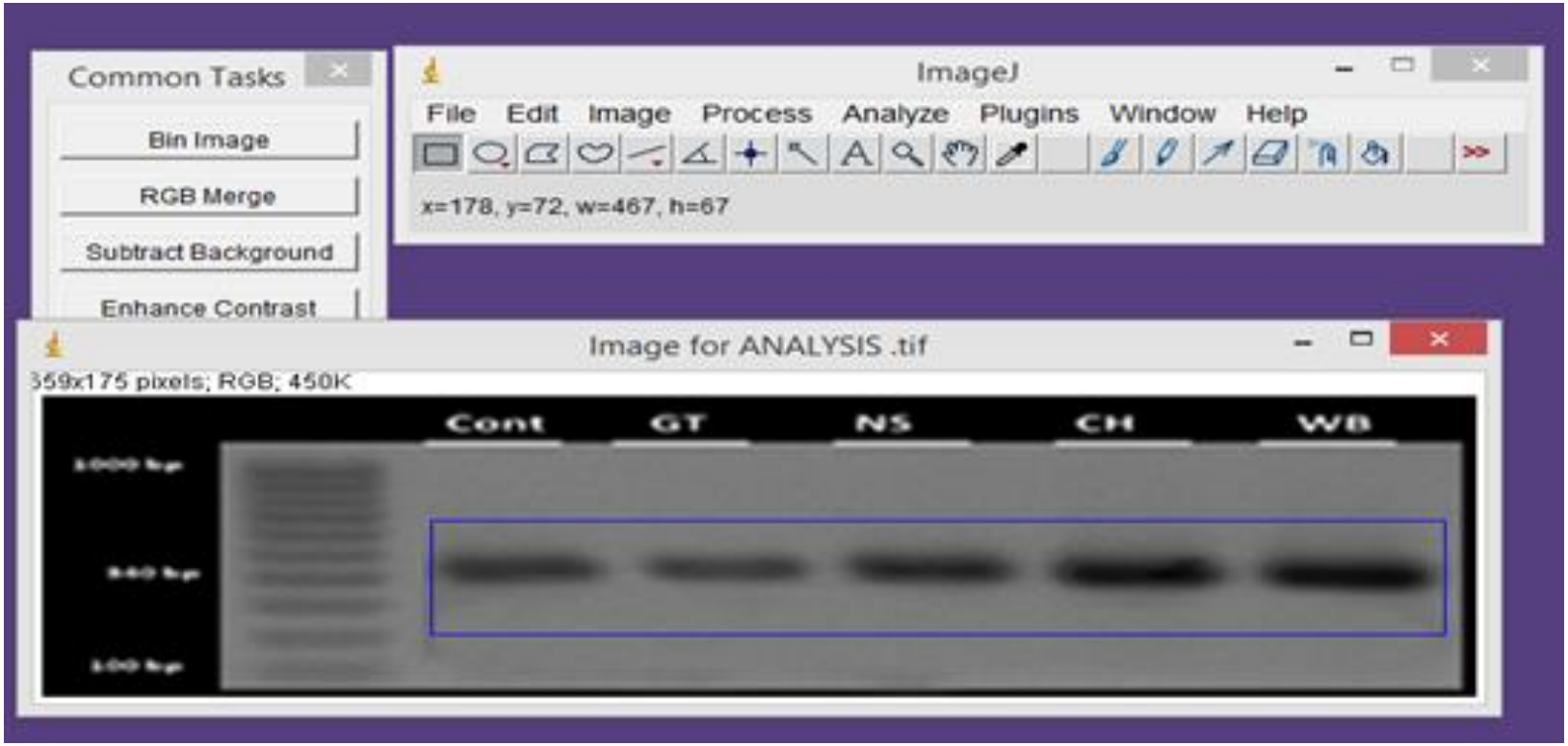


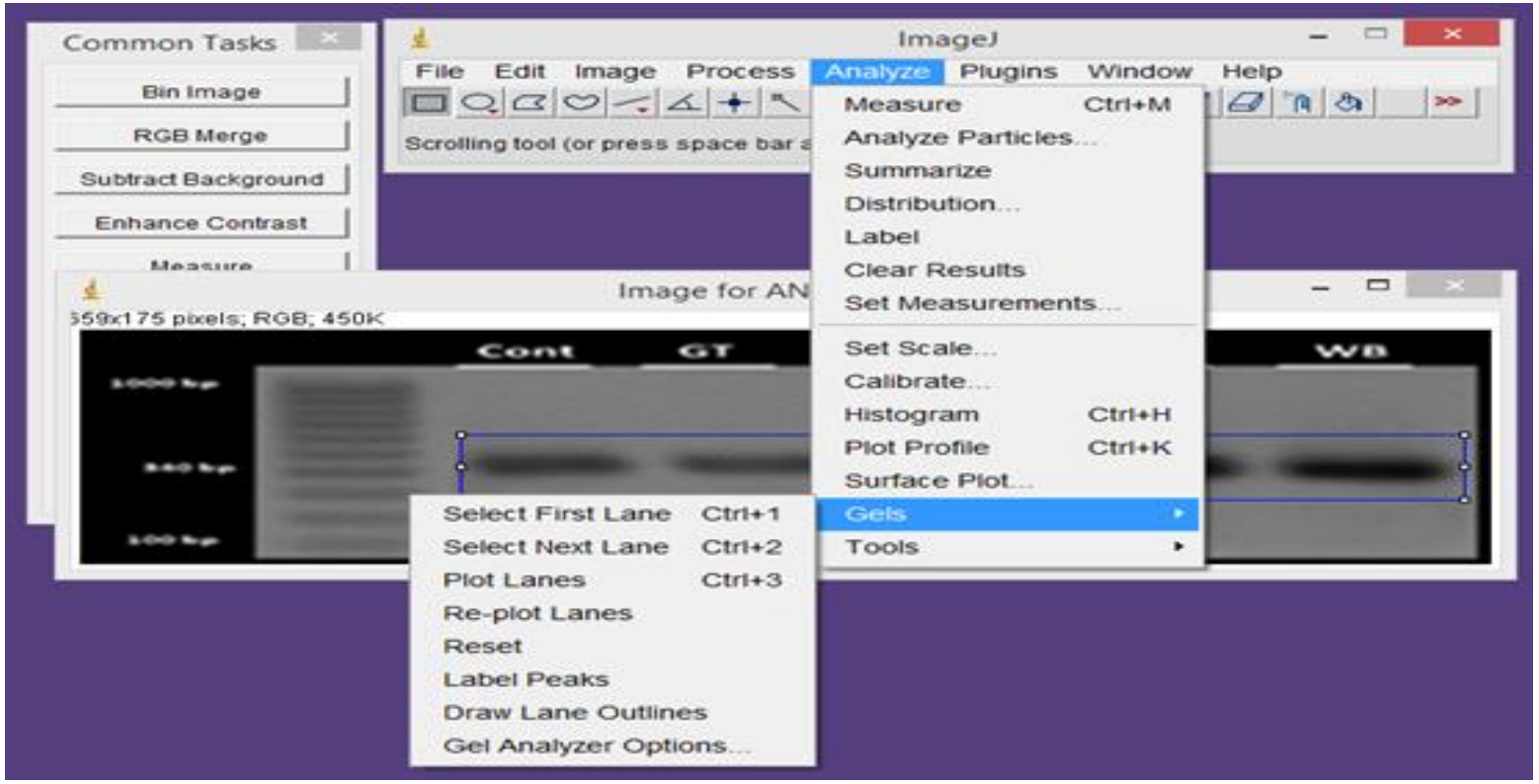
Photo after color inversion……bands will appear in black



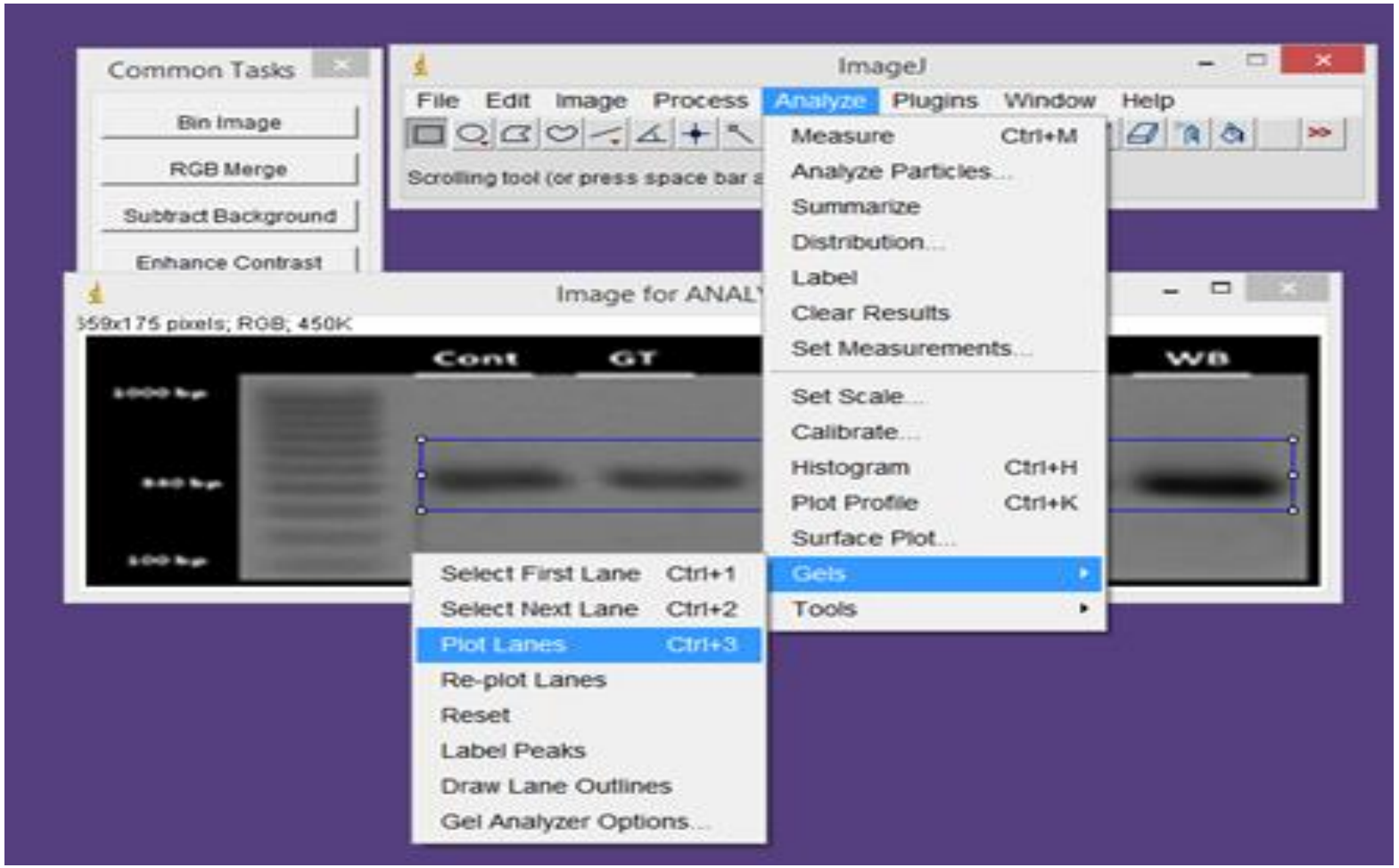
Select the area of bands using rectangular selection

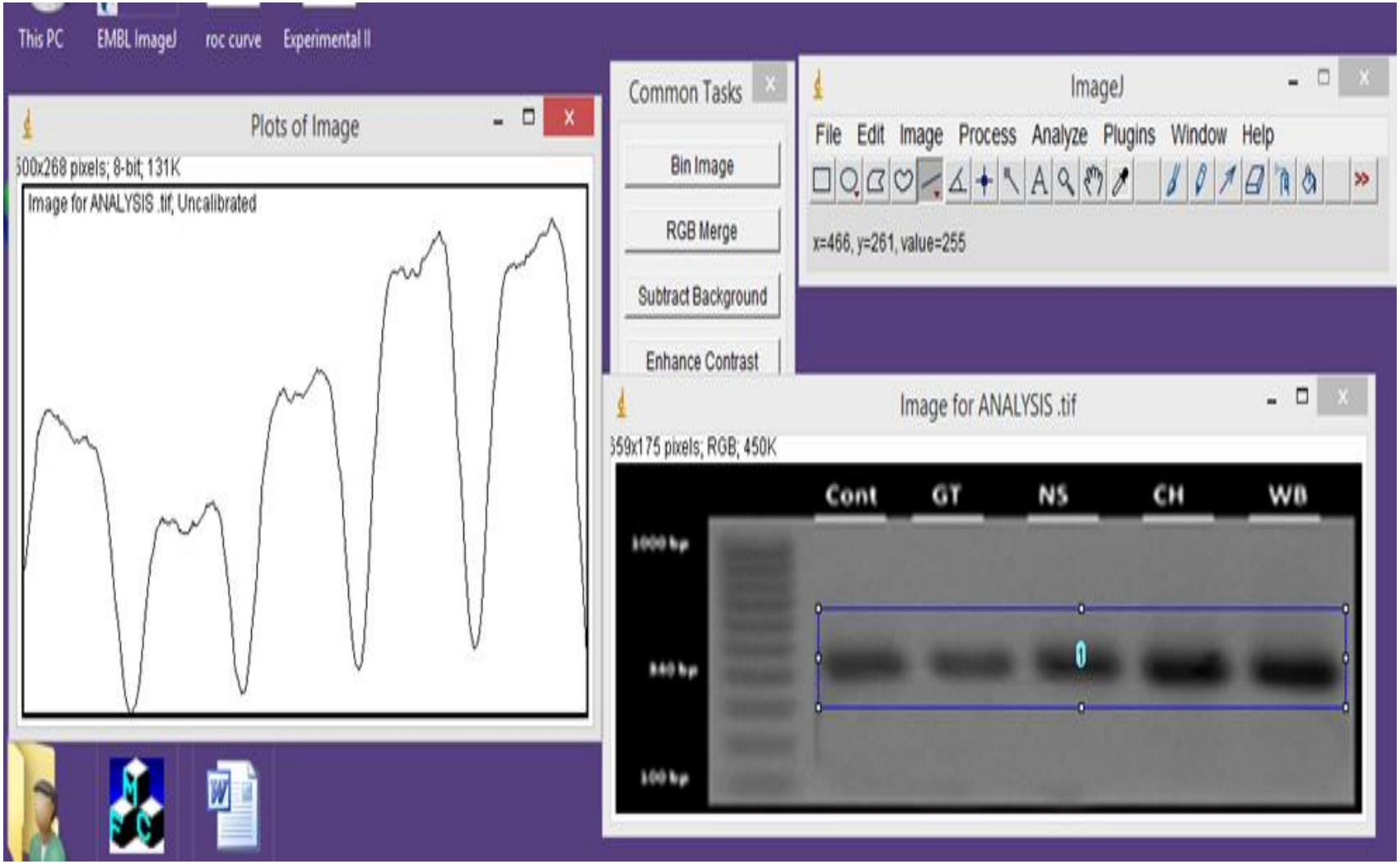


Analyze ….gels …..select the first lane

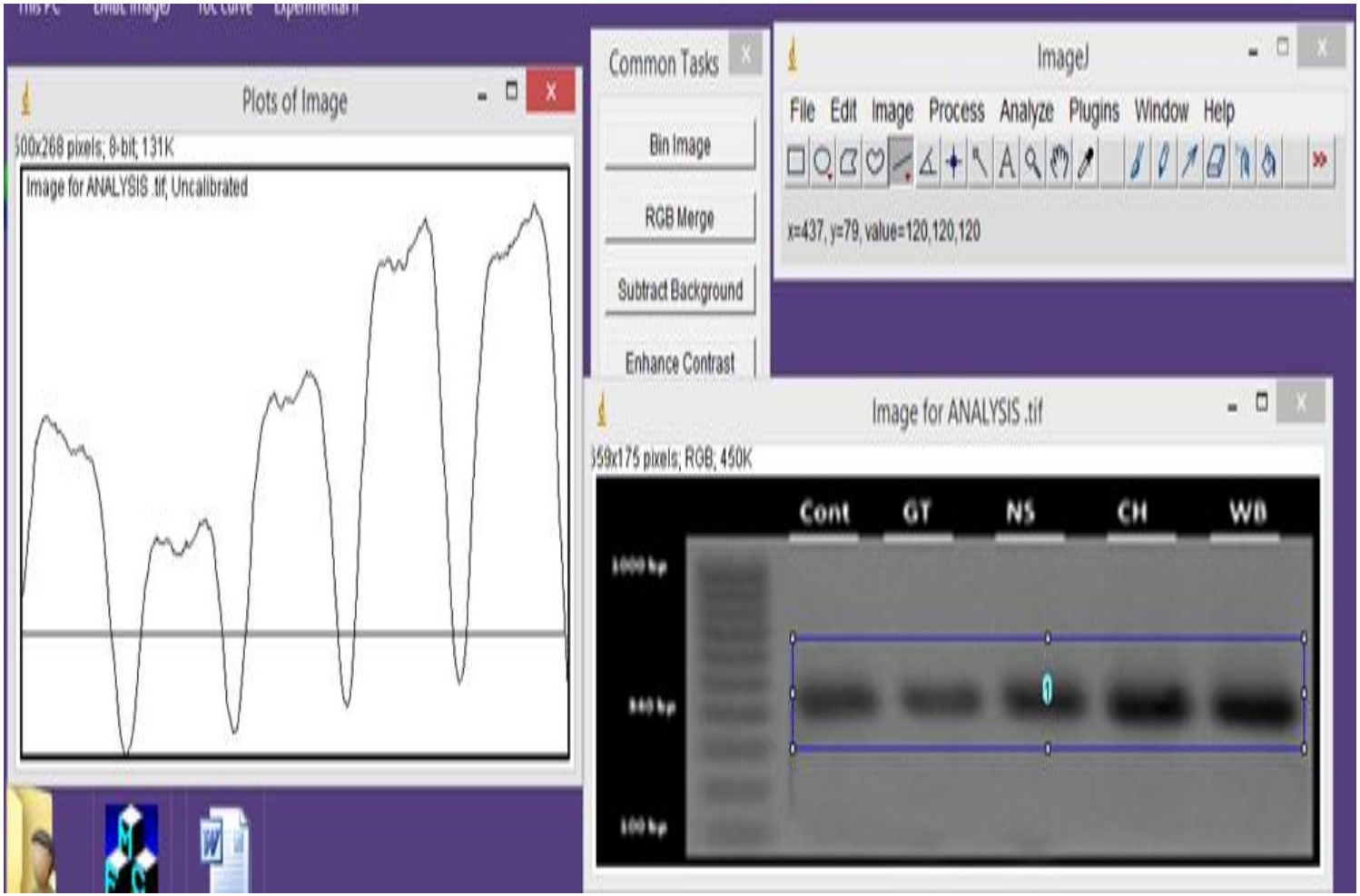


Analyze-gel-plot lanes

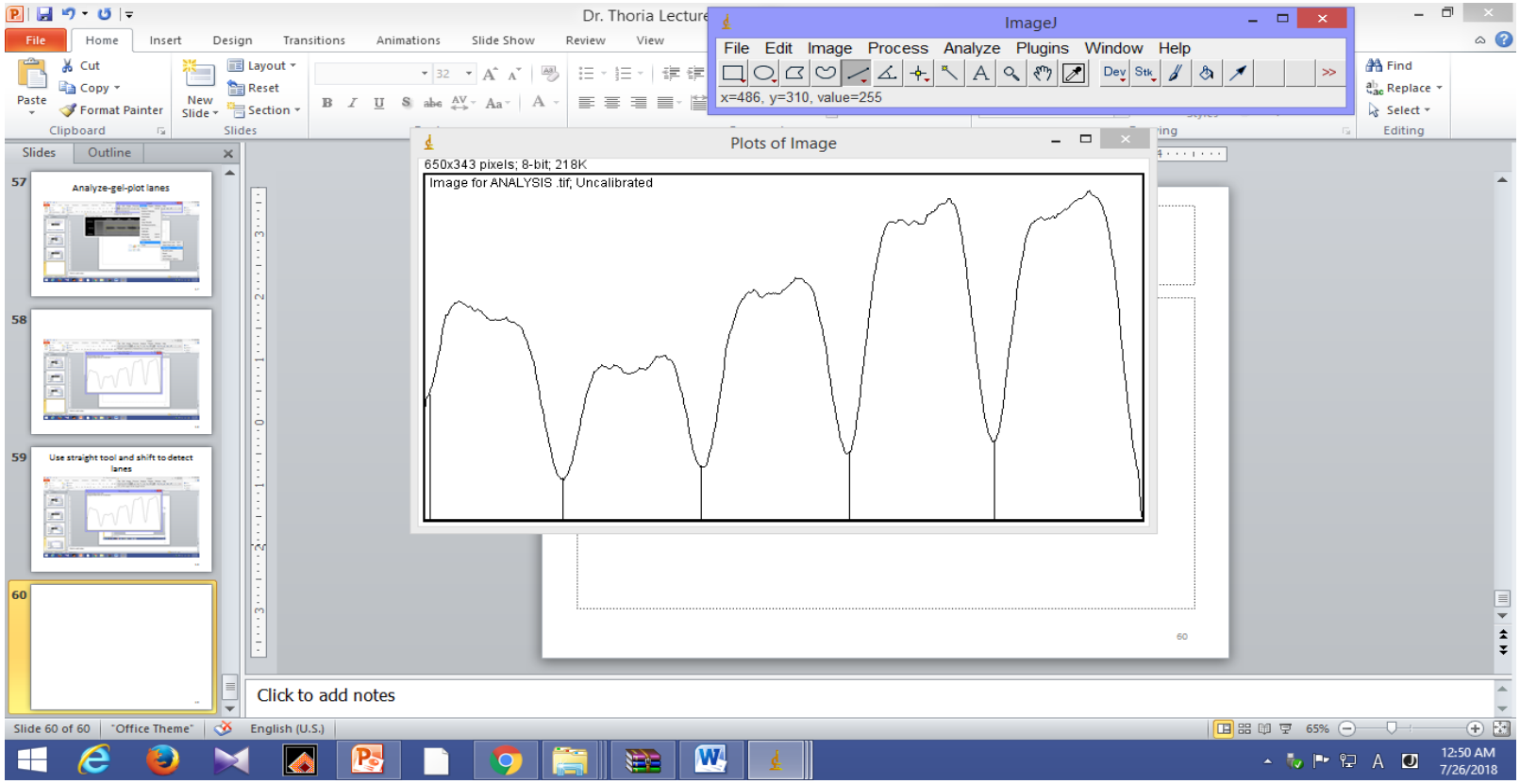



This PC
EMBL ImageJ
roc curve
Experimental II
Plots of Image
500x268 pixels; 8-bit; 131K
Image for ANALYSIS .tif; Uncalibrated
Common Tasks
Bin Image
RGB Merge
Subtract Background
Enhance Contrast
ImageJ
File Edit Image Process Analyze Plugins Window Help
x=466, y=261, value=255
Image for ANALYSIS .tif
359x175 pixels; RGB; 450K
Cont
GT
NS
CH
WB
1000 bp
100 bp

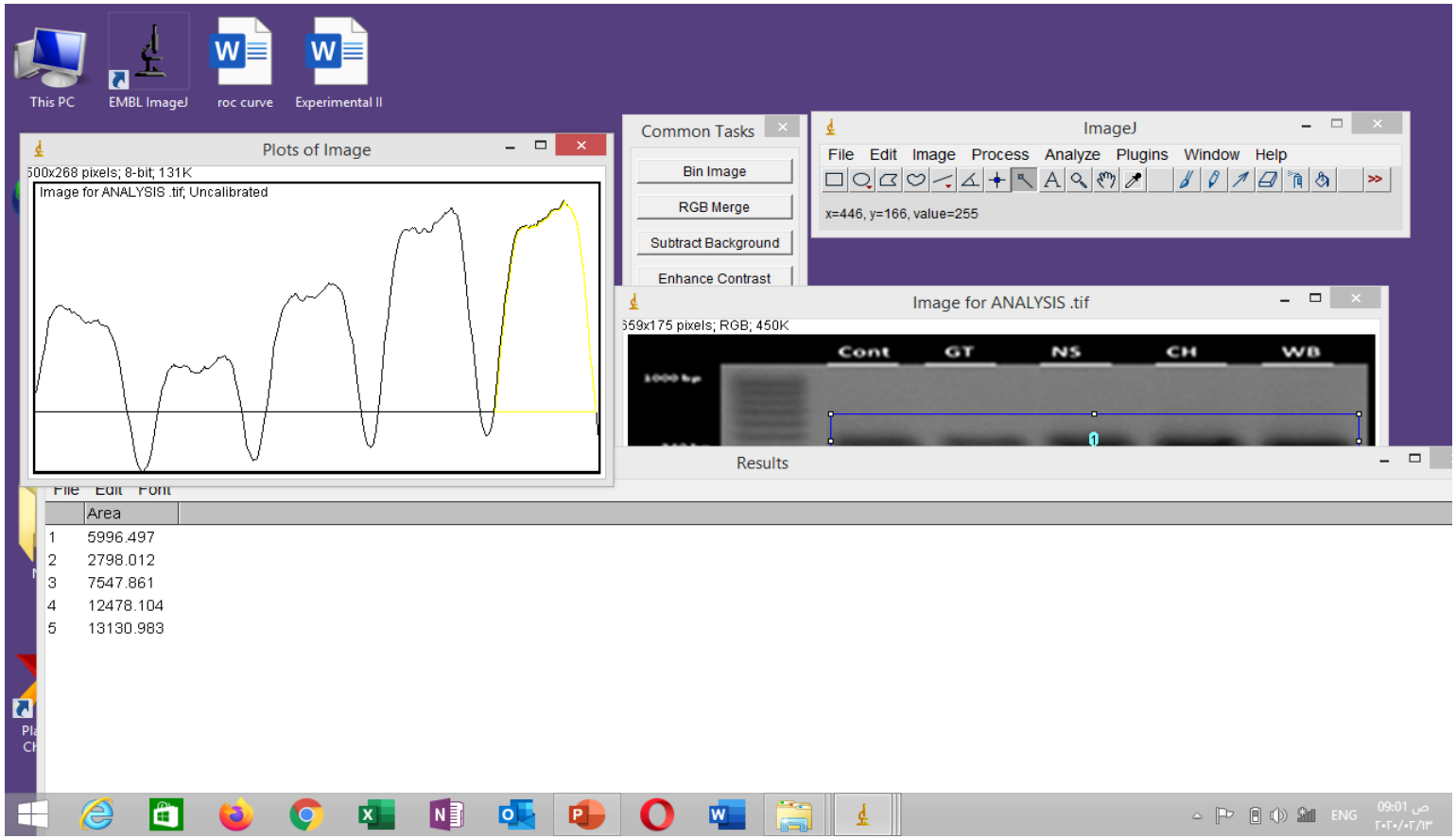
Use straight line tool and shift to detect bands



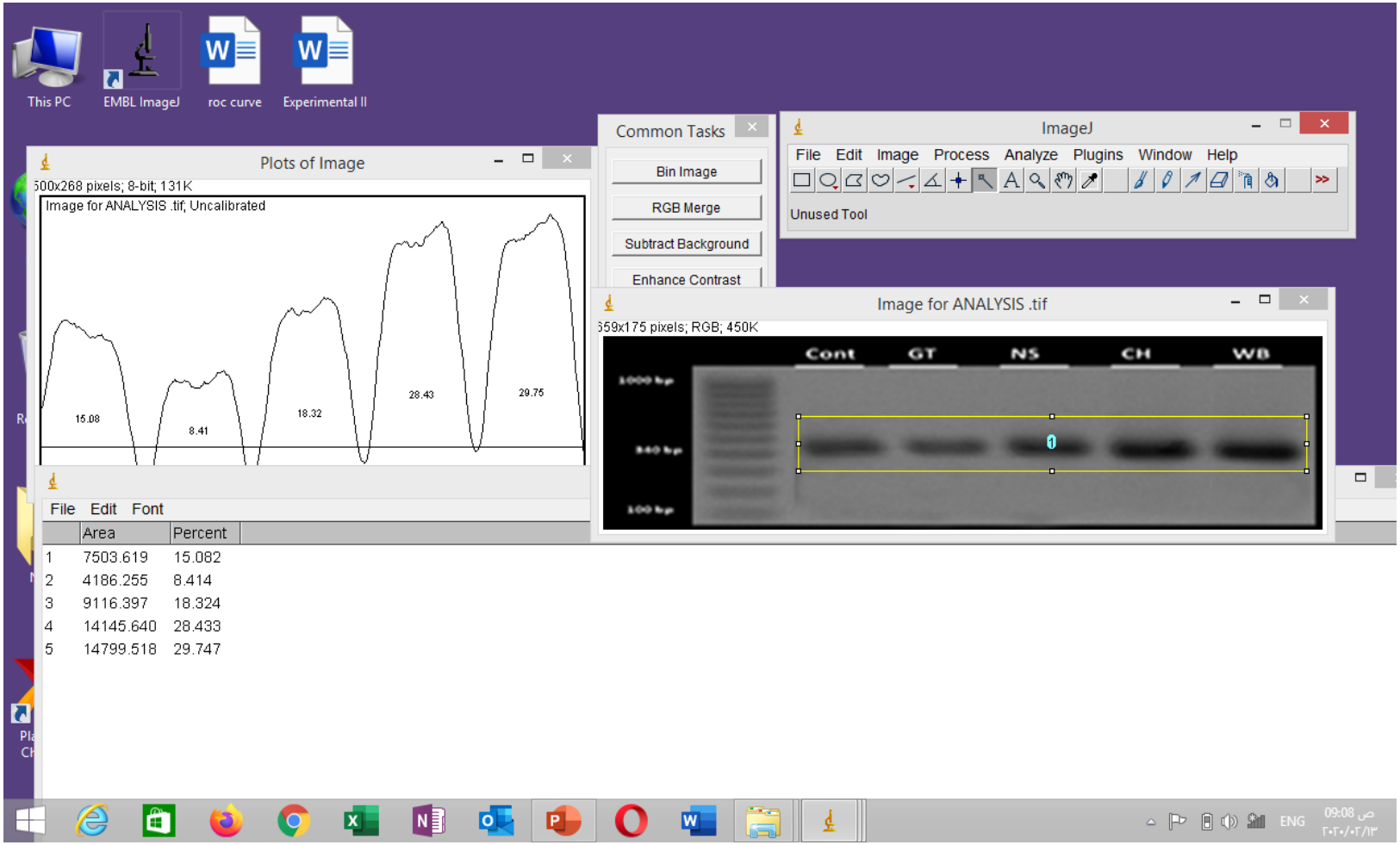
Another option to select area of each band separately



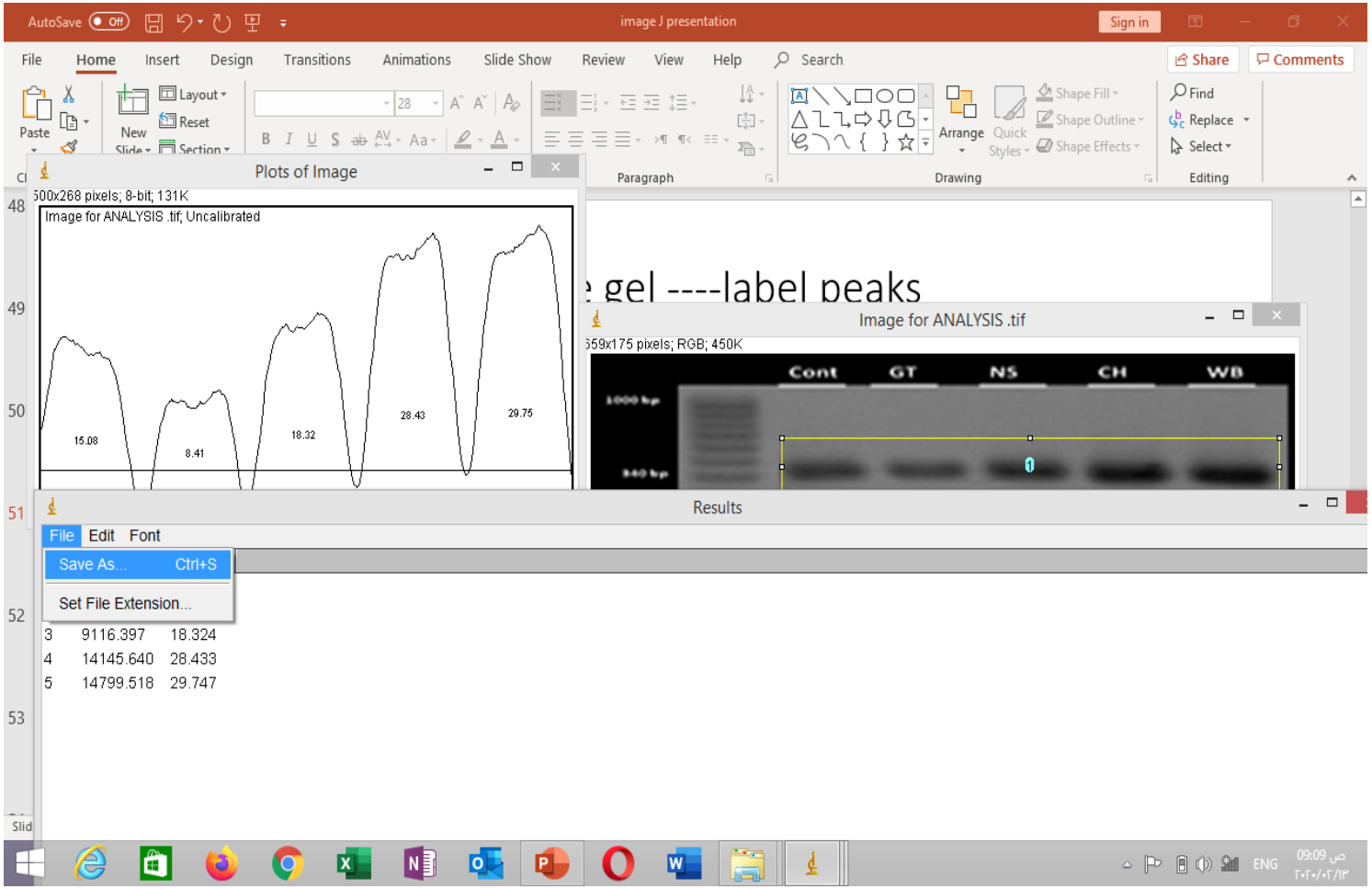
Use wand tool to detect areas of bands



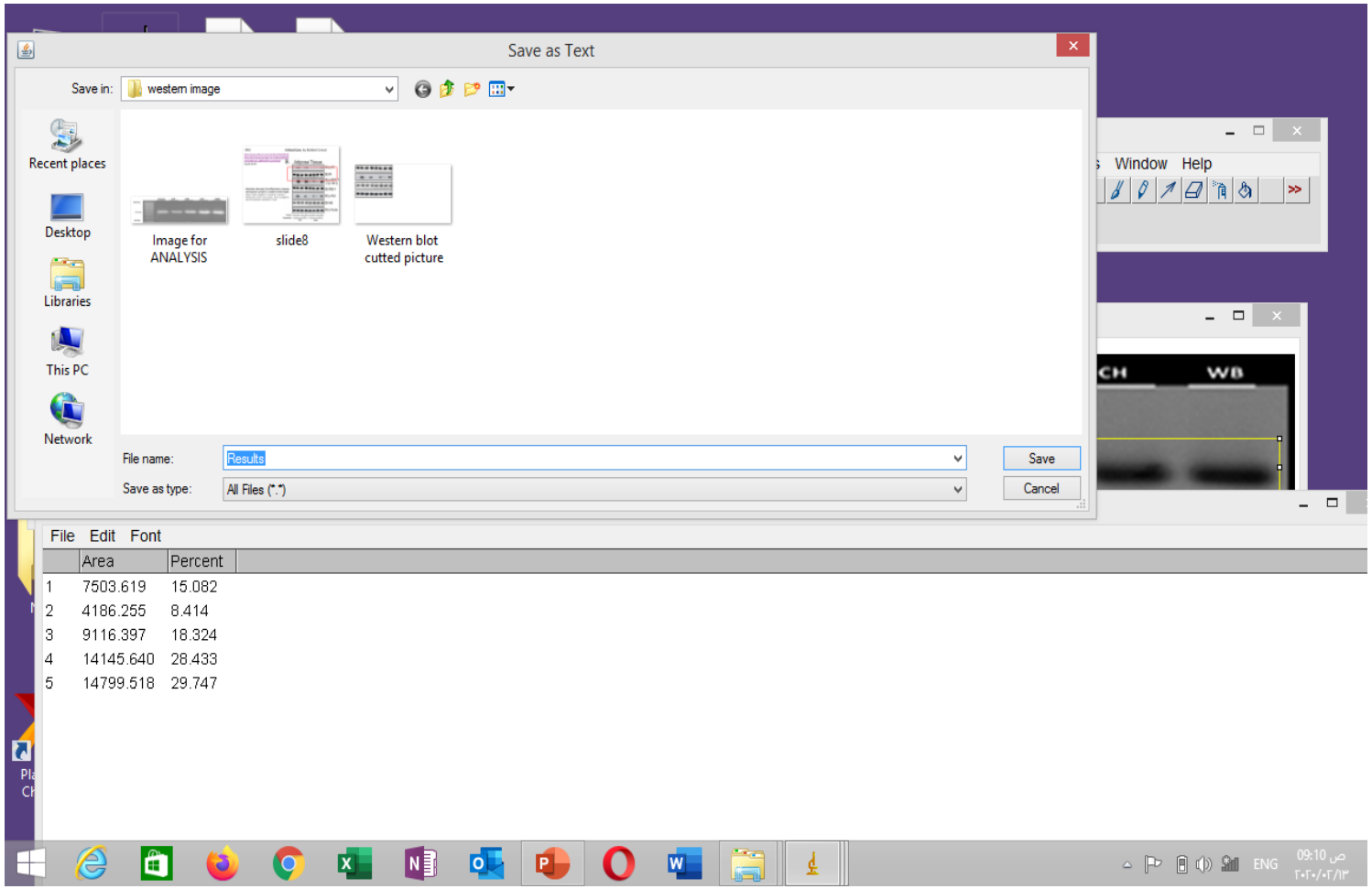
Click analyze gel ----label peaks



You can save your results by clicking file ….save as in results window

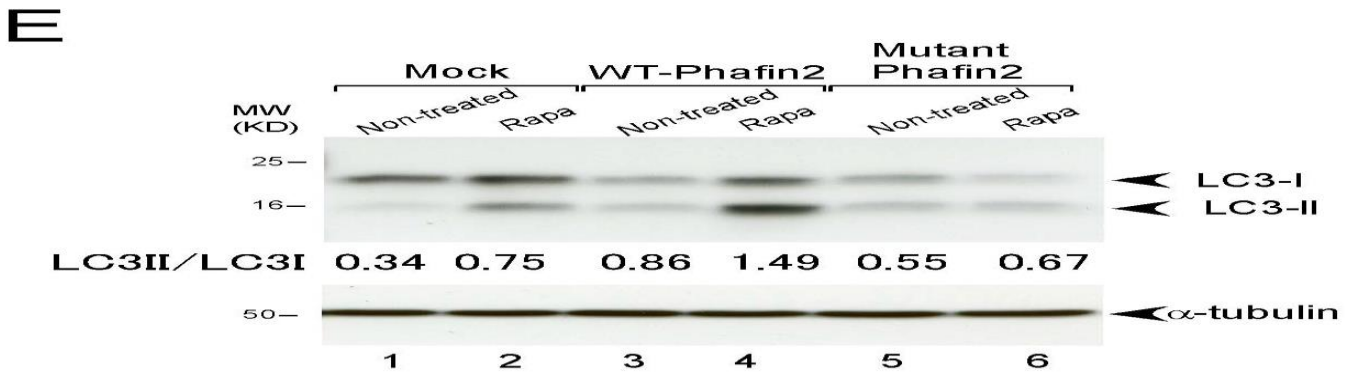


Then open in excel and analyze the results



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

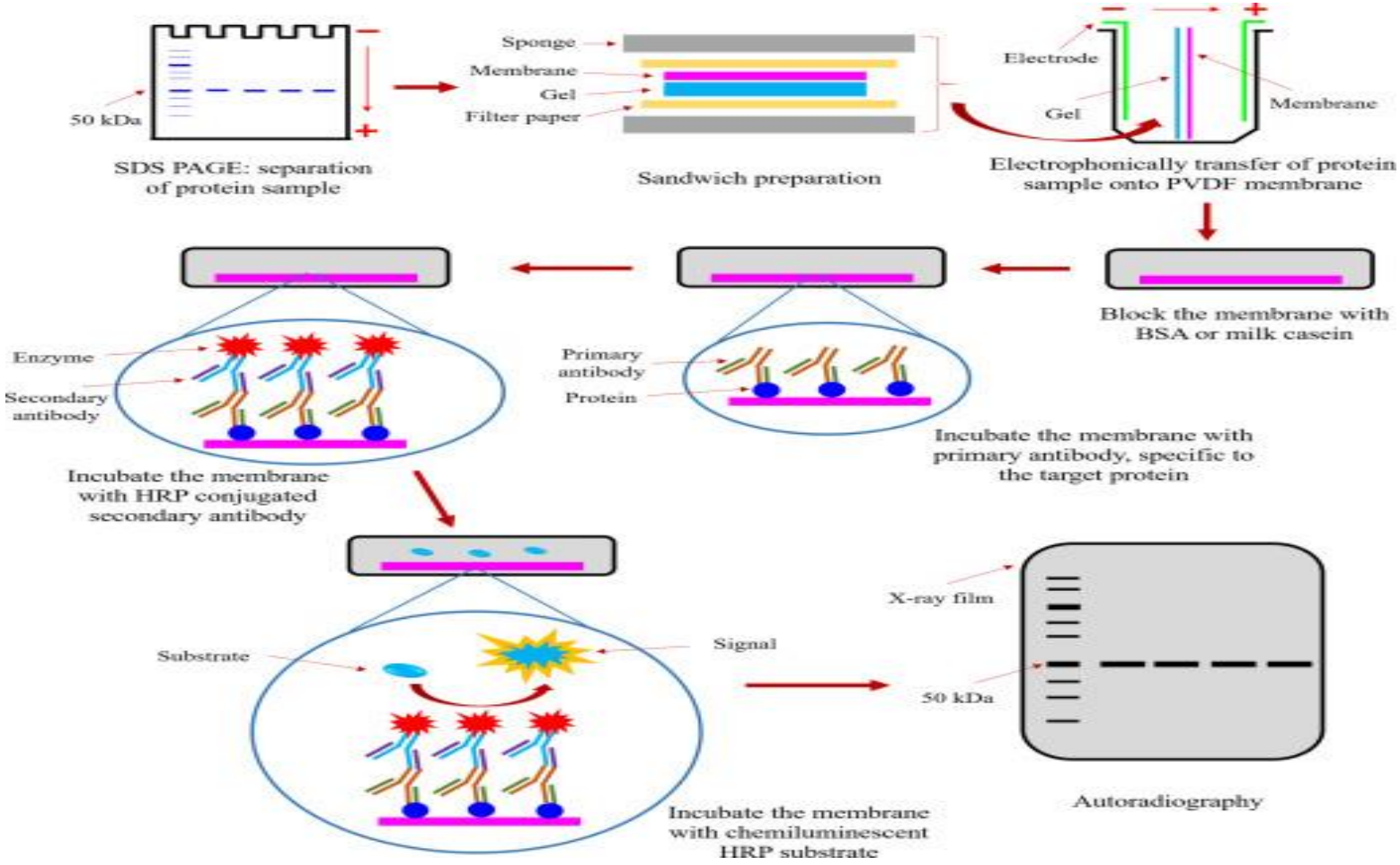
- You can calculate the fold change by dividing over the area or percent of each group or band on that of control
- You can add the values on each band in the image that will be added in paper. Something like in this



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

Analysis of western blot image

Western blot overview



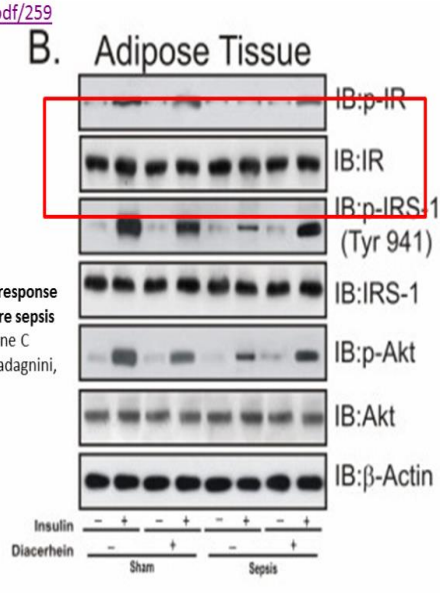
Harry Towbin, in Encyclopedia of Immunology (Second Edition), 1998

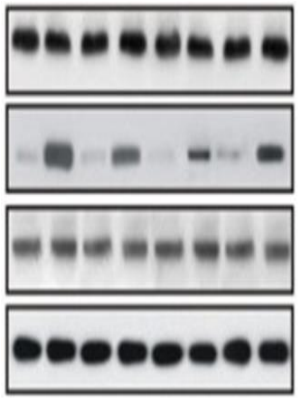
Image for western blot

2012 **Critical Care**, by BioMed Central

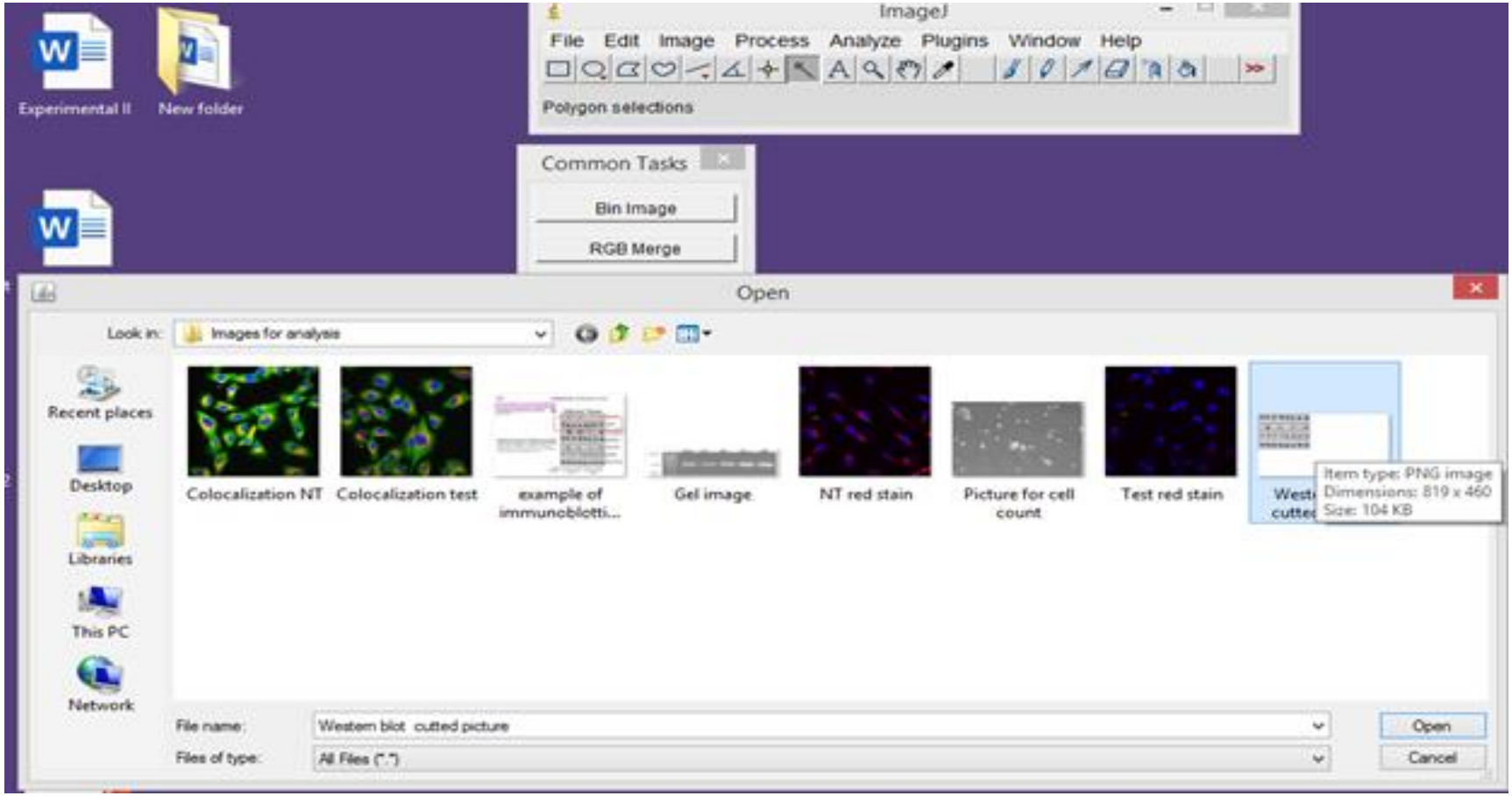
http://www.ccforum.com/content/16/4/R158
http://download.springer.com/static/pdf/259/art%253A10.1186%252Fcc11478.pdf
Fig 4B (IB:IR)

Diacerhein attenuates the inflammatory response and improves survival in a model of severe sepsis
Kelly L Calisto, Angélica C Camacho, Francine C Mittestainer, Bruno M Carvalho, Dioze Guadagnini, José B Carvalheira and Mario J Saad

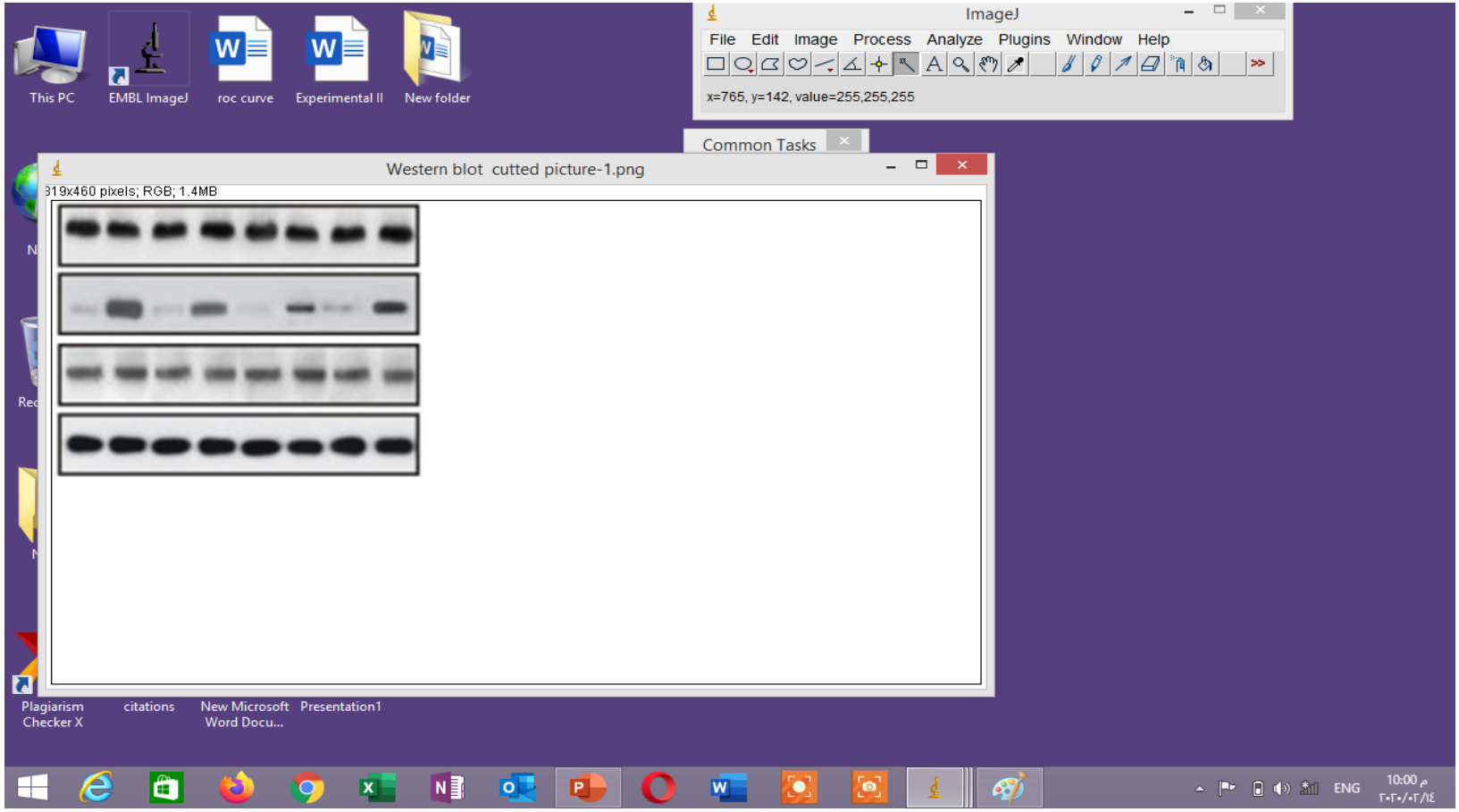




Open the image in image J



As there is too much free space crop the image



Rectangular selection then image crop

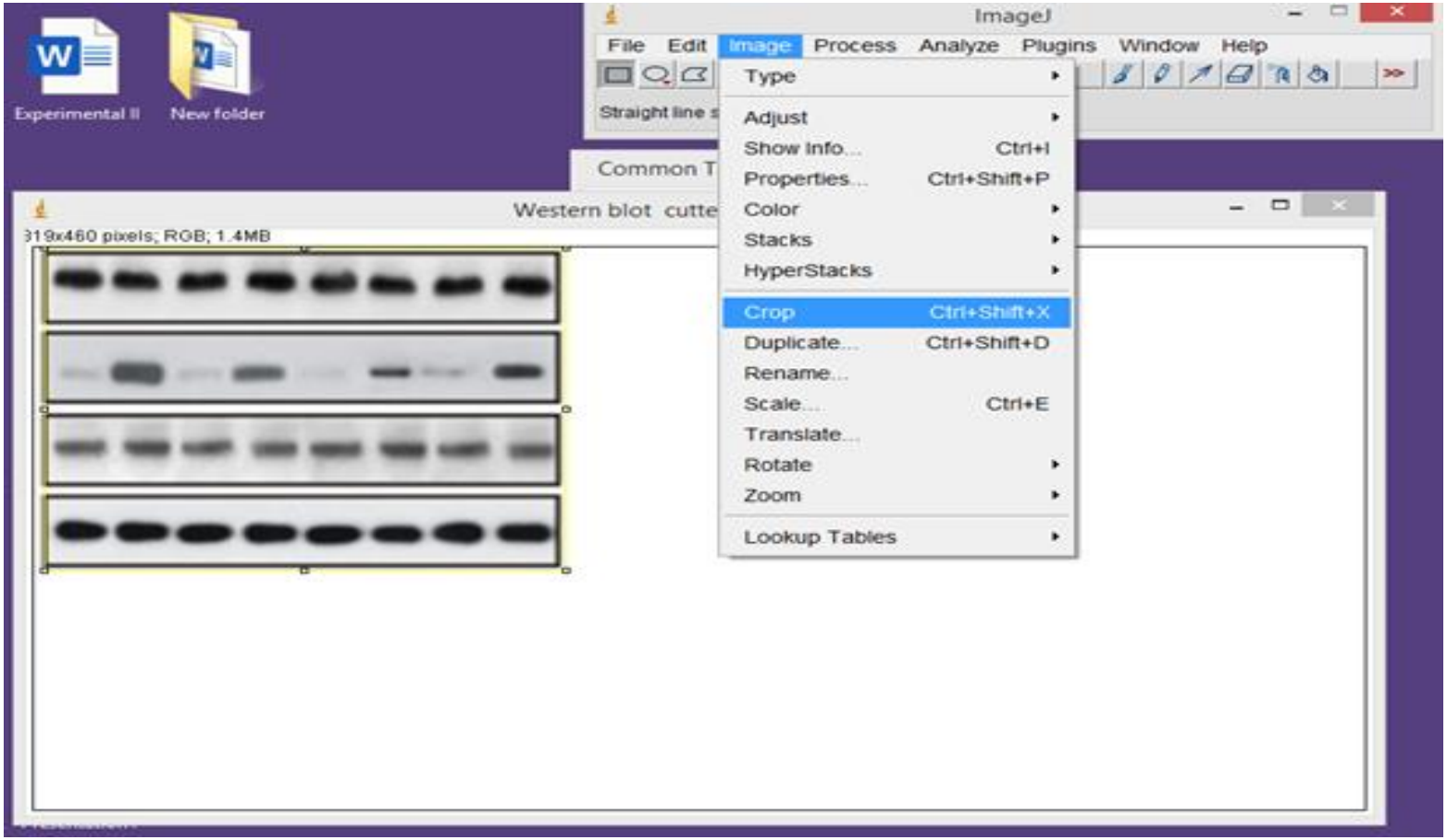
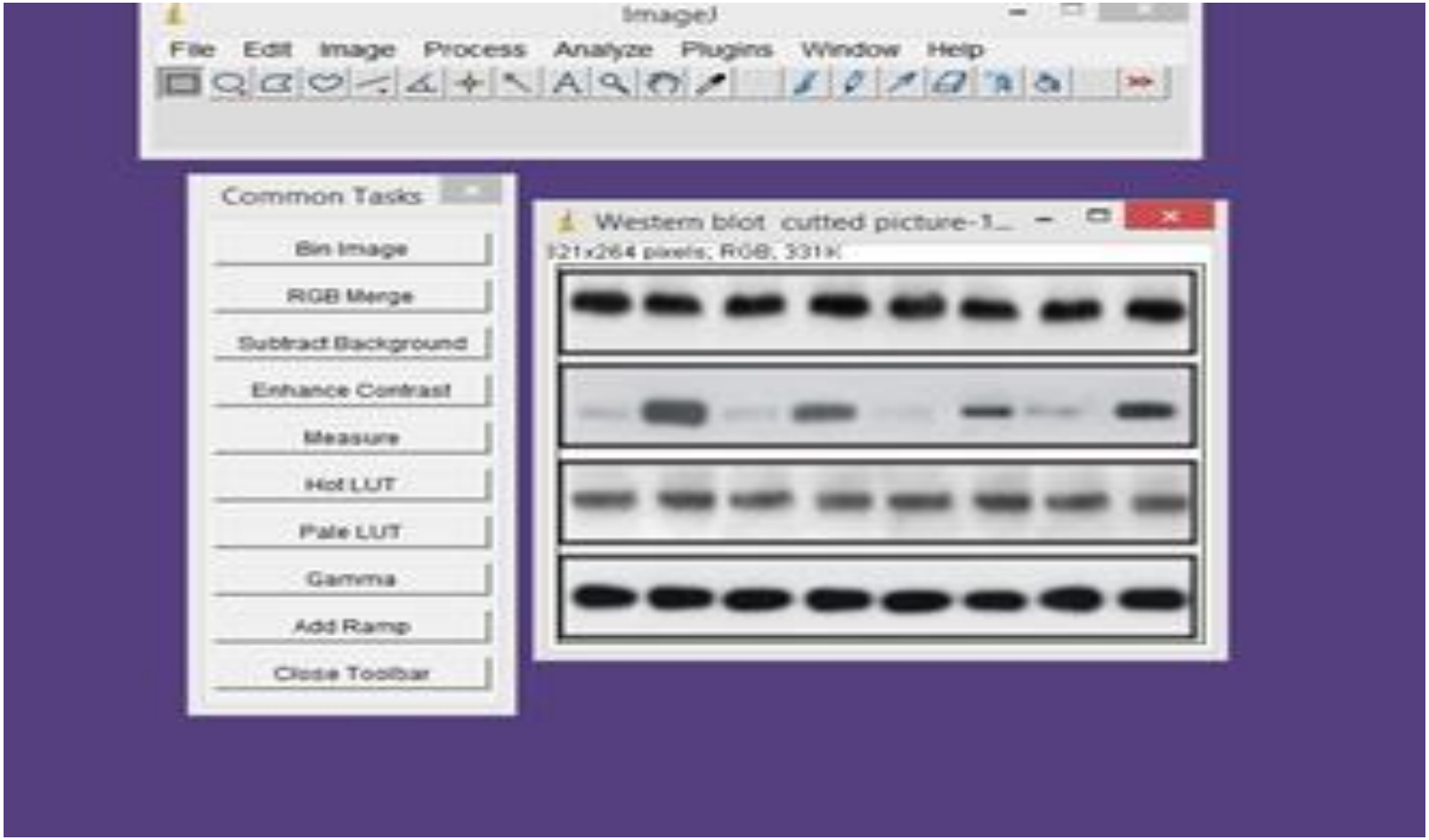
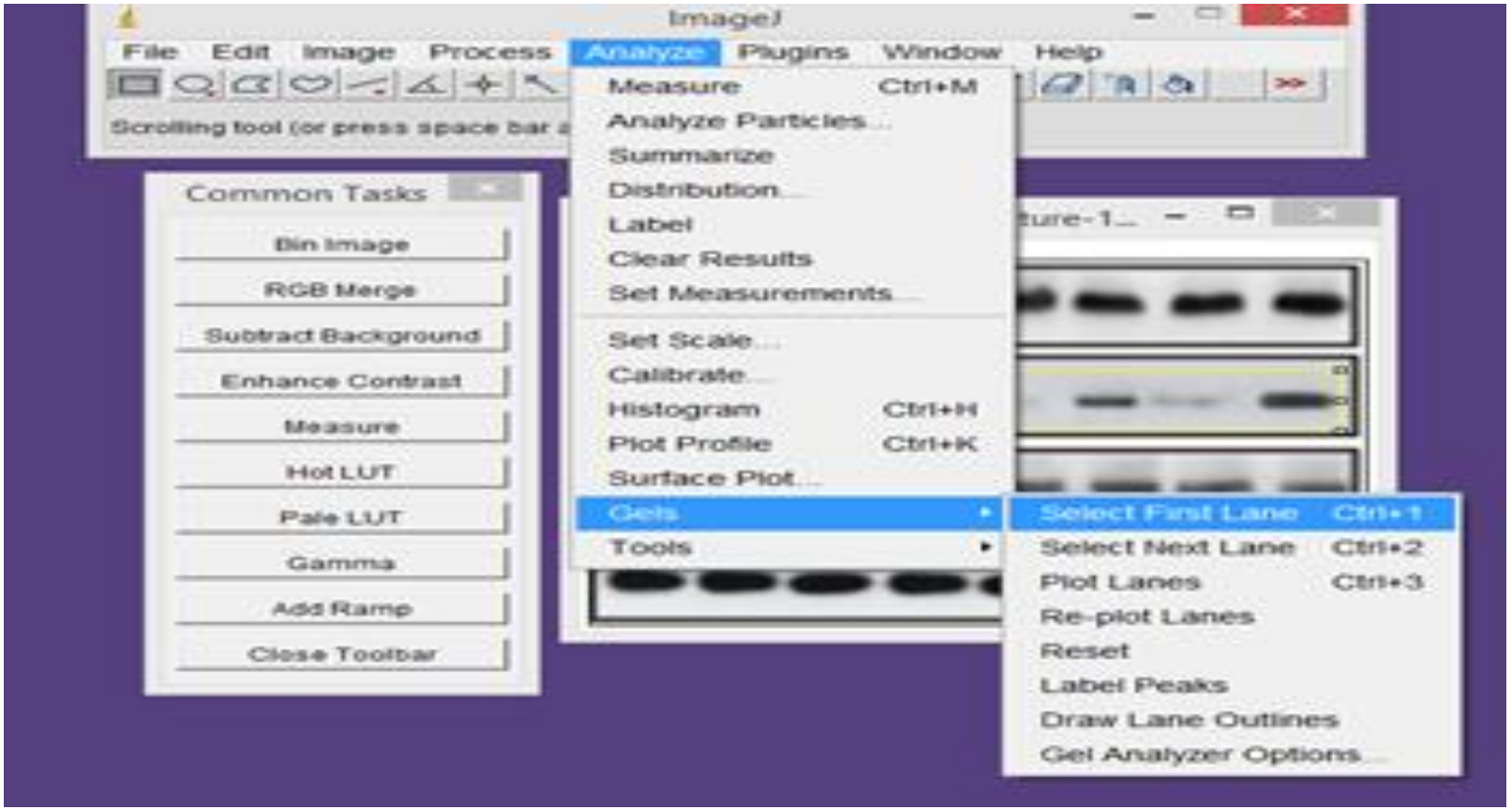


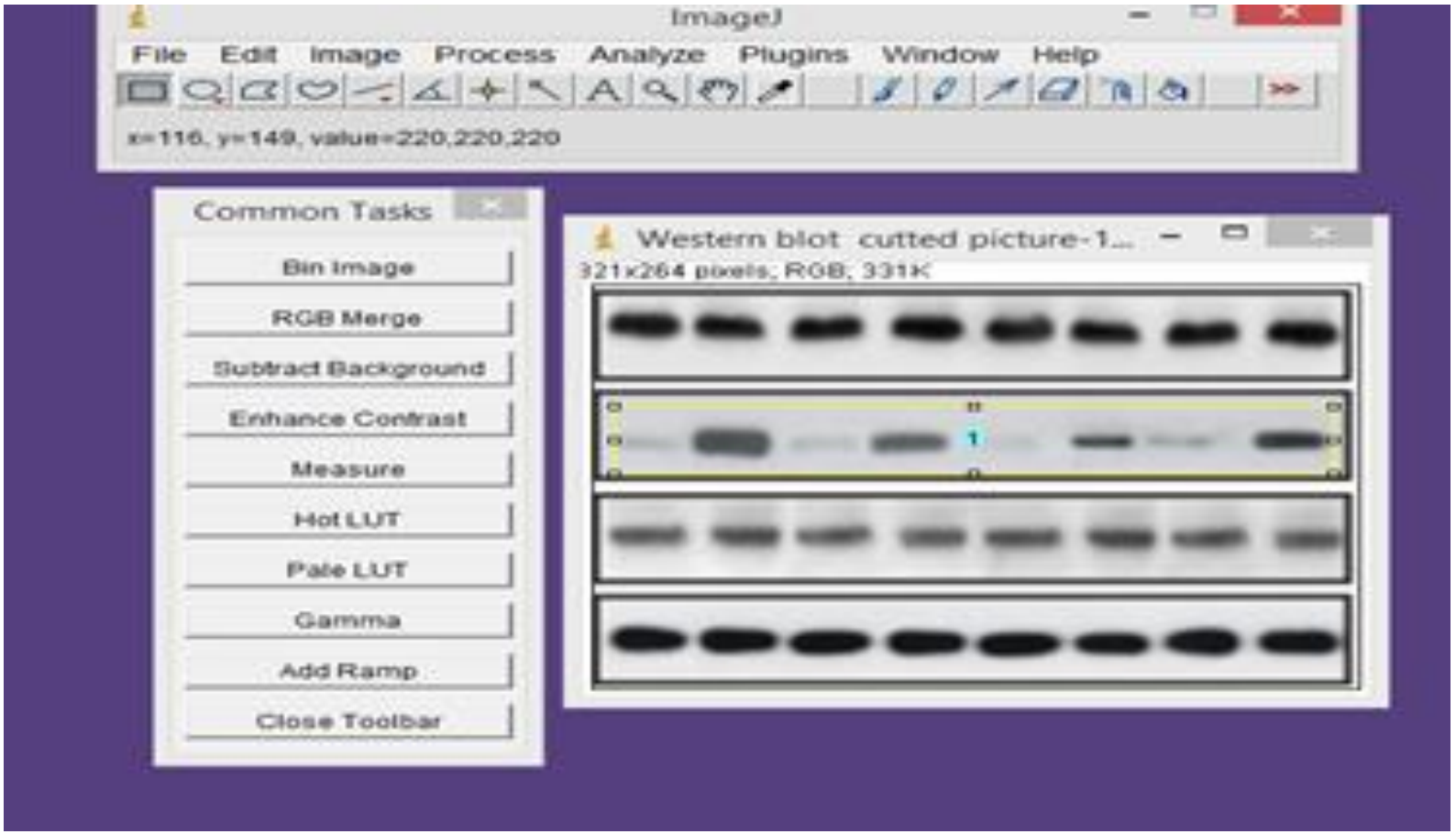
Image became as in view



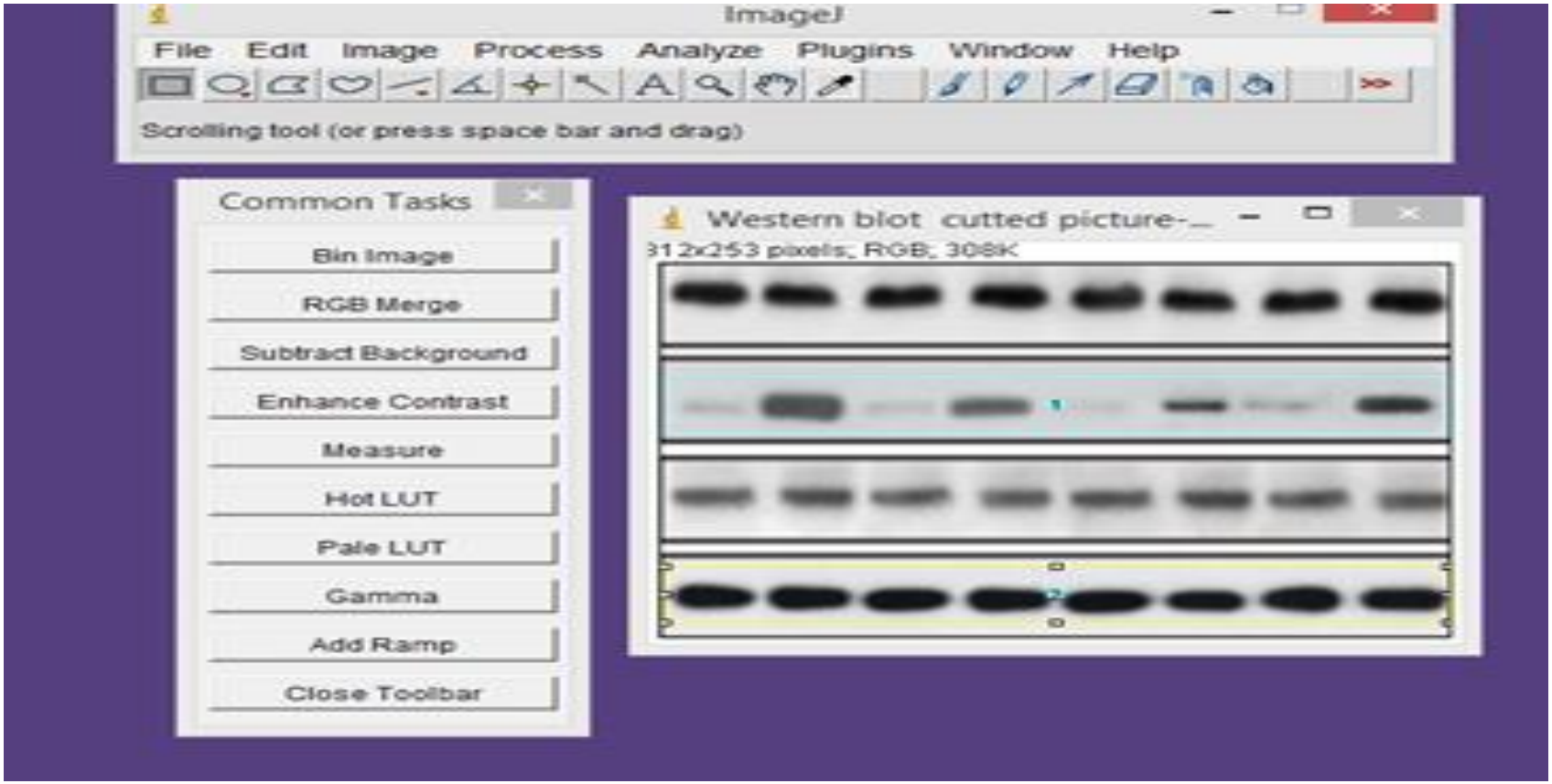
As in this image select one lane and the last one which is internal control as following



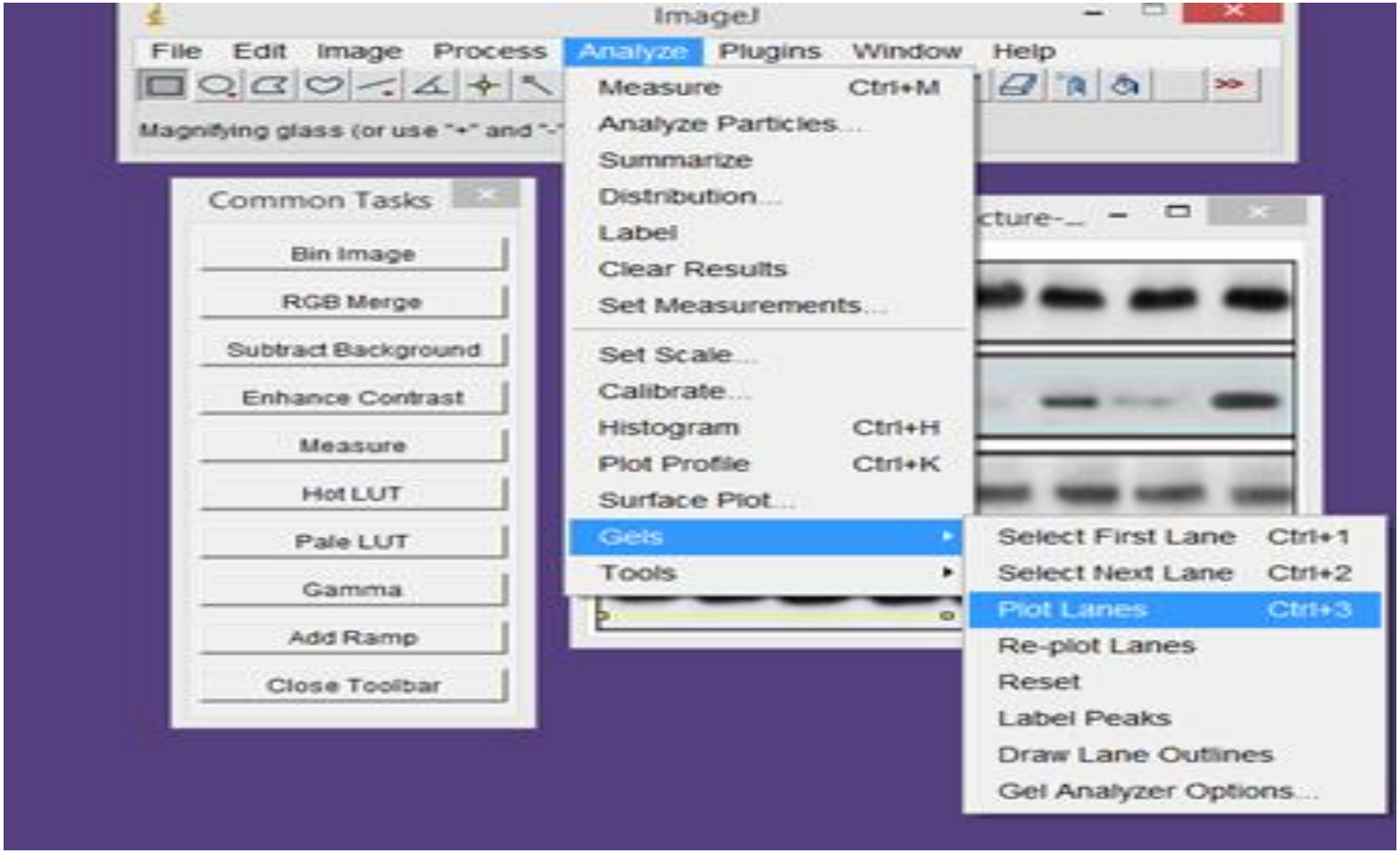
It will be selected with no. 1 in the middle



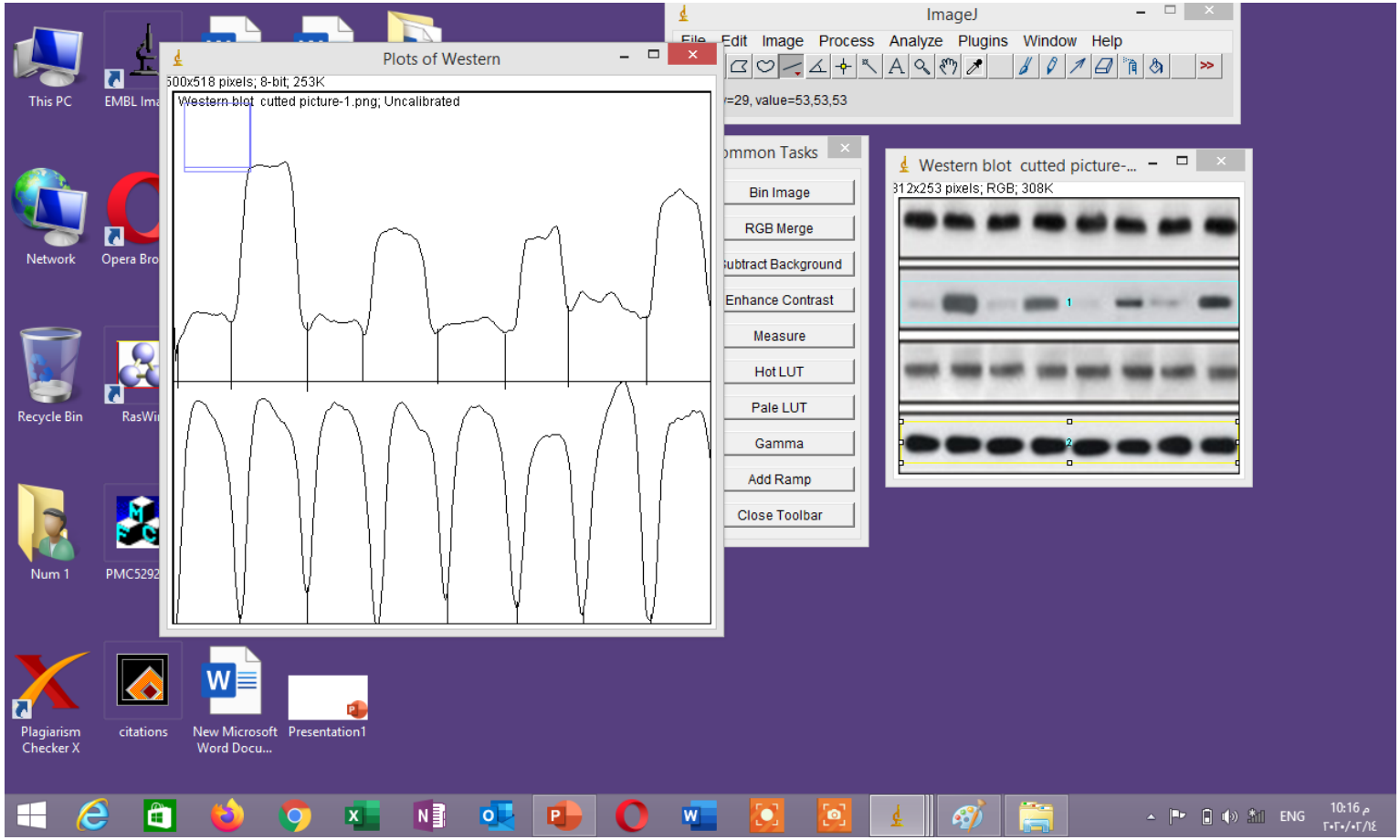
Move the selection to the last lane and analyze gel select next lane and no.2 will be added on this lane



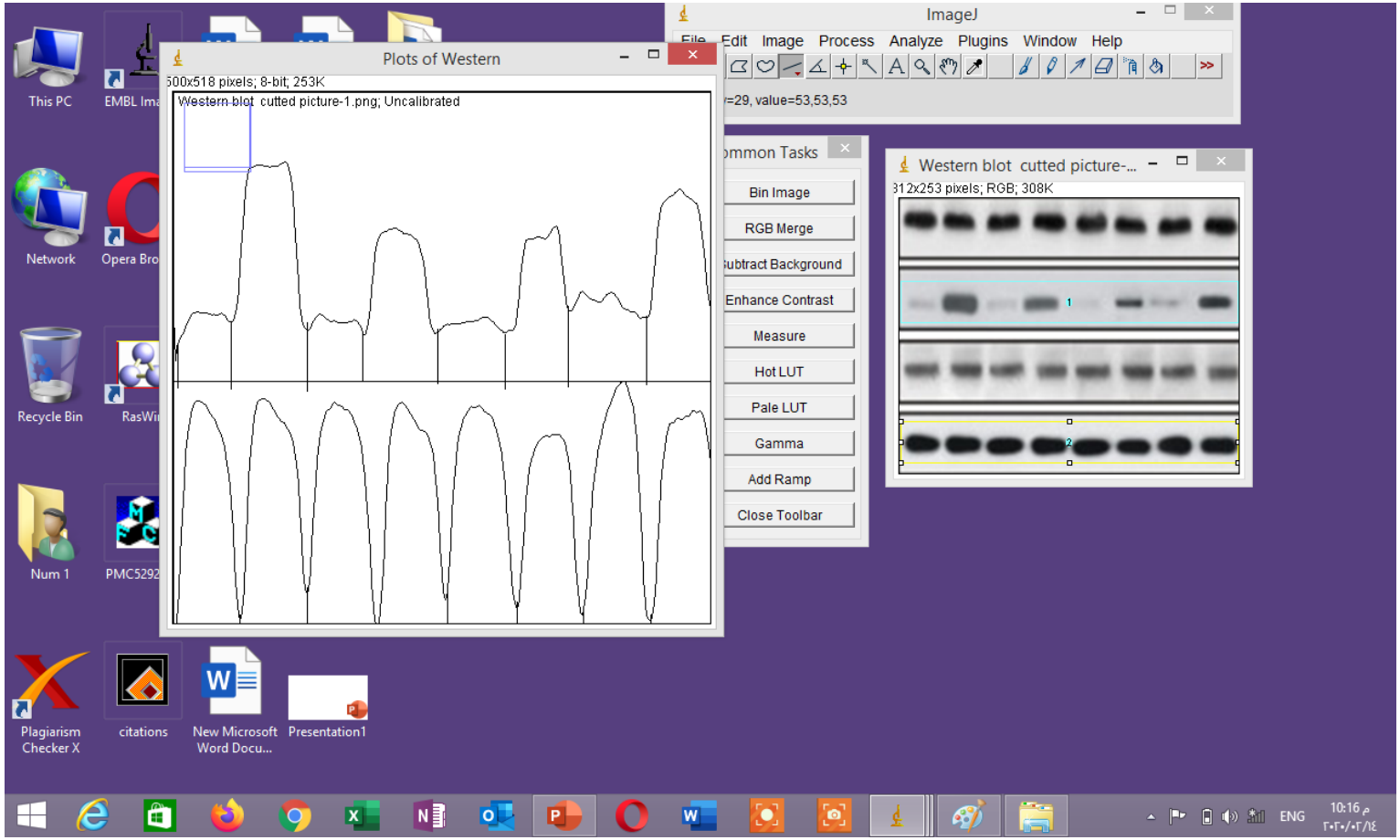
Analyze …gel …plot lanes



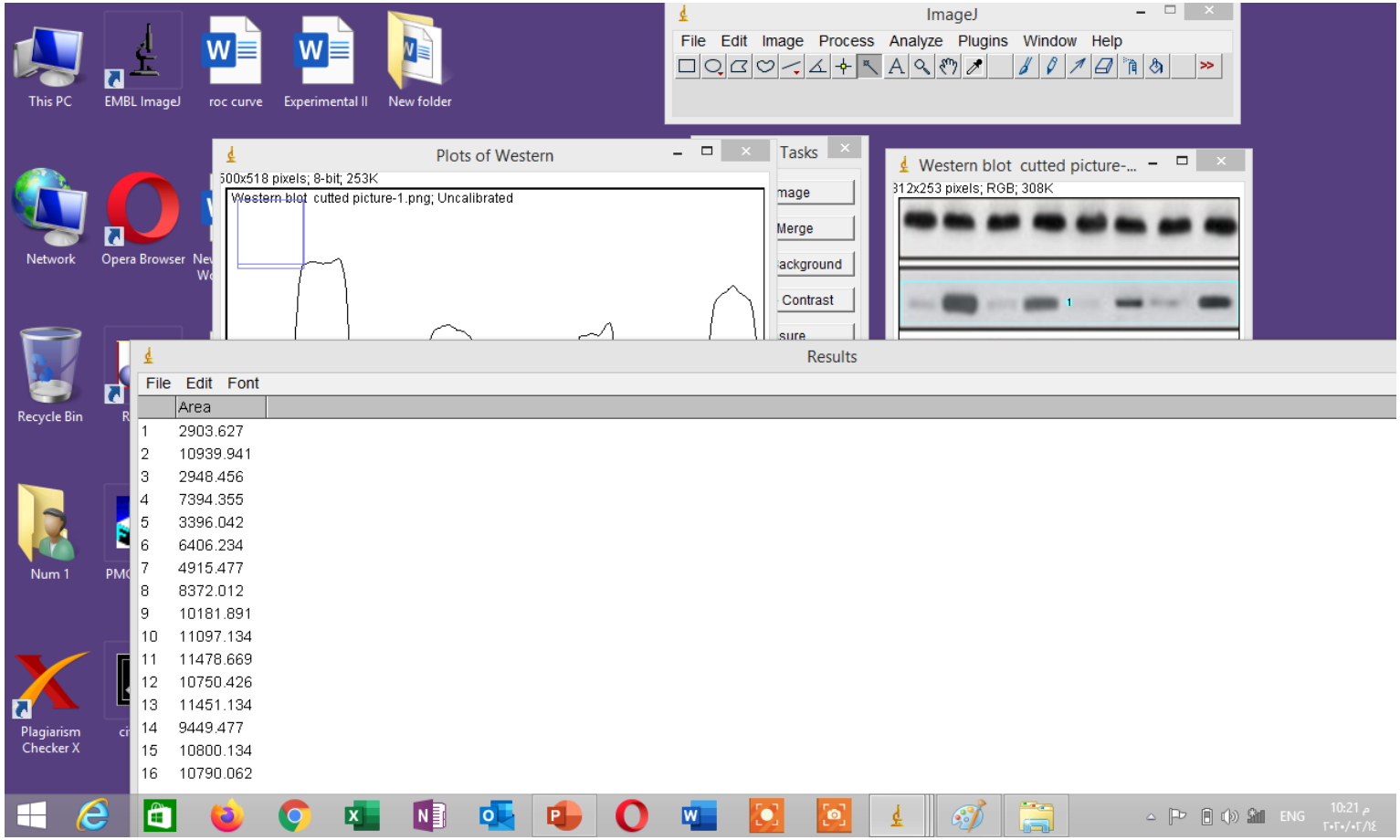
Make selection like this using shift and line tool



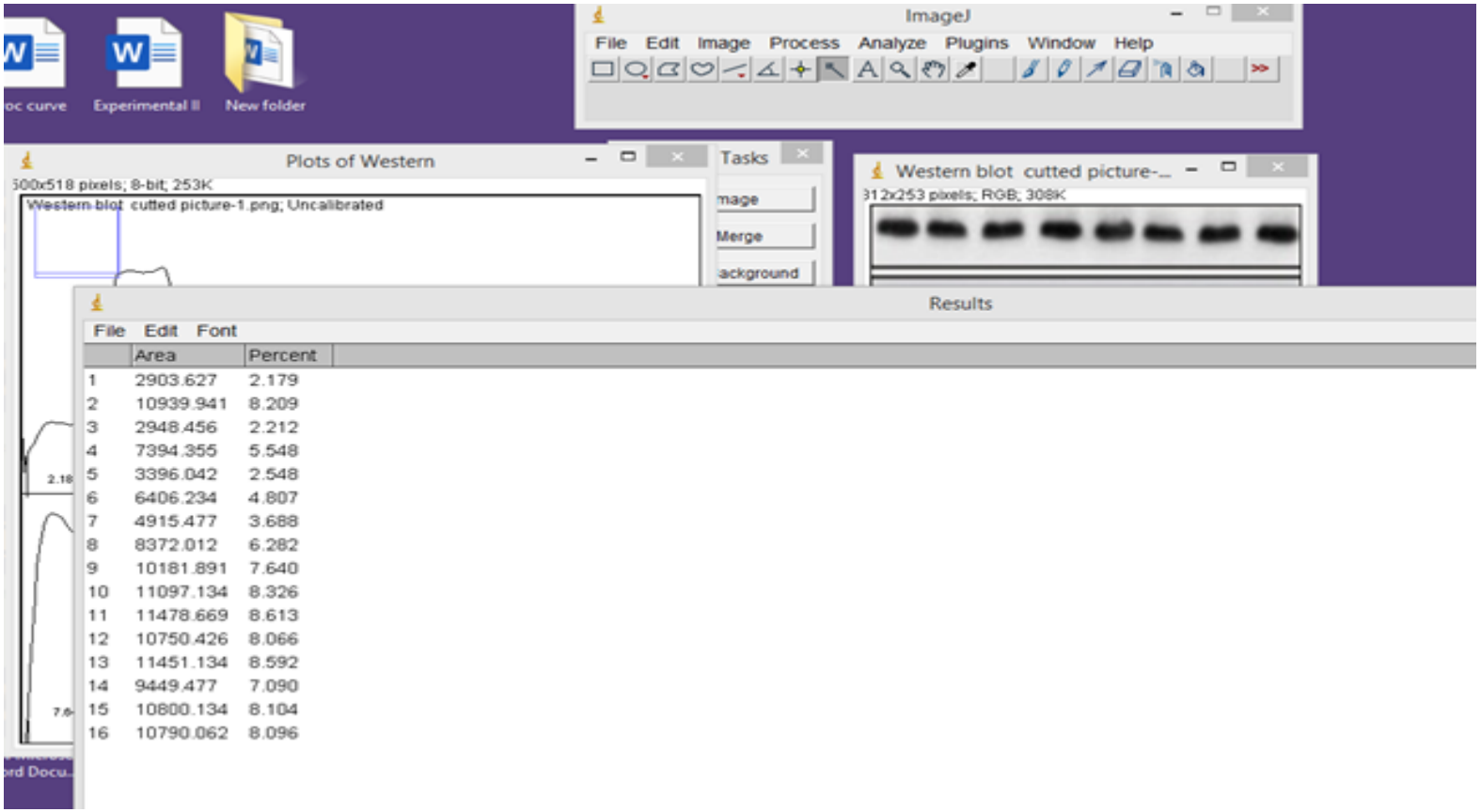
Use wand tool to analyze area of each band



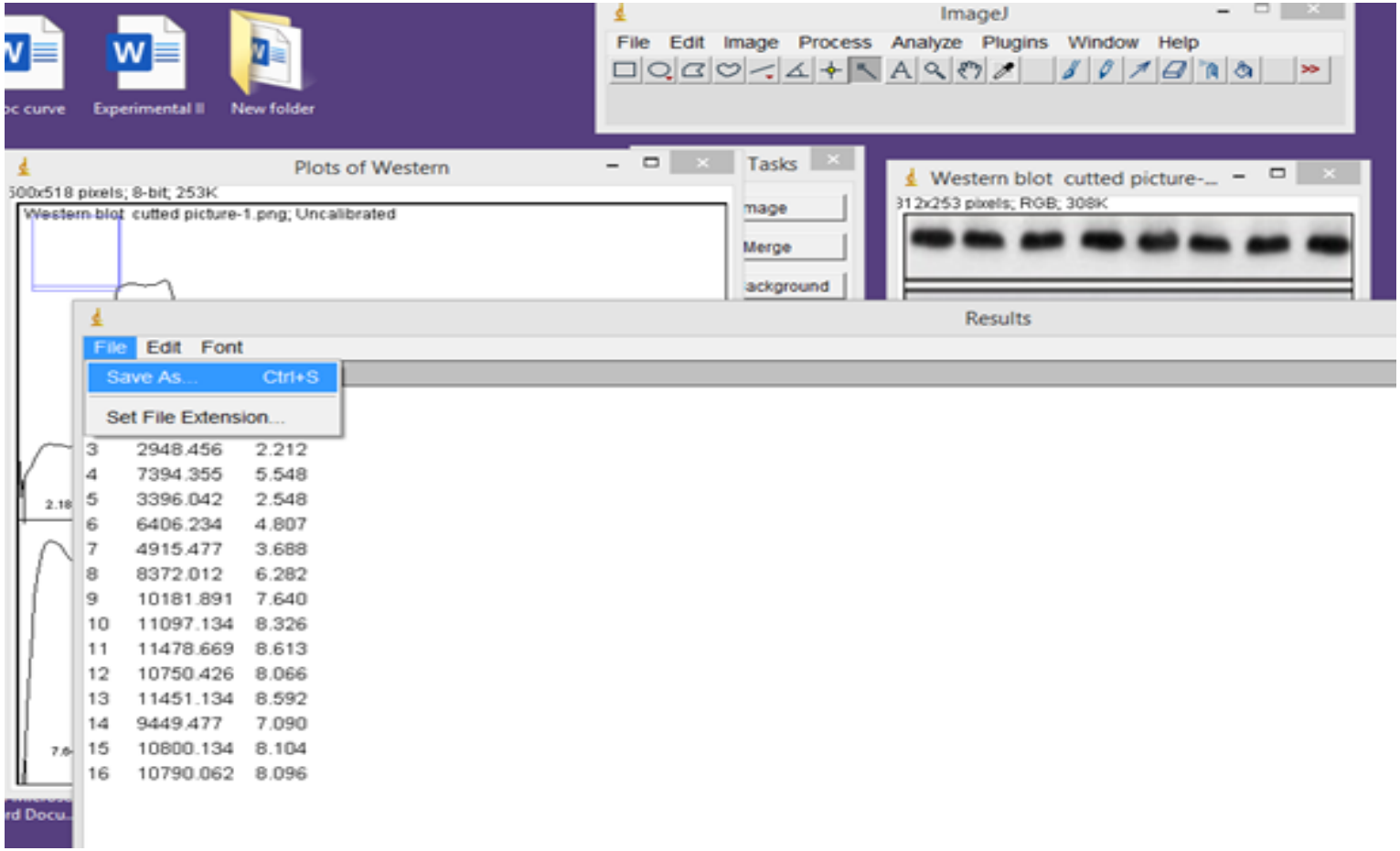
You just click wand tool and in the middle of area click you will get the area size in results file



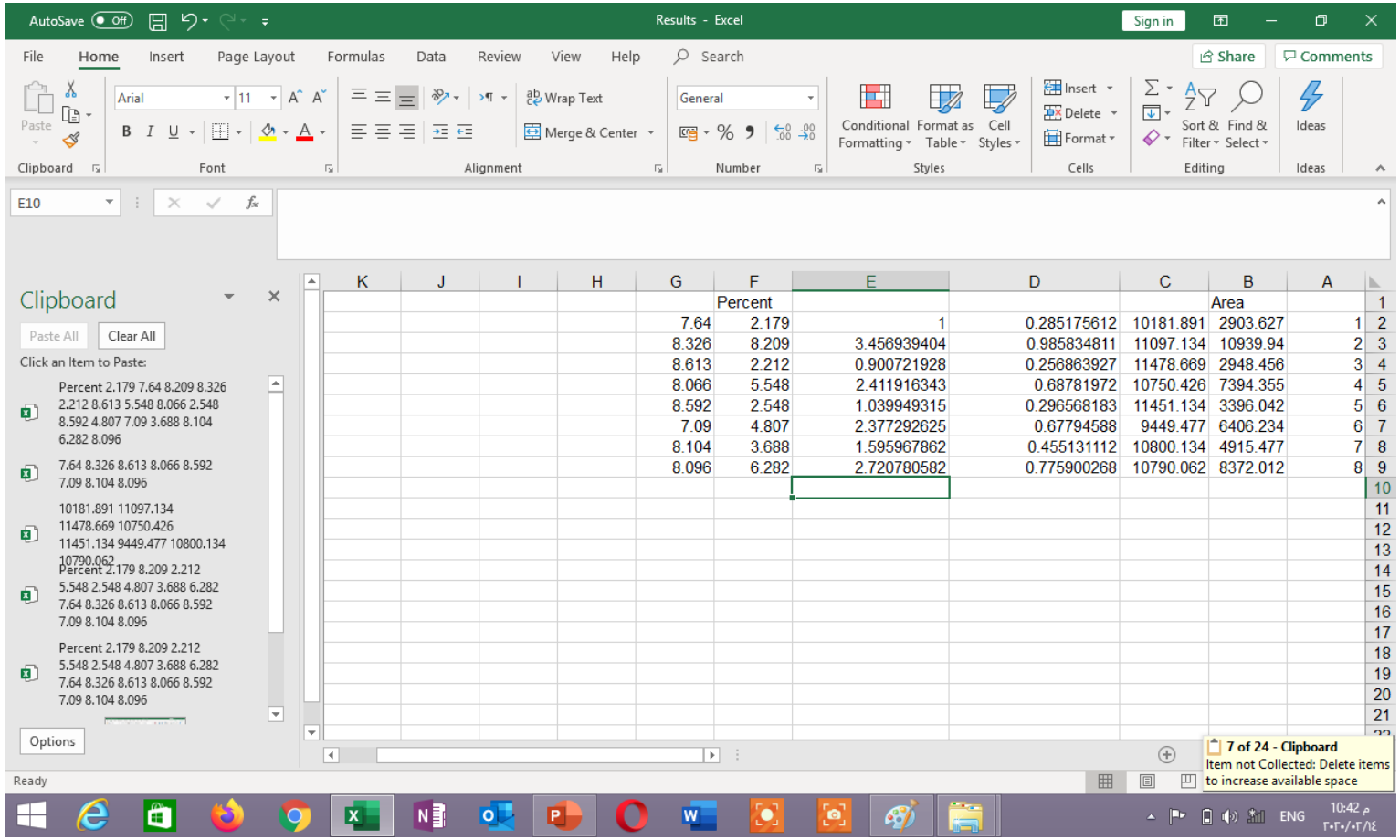
Click analyze gels then label peaks you will get the percent of area



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

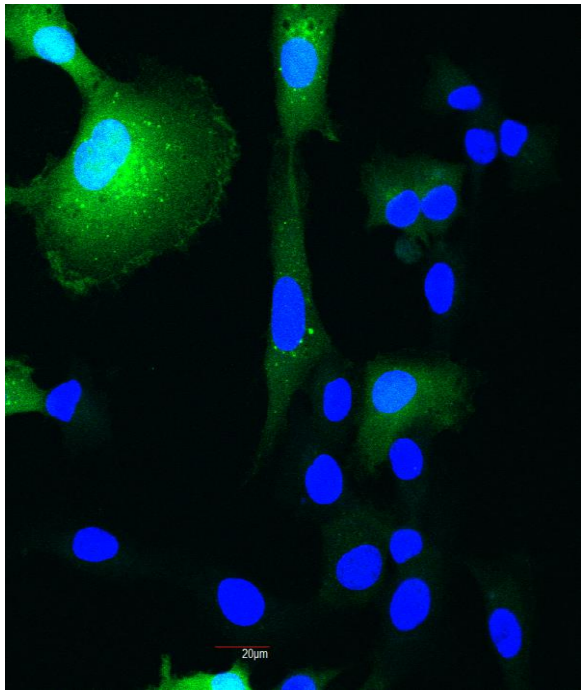


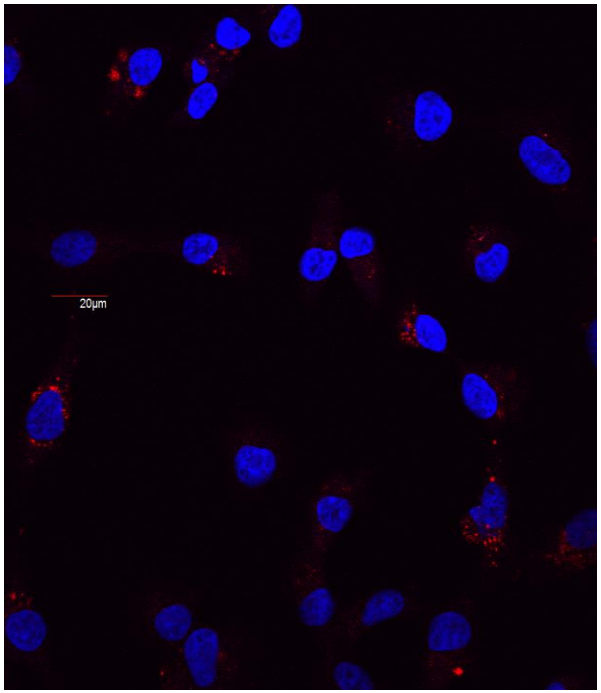
To get the fold change of each band first divide over that of each internal control then the resulted value on control value (non treated)

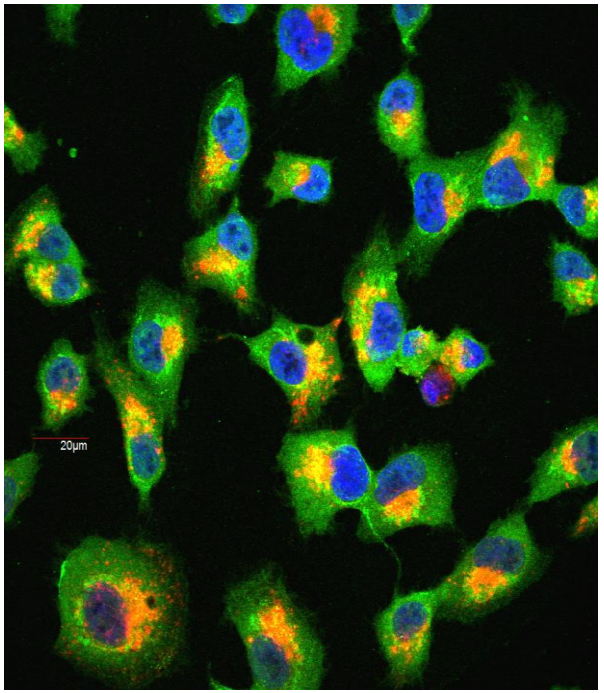


	G	F	E	D	C	B	A
1		Percent				Area	
2	7.64	2.179	1	0.285175612	10181.891	2903.627	1
3	8.326	8.209	3.456939404	0.985834811	11097.134	10939.94	2
4	8.613	2.212	0.900721928	0.256863927	11478.669	2948.456	3
5	8.066	5.548	2.411916343	0.68781972	10750.426	7394.355	4
6	8.592	2.548	1.039949315	0.296568183	11451.134	3396.042	5
7	7.09	4.807	2.377292625	0.67794588	9449.477	6406.234	6
8	8.104	3.688	1.595967862	0.455131112	10800.134	4915.477	7
9	8.096	6.282	2.720780582	0.775900268	10790.062	8372.012	8

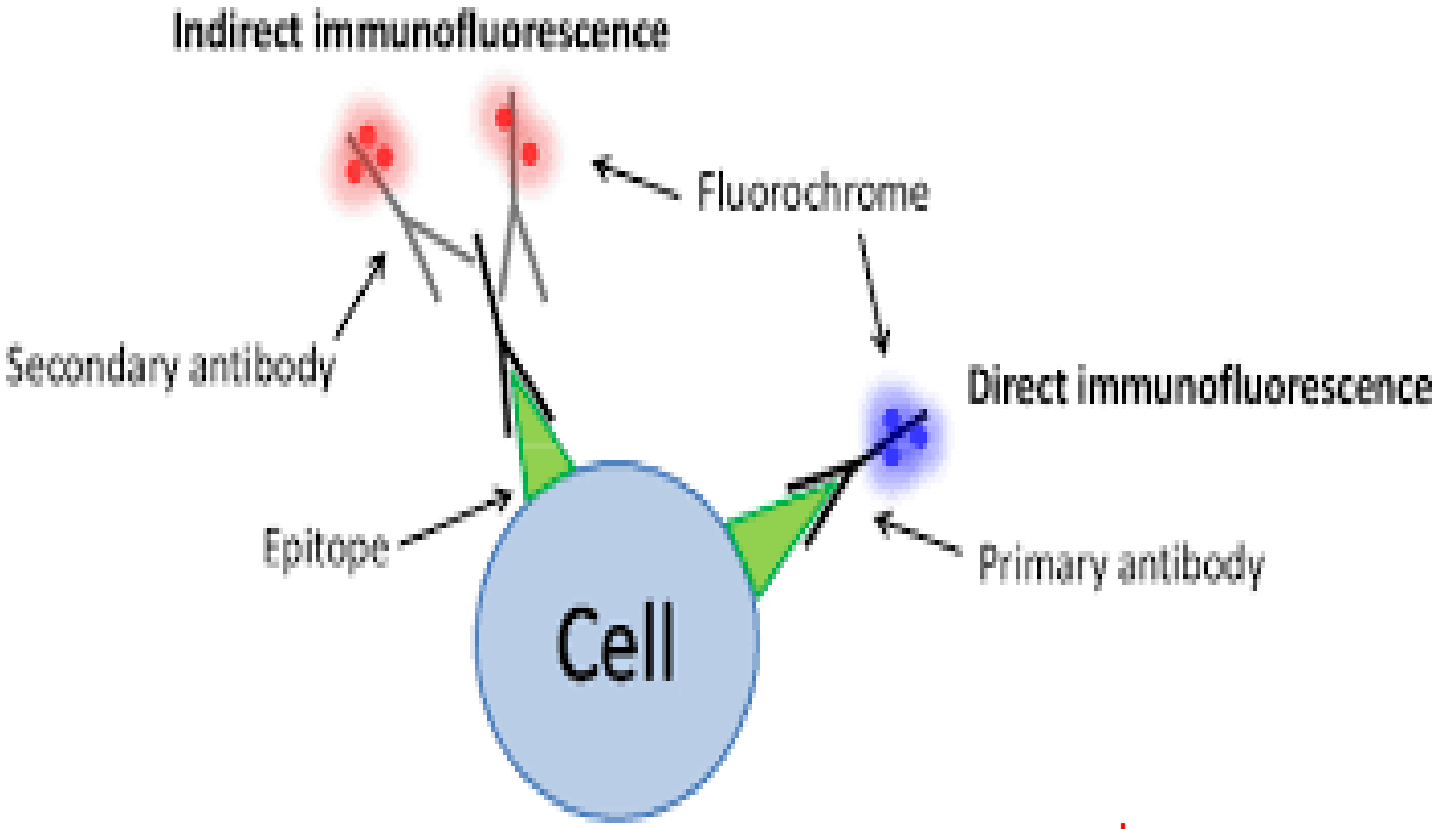
Analysis of fluorescence signal you get from confocal microscopy





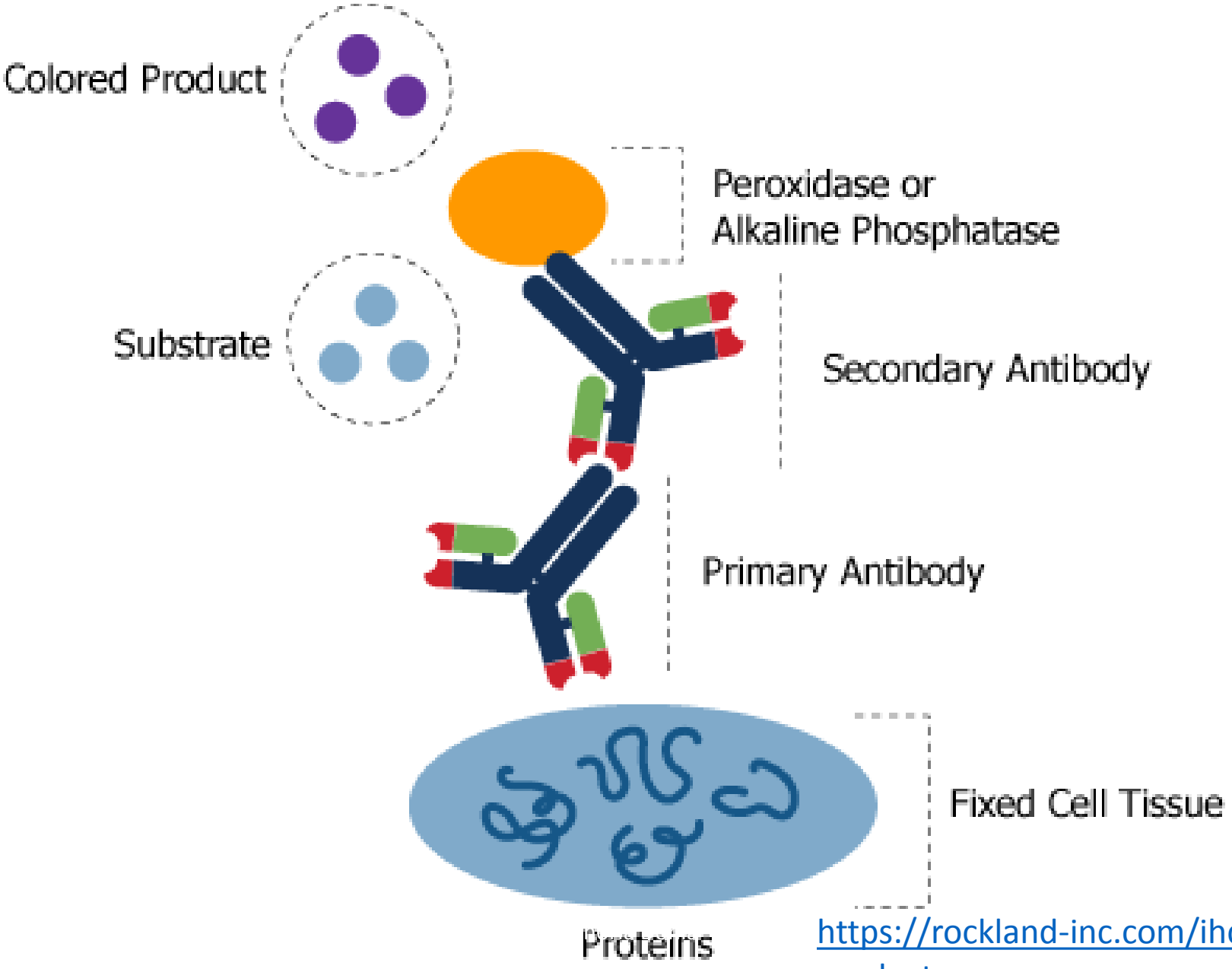


Immunofluorescence



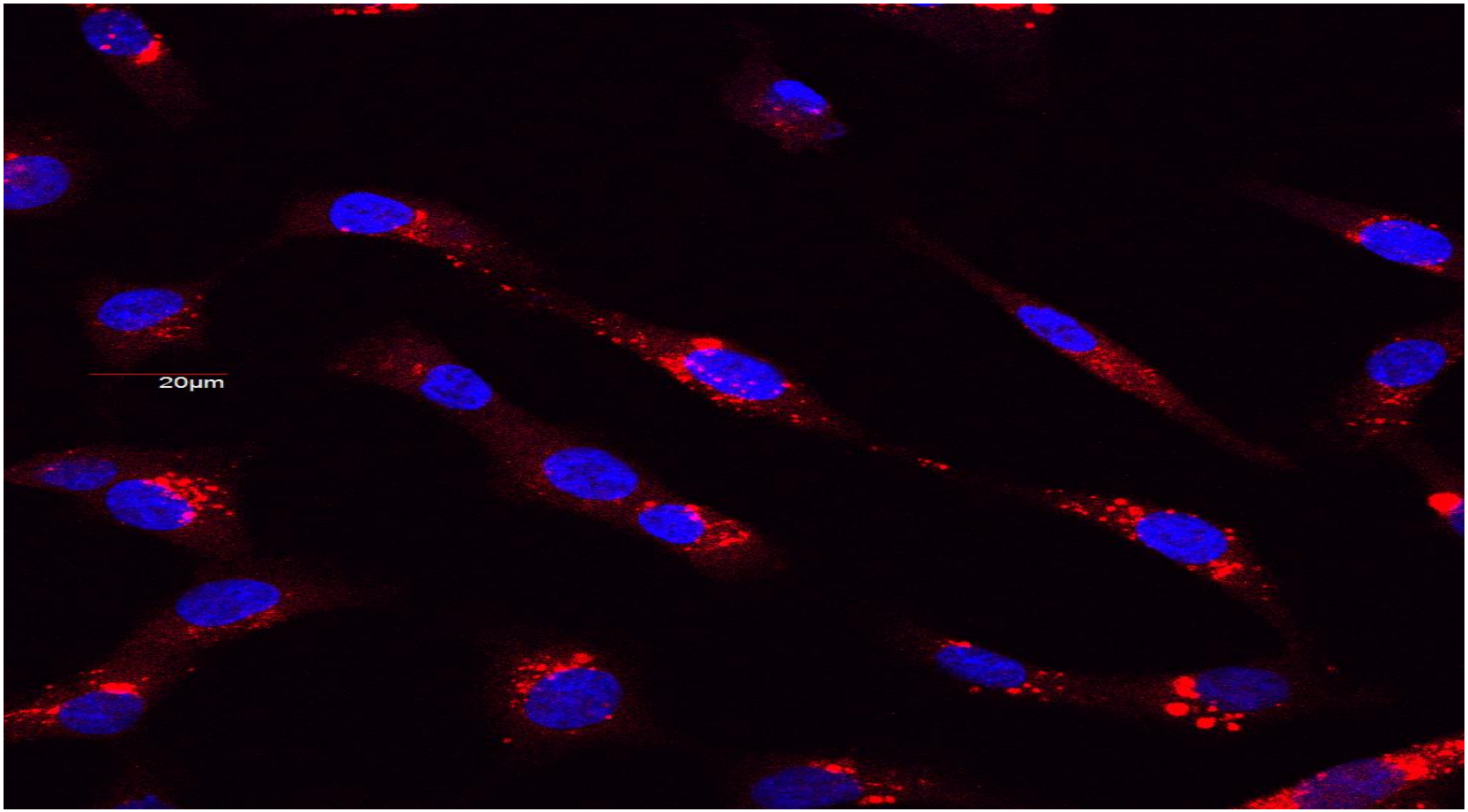
Jove.com

Immunohistochemistry Schematic

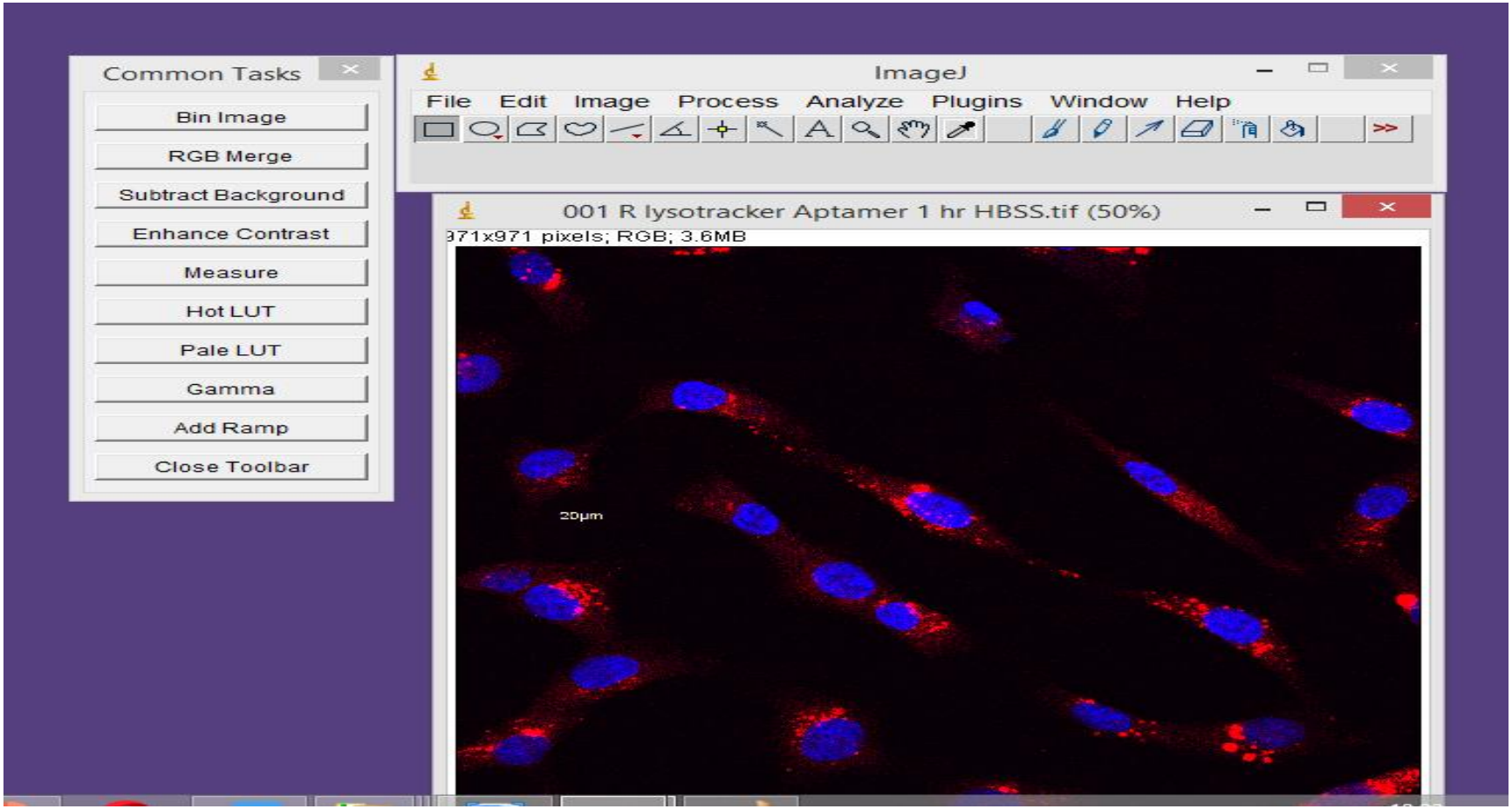


https://rockland-inc.com/ihc-products.aspx

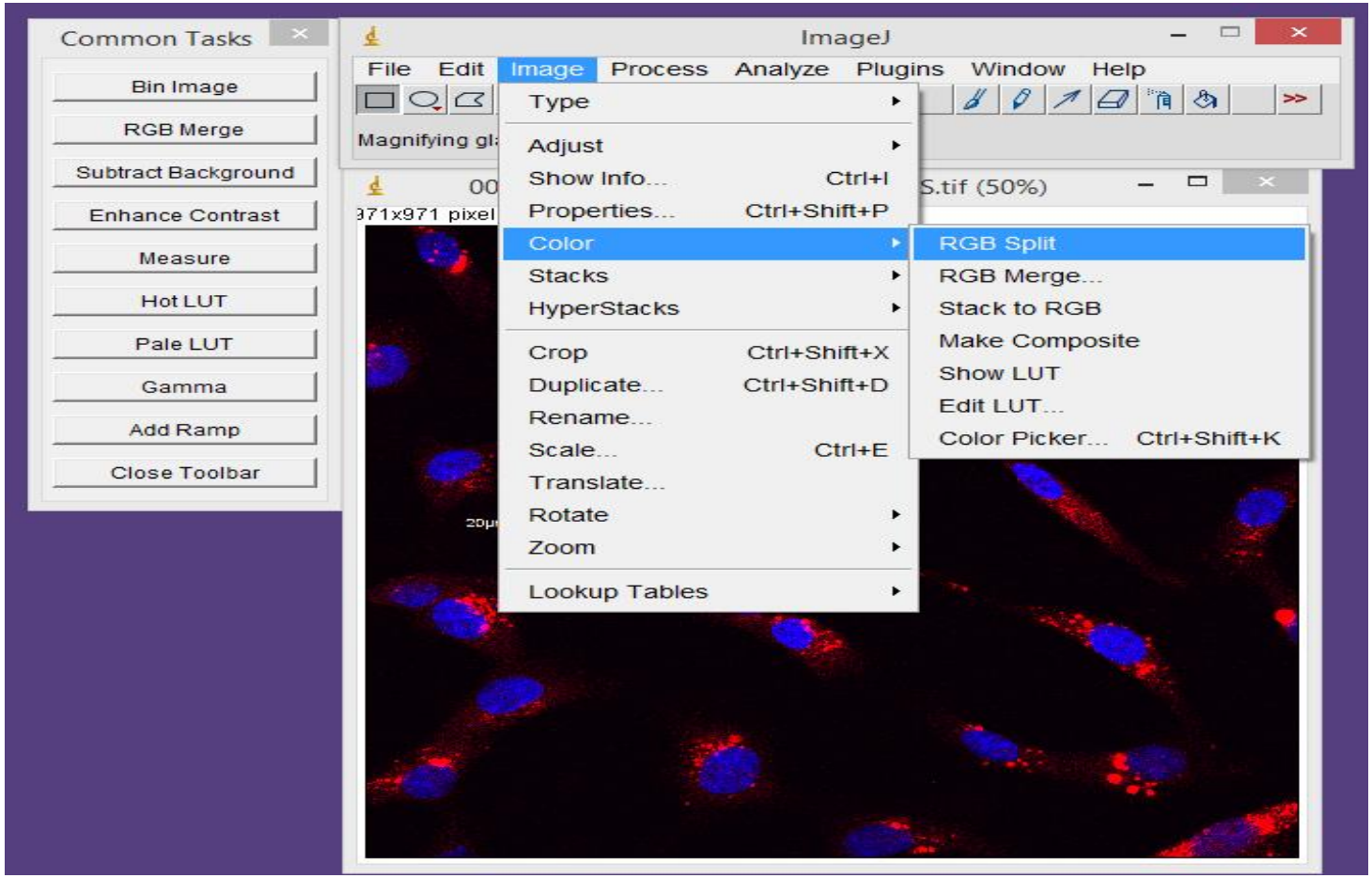
Analyzing fluorescence in this image (lysotracker staining)



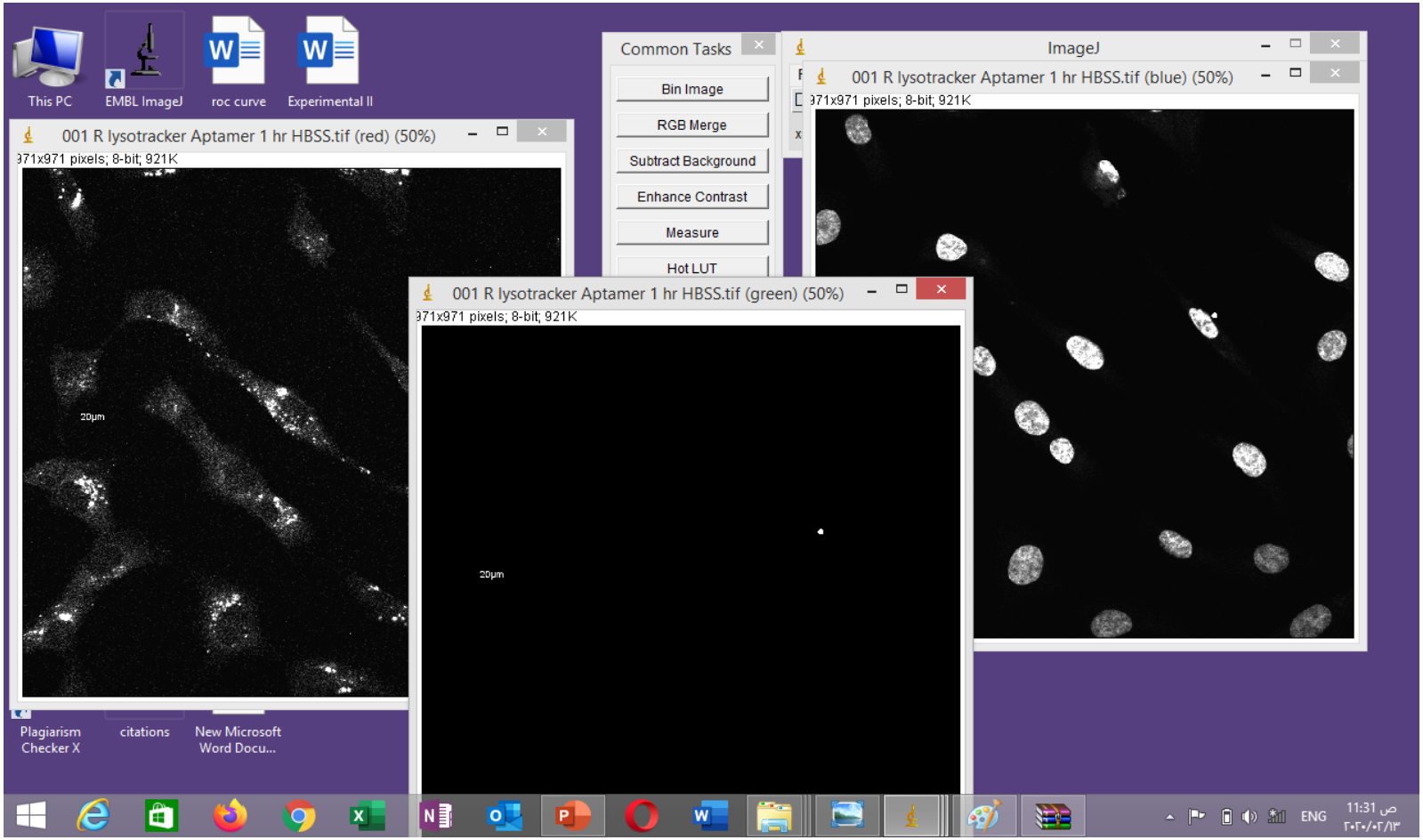
Open the image in image J



Split colors in this image as following



You will get three images with different color layers

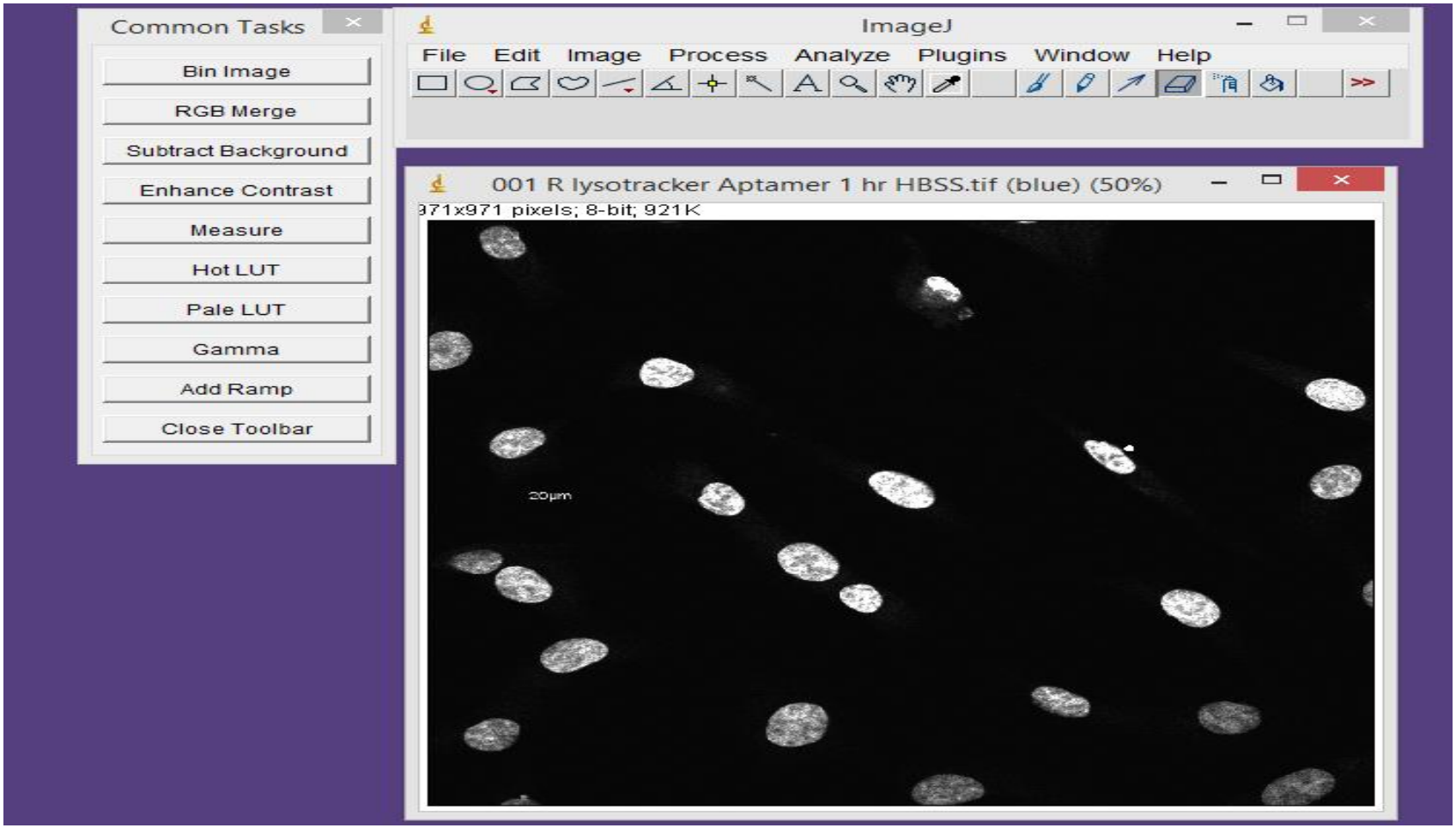


Note:

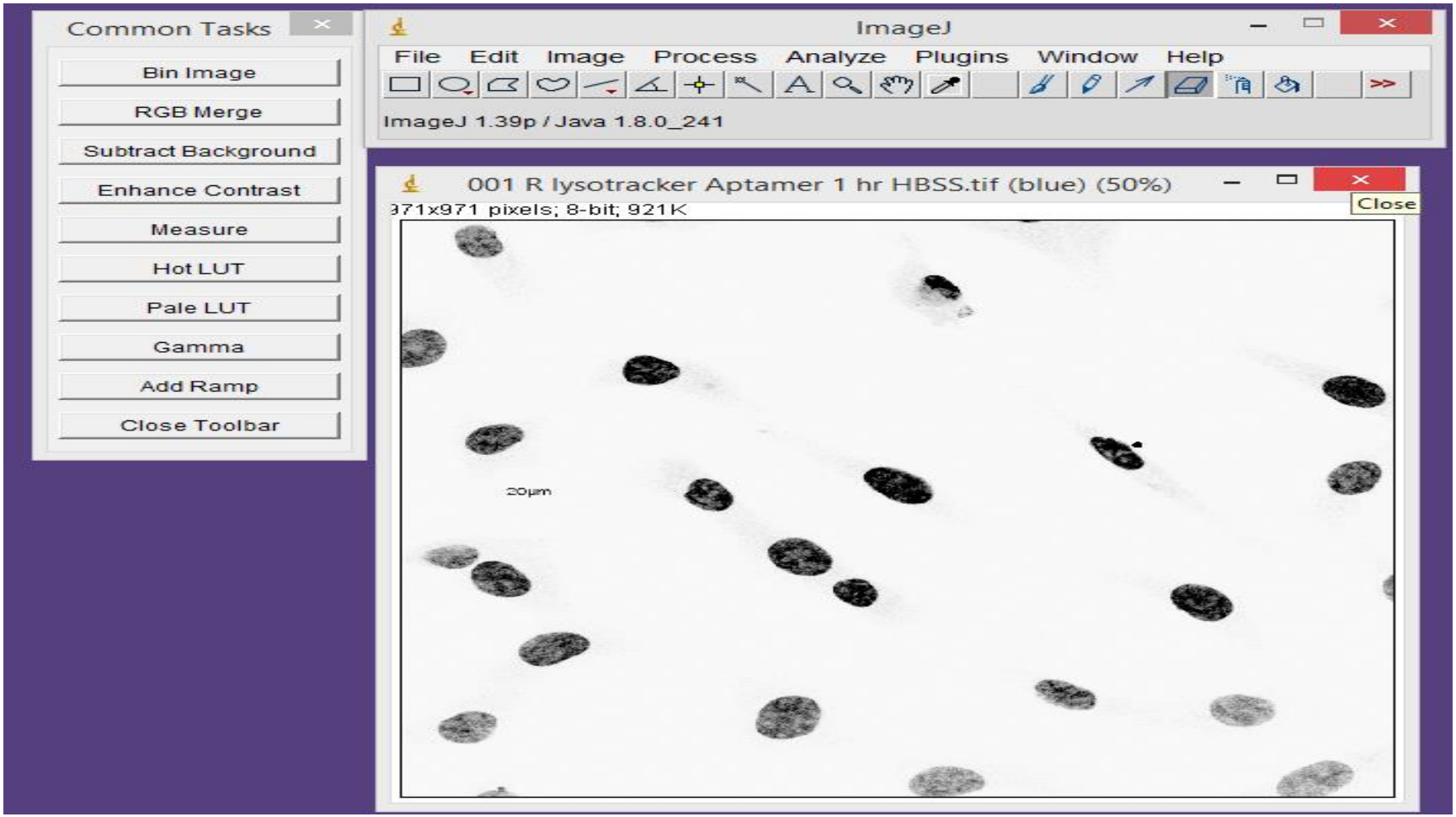
- **The green layer is not included as you have only red fluorescence and blue for nucleus and we will use blue layer later in counting cells.**

- 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



Edit …..invert


Common Tasks
Bin Image
RGB Merge
Subtract Background
Enhance Contrast
Measure
Hot LUT
Pale LUT
Gamma
Add Ramp
Close Toolbar
ImageJ
File Edit Image Process Analyze Plugins Window Help
ImageJ 1.39p / Java 1.8.0_241
001 R lysotracker Aptamer 1 hr HBSS.tif (blue) (50%)
971x971 pixels; 8-bit; 921K
Close
20µm

Remove the diameter of cell (as no.) by eraser

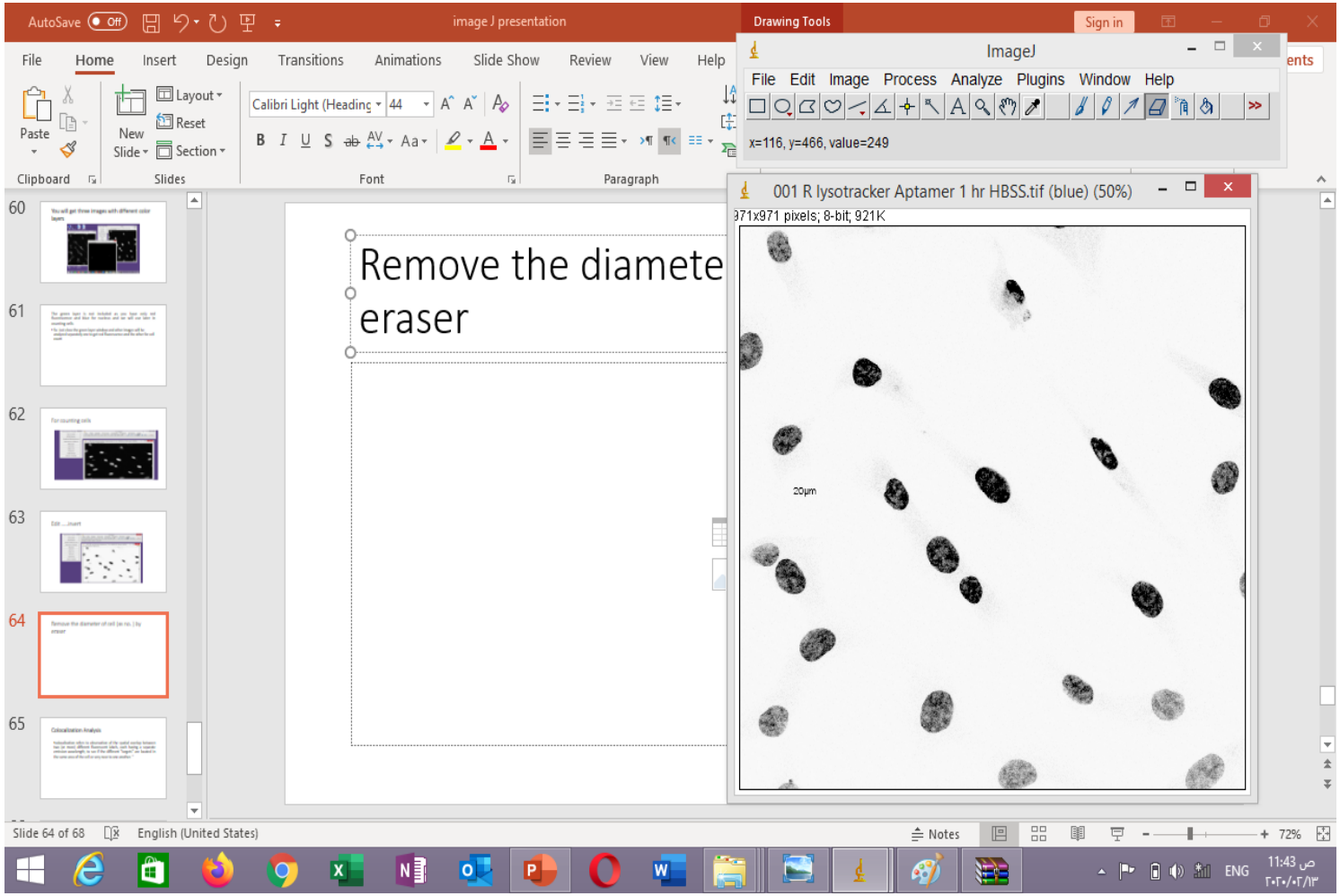
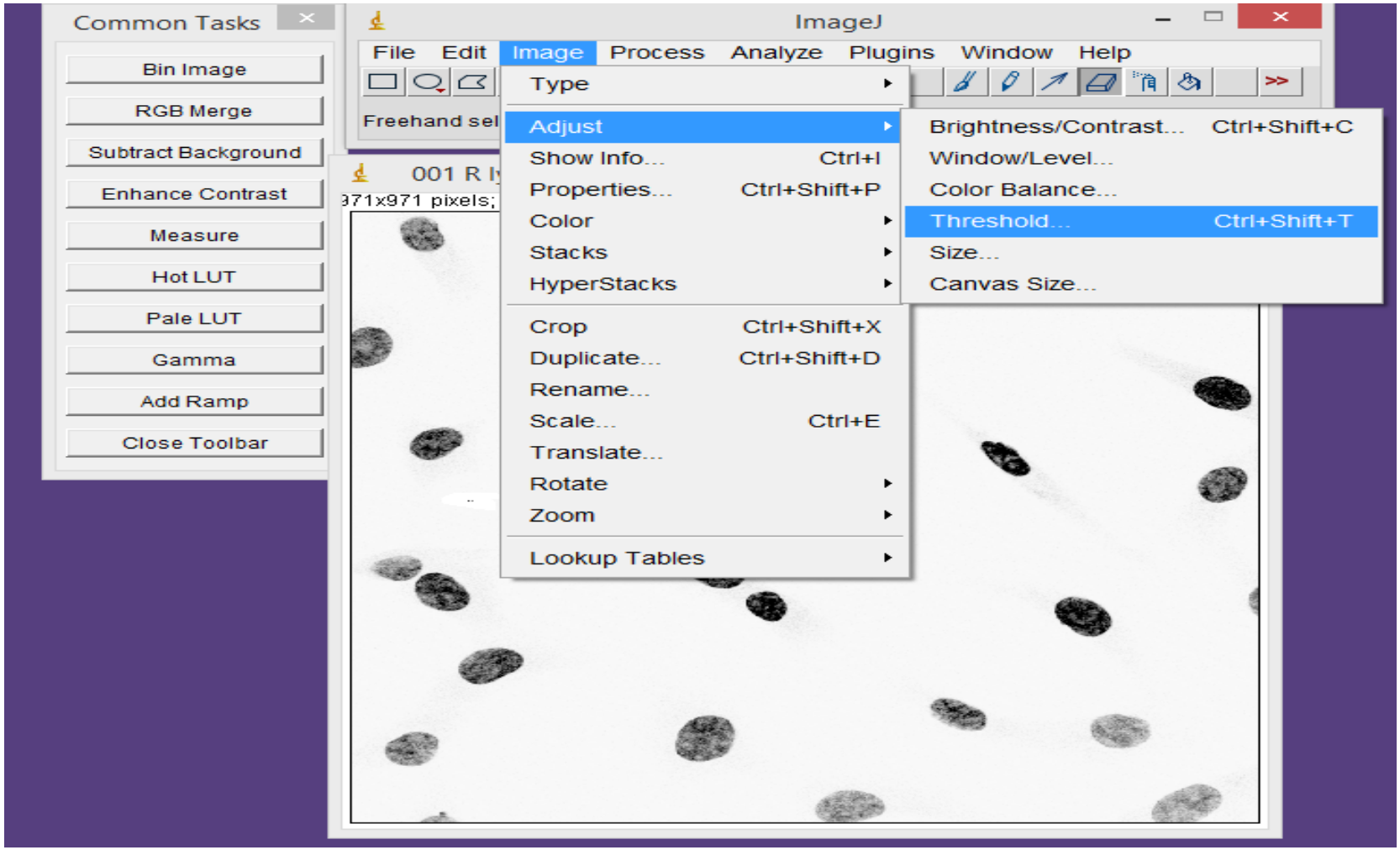
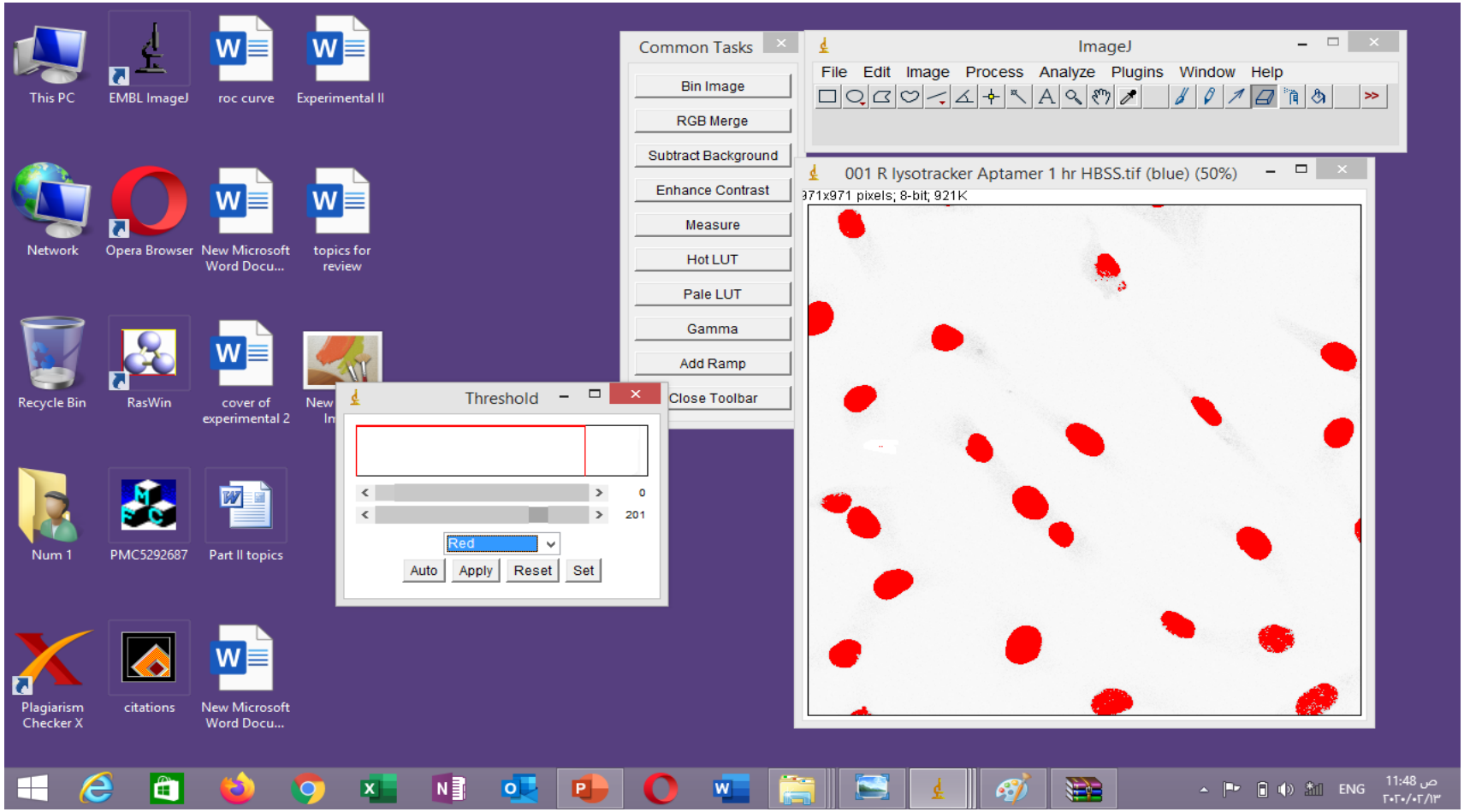


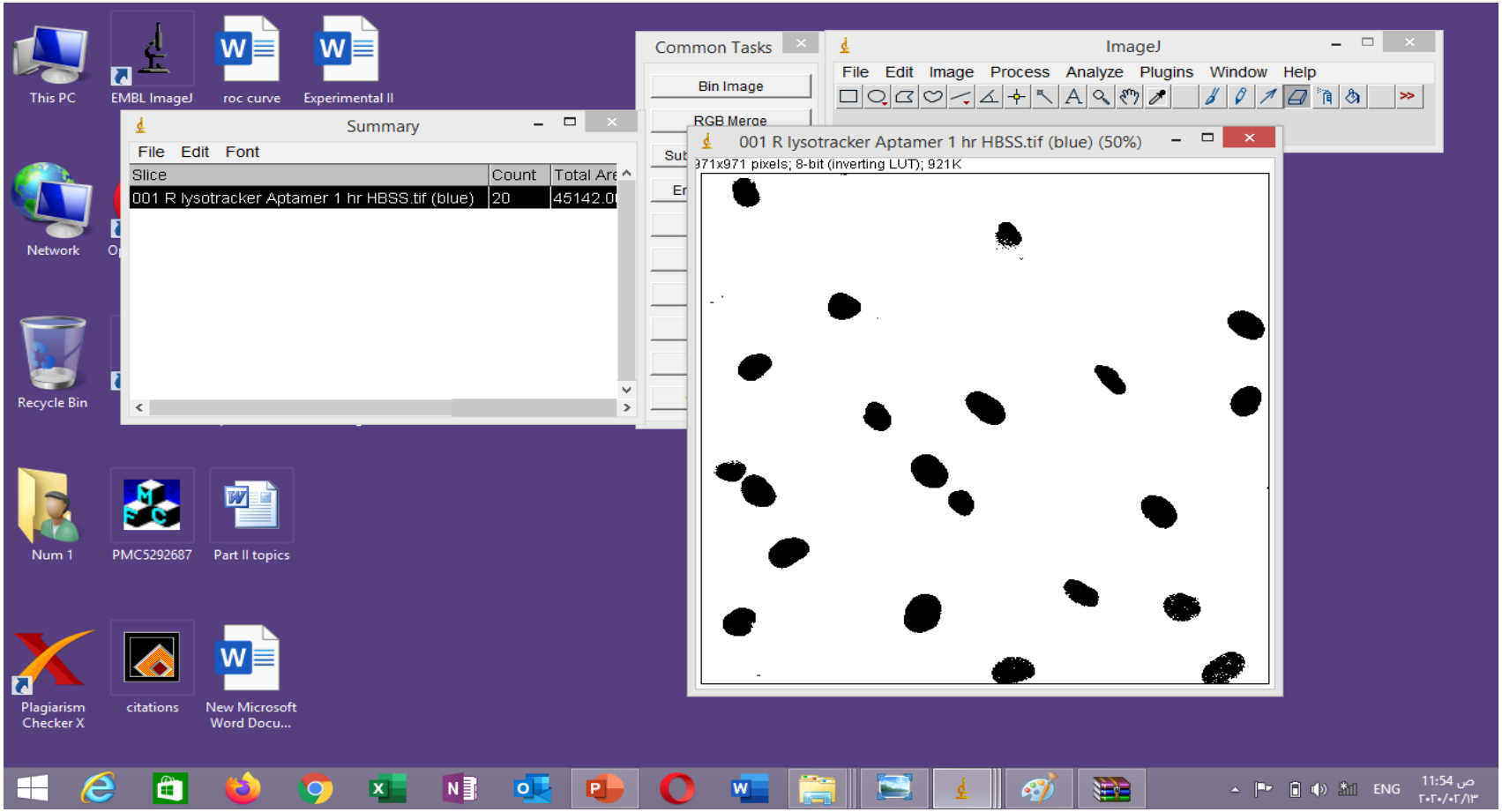
Image -----adjust-----threshold



Move the down scroll of threshold window till adjusting nuclei view



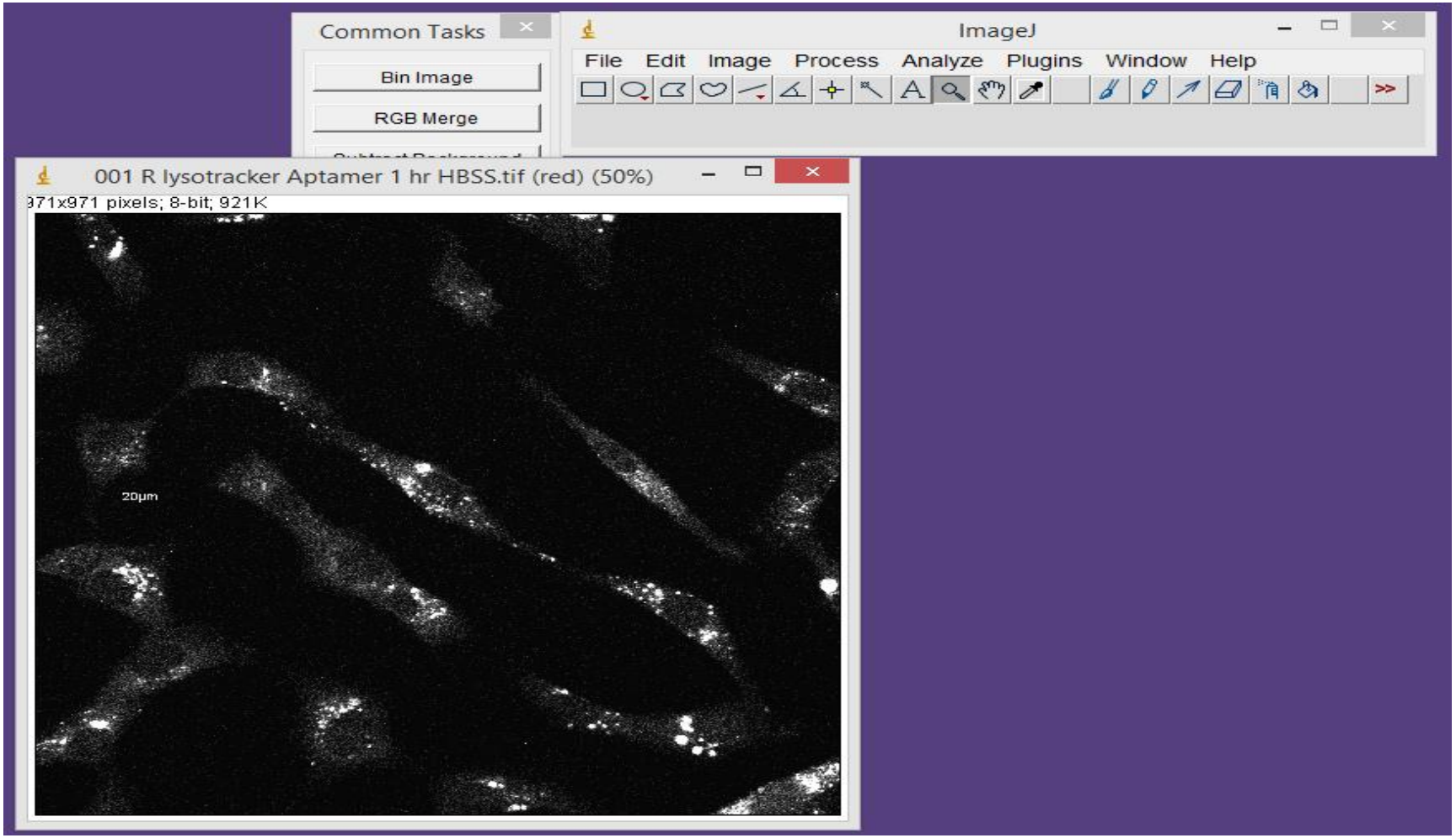
Click apply and analyze---particles ...60-infinity



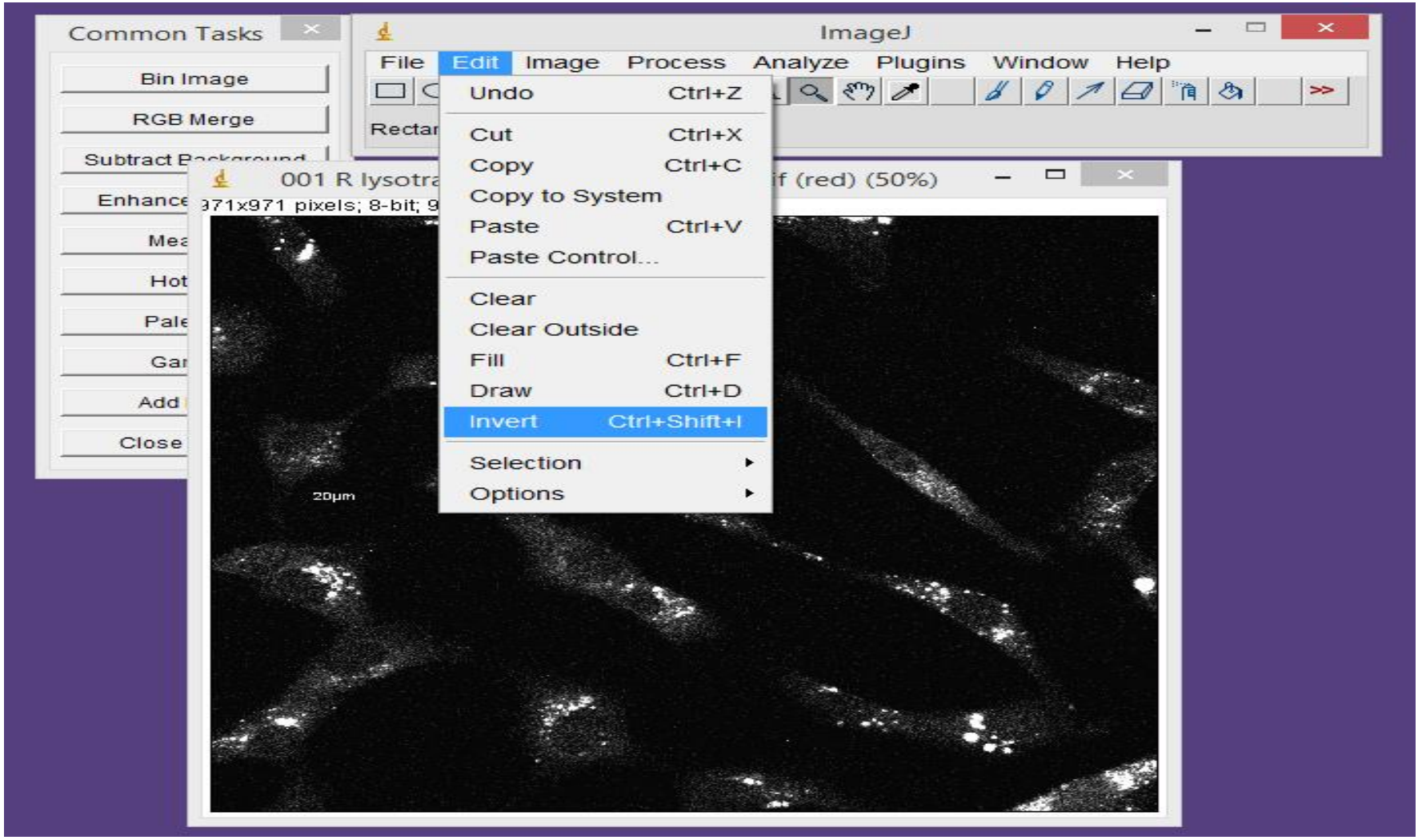
Important

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

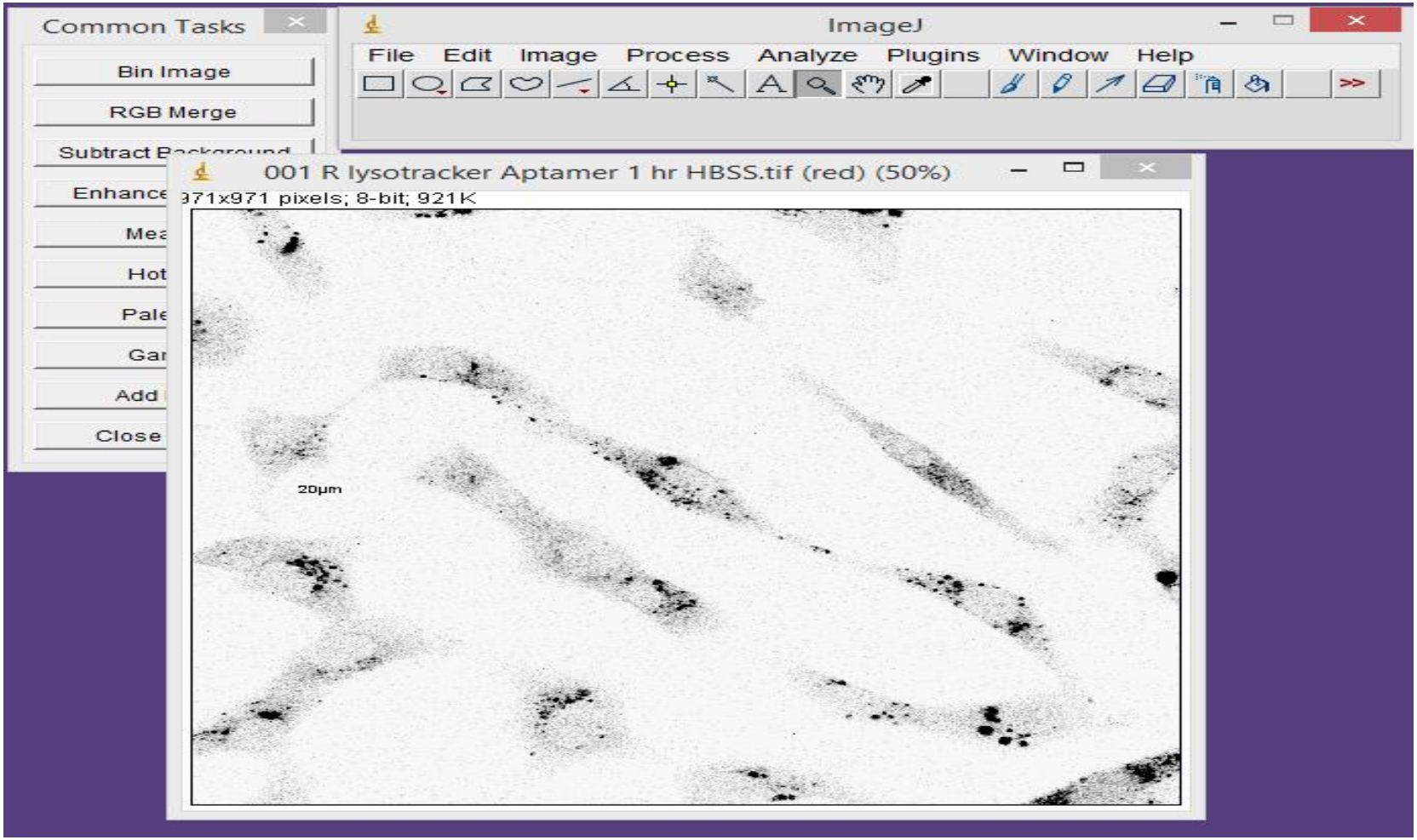
Analysis of red fluorescence



Edit ------invert


Common Tasks
Bin Image
RGB Merge
Subtract Background
Enhance
Mea
Hot
Pale
Gar
Add
Close
ImageJ
File
Edit
Image
Process
Analyze
Plugins
Window
Help
Undo Ctrl+Z
Cut Ctrl+X
Copy Ctrl+C
Copy to System
Paste Ctrl+V
Paste Control...
Clear
Clear Outside
Fill Ctrl+F
Draw Ctrl+D
Invert Ctrl+Shift+I
Selection
Options
001 R lysotra
971x971 pixels; 8-bit; 9
if (red) (50%)
20µm

You will get white background and black fluorescent signal



Remove diameter by eraser

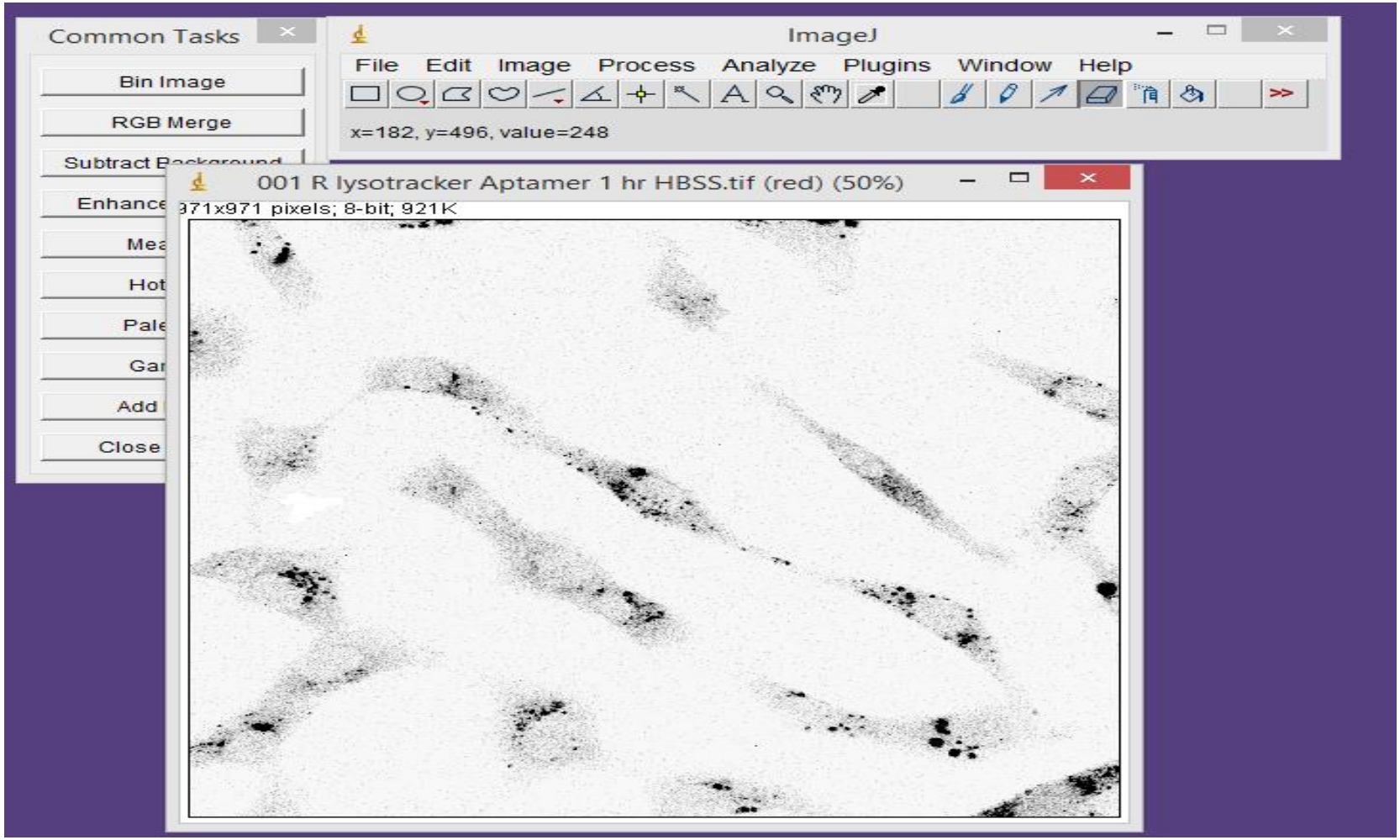
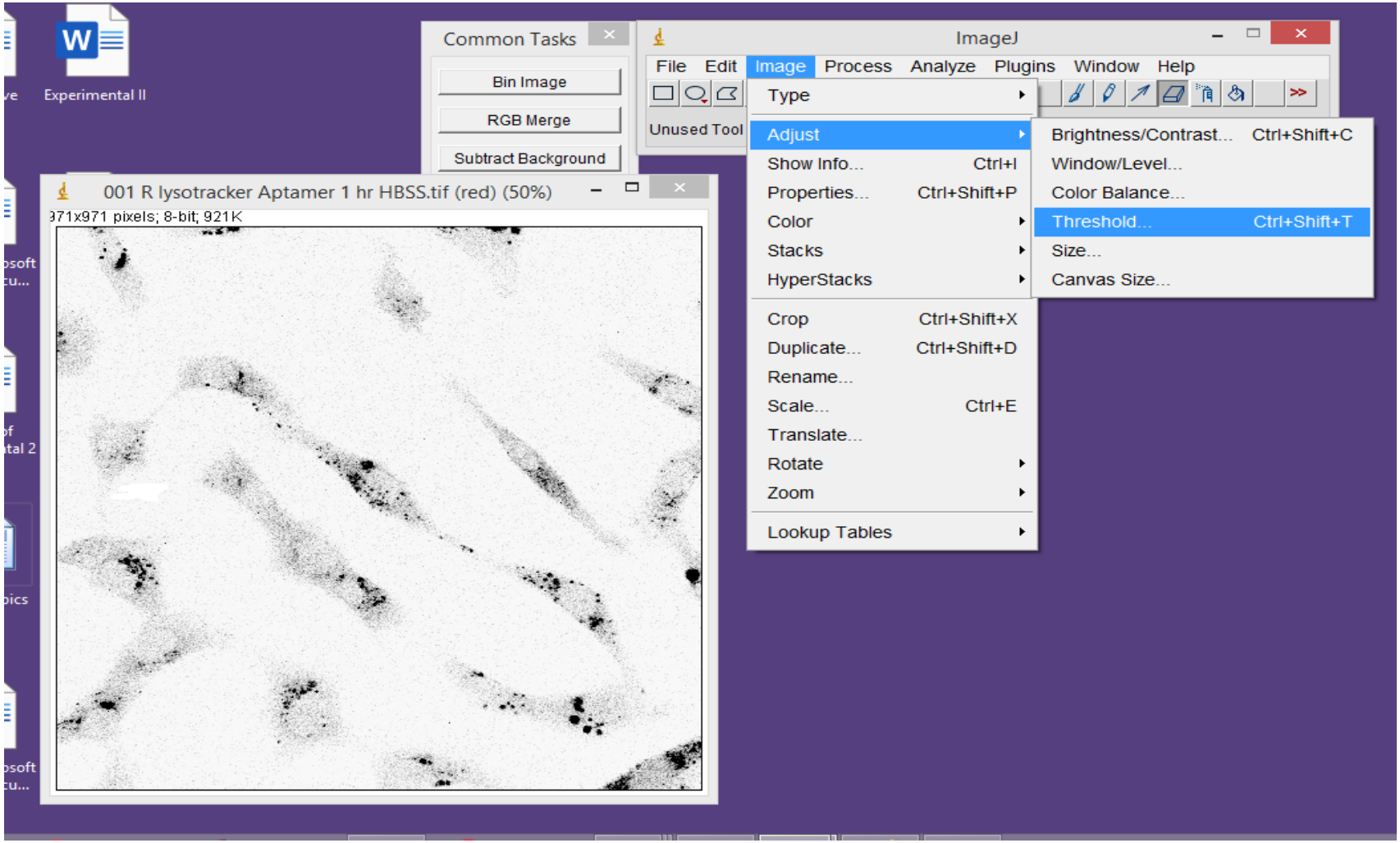
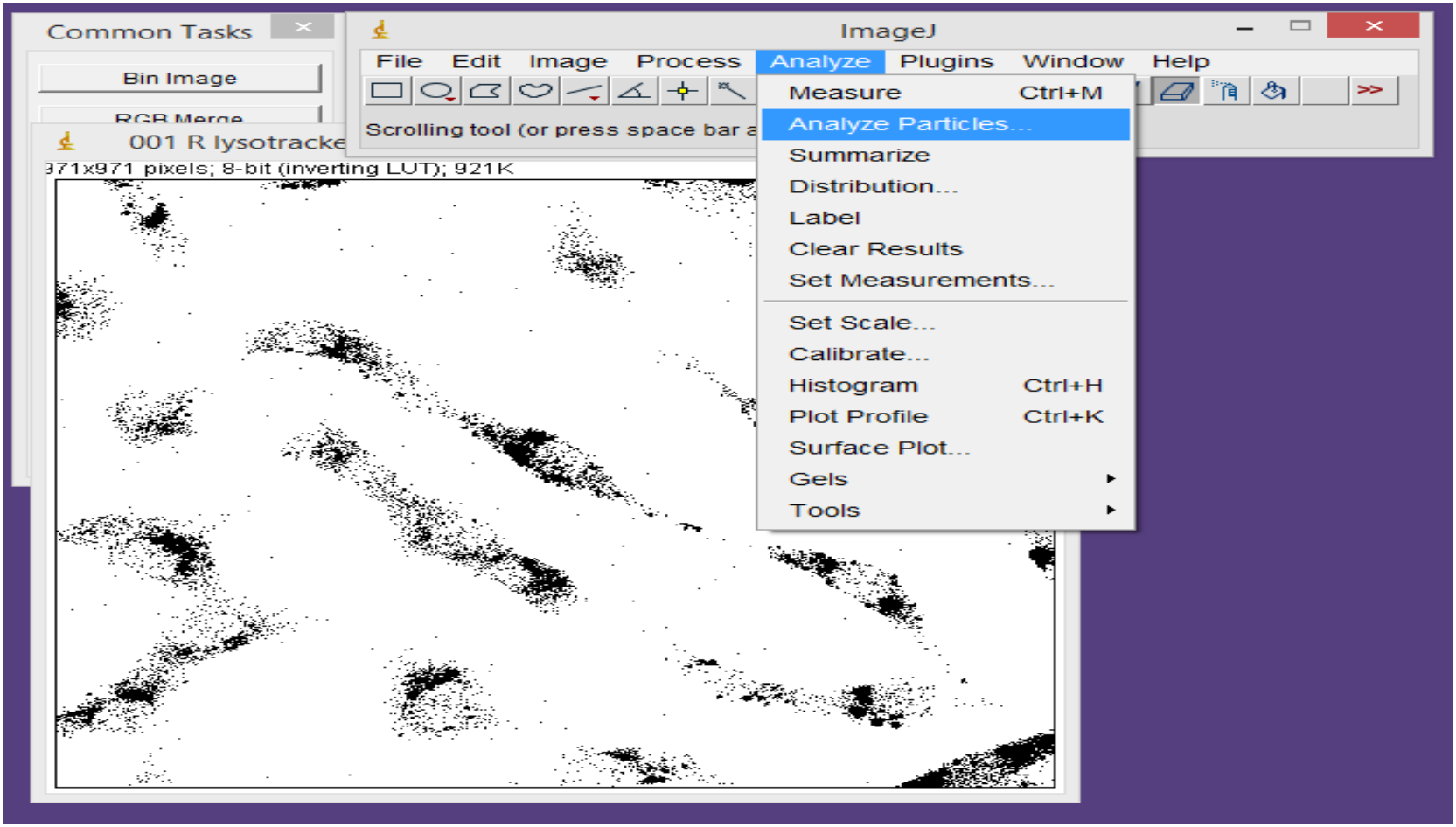


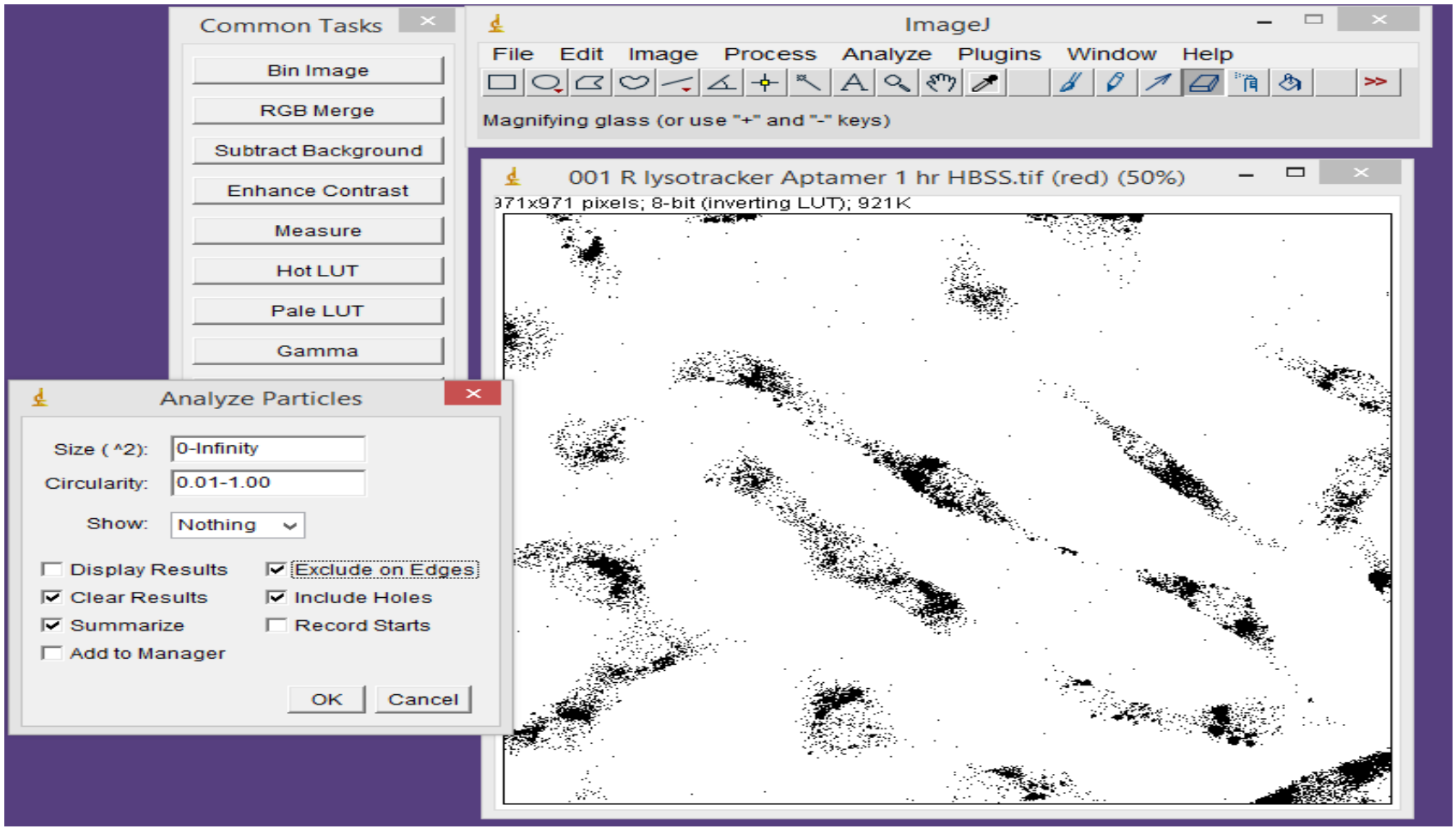
Image----adjust-----threshold



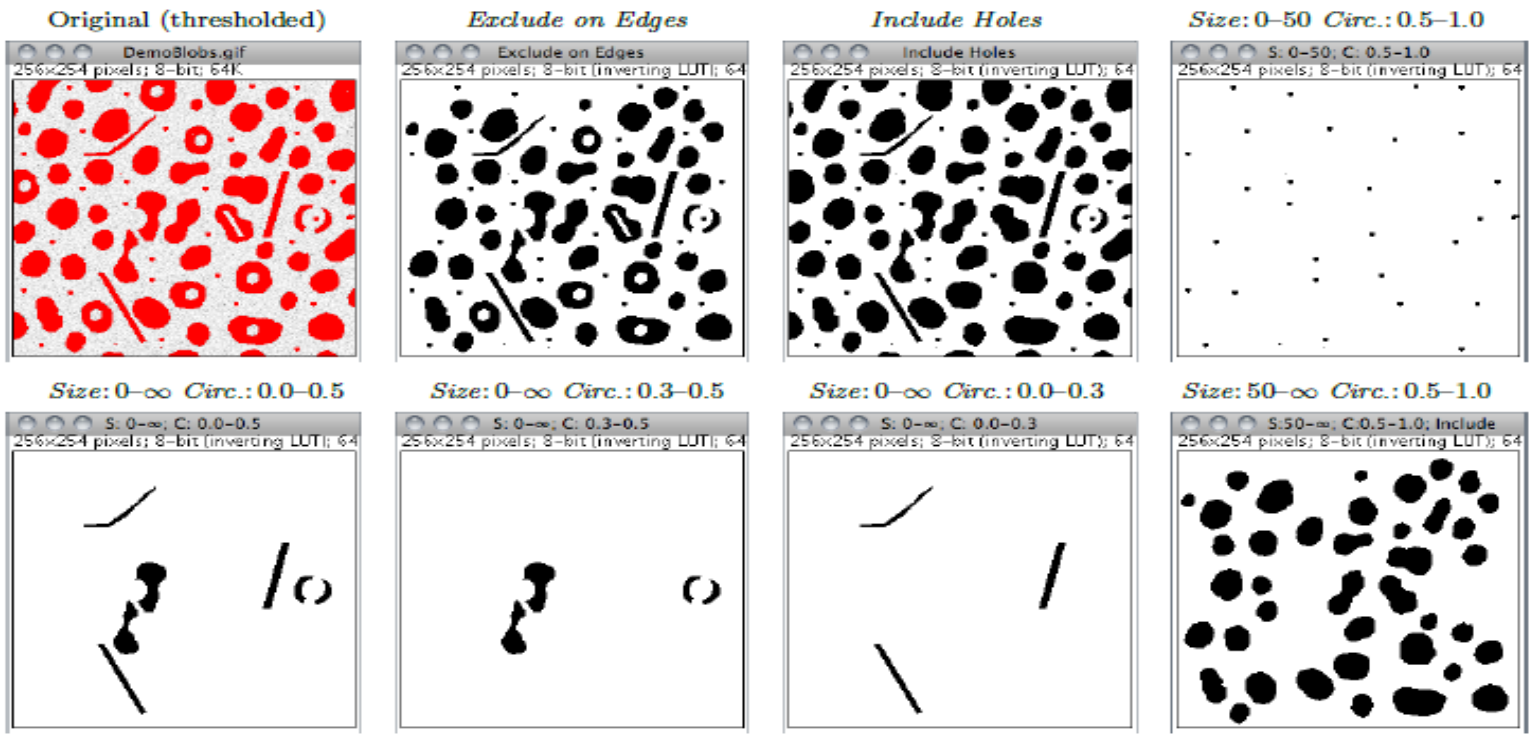
Click apply or move the down scroll to clarify the fluorescence then analyze …..analyze particles



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



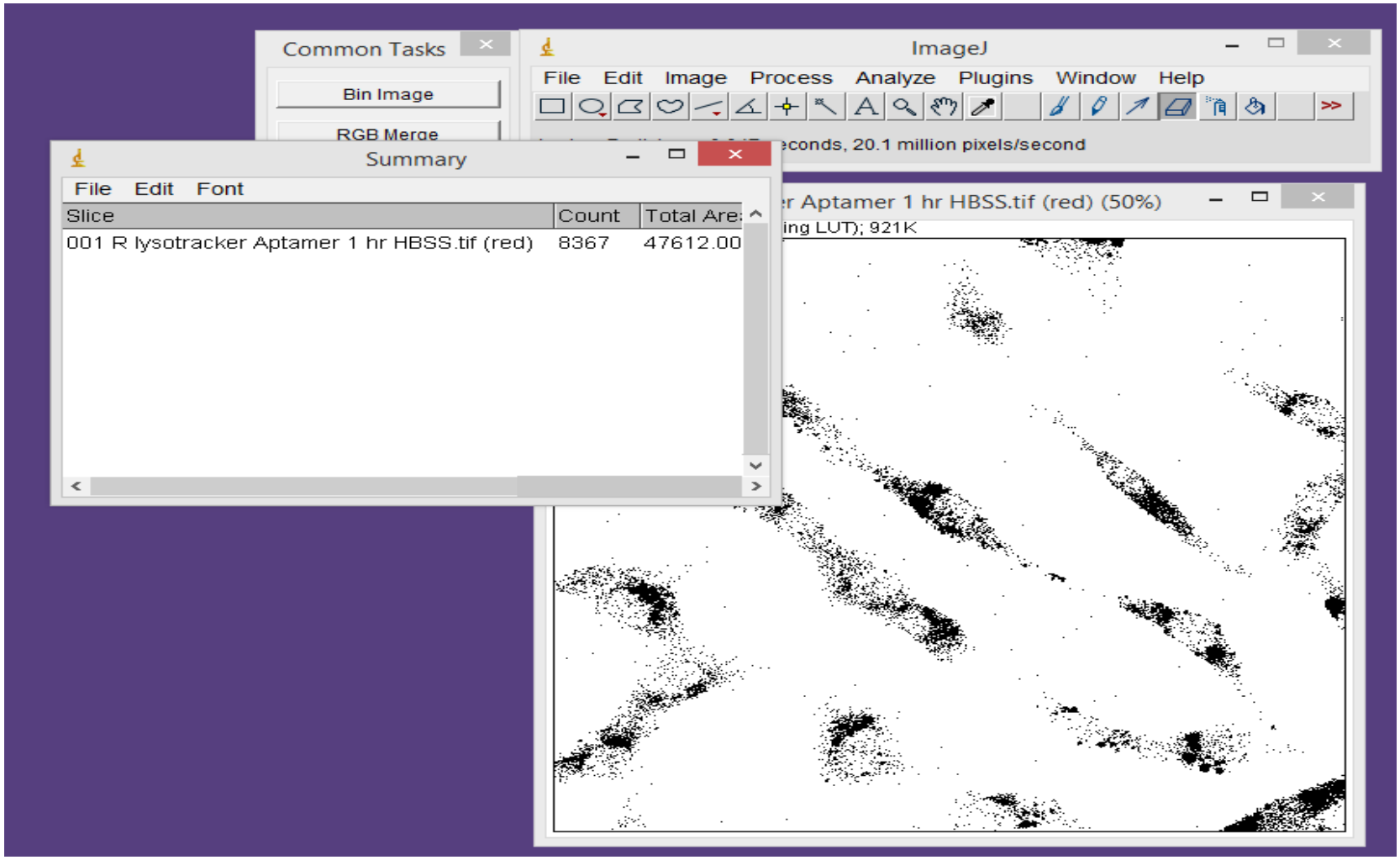
Important note:



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

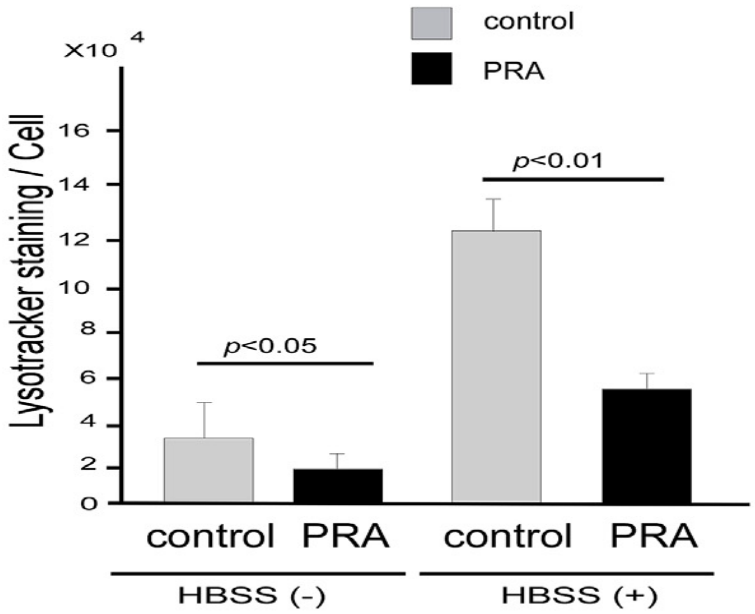
Image J guide book

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



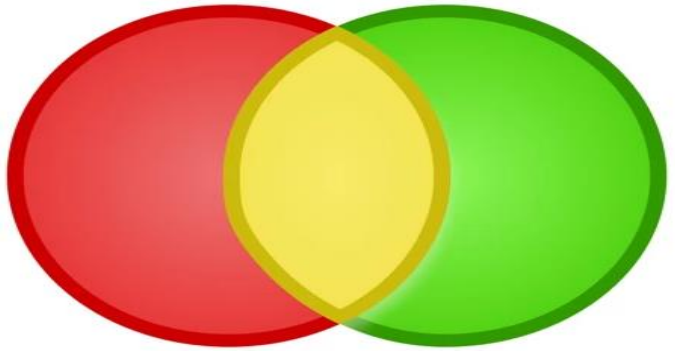
T. Donia et 150 al. / Biochemical and Biophysical Research Communications 517 (2019) 146e154

Important note:

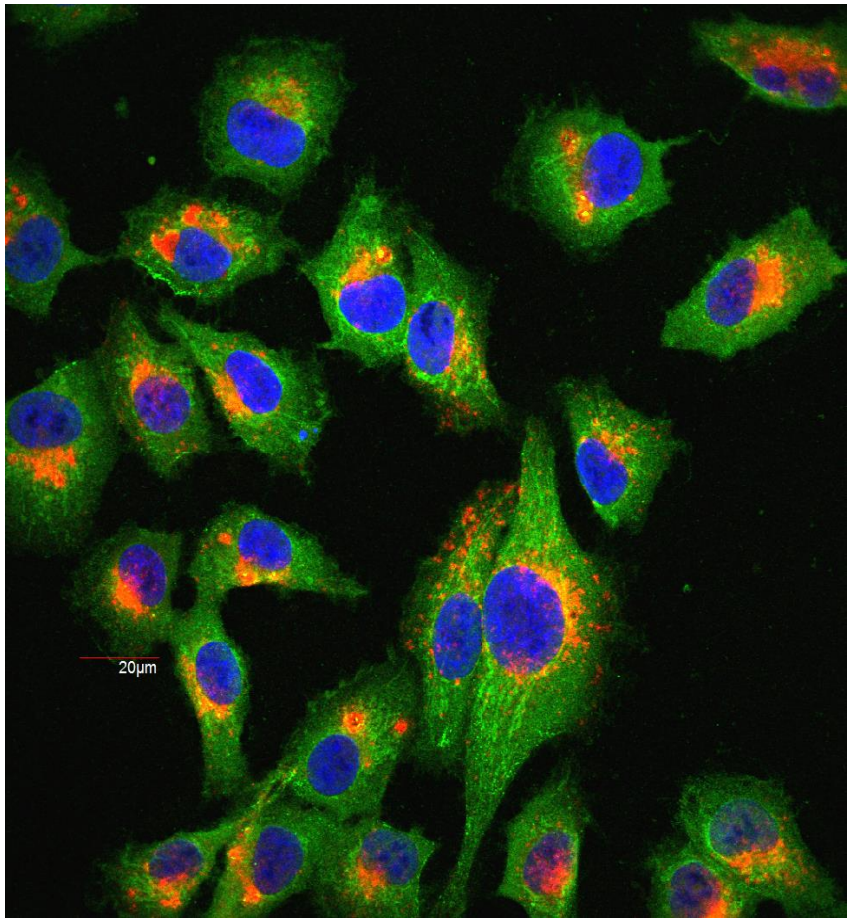
- Thresholding tool setting in a positive control specimen should then be duplicated in every image to be compared

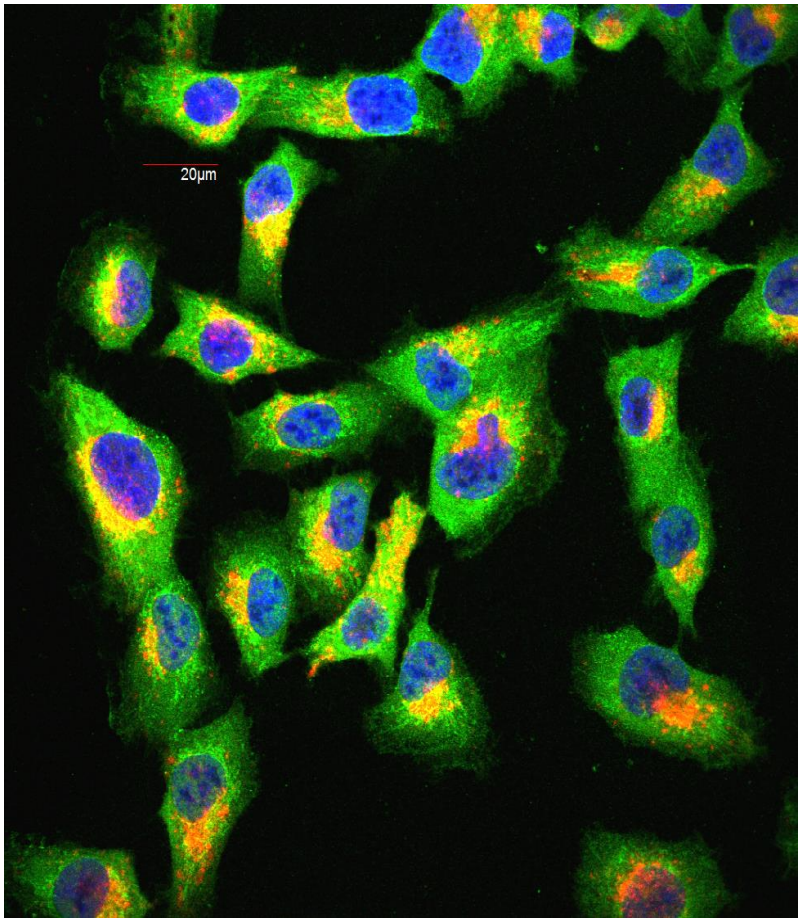
Colocalization Analysis

Colocalization .....observation 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. ”

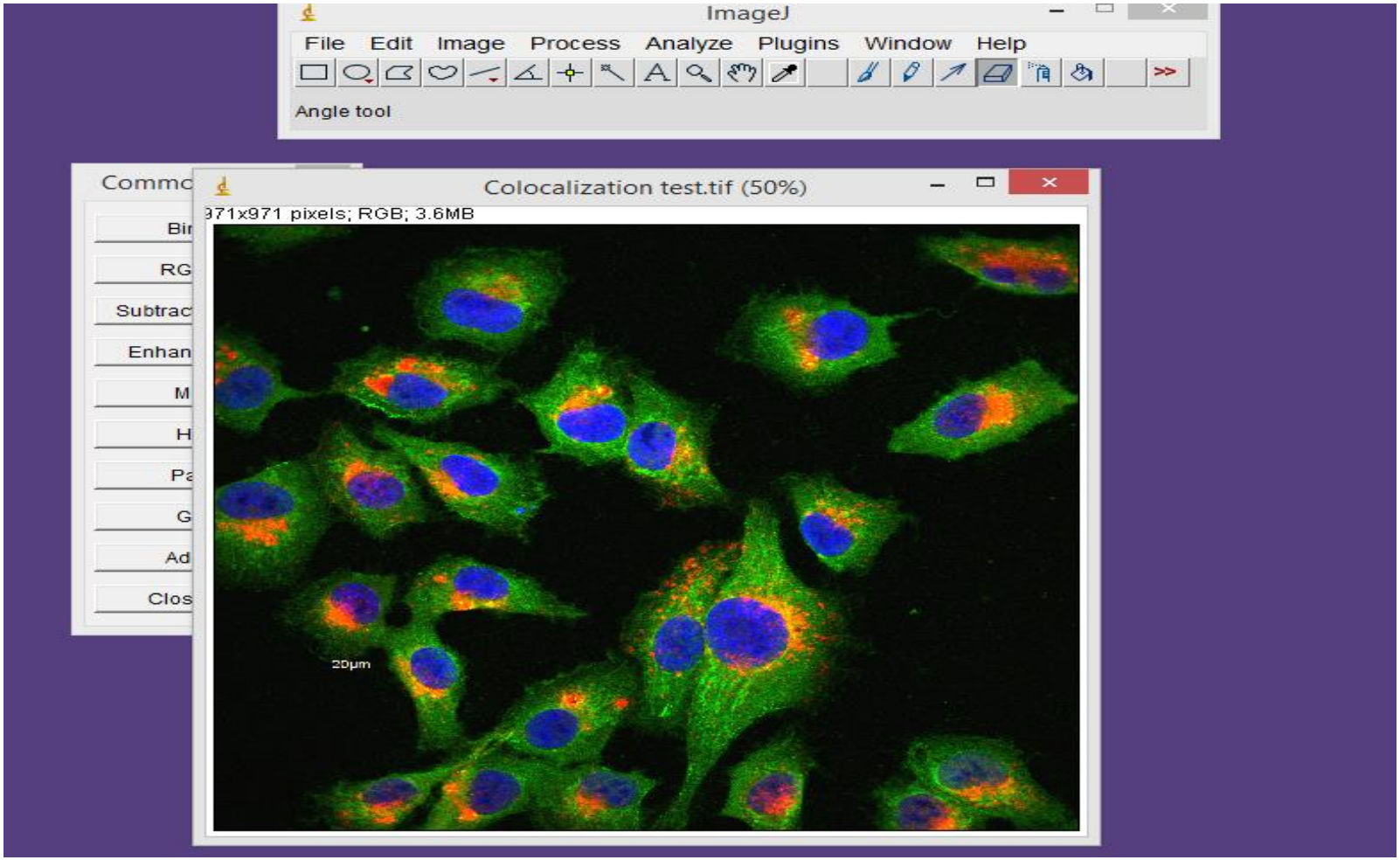


Images for analysis of colocalization

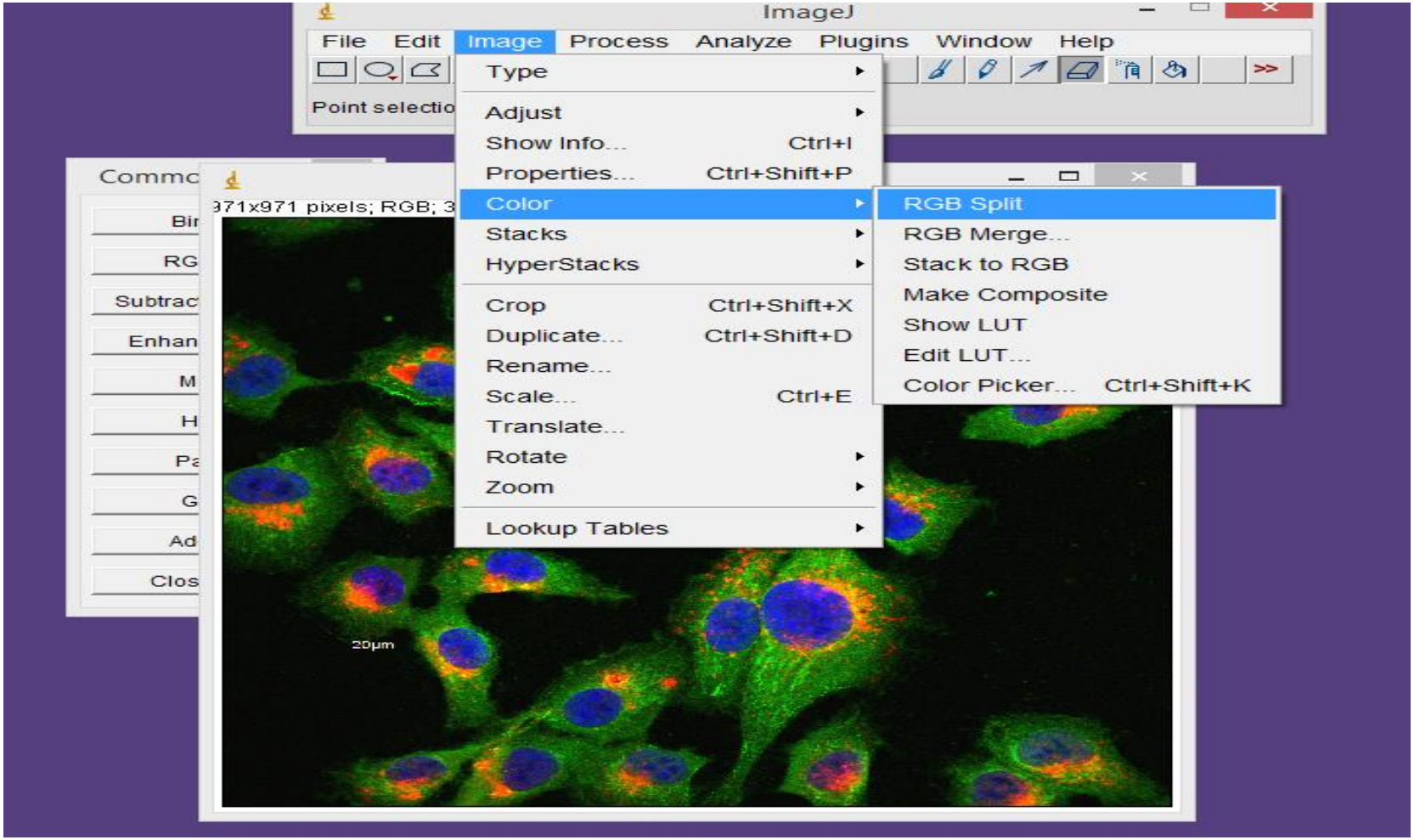




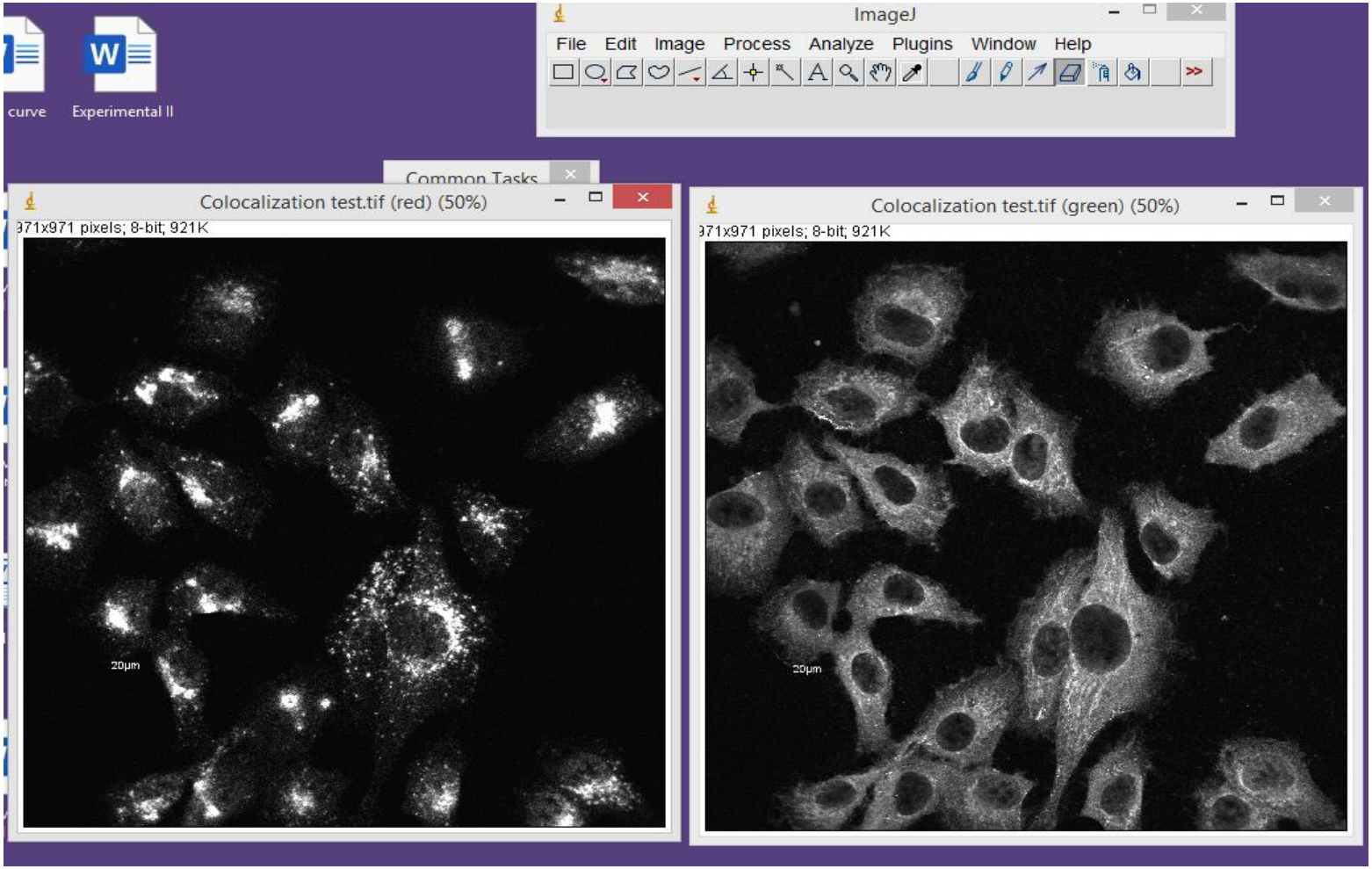
Open the image in image J



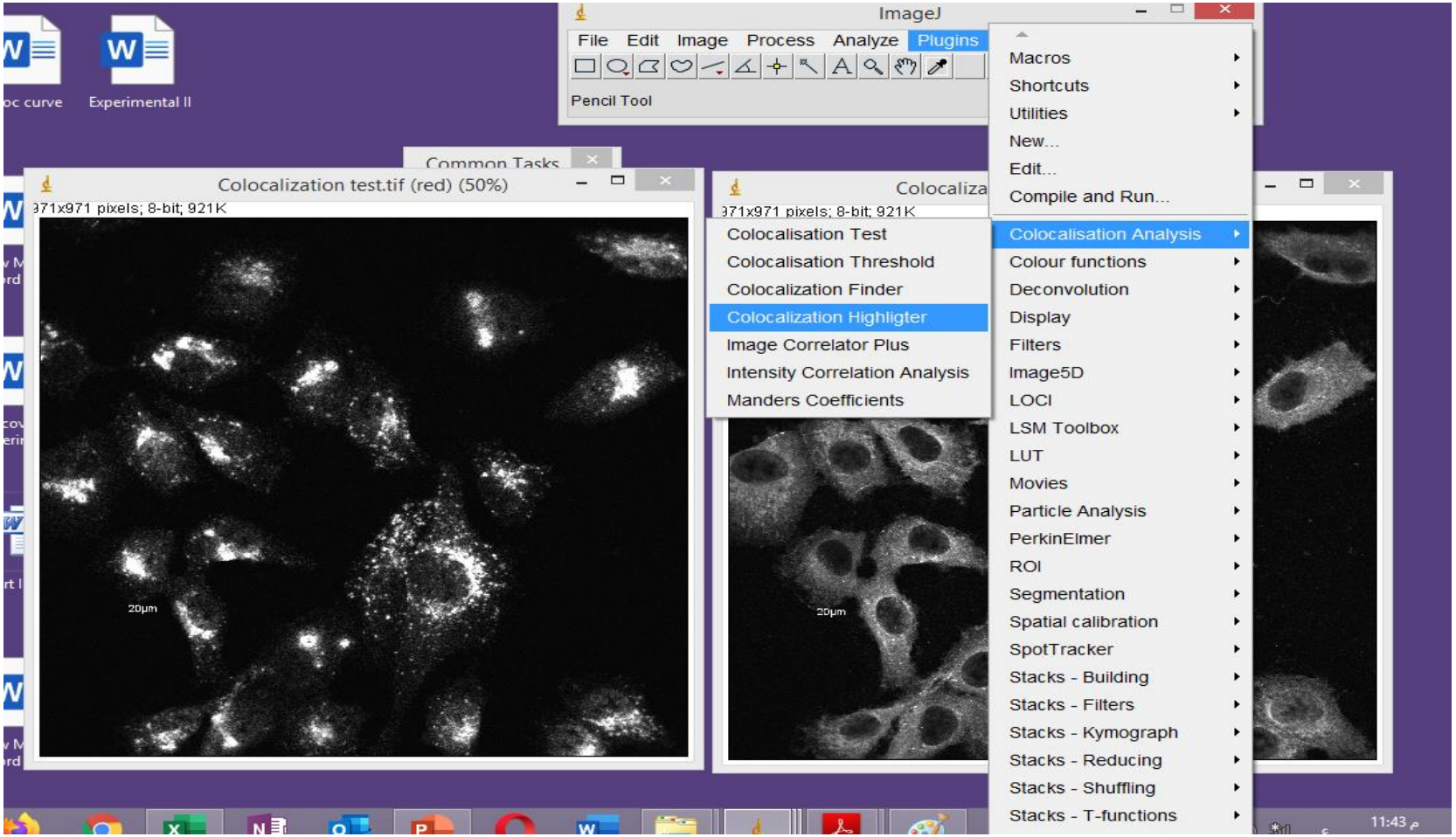
Split image colors



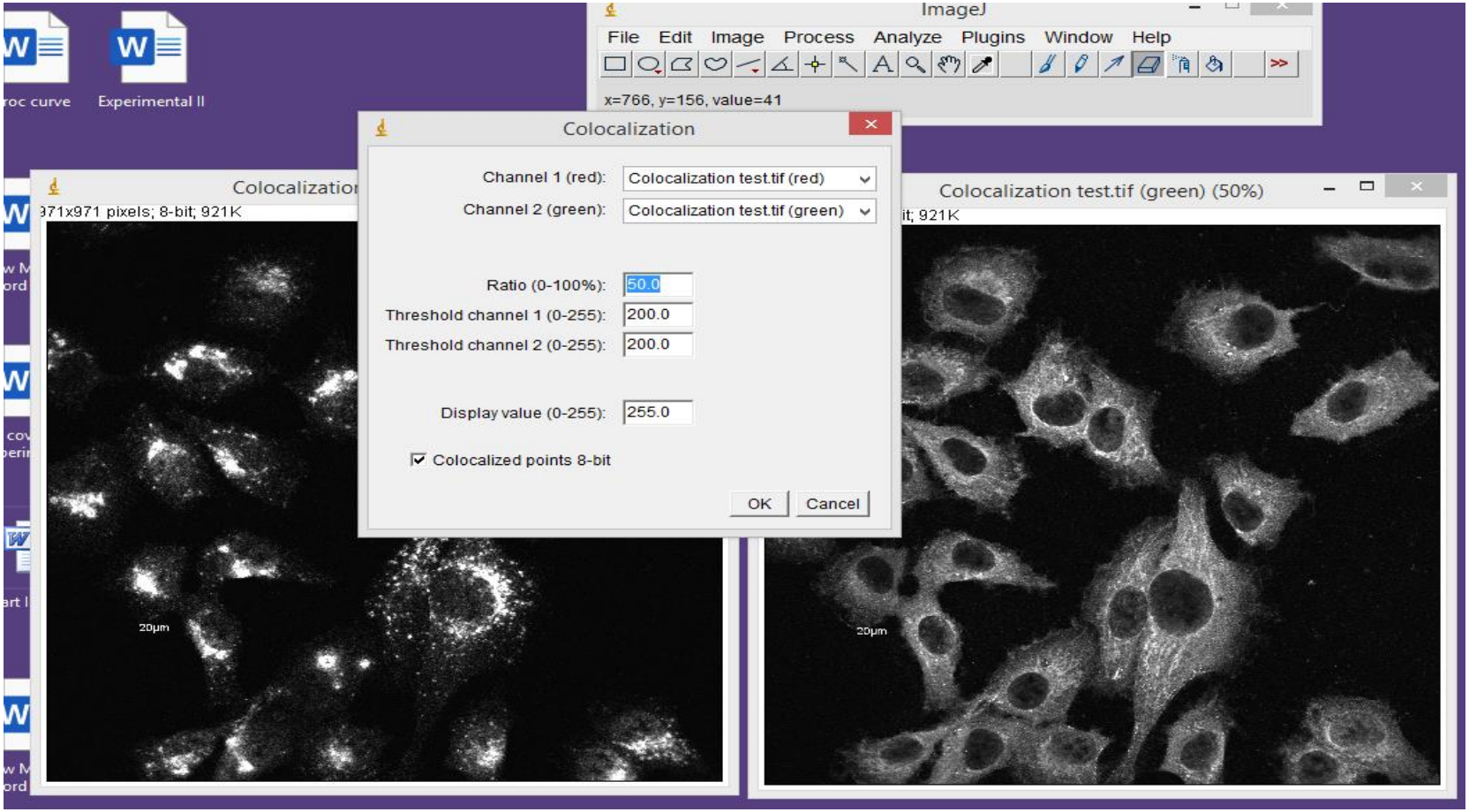
Delete the blue color image for DAPI



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

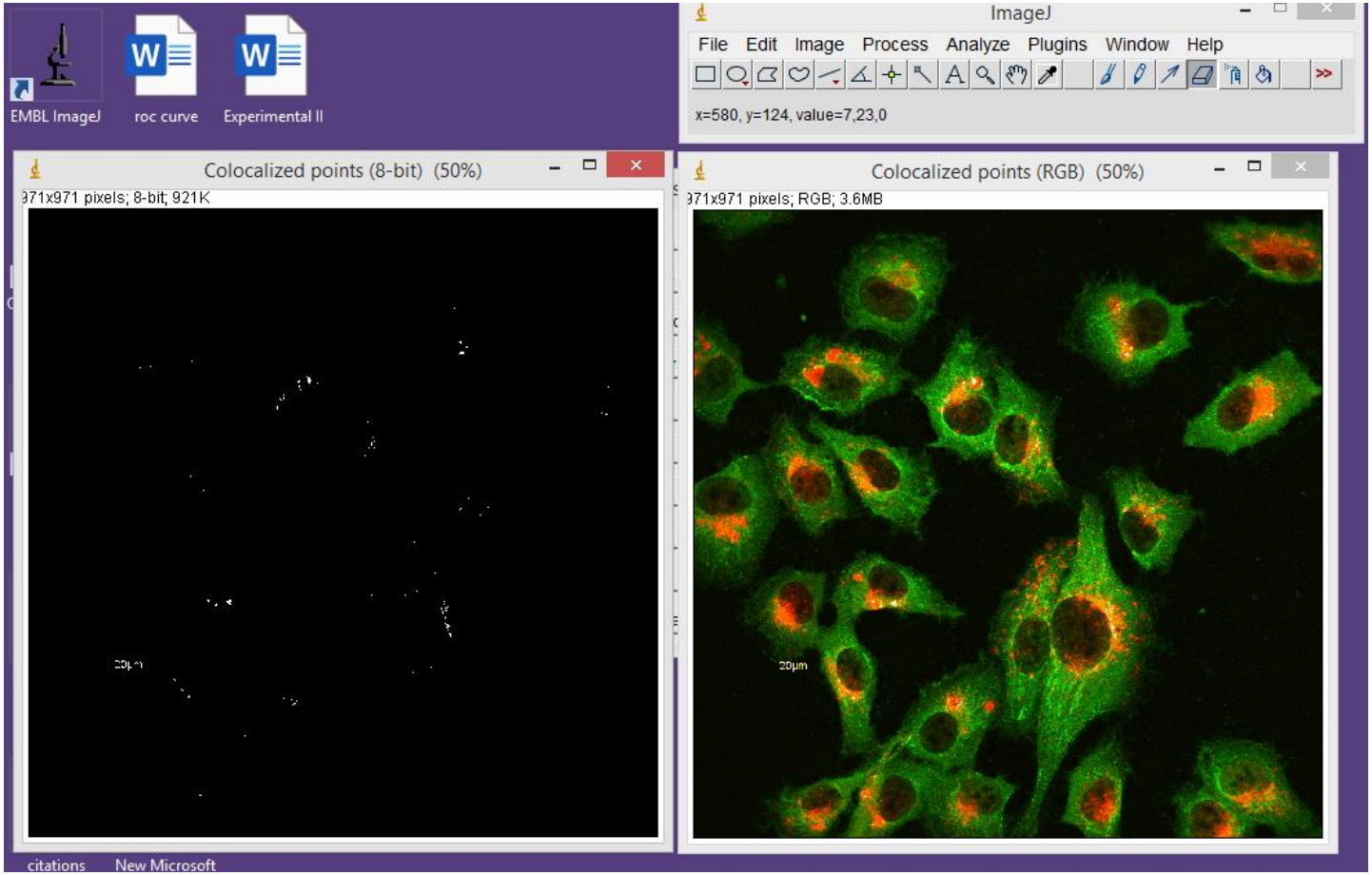


Record the settings you used to apply the same for all studied images

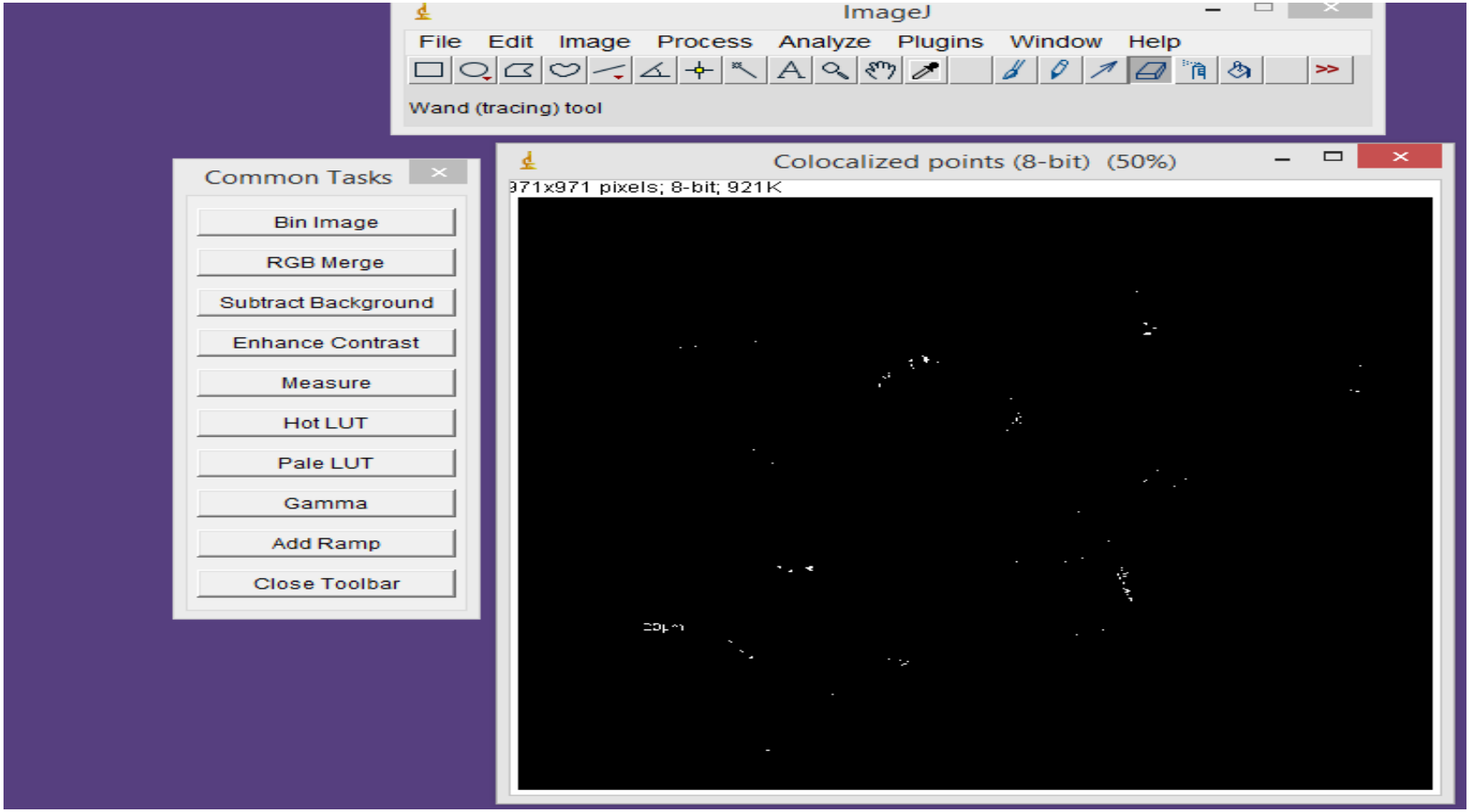


You will get two images

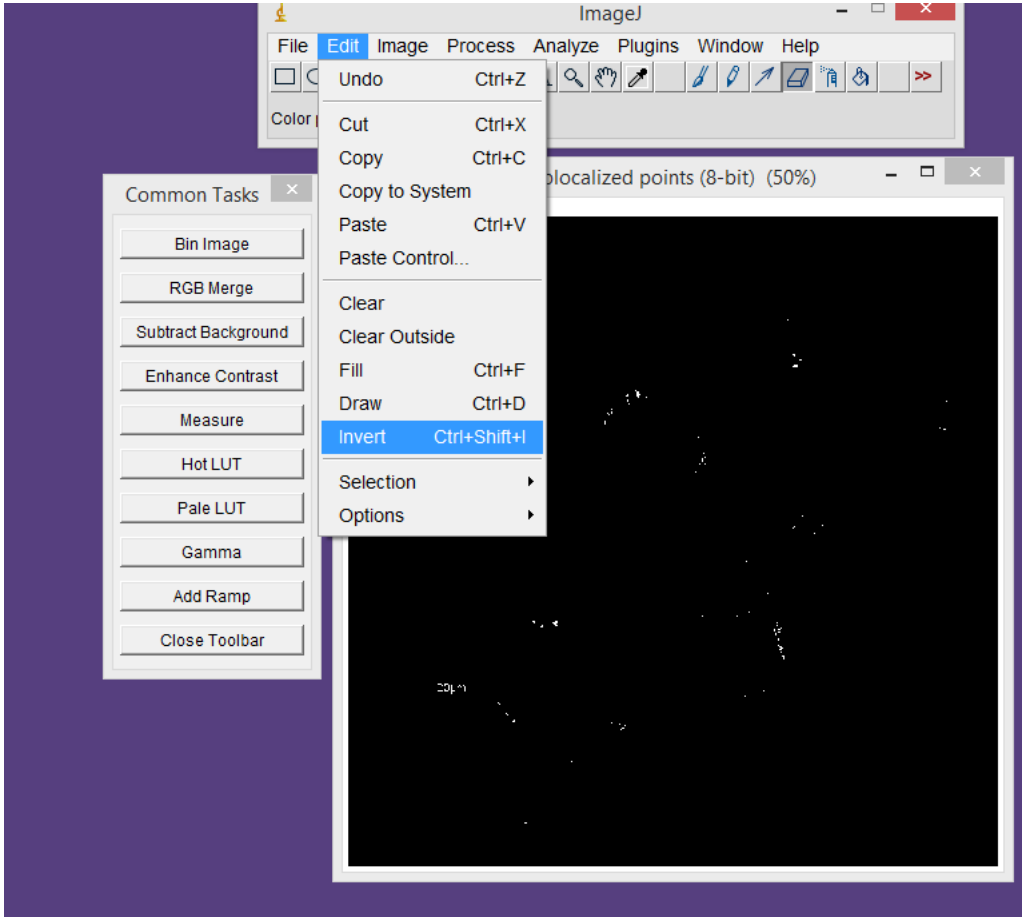
- One for colocalized points in 8 bit and the other in RGB
- You can save them and the image for analysis will be 8 bit


EMBL ImageJ
roc curve
Experimental II
ImageJ
File Edit Image Process Analyze Plugins Window Help
x=580, y=124, value=7,23,0
Colocalized points (8-bit) (50%)
971x971 pixels; 8-bit; 921K
Colocalized points (RGB) (50%)
971x971 pixels; RGB; 3.6MB
20µm
citations
New Microsoft

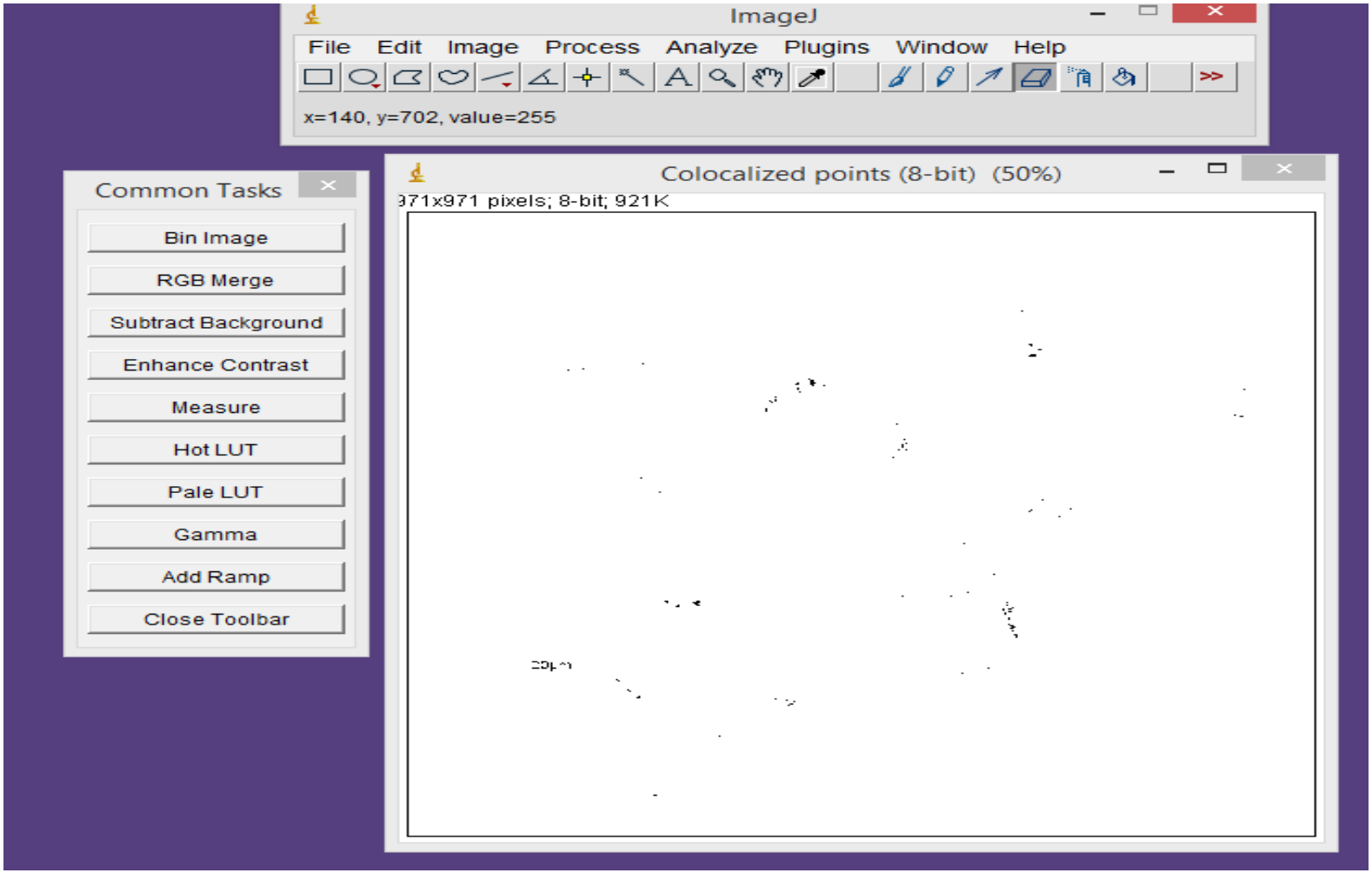
Colocalized point (8 bit)



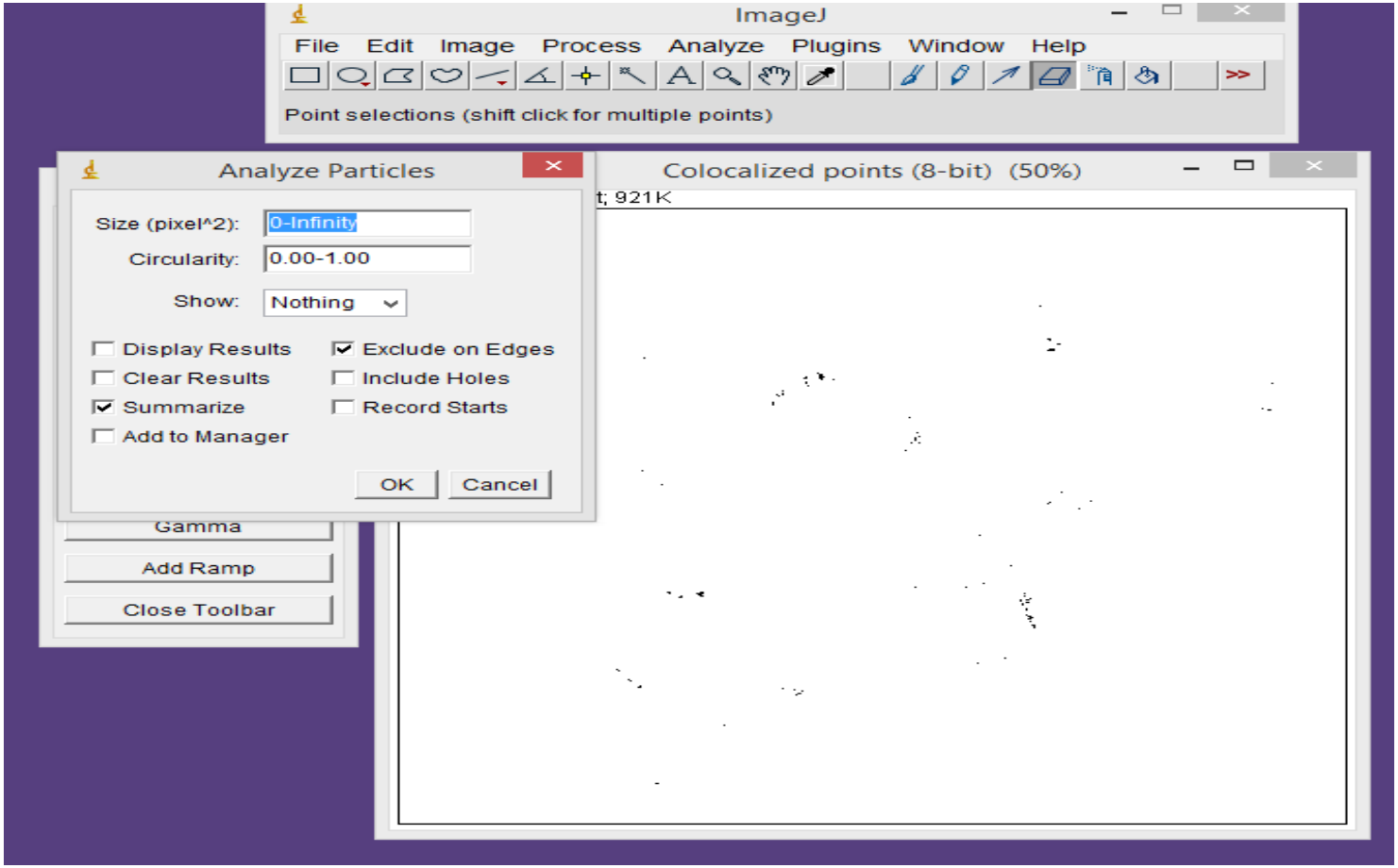
Edit …..invert


ImageJ
File
Edit
Image
Process
Analyze
Plugins
Window
Help
Undo Ctrl+Z
Cut Ctrl+X
Copy Ctrl+C
Copy to System
Paste Ctrl+V
Paste Control...
Clear
Clear Outside
Fill Ctrl+F
Draw Ctrl+D
Invert Ctrl+Shift+I
Selection
Options
Common Tasks
Bin Image
RGB Merge
Subtract Background
Enhance Contrast
Measure
Hot LUT
Pale LUT
Gamma
Add Ramp
Close Toolbar
localized points (8-bit) (50%)

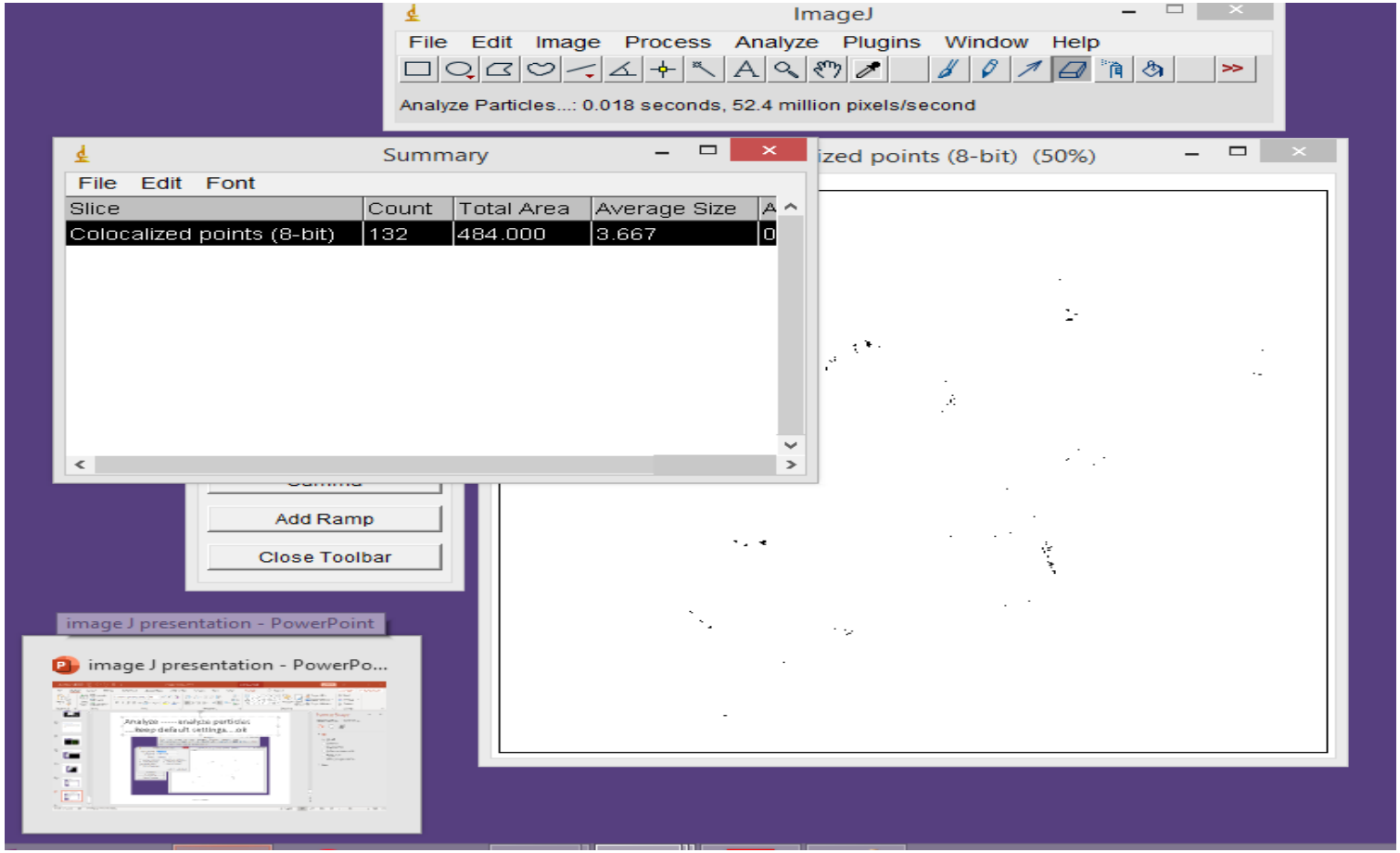
Erase the diameter



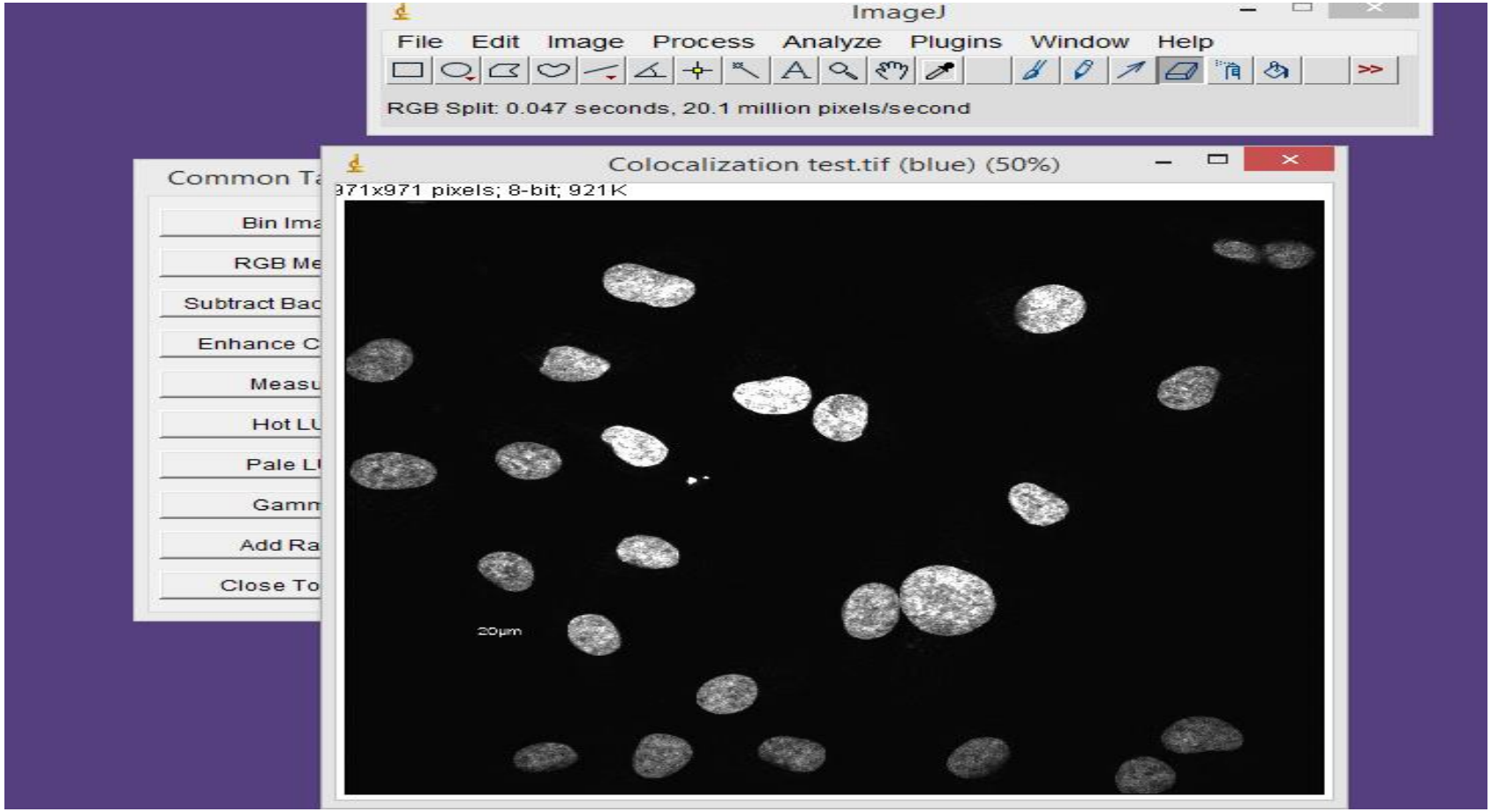
Analyze -----analyze particles ….keep default settings….ok



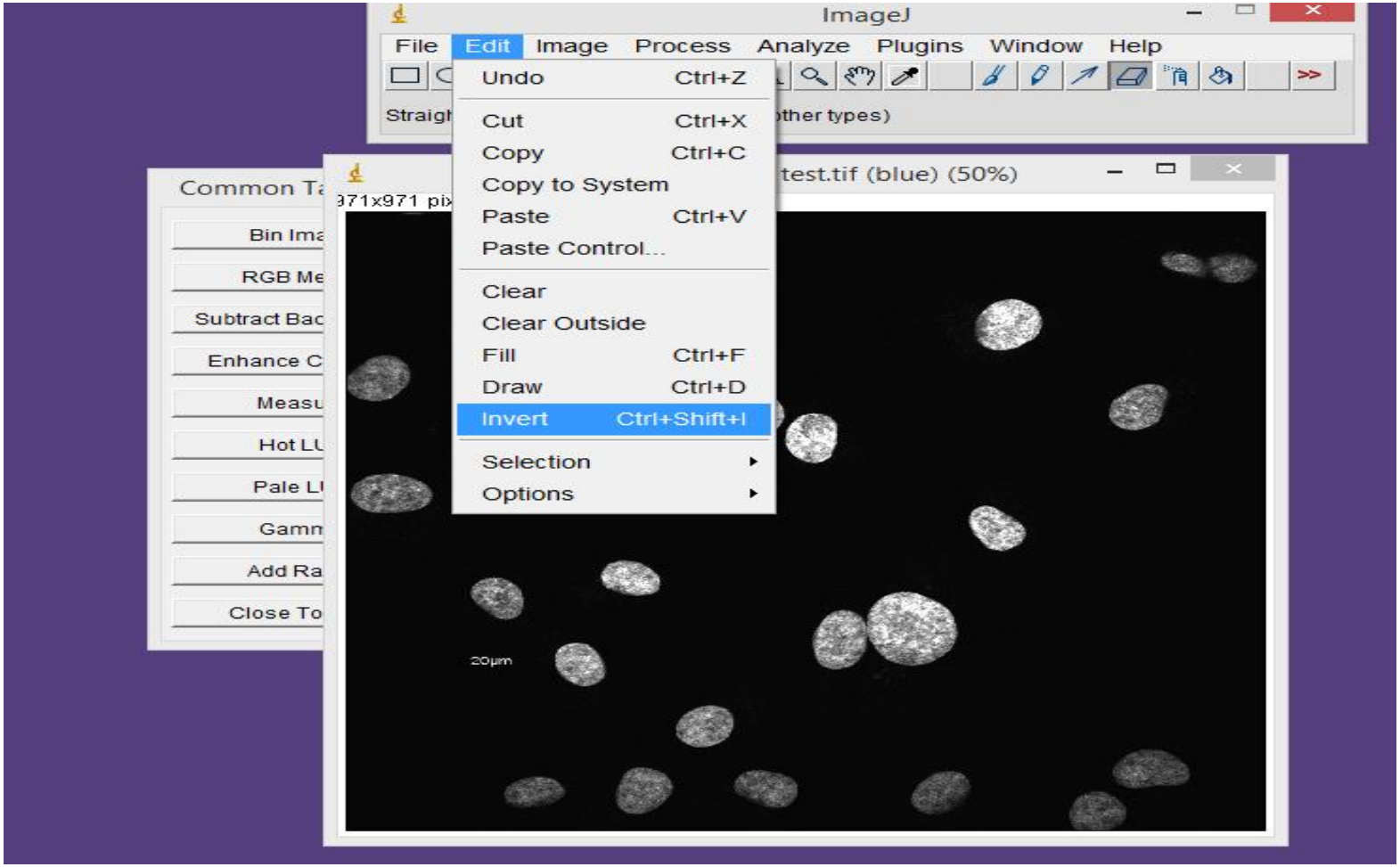
We just focus on count column which expresses colocalization



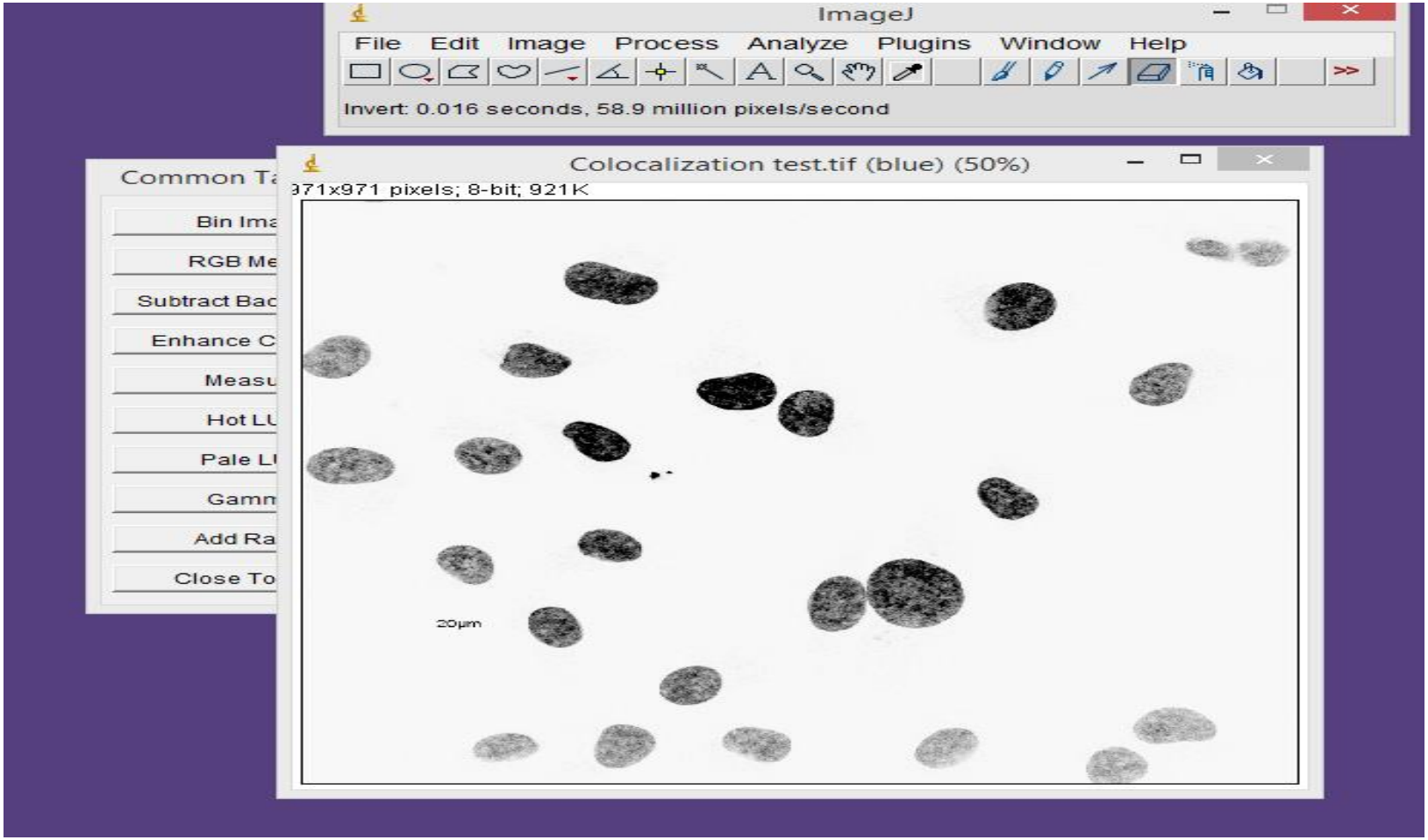
Count the number of cells by splitting the image colors and keeping blue one.



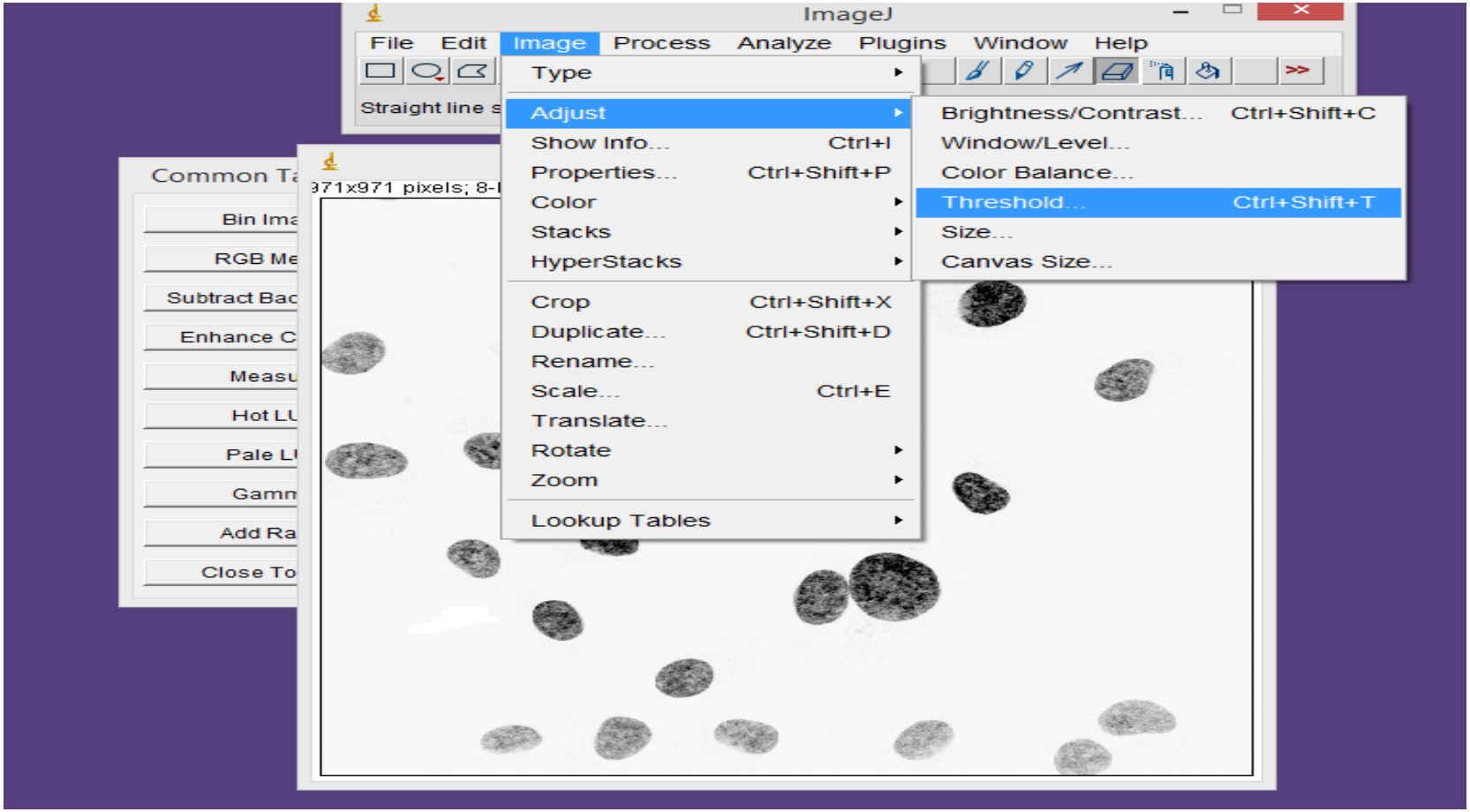
Edit …invert


ImageJ
File
Edit
Image
Process
Analyze
Plugins
Window
Help
Undo Ctrl+Z
Cut Ctrl+X
Copy Ctrl+C
Copy to System
Paste Ctrl+V
Paste Control...
Clear
Clear Outside
Fill Ctrl+F
Draw Ctrl+D
Invert Ctrl+Shift+I
Selection
Options
test.tif (blue) (50%)
20µm

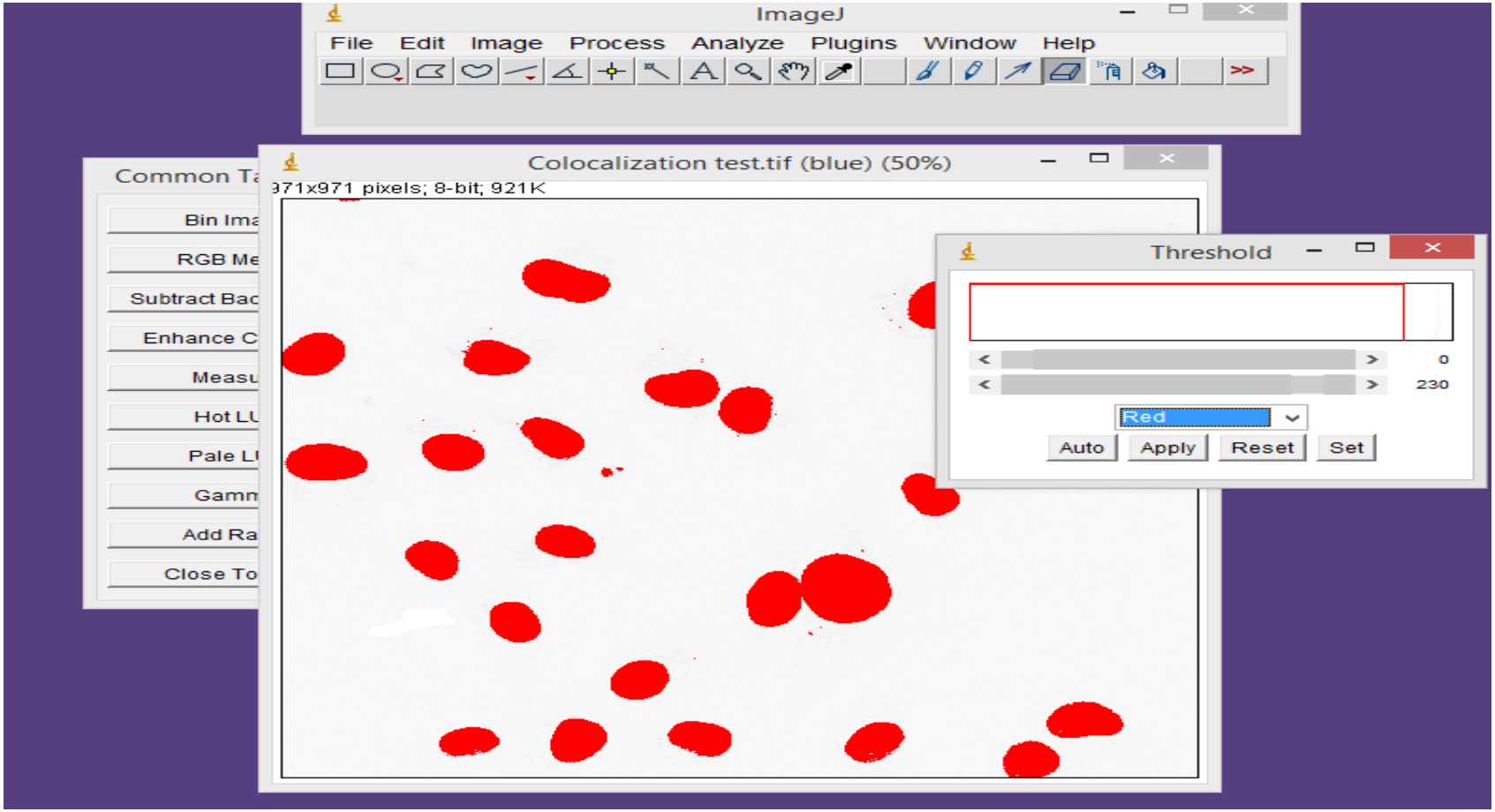
Then remove the diameter by eraser



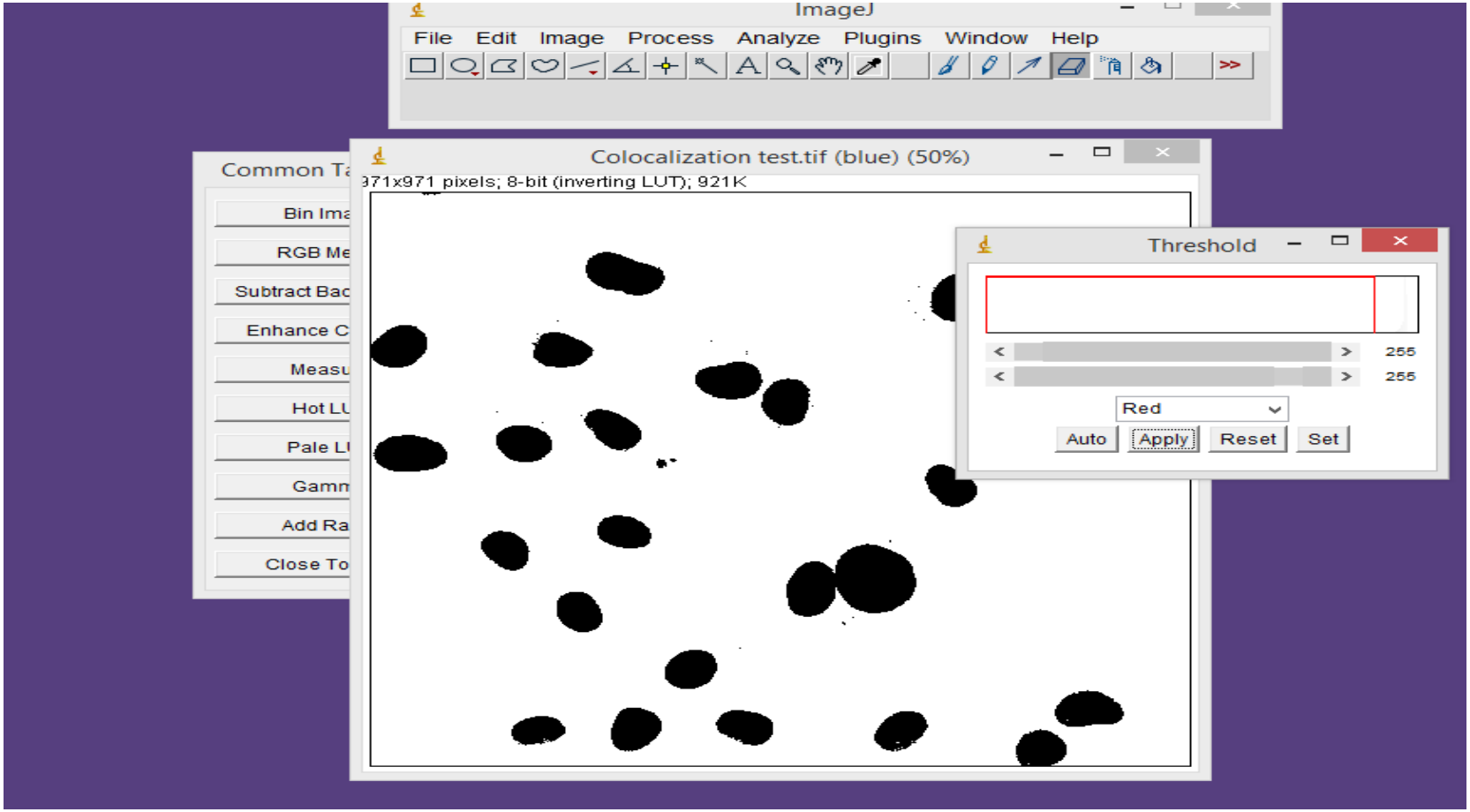
Adjust …..threshold



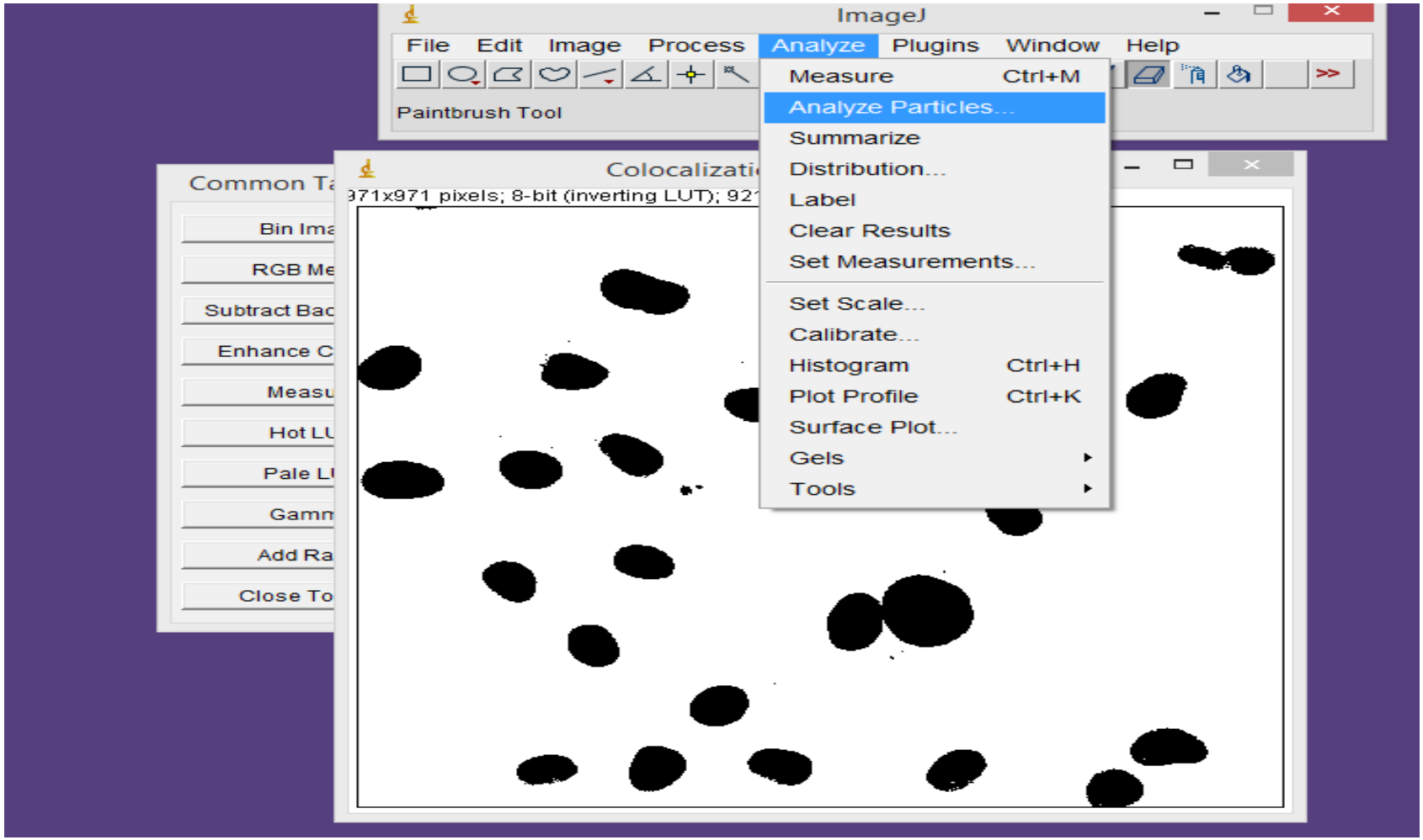
Adjust the down scroll to right till you get the clear nuclei



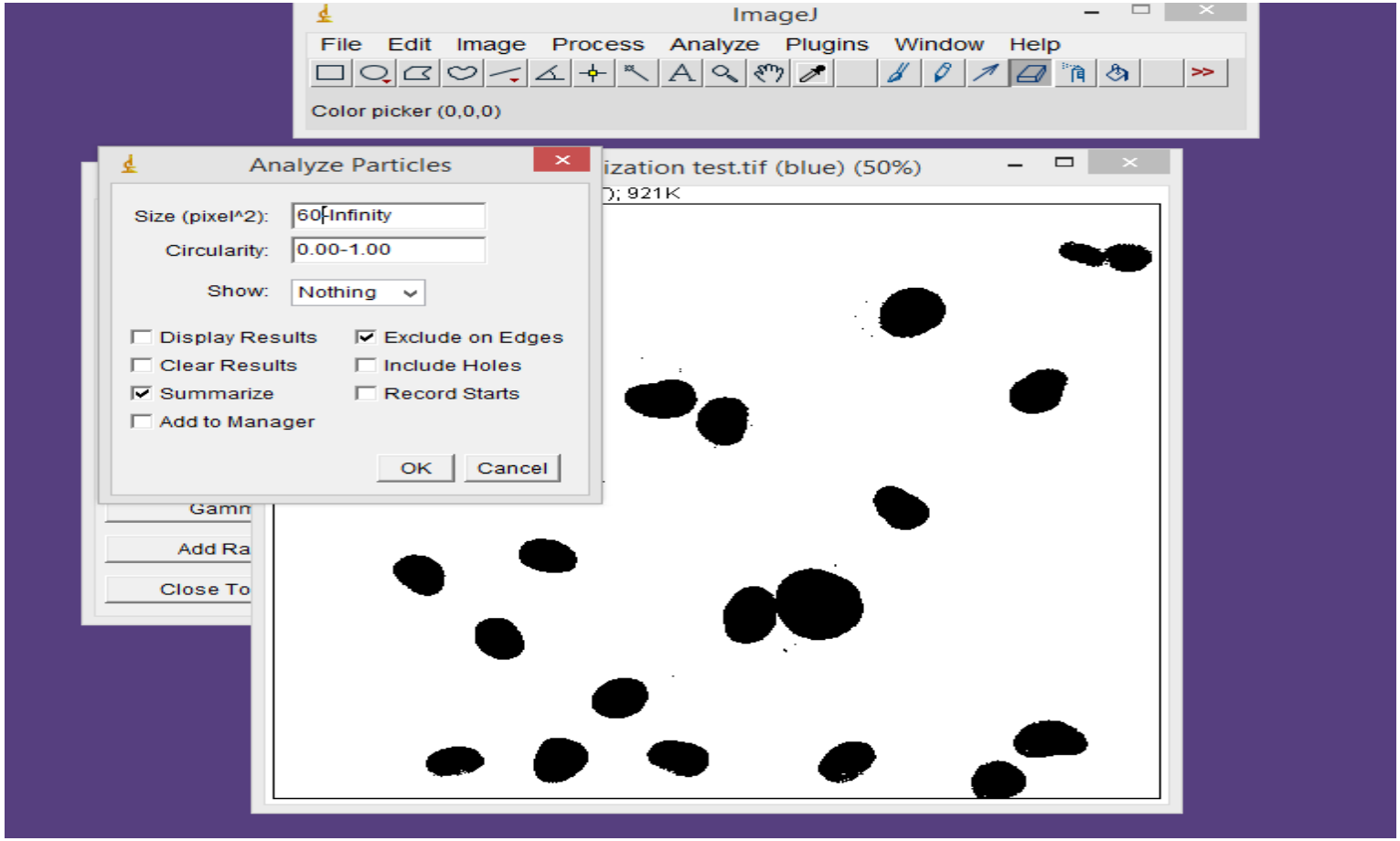
Apply to get such view



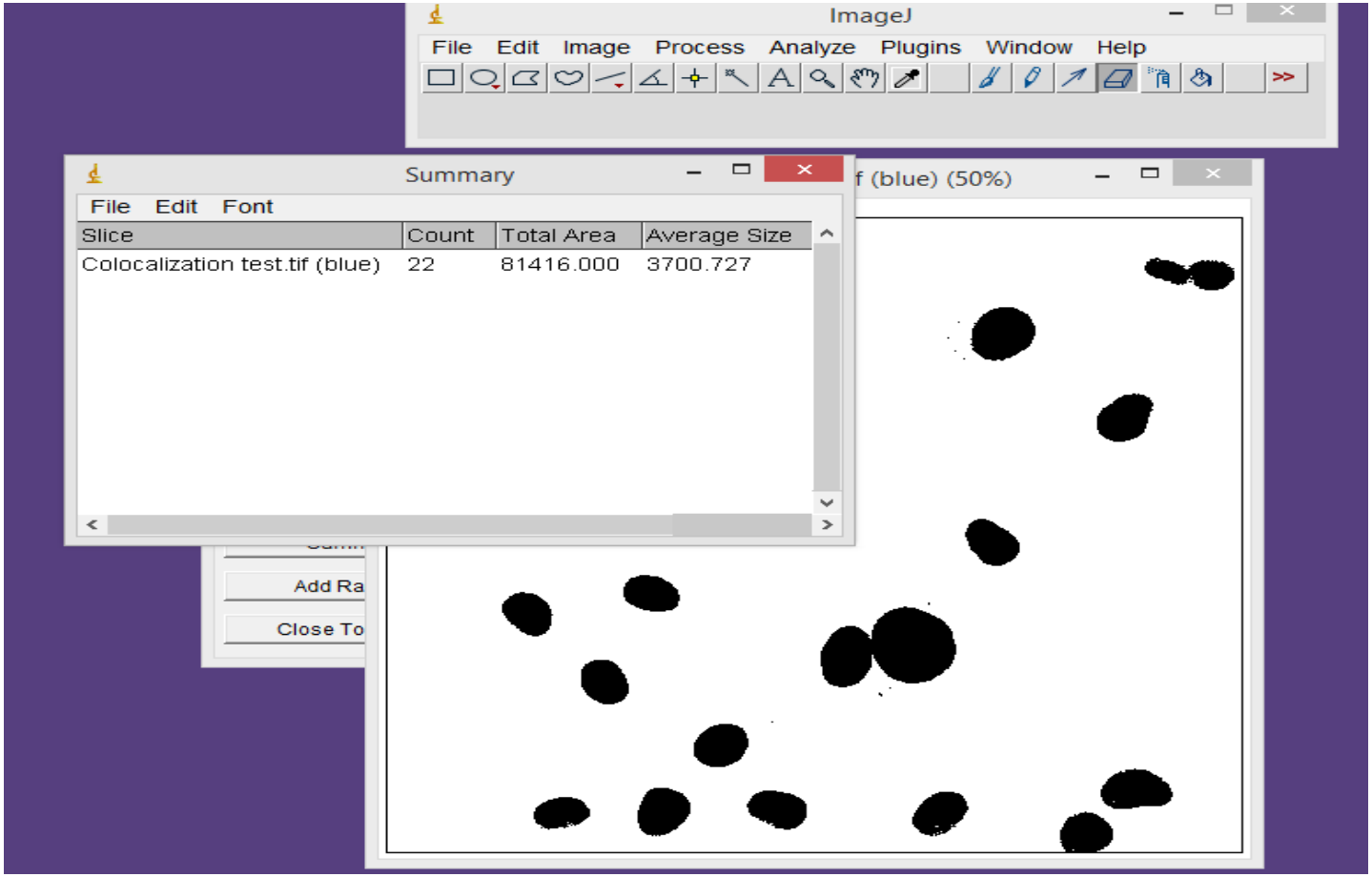
Analyze ….analyze particles



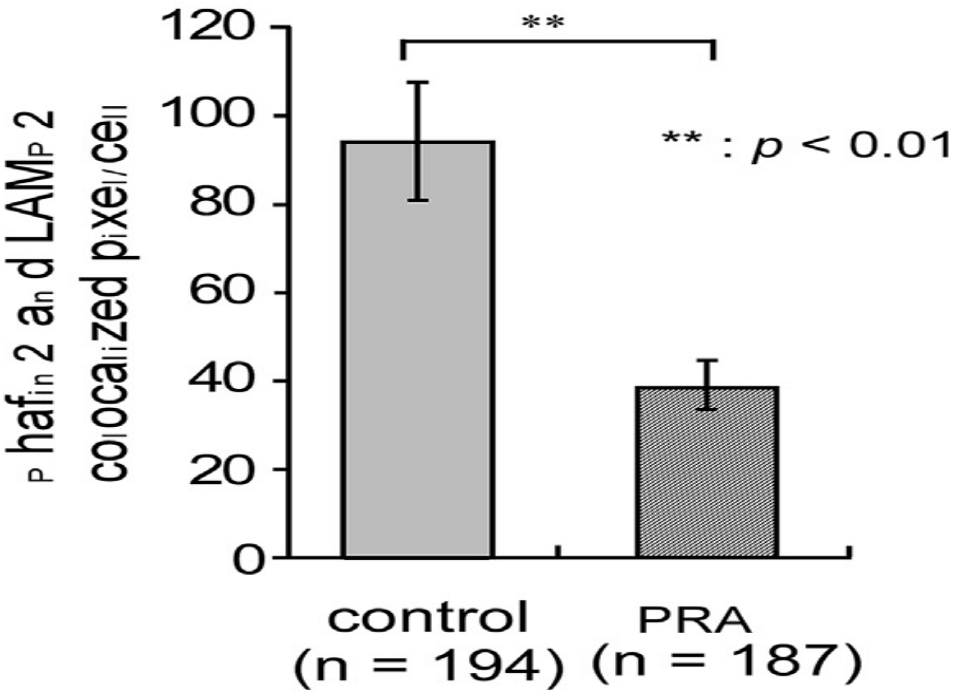
Keep all settings in default form with changing size to be 60- infinity



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

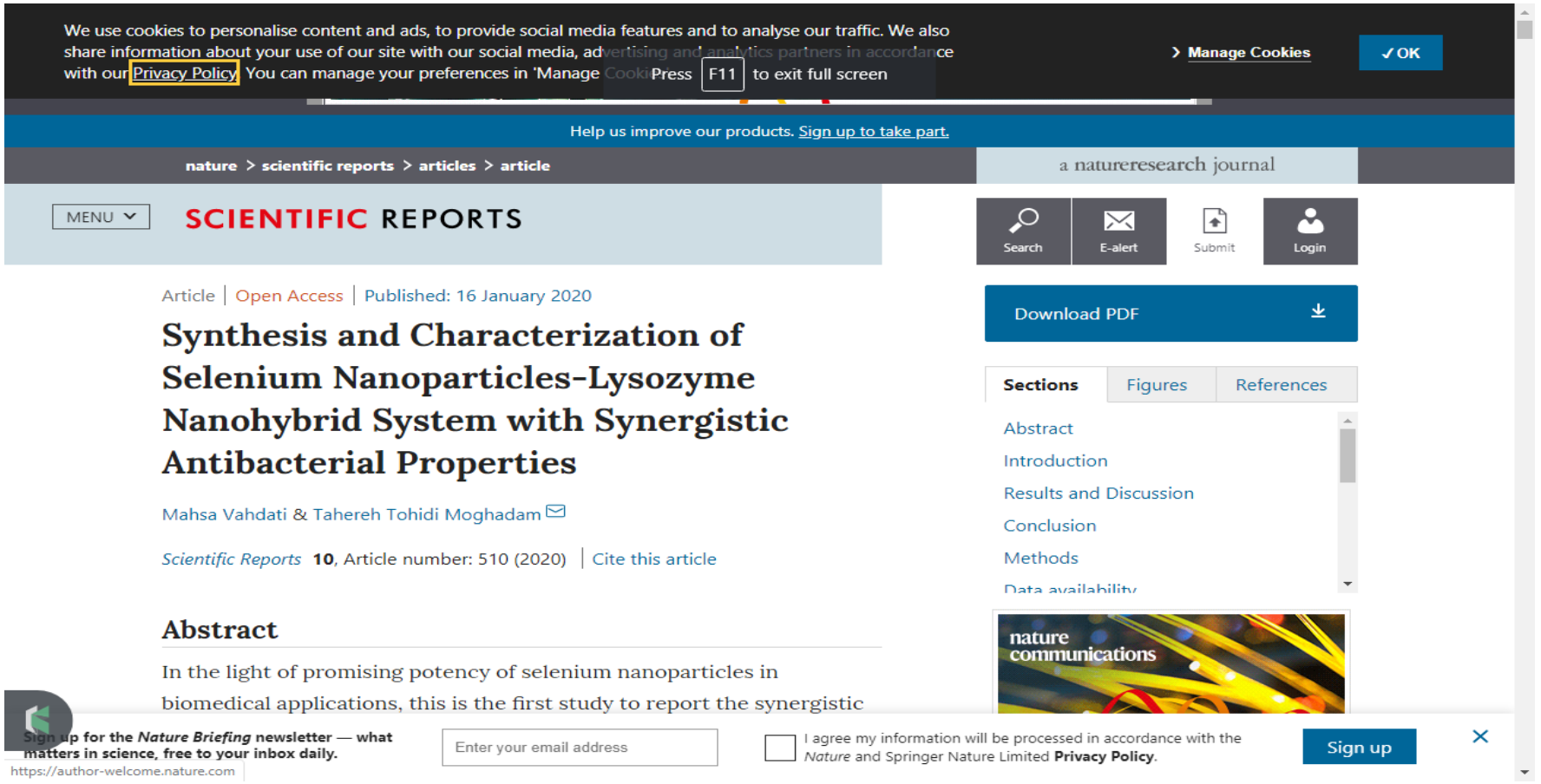


T. Donia et 150 al. / Biochemical and Biophysical Research Communications 517 (2019) 146e154

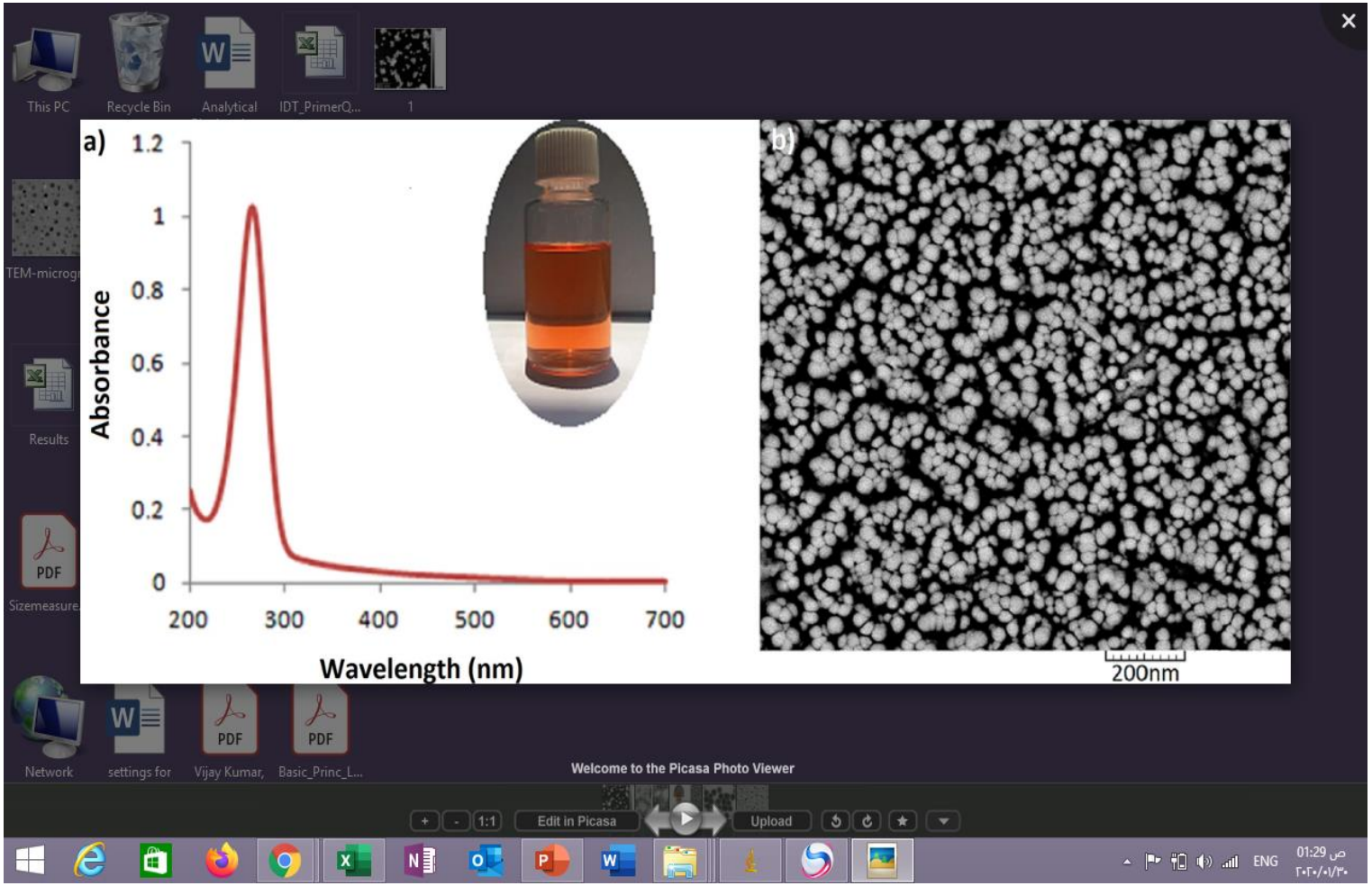
Nanoparticle size distribution analysis by image J

Thoria Donia

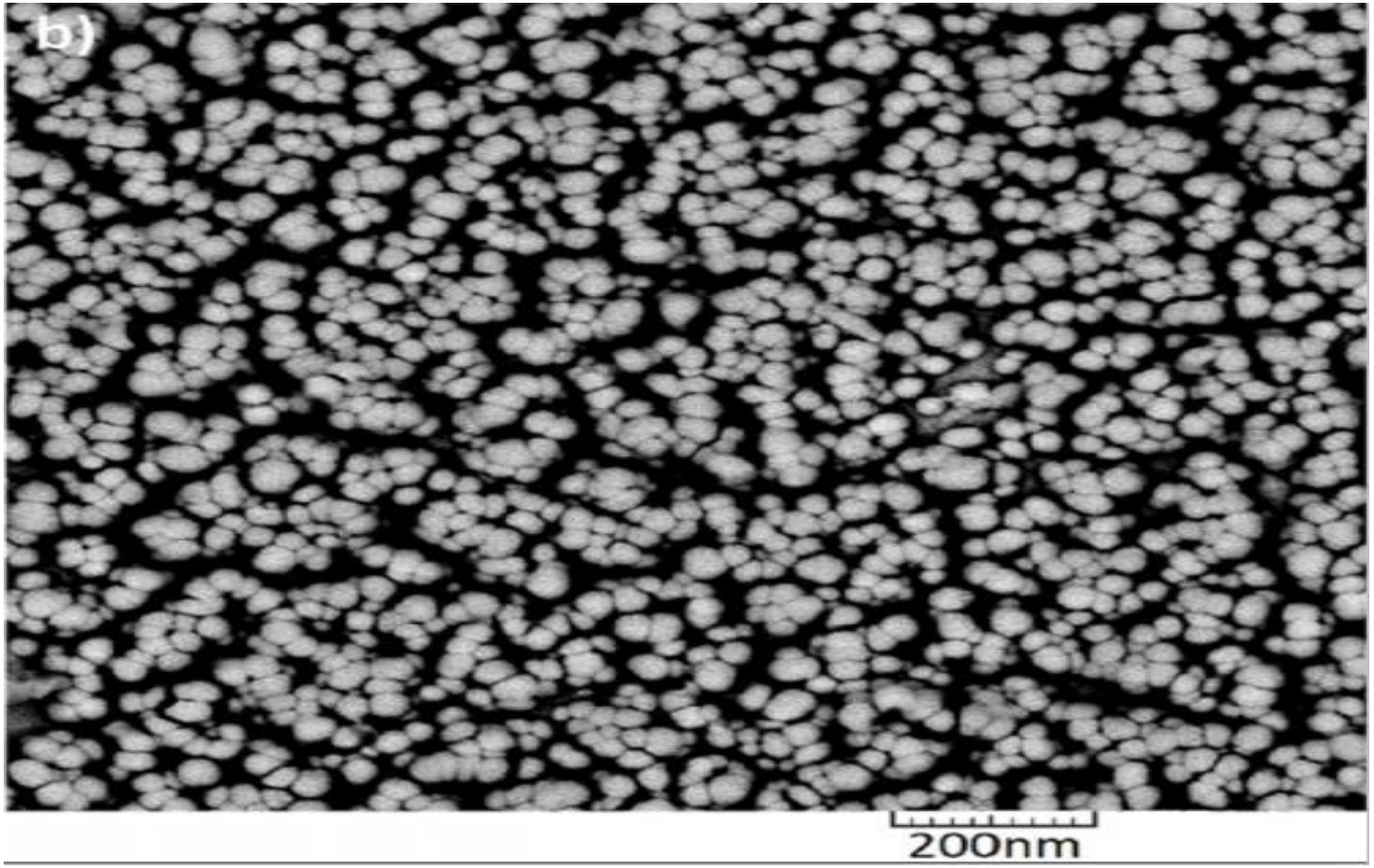
Published paper



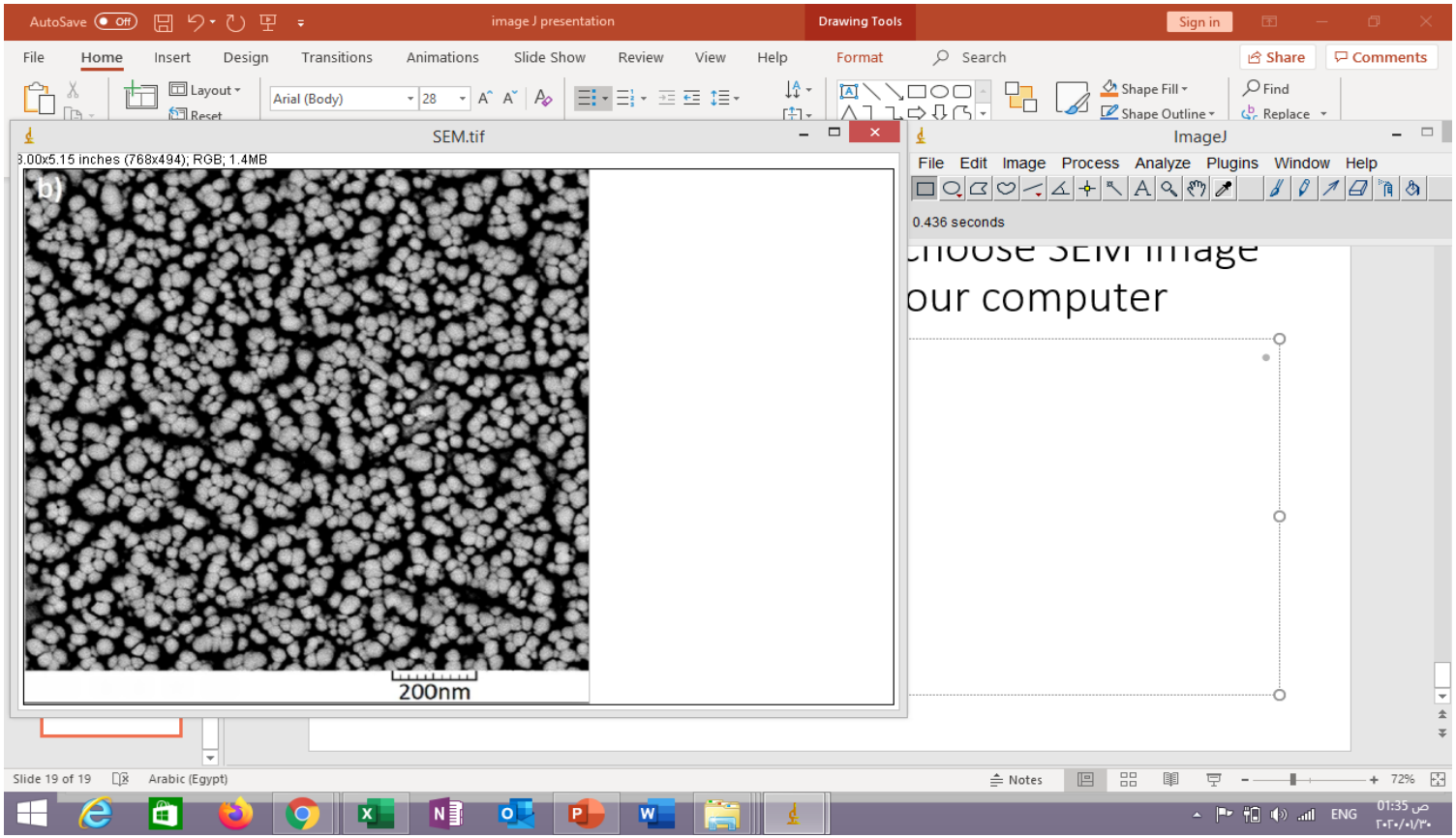
Picture containing SEM analysis



I separate the picture in paint and saved as TIFF file



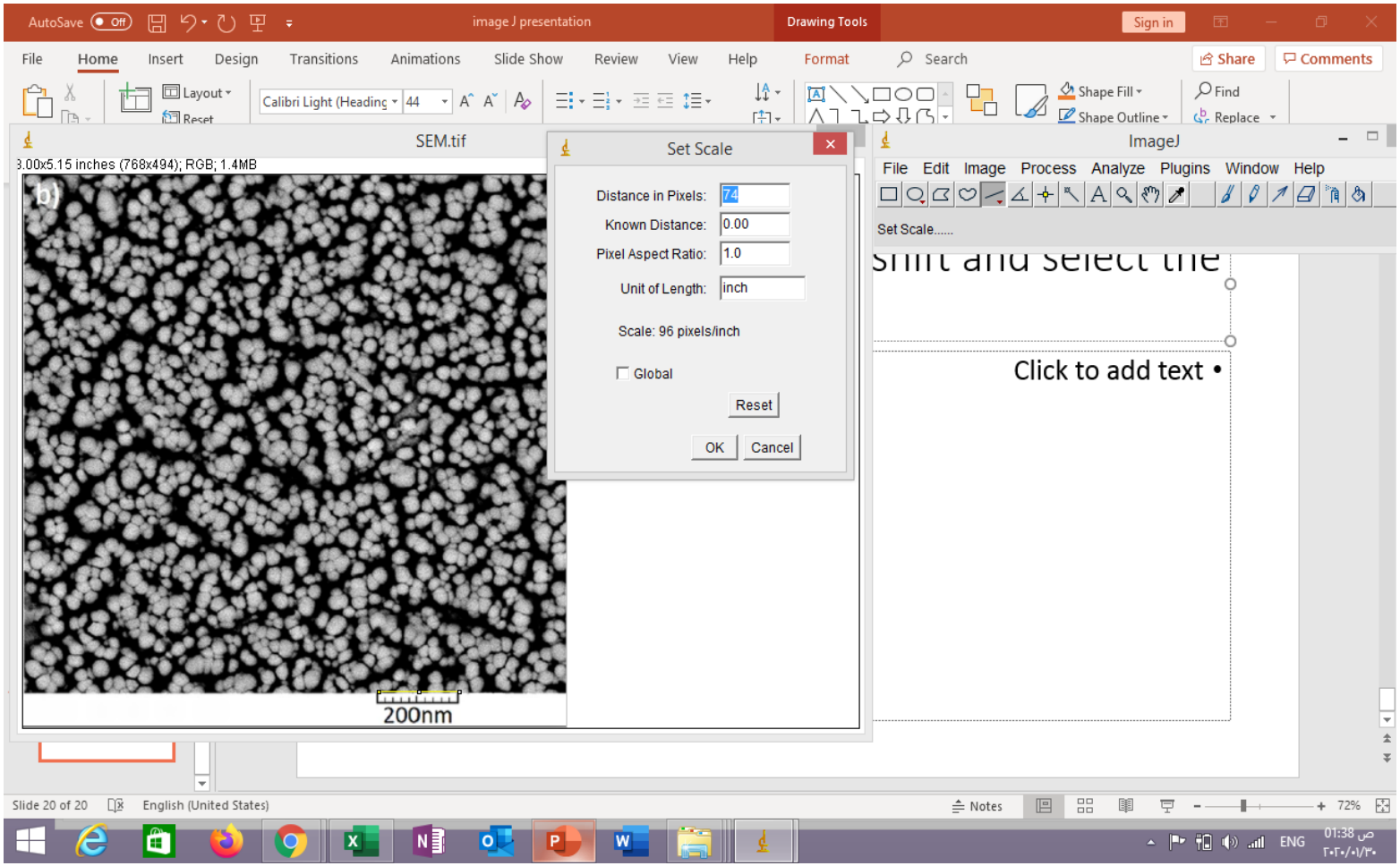
In image J file -----open ----choose SEM image from specific partition on your computer



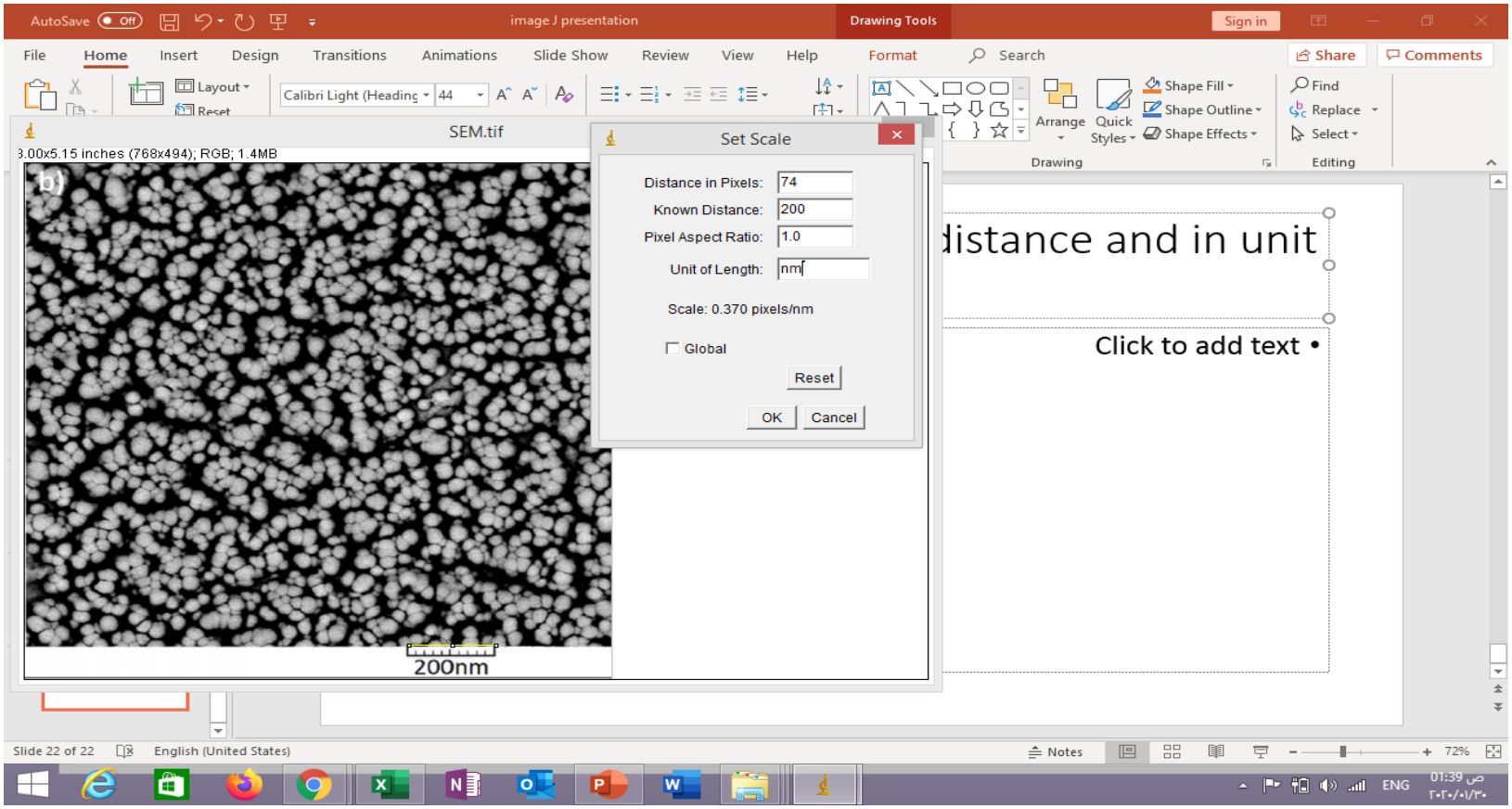
Click straight line tool with shift and select the scale at the end of picture

- 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

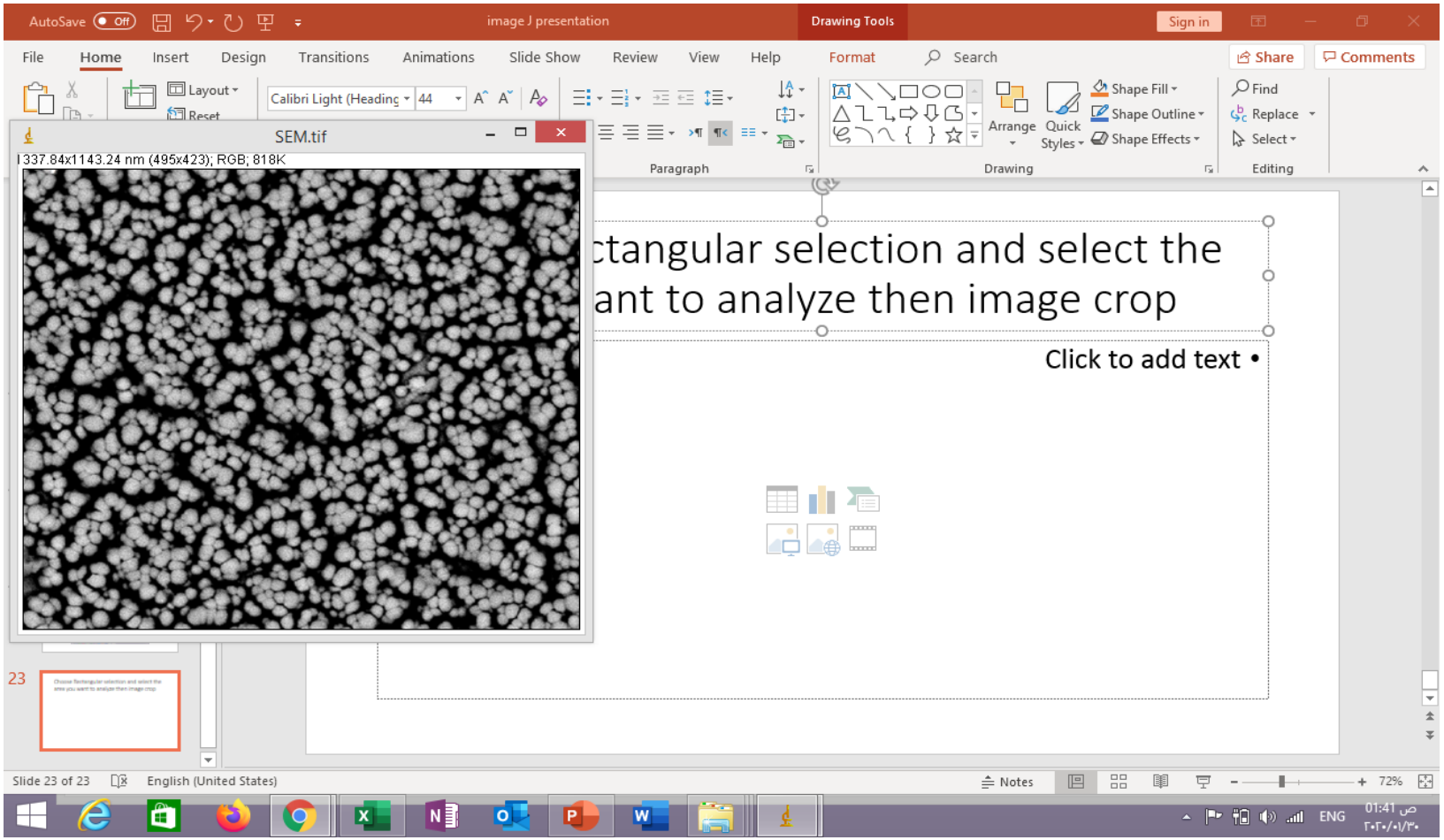
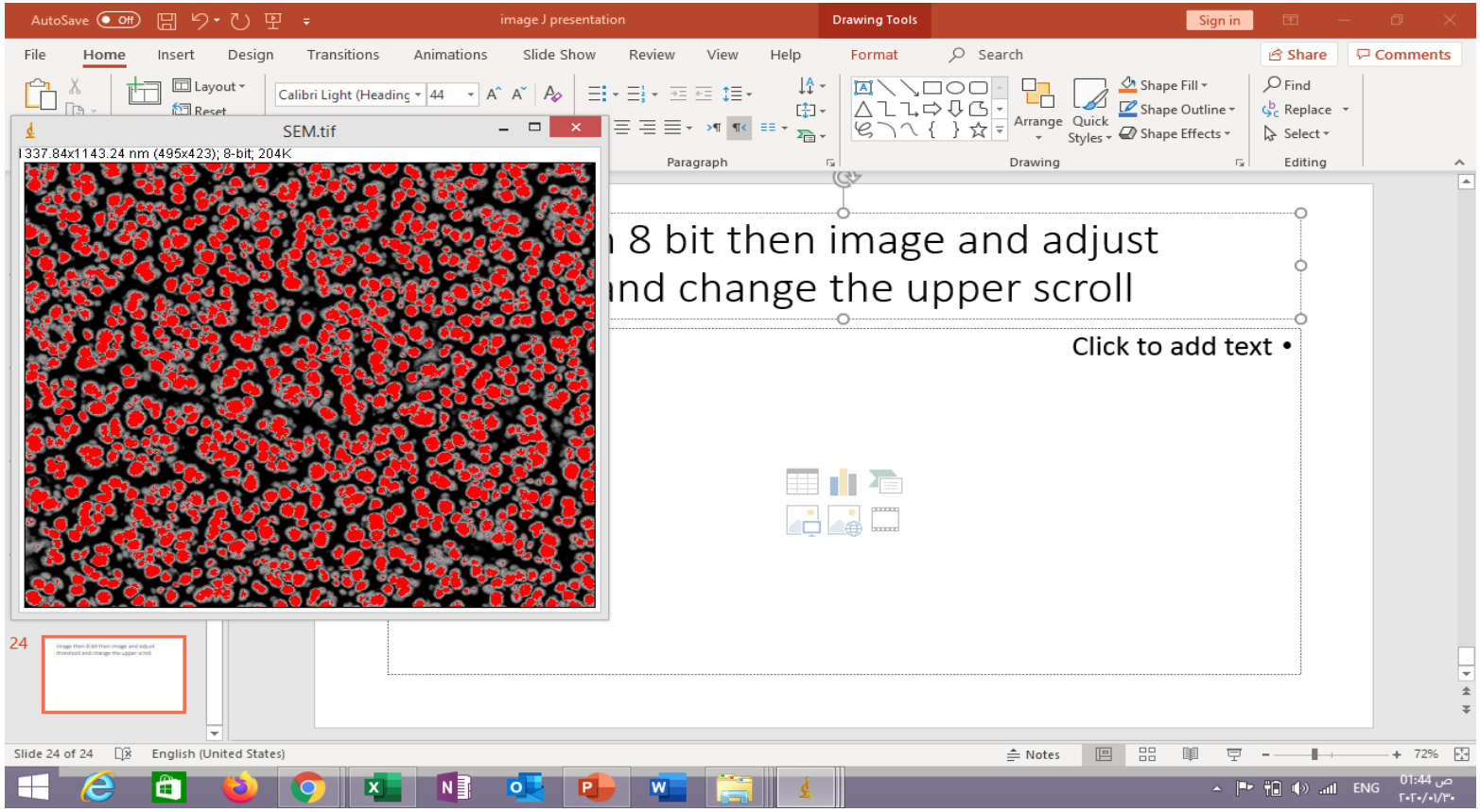
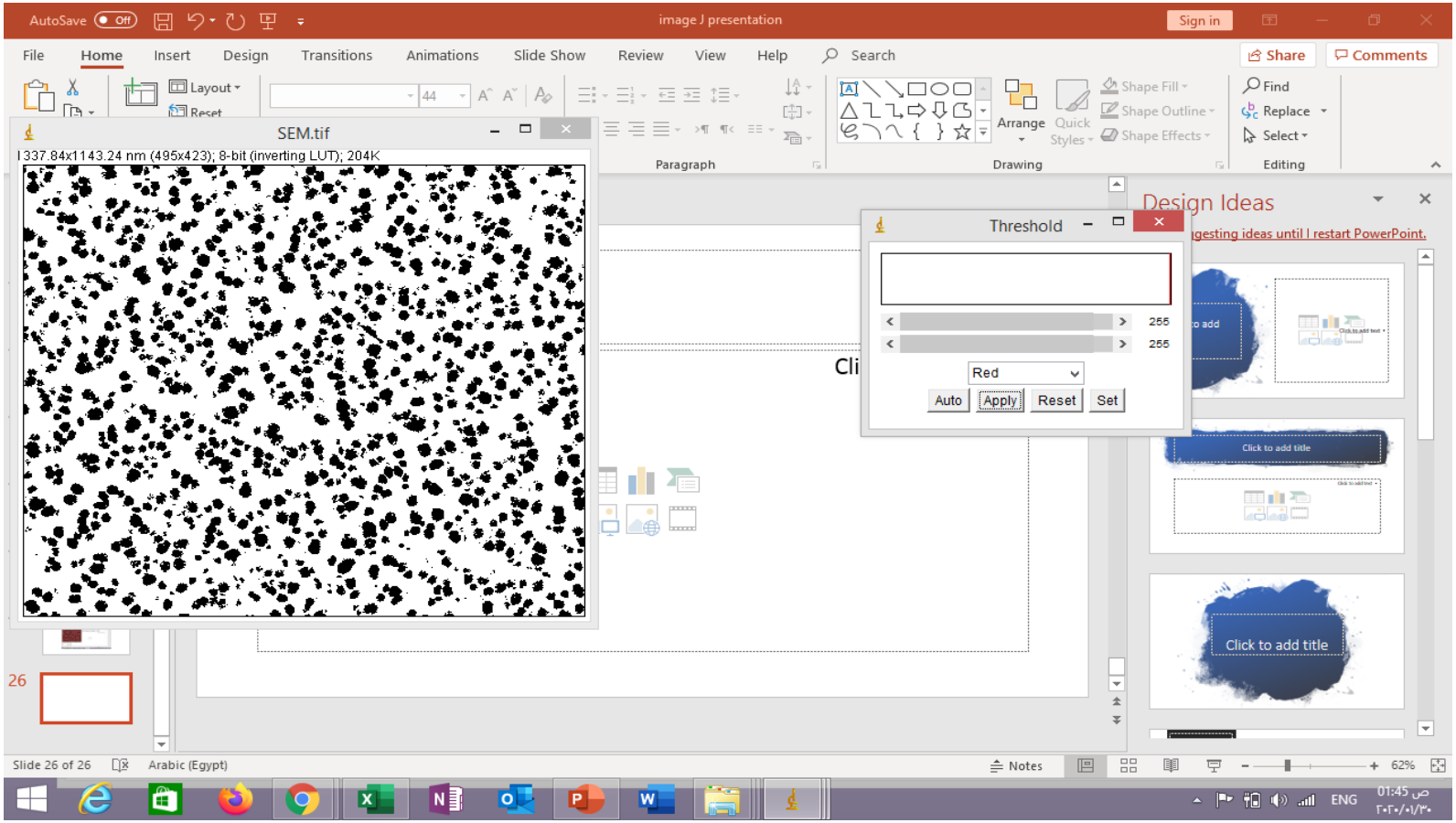


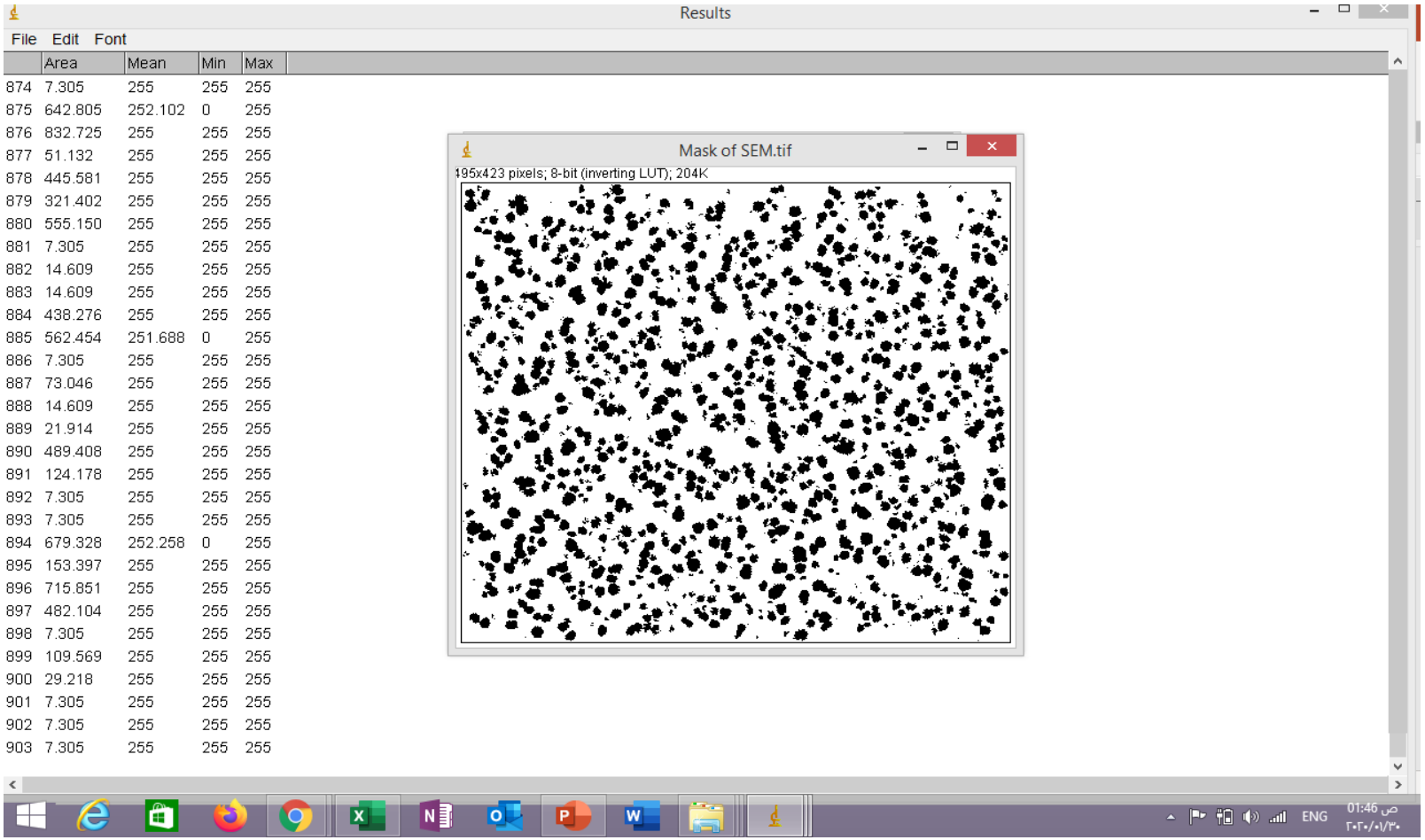
Image then 8 bit then image and adjust threshold and change the upper scroll then apply



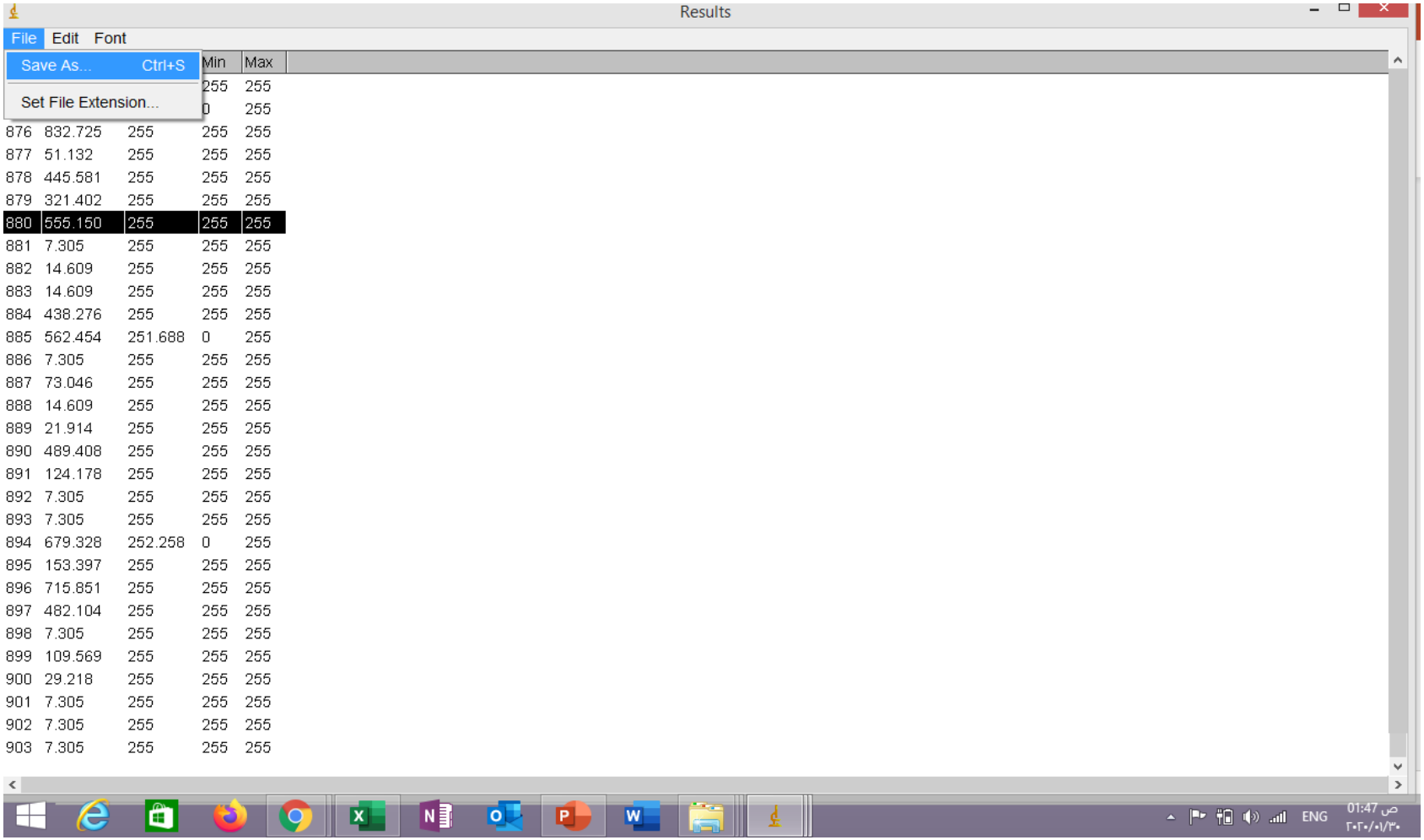
You will get such view



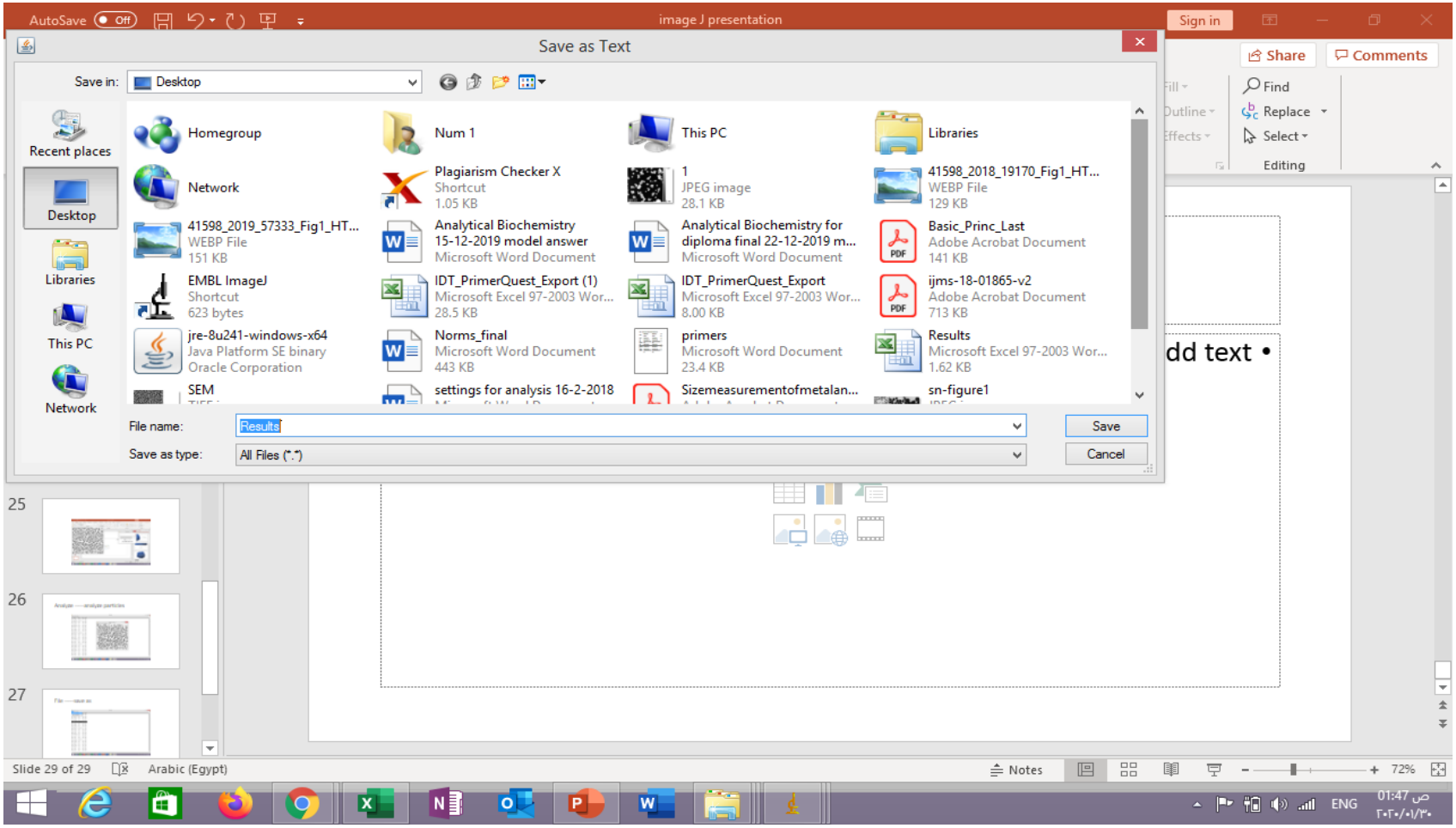
Analyze -----analyze particles and in show choose masks and you can save the new image



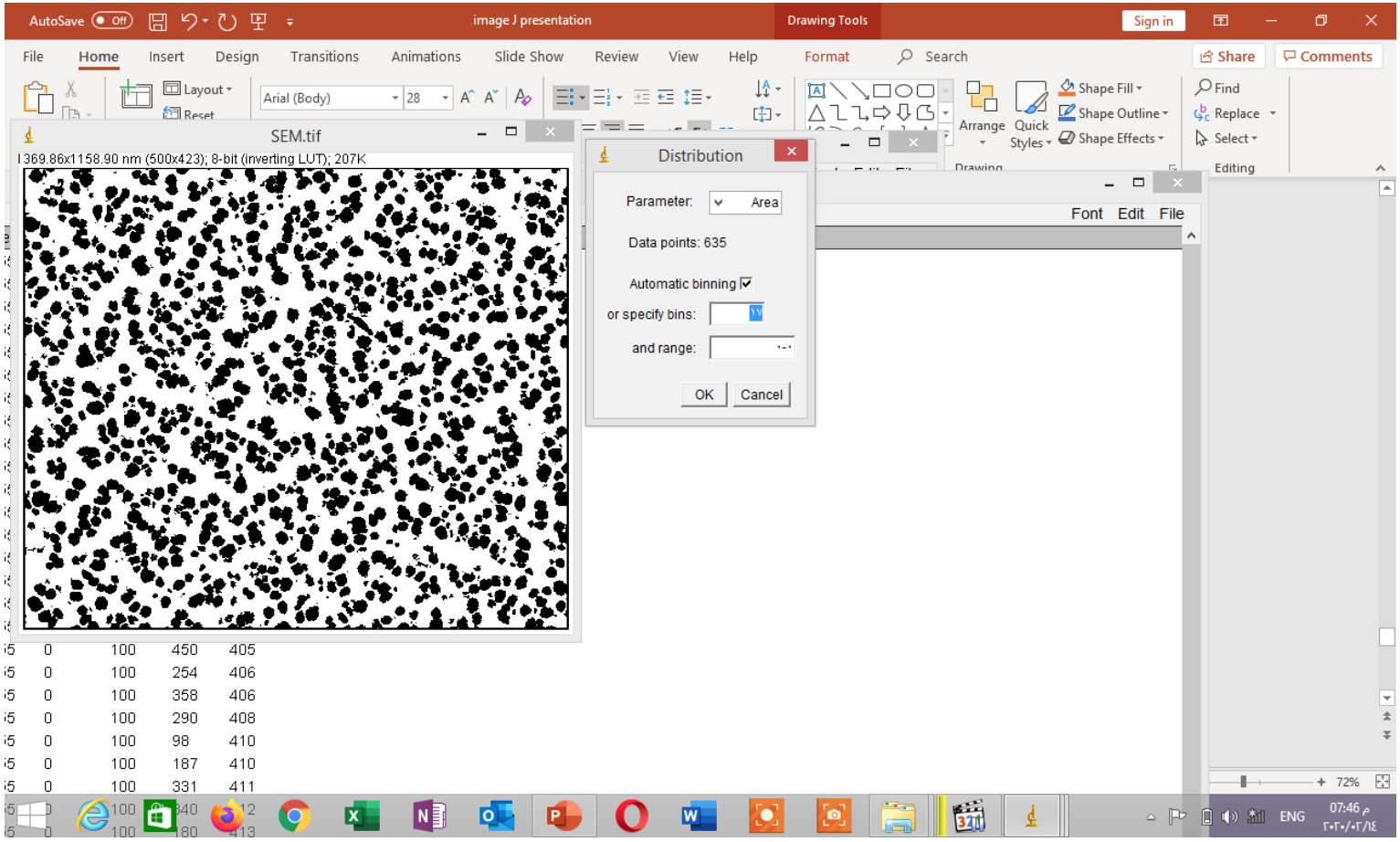
File -----save as


Results
File
Edit
Font
Save As... Ctrl+S
Set File Extension...
Min
Max
255 255
0 255
876 832.725 255 255 255
877 51.132 255 255 255
878 445.581 255 255 255
879 321.402 255 255 255
880 555.150 255 255 255
881 7.305 255 255 255
882 14.609 255 255 255
883 14.609 255 255 255
884 438.276 255 255 255
885 562.454 251.688 0 255
886 7.305 255 255 255
887 73.046 255 255 255
888 14.609 255 255 255
889 21.914 255 255 255
890 489.408 255 255 255
891 124.178 255 255 255
892 7.305 255 255 255
893 7.305 255 255 255
894 679.328 252.258 0 255
895 153.397 255 255 255
896 715.851 255 255 255
897 482.104 255 255 255
898 7.305 255 255 255
899 109.569 255 255 255
900 29.218 255 255 255
901 7.305 255 255 255
902 7.305 255 255 255
903 7.305 255 255 255
ENG
01:47 ص
٢٠٢٠/٠١/٣٠

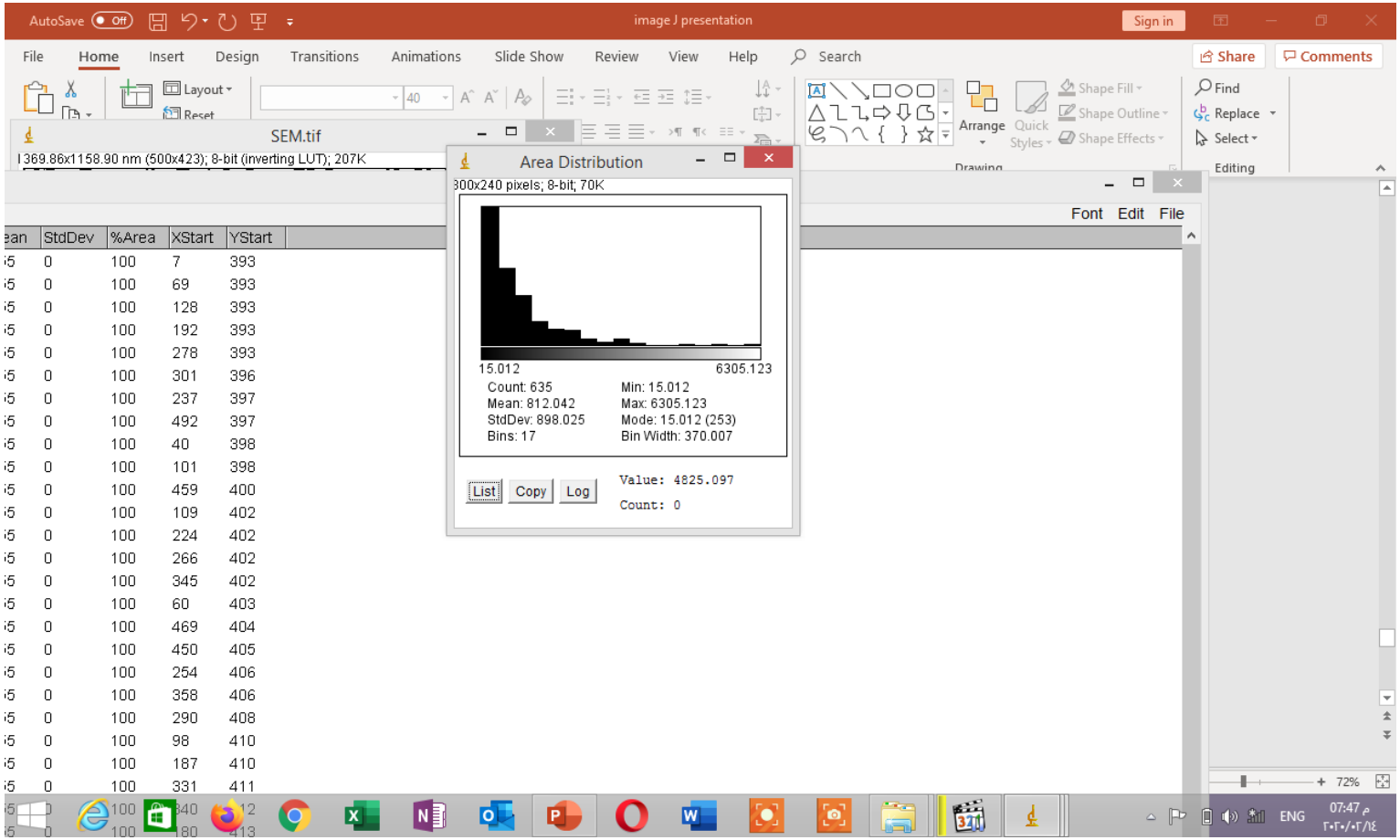
Select specific partition and analyze your results using excel



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



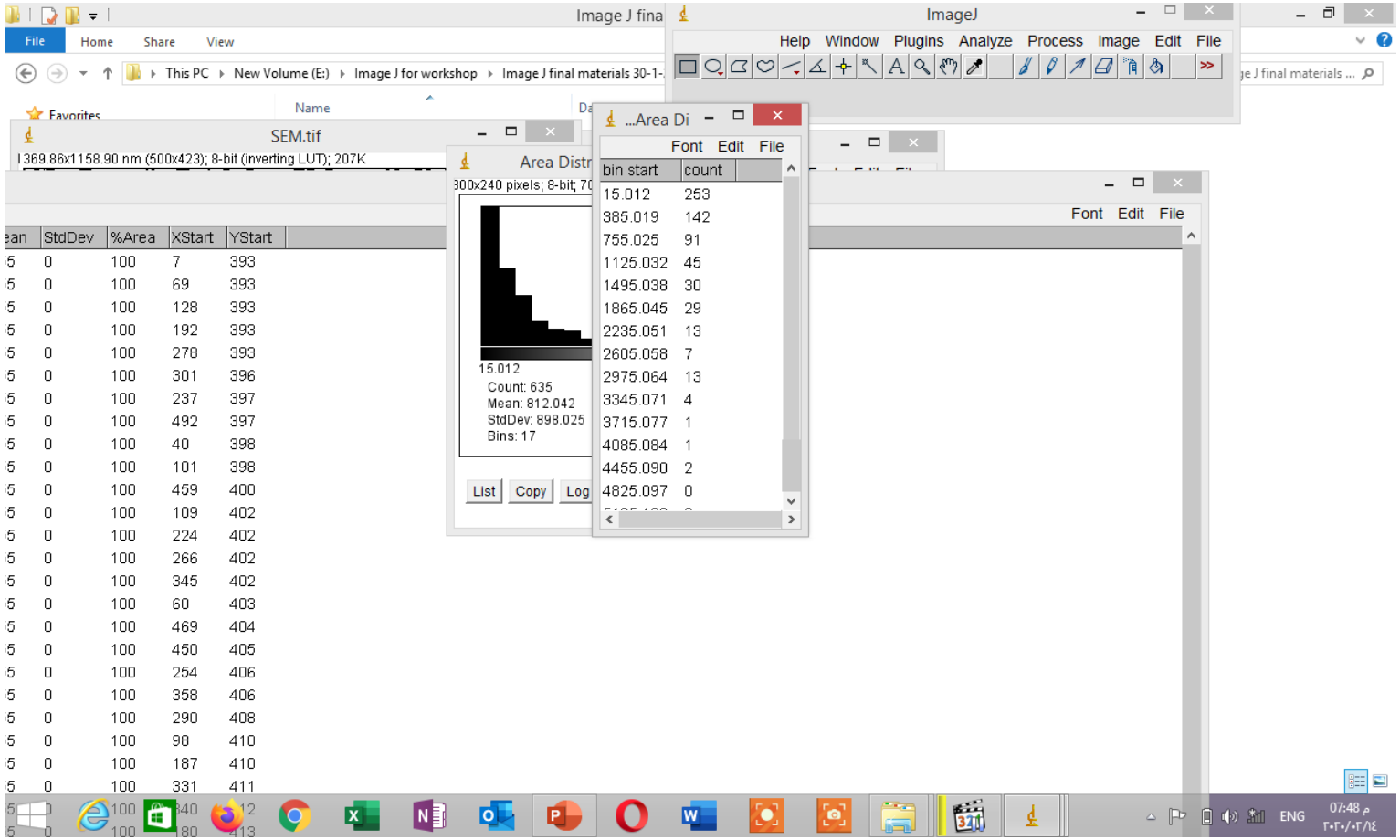
You will get area distribution graph as following by clicking on list you will get the next window



Distribution histogram

- Size range ….split into small size classes or bins and the no. of particles contained in each size bin are counted.

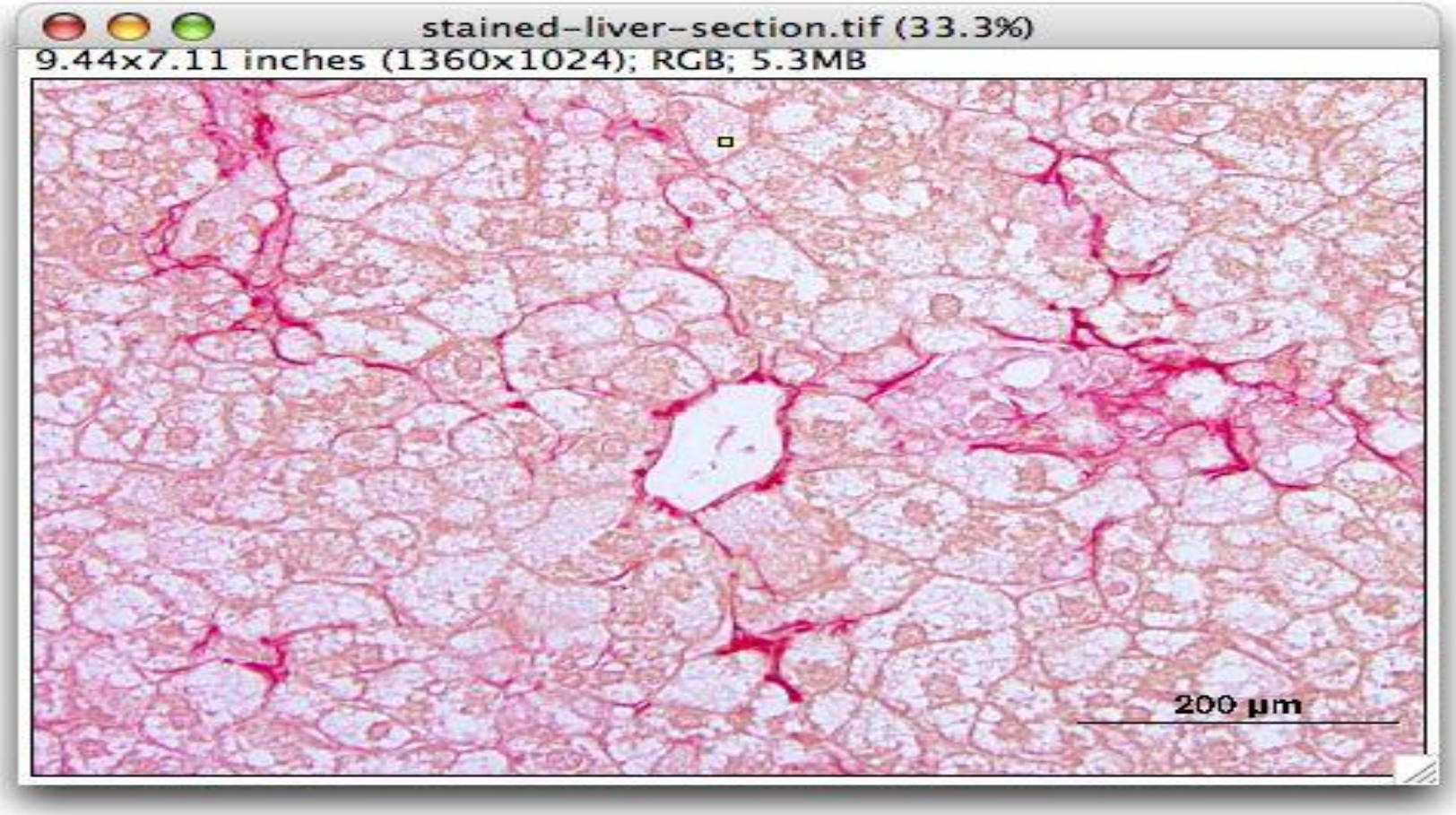
You can save data for area distribution for further analysis



Calculate the Diameter of a Circle, from Its Area

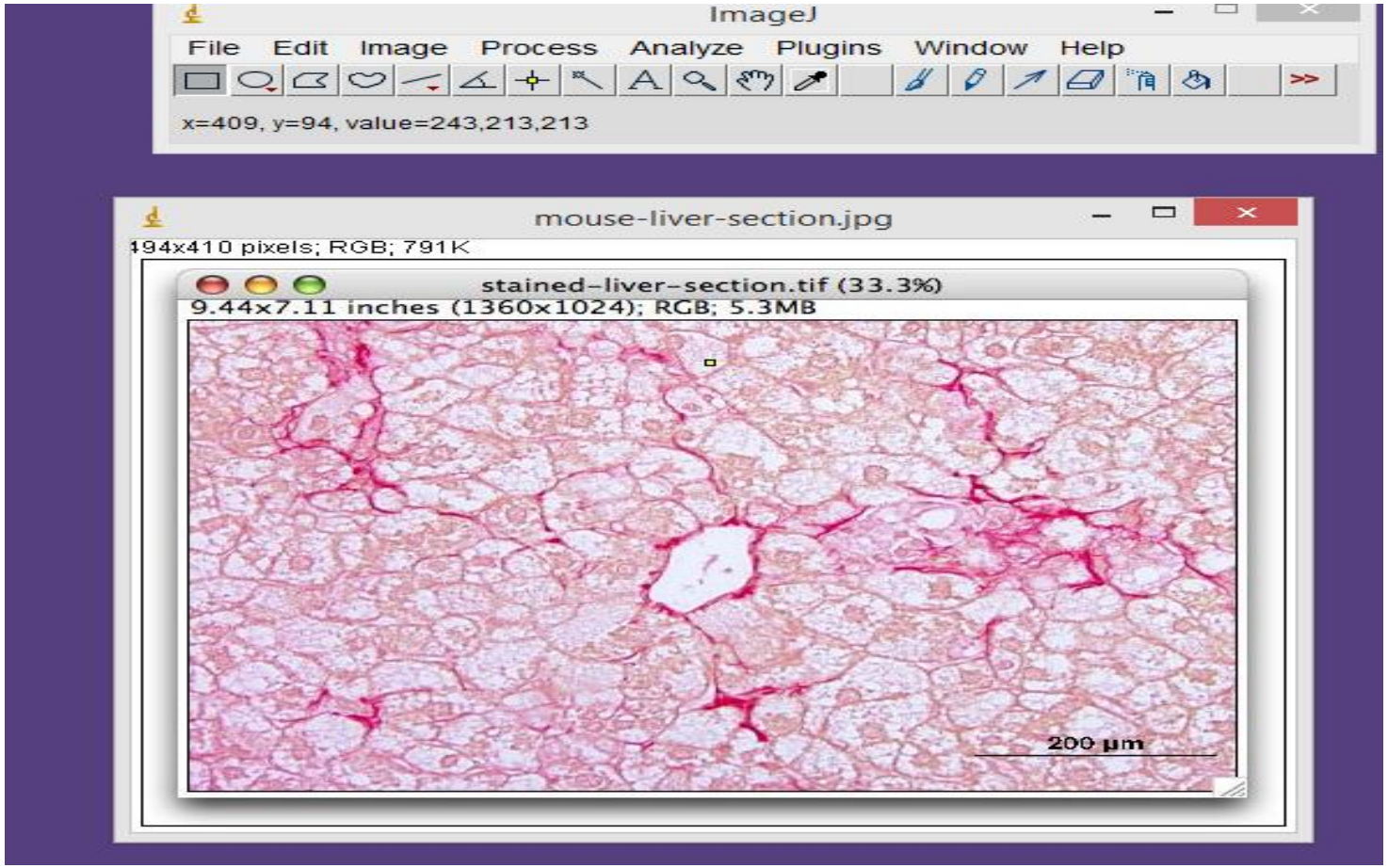
1. Divide the **area** (in square units) by Pi (approximately 3.14159). Example: 303,000/3.14159 = 96447.98.
2. Take the square root of the result (Example: 310.56). This is the radius.
3. Now double the radius to get the **diameter** (Example: 621.12 meters).

Quantifying Stained Liver Tissue

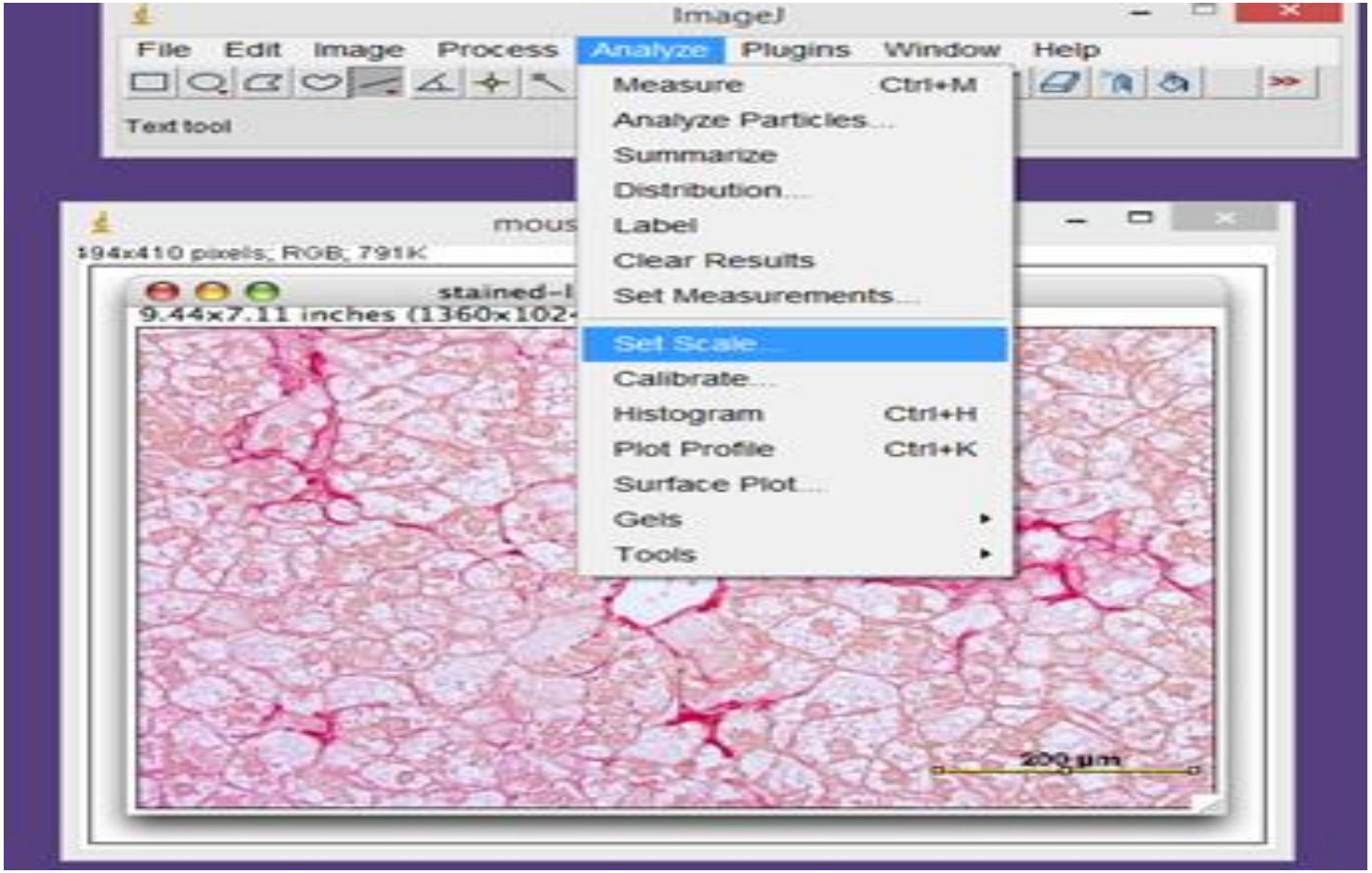


Open the image in image J

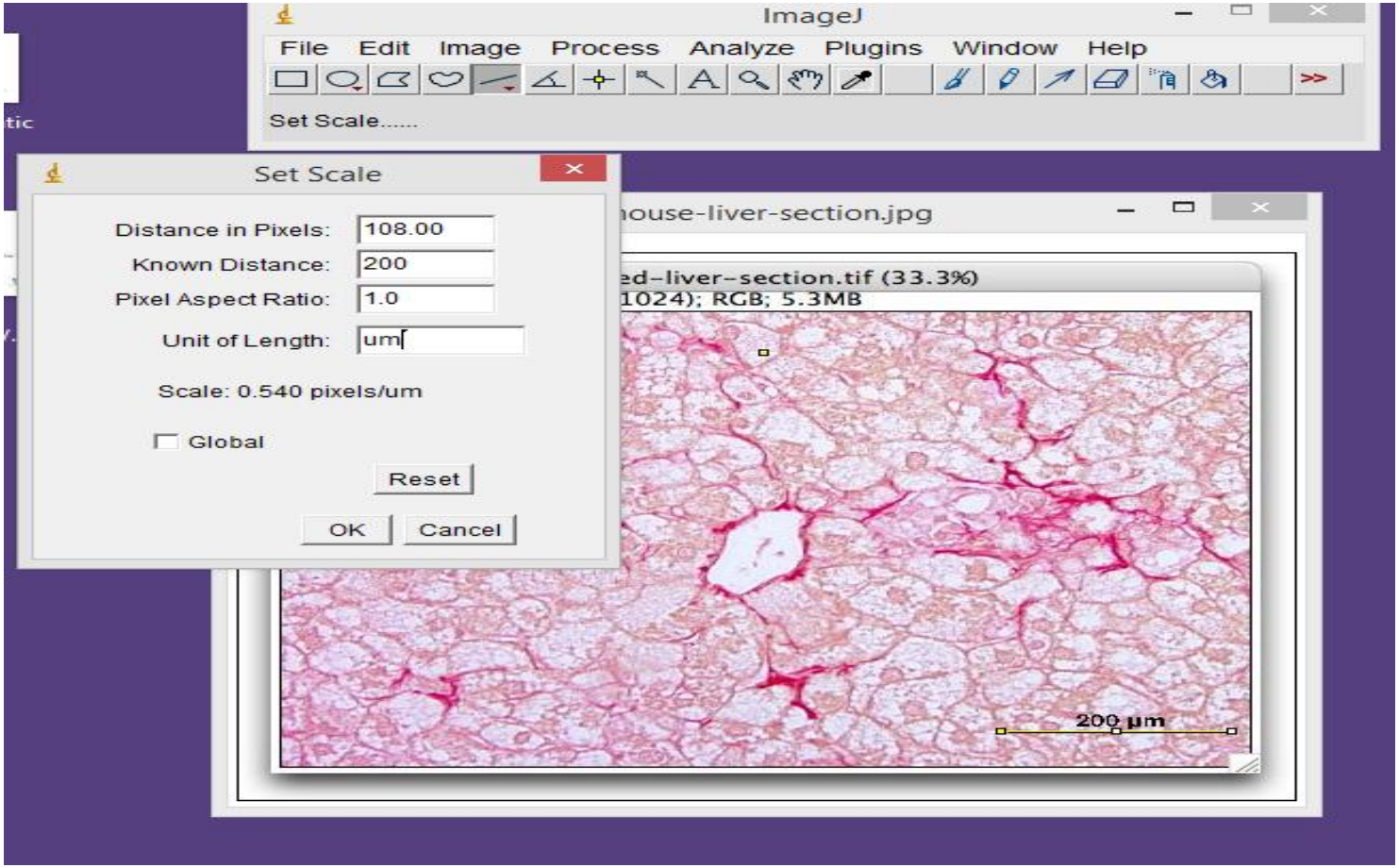
- **File ….open …..choose its location…..open**



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".



Dimensions changed to um scale

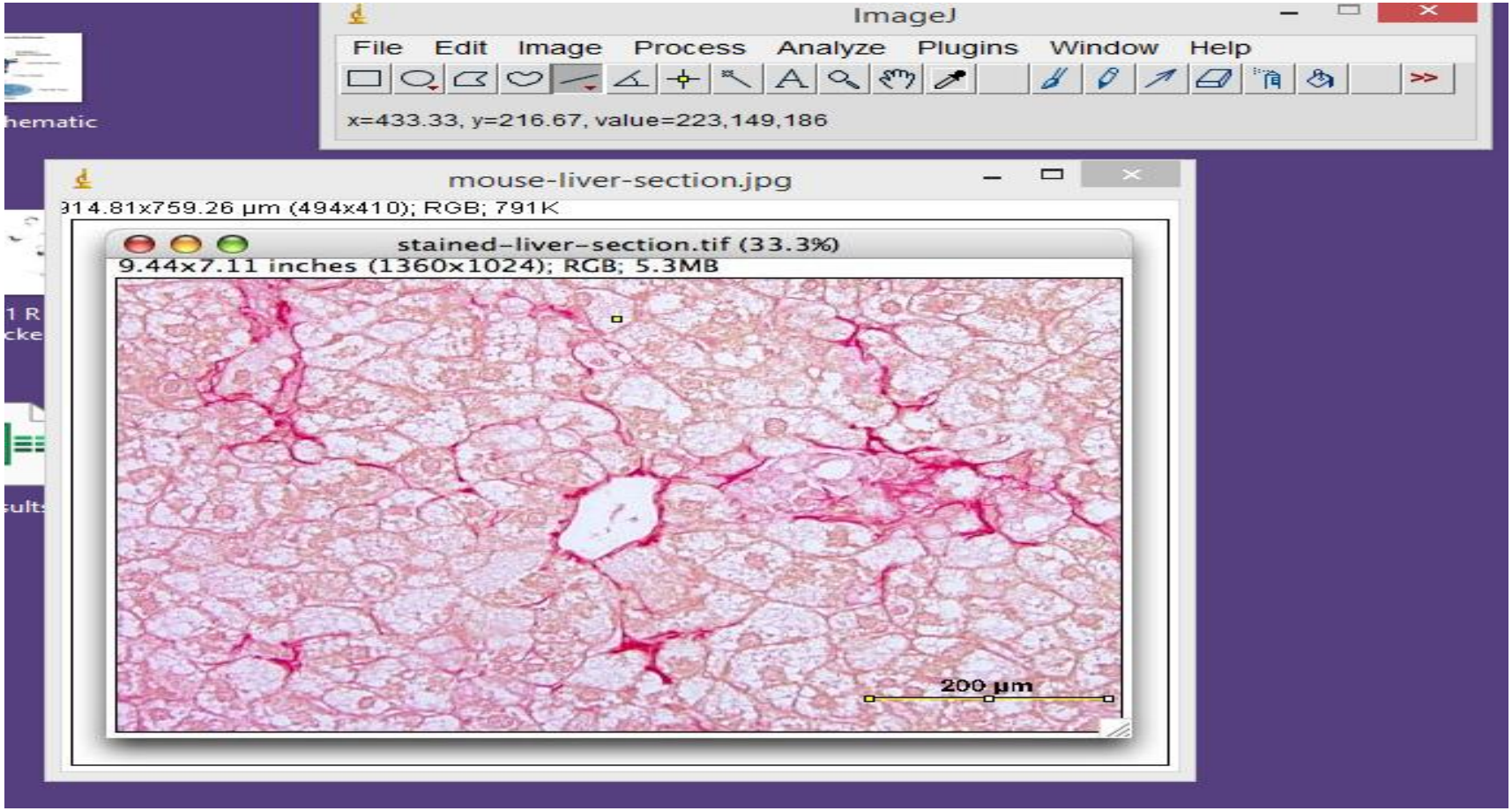
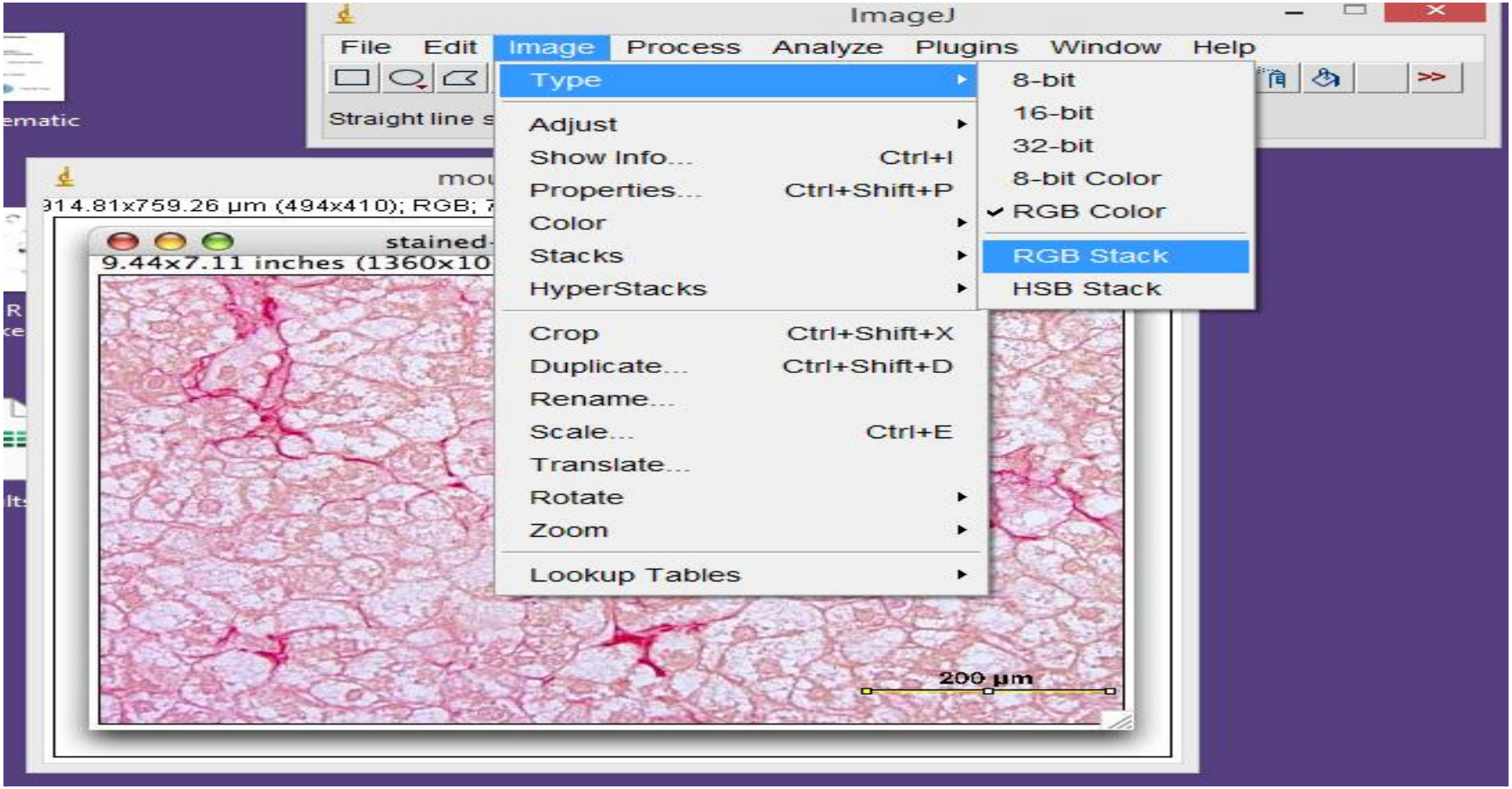
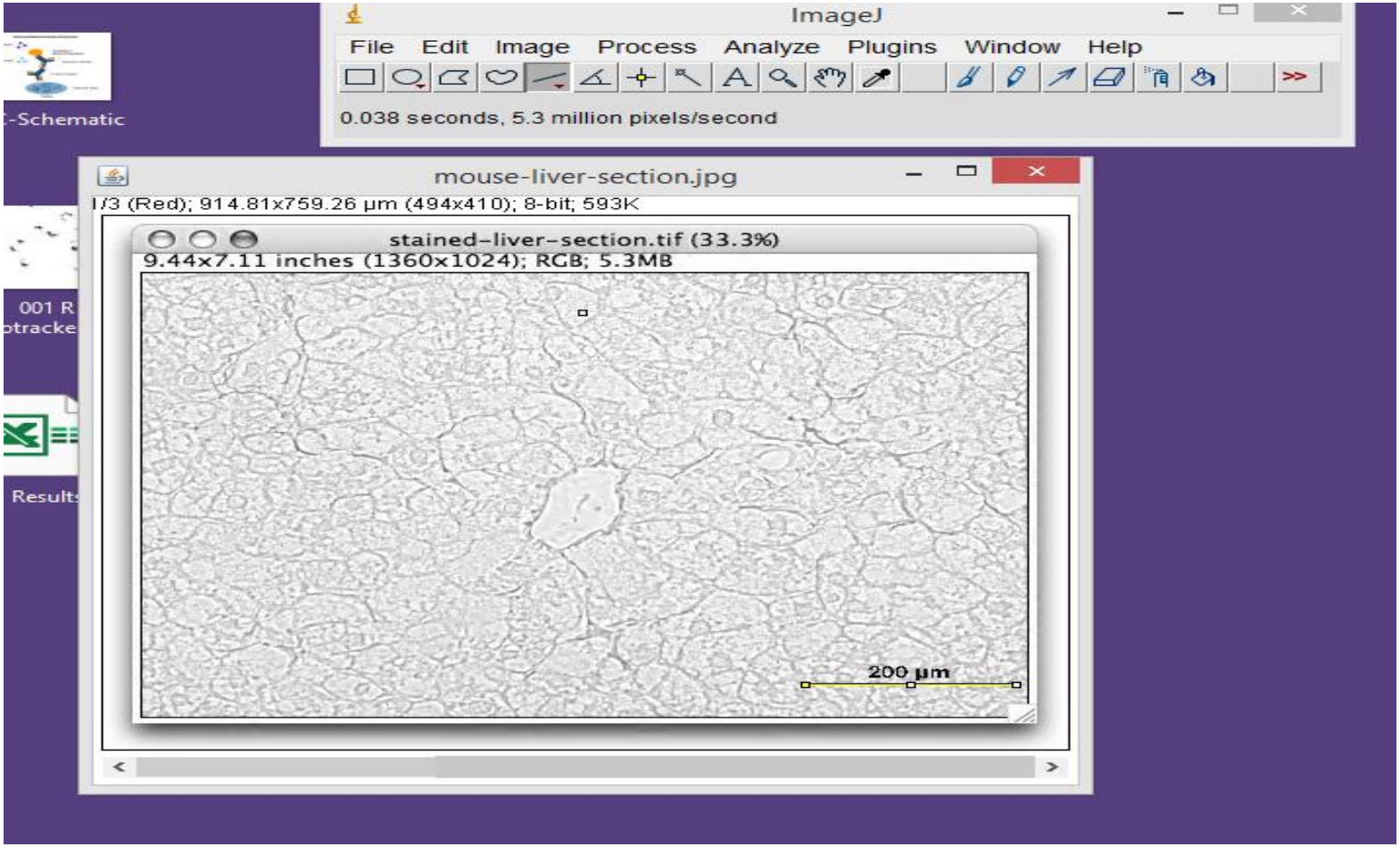


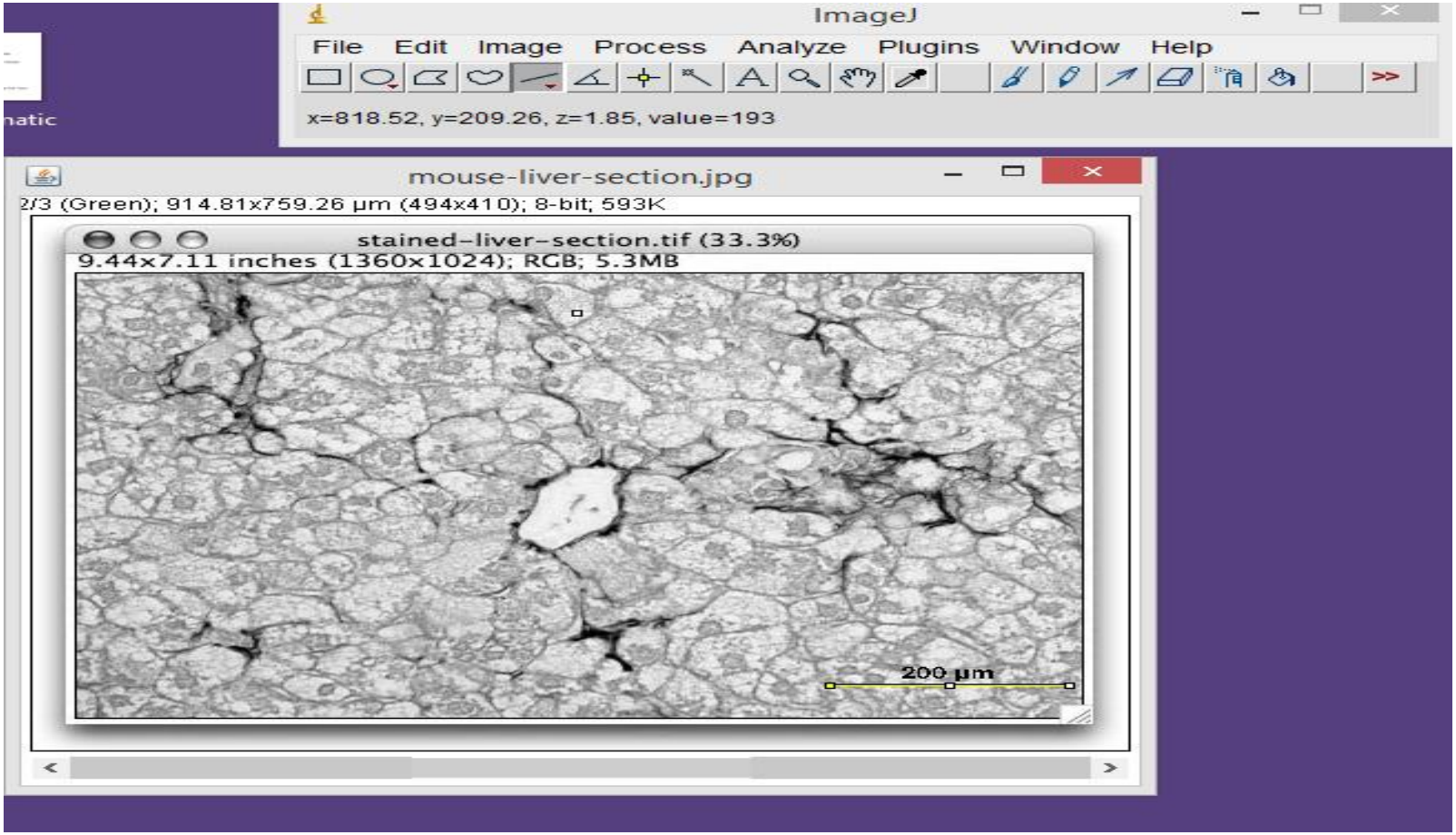
Image ----type ---RGB stack to split the image into red, green and blue channels.



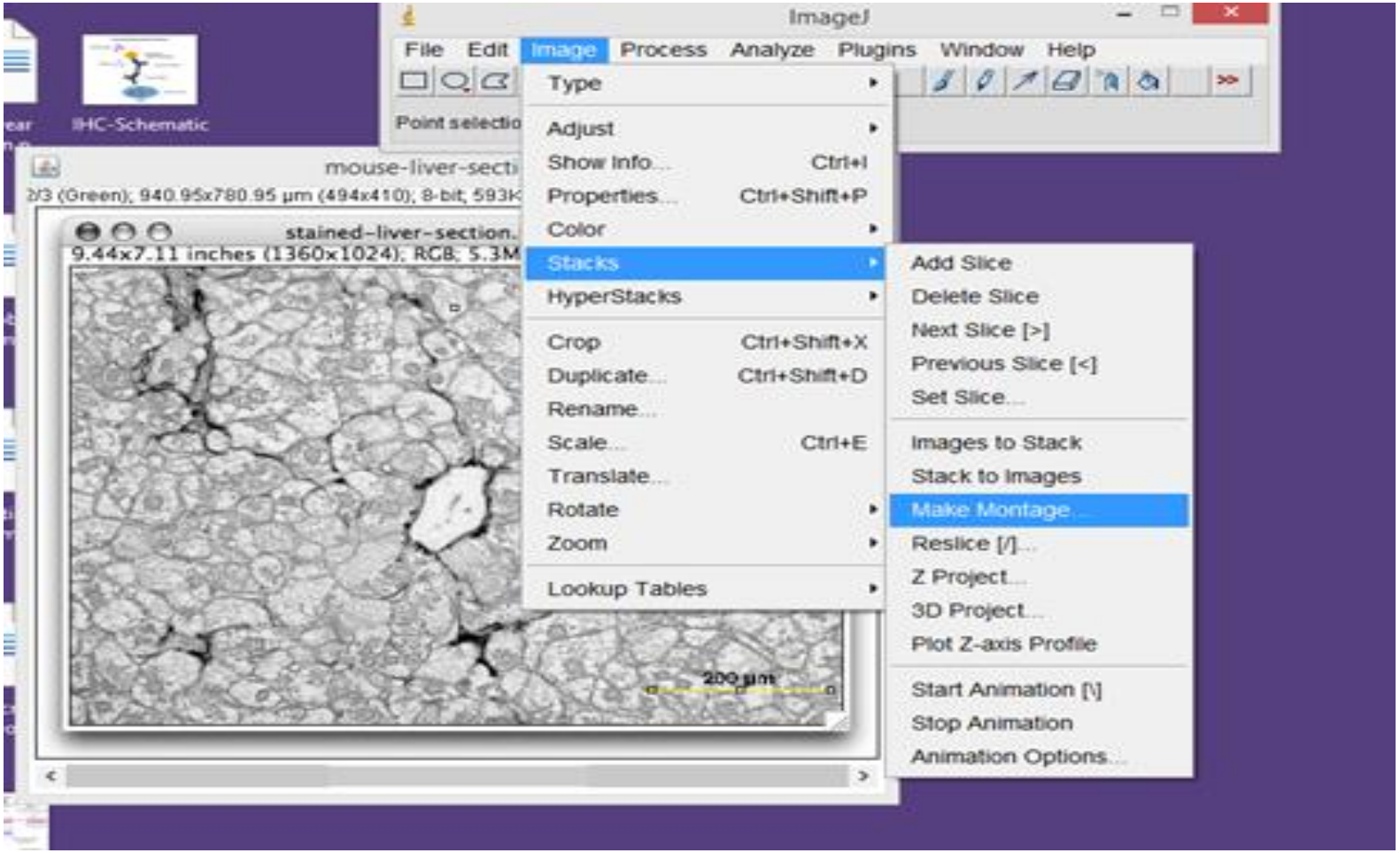
You will get such view



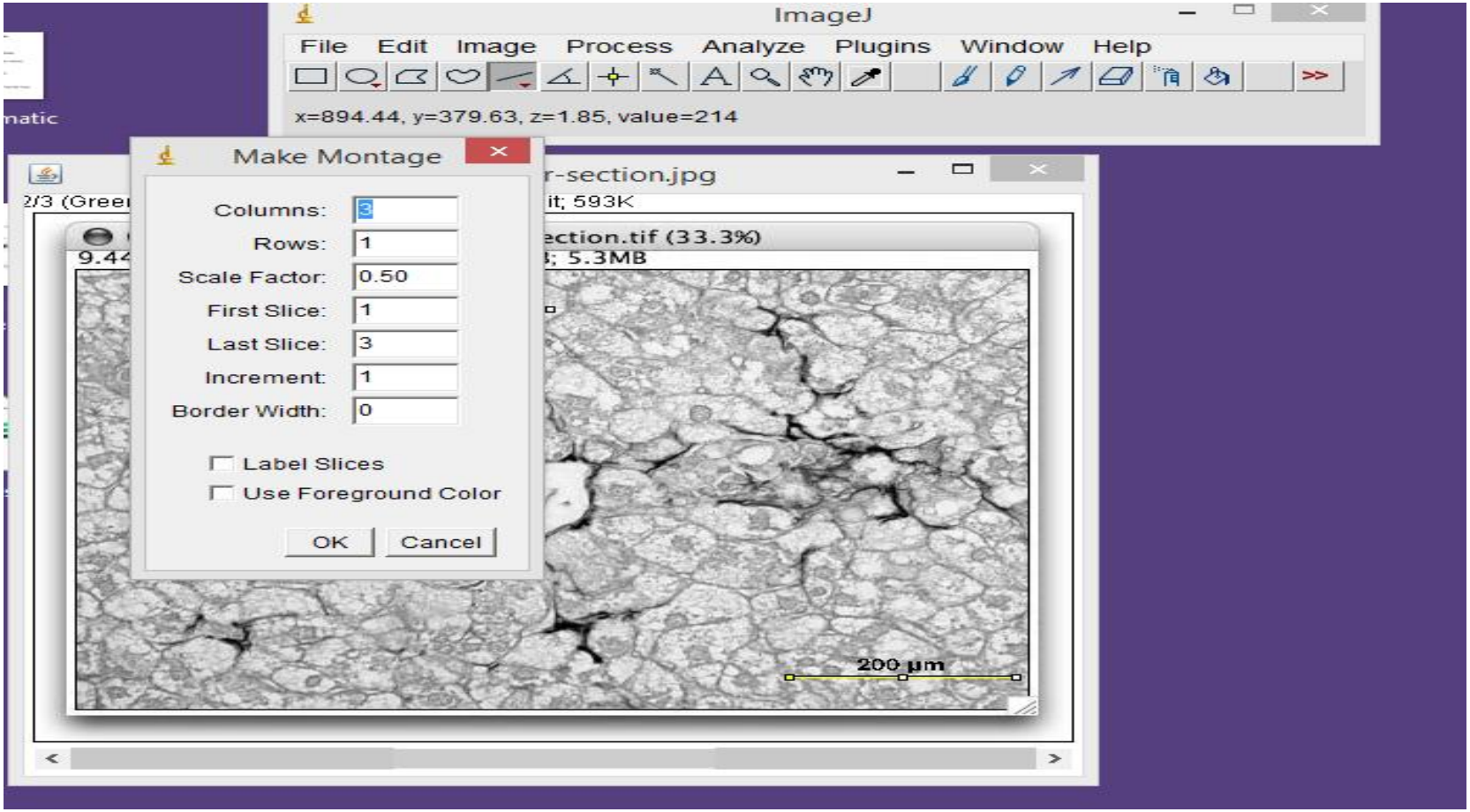
Move the slider to view each of the channels. Notice that the green channel has the best separation.



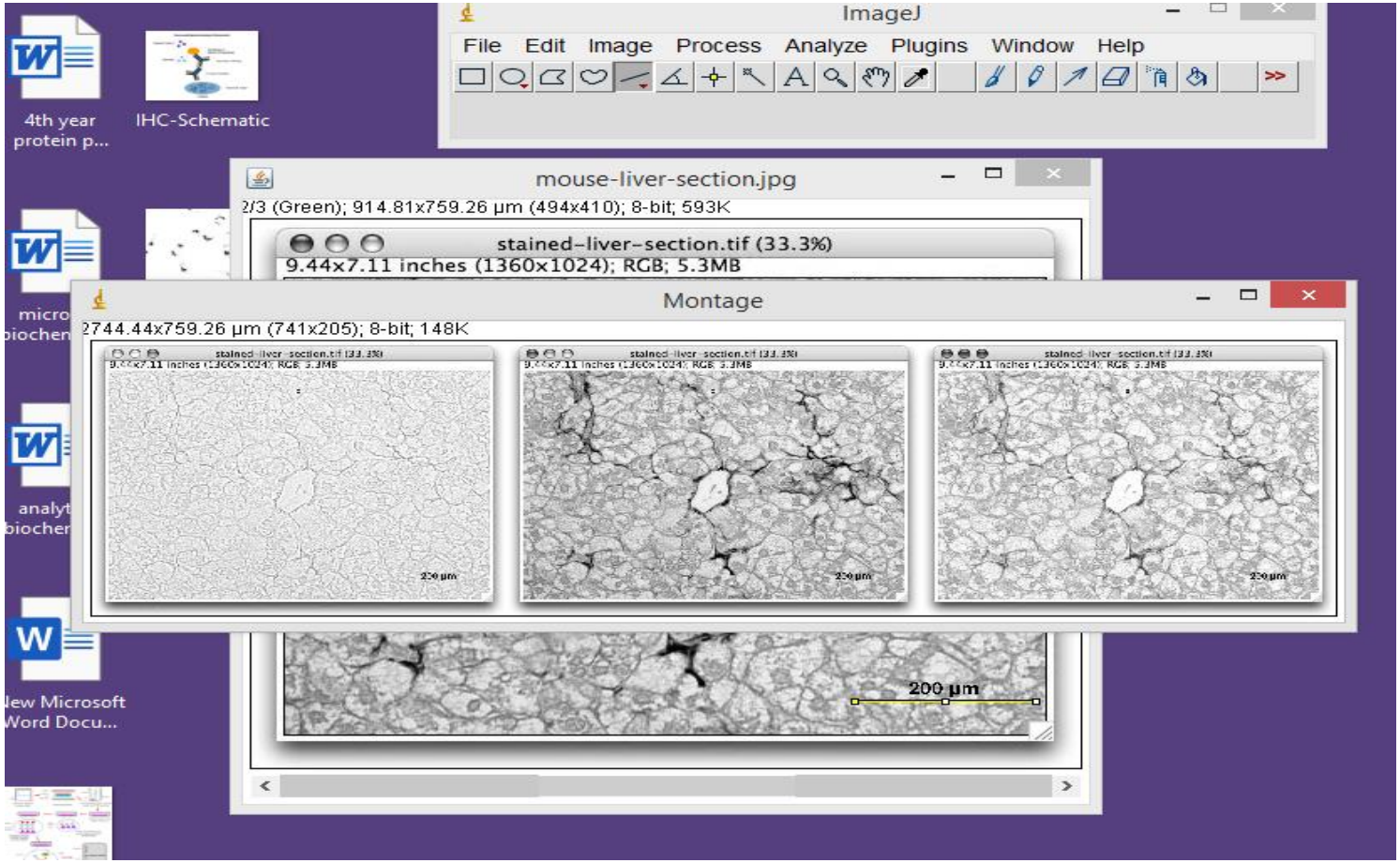
Image>Stacks>Make Montage to view all three channels at the same time.



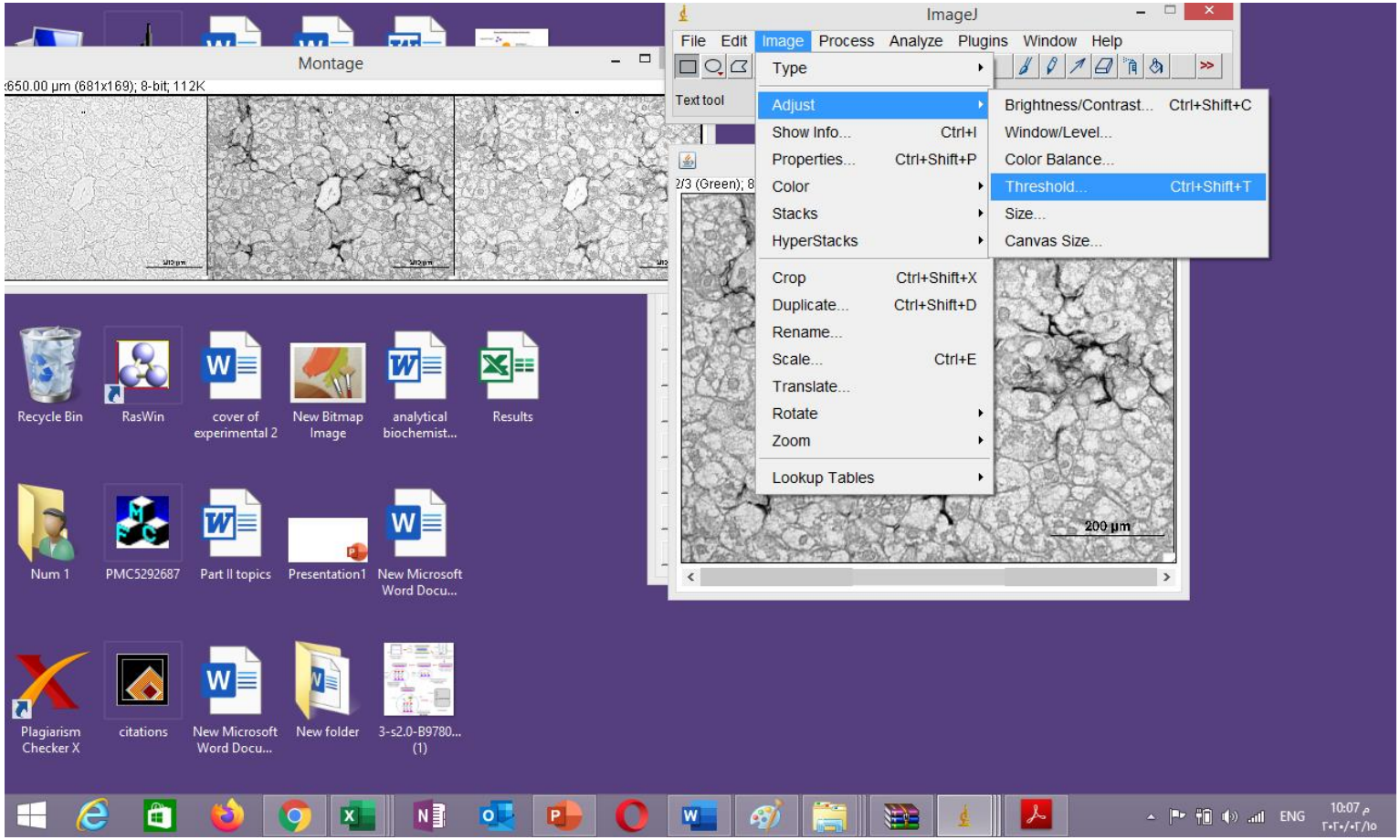
Click ok in make montage box



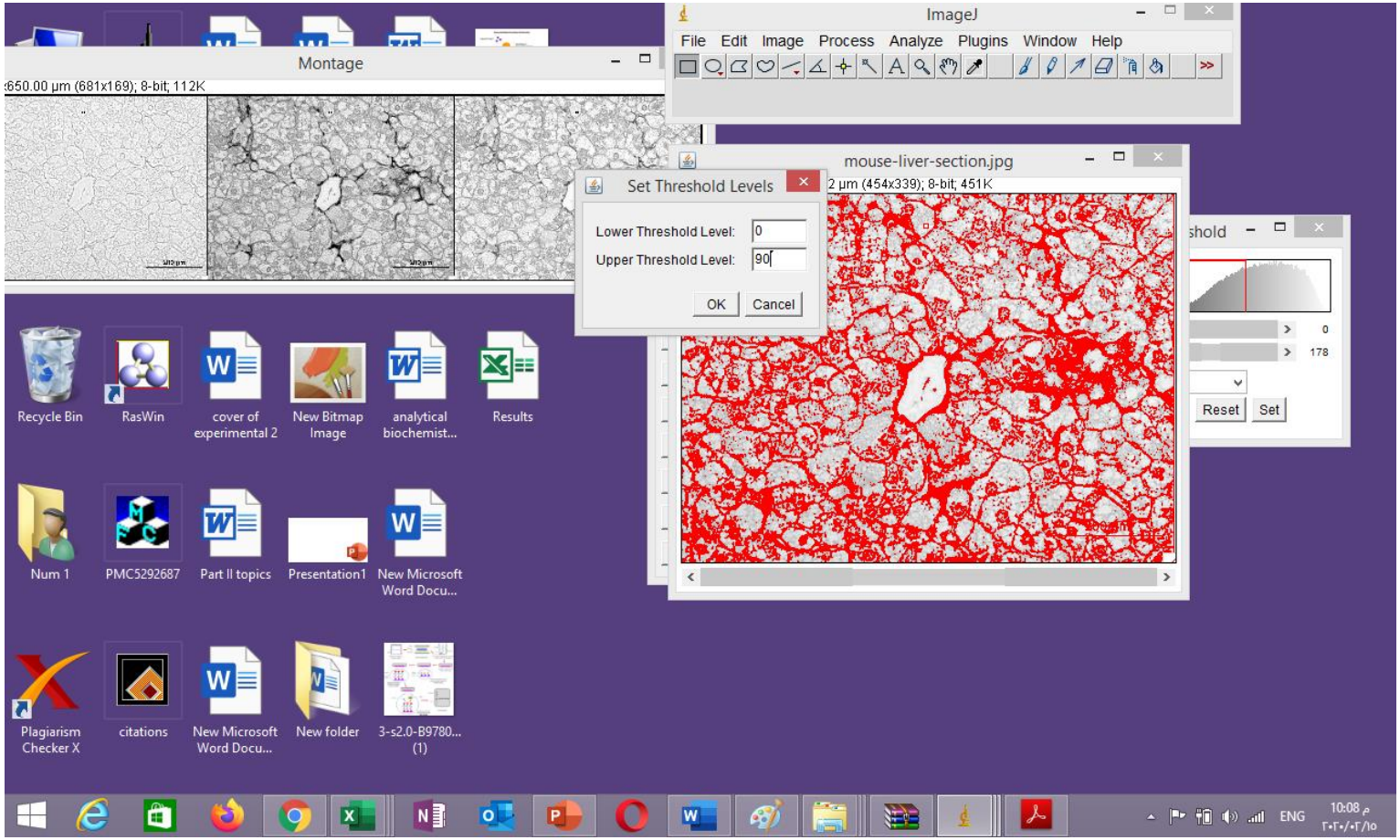
All three channels will appear at the same time appear



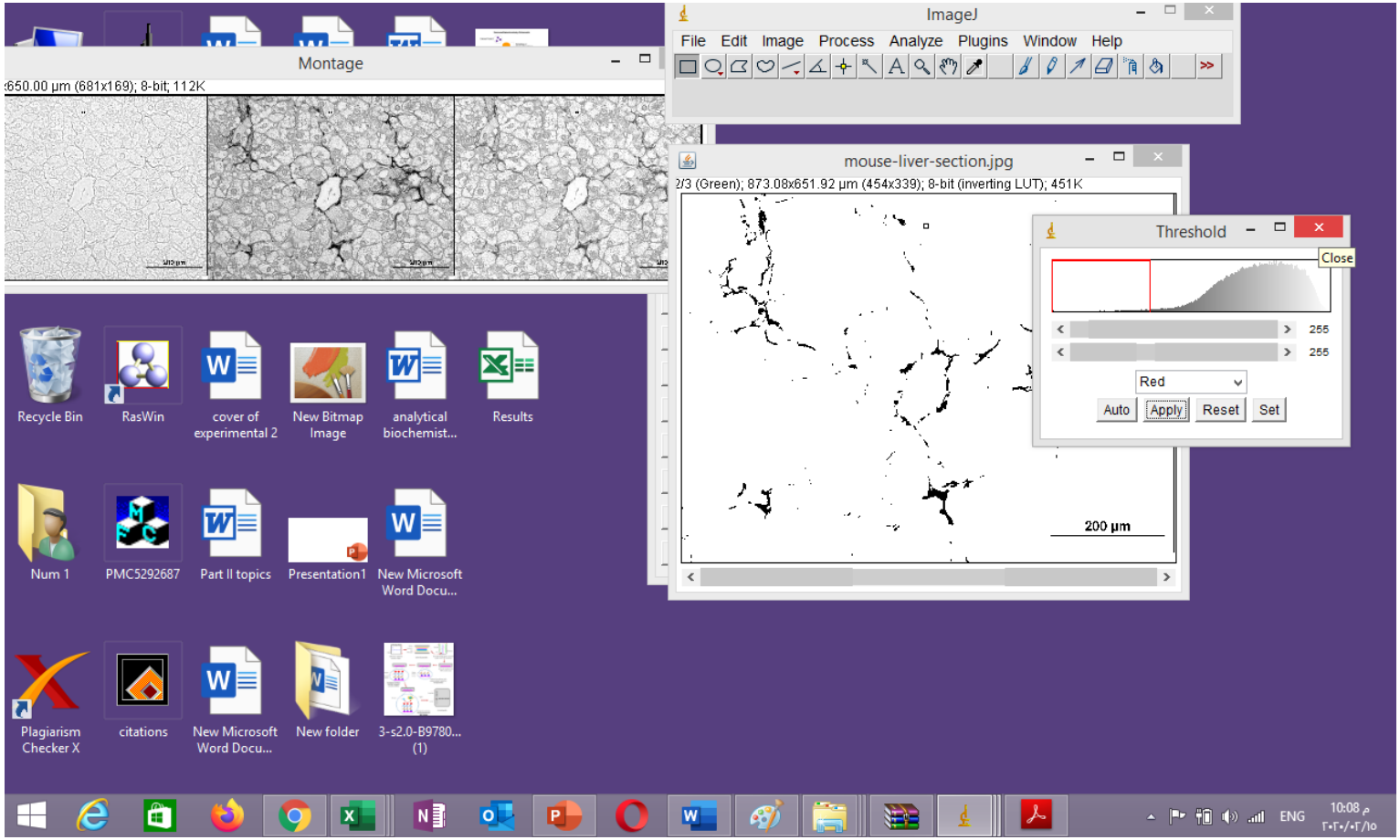
Select the RGB stack (with the Green channel selected) and press shift-t (*Image>Adjust>Threshold*).



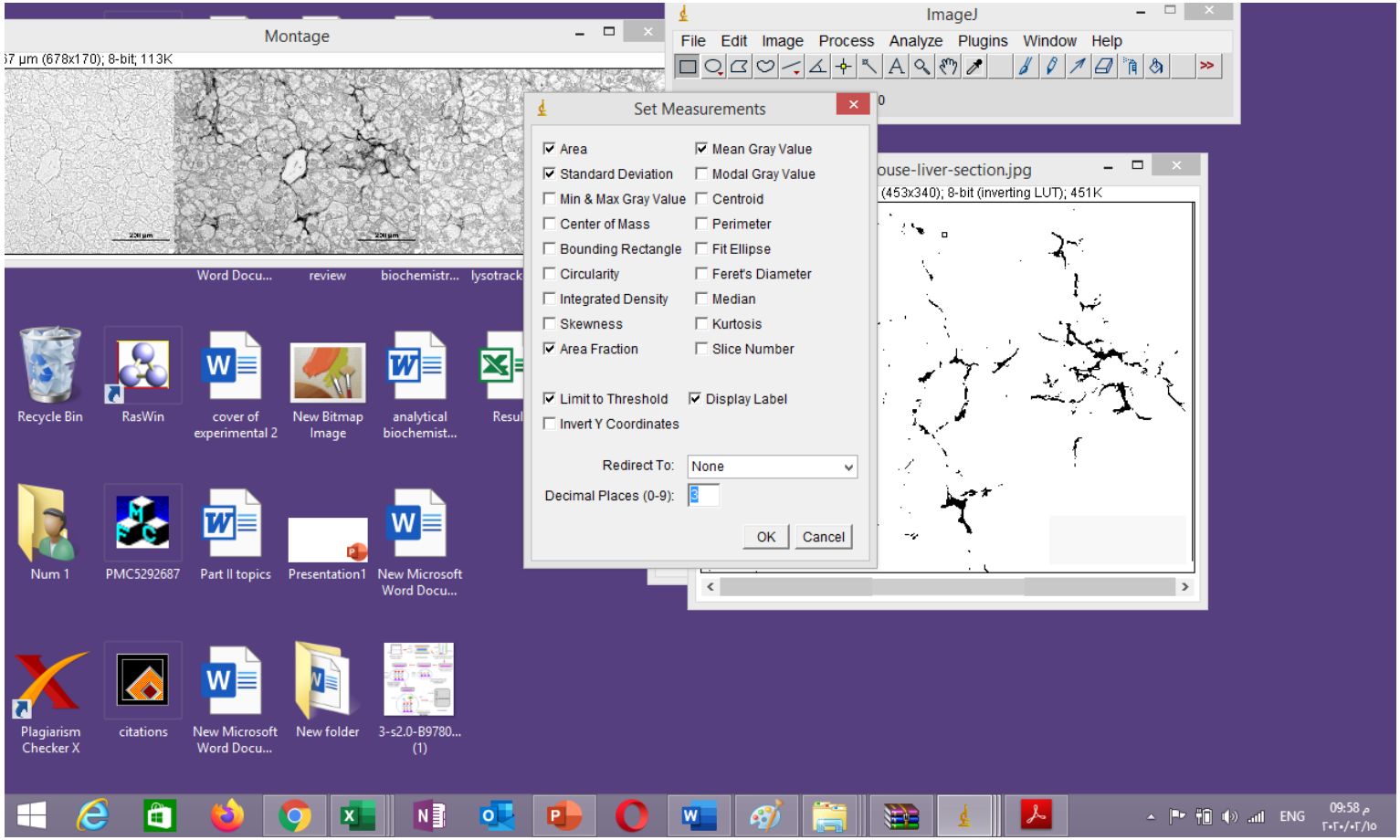
Set threshold level in the upper threshold level



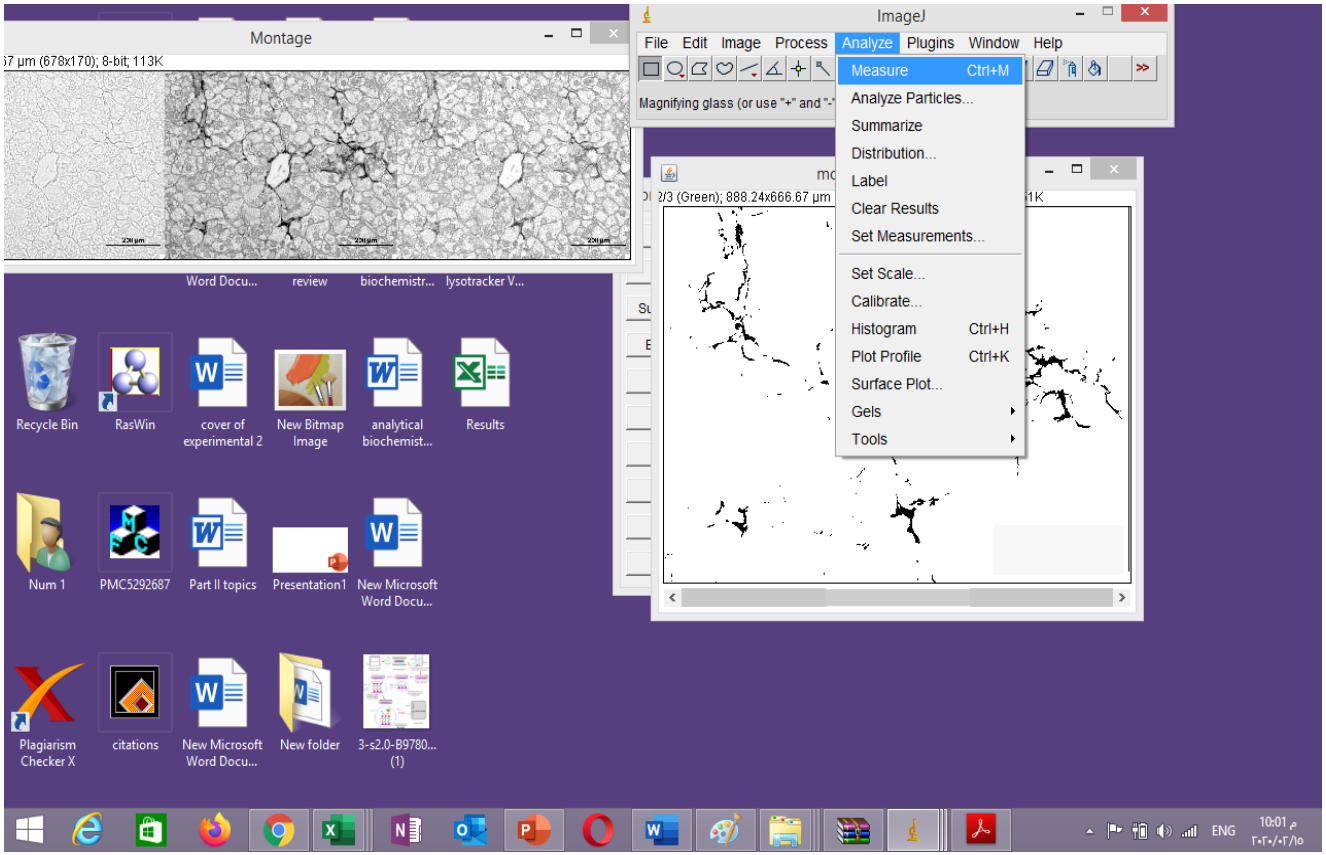
Click apply


ImageJ
File Edit Image Process Analyze Plugins Window Help
Montage
650.00 µm (681x169); 8-bit; 112K
mouse-liver-section.jpg
2/3 (Green); 873.08x651.92 µm (454x339); 8-bit (inverting LUT); 451K
200 µm
Threshold
Close
255
255
Red
Auto
Apply
Reset
Set
Recycle Bin
RasWin
cover of experimental 2
New Bitmap Image
analytical biochemist...
Results
Num 1
PMC5292687
Part II topics
Presentation1
New Microsoft Word Docu...
Plagiarism Checker X
citations
New Microsoft Word Docu...
New folder
3-s2.0-B9780... (1)
ENG
10:08

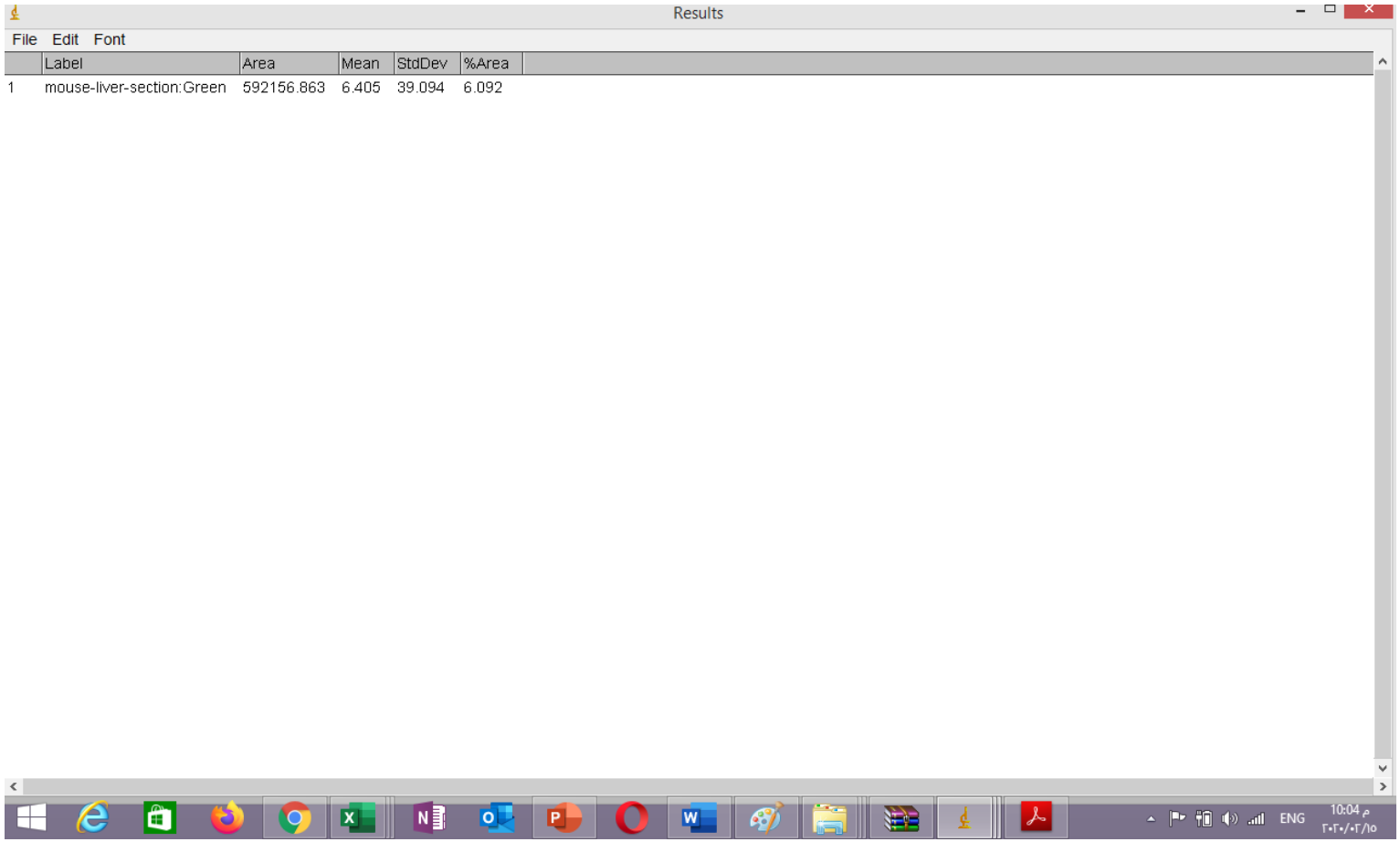
Analyze>Set Measurements dialog and checking "Area", "Area Fraction", "Limit to Threshold" and "Display Label"



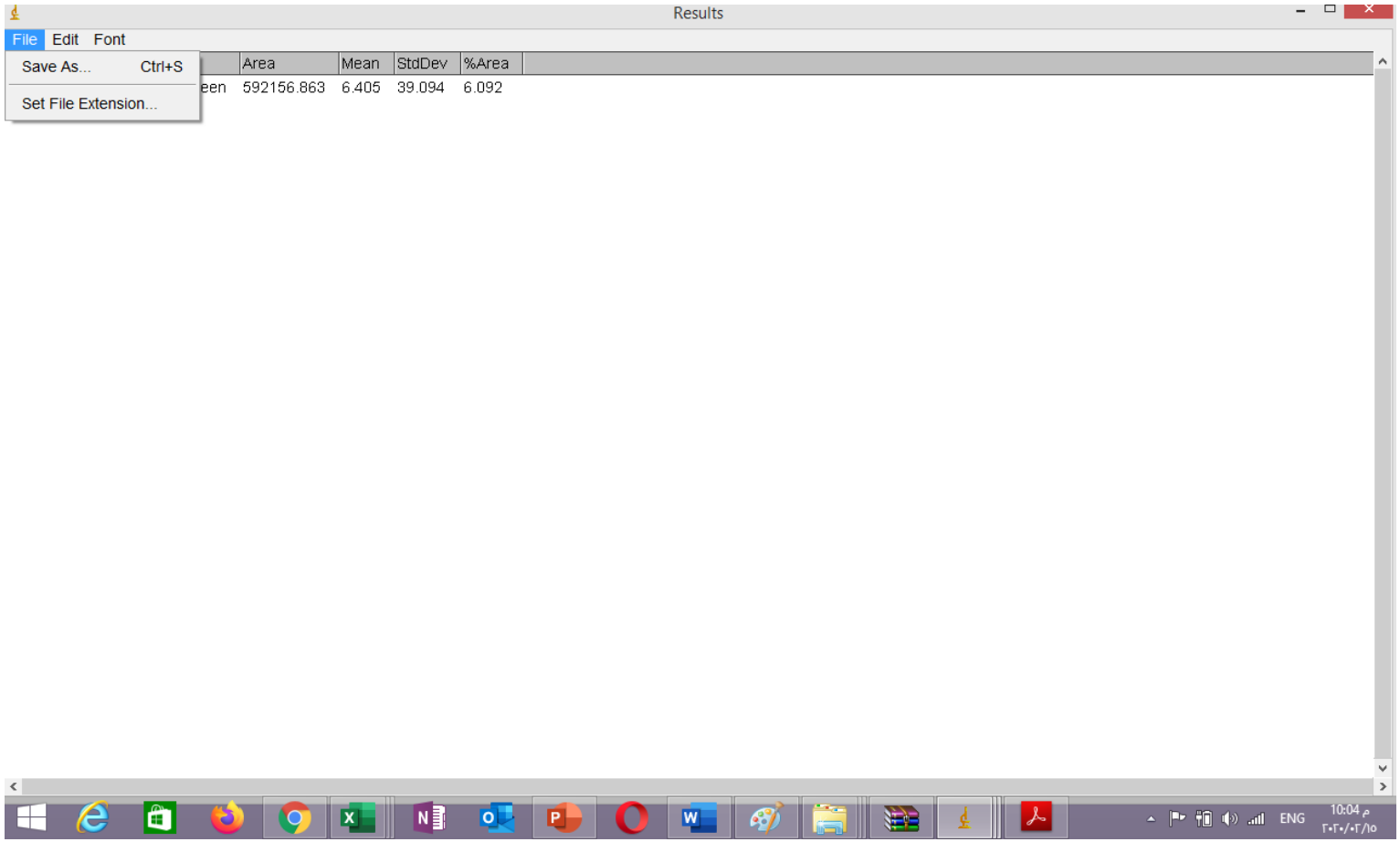
(*Analyse>Measure*)



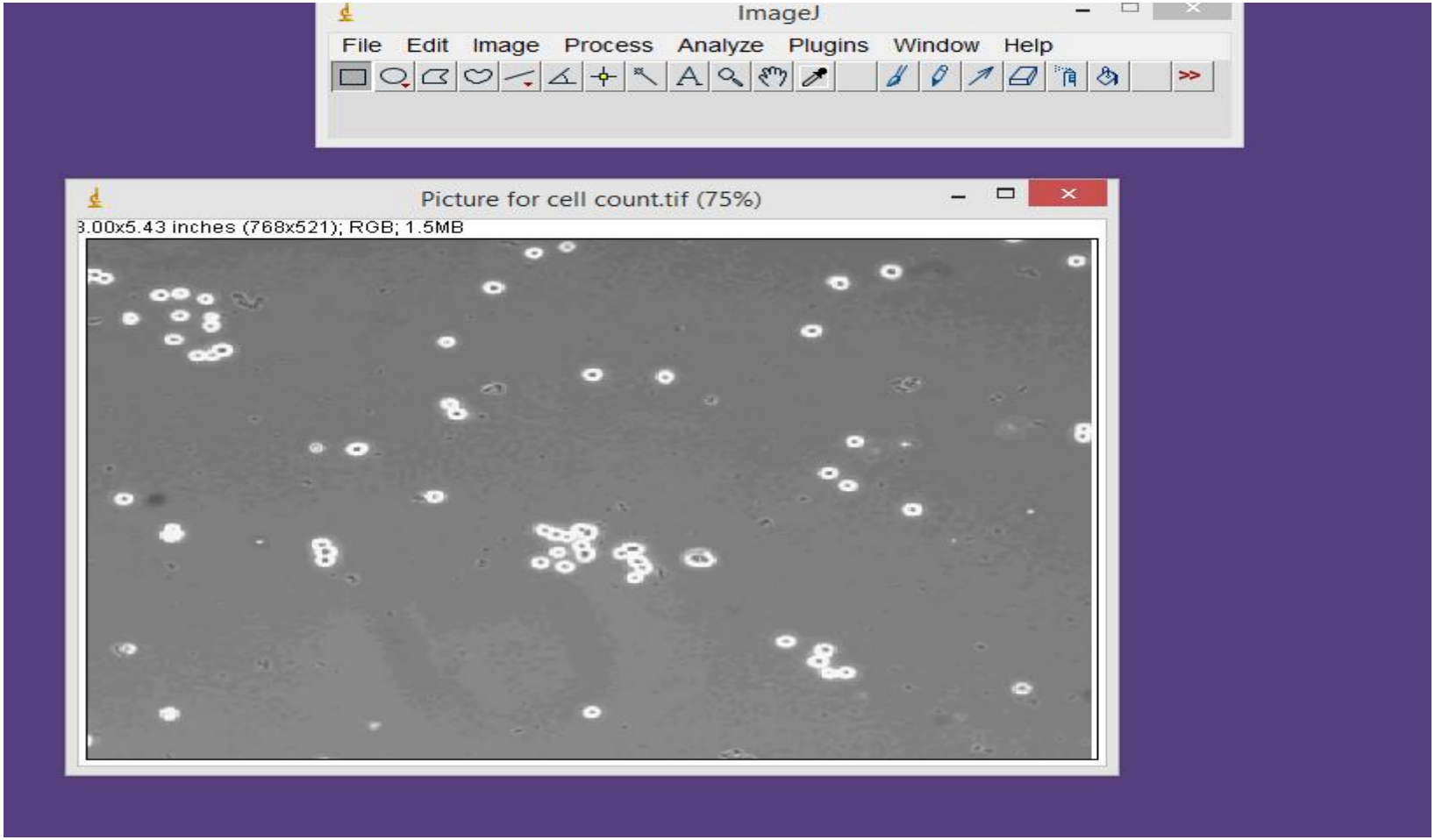
The area and percent area will be displayed in the "Results" window



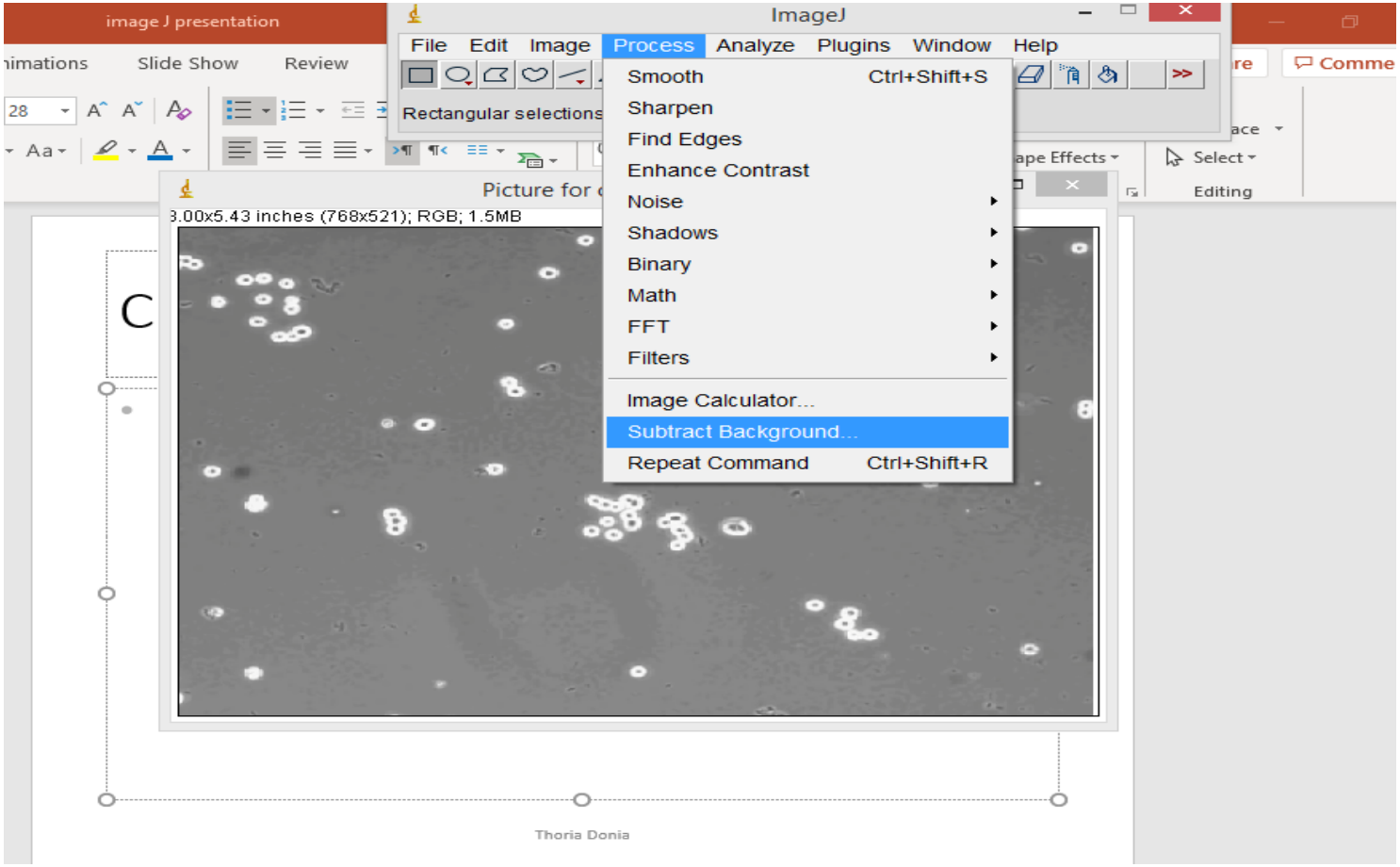
Save results by clicking Save As"



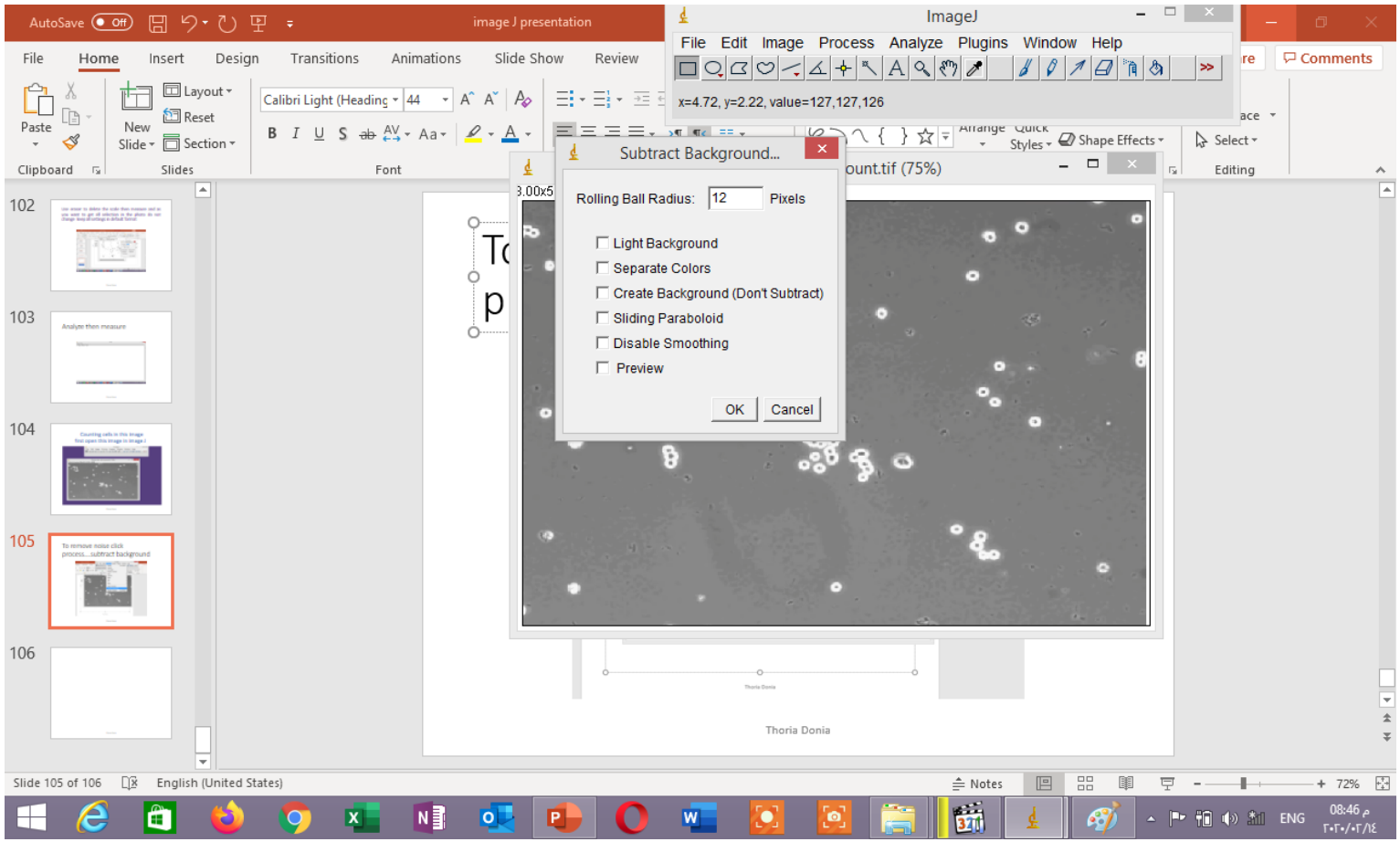
Counting cells in this image first open this image in image J



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

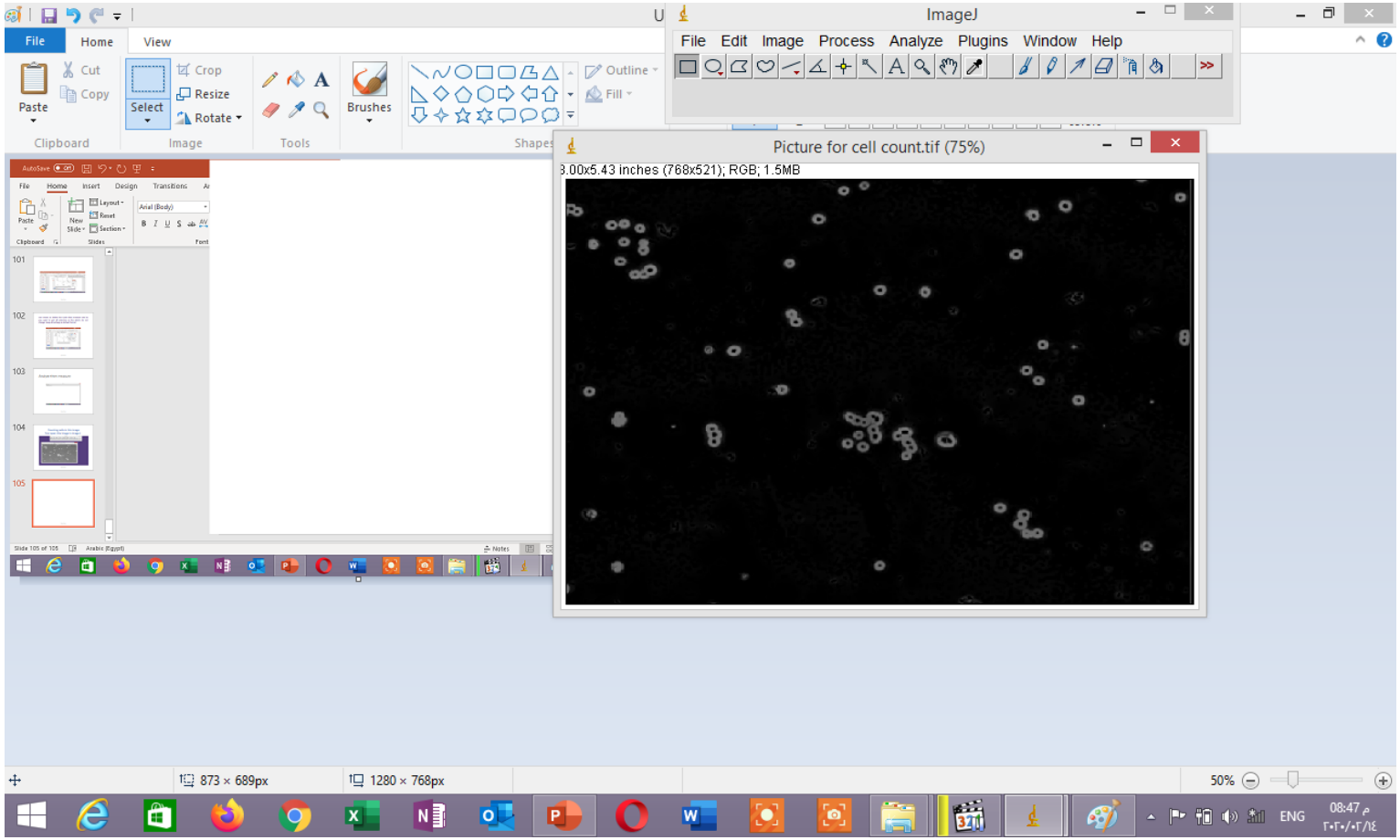
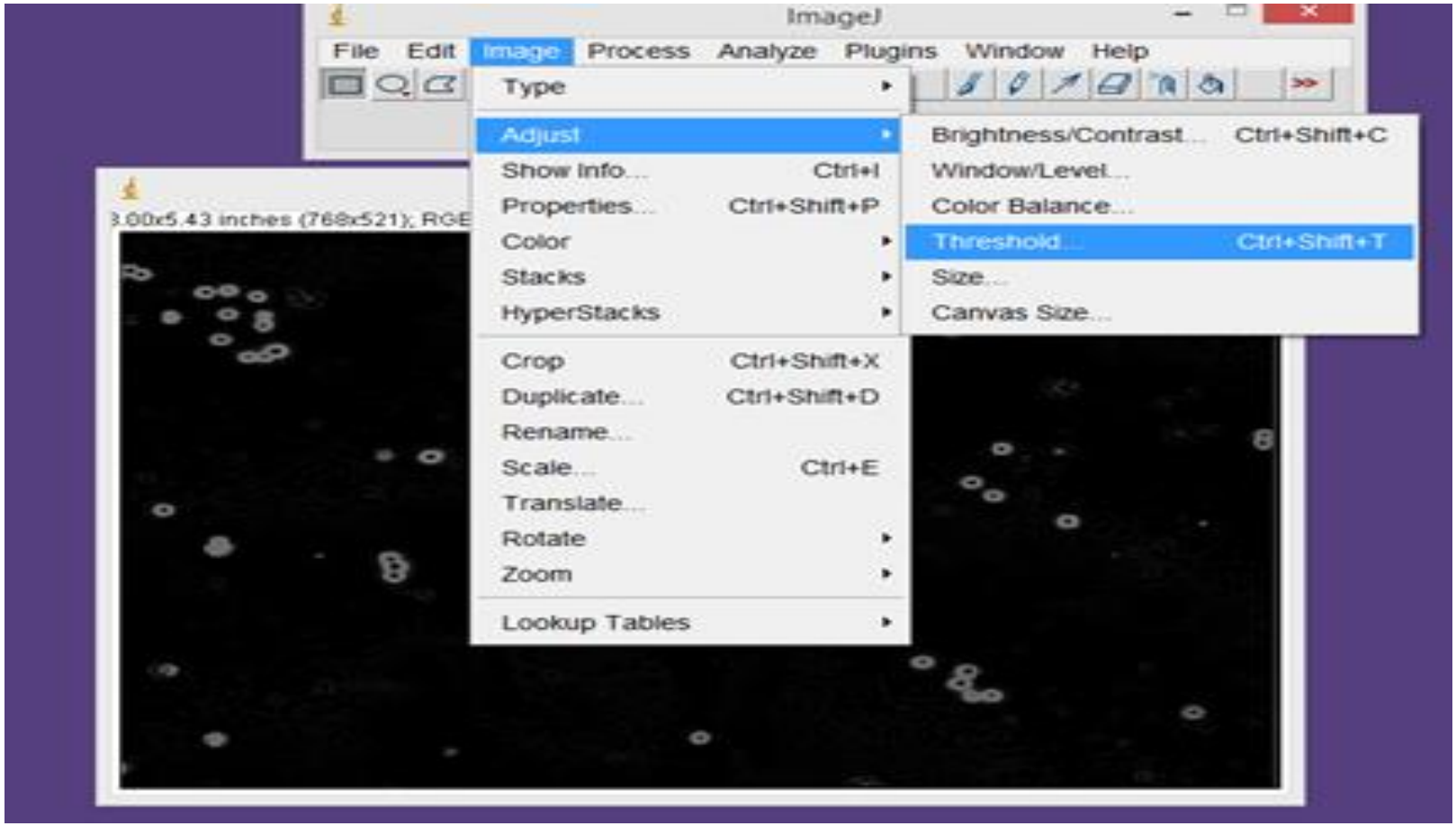
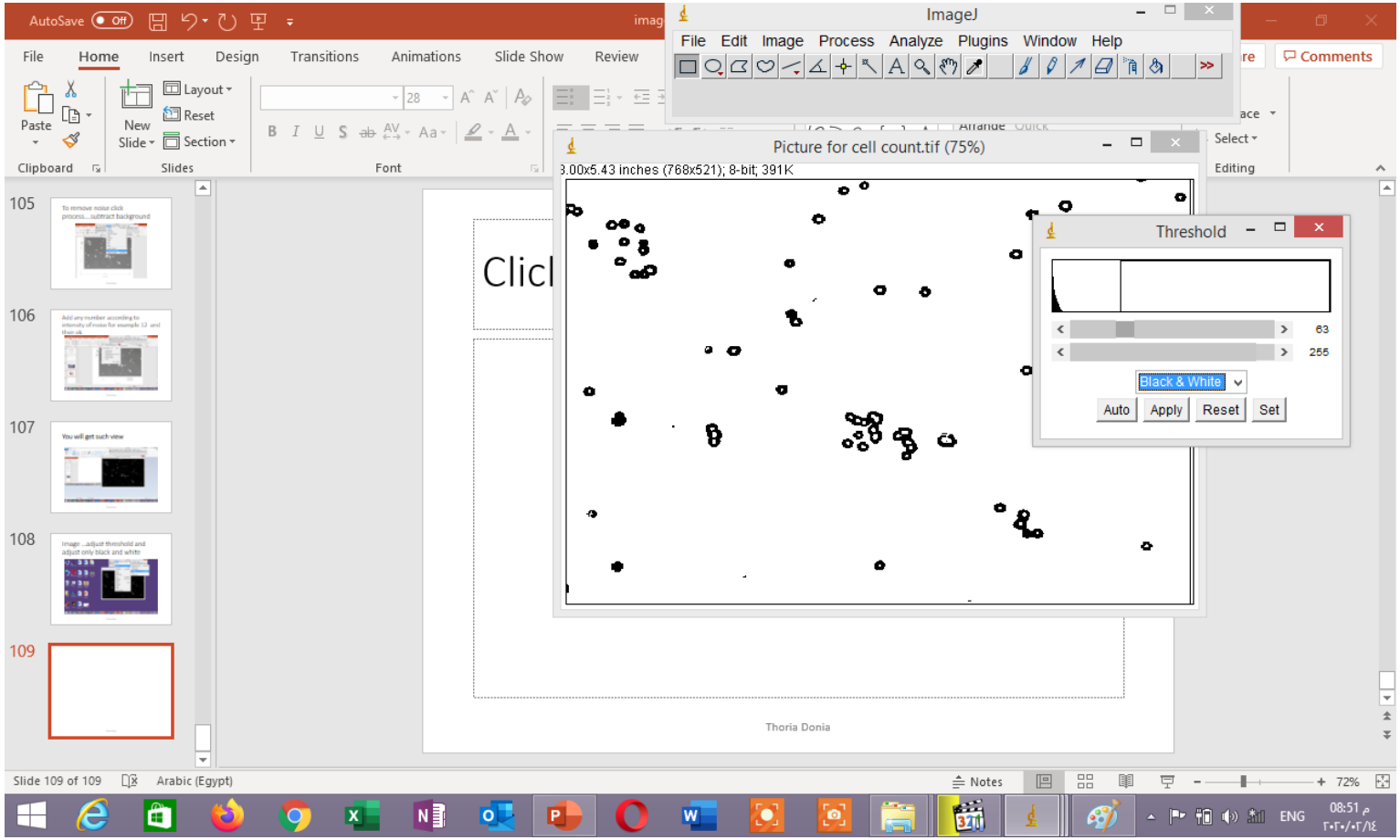


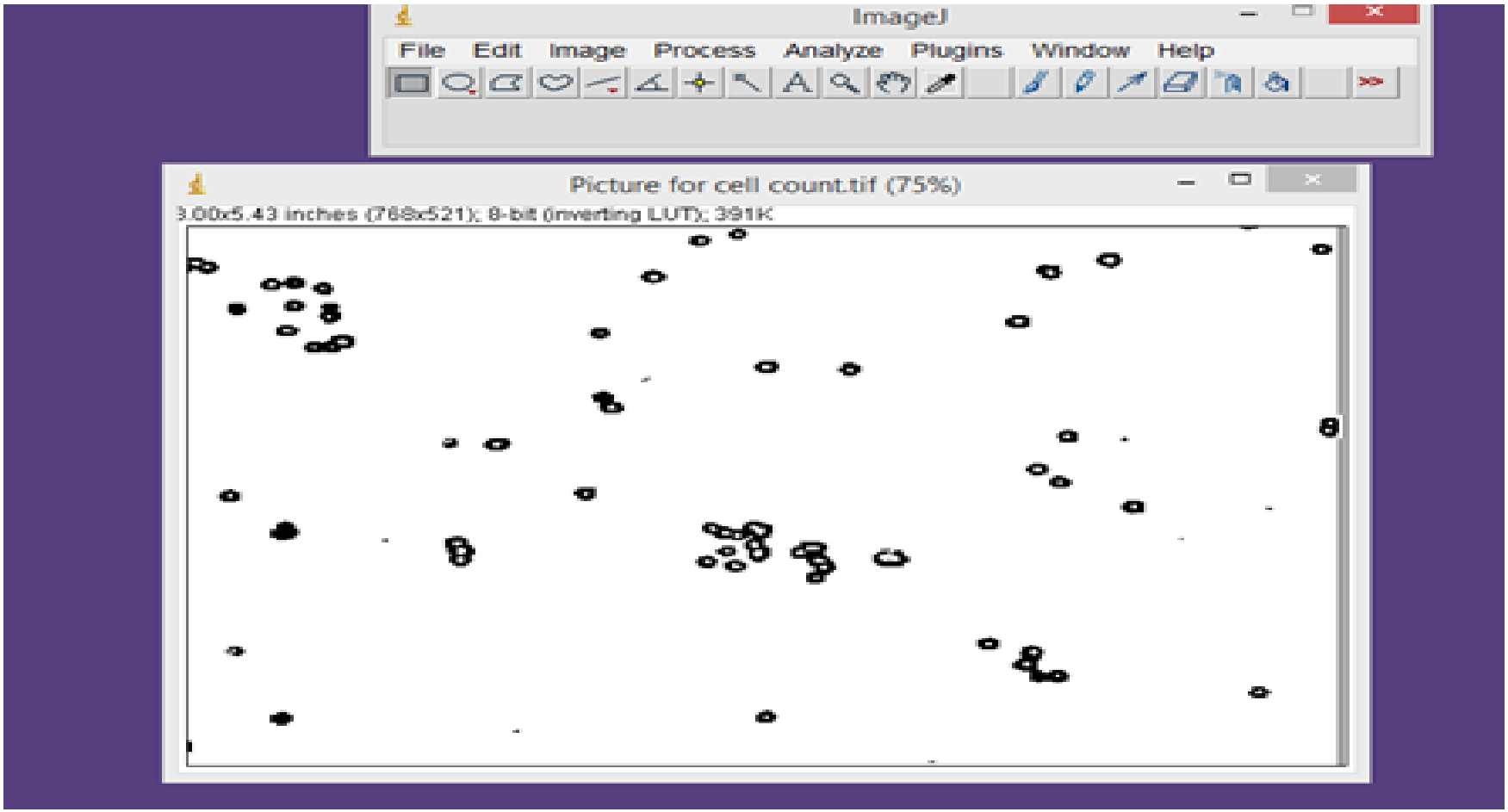
Image …adjust threshold and adjust only black and white



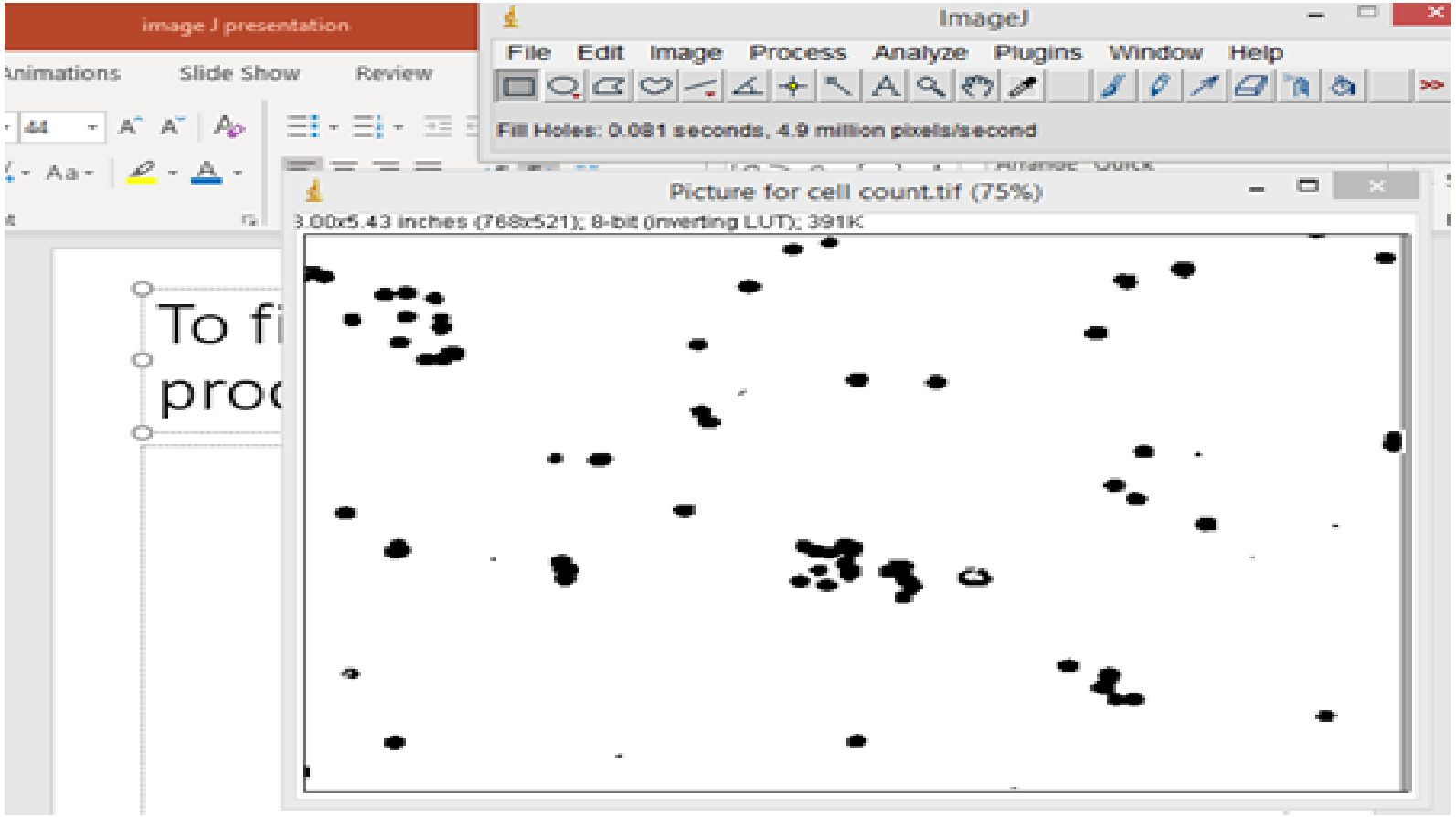
If nothing happen just make your image 8 bit and then change the upper scroll till you get clear cells



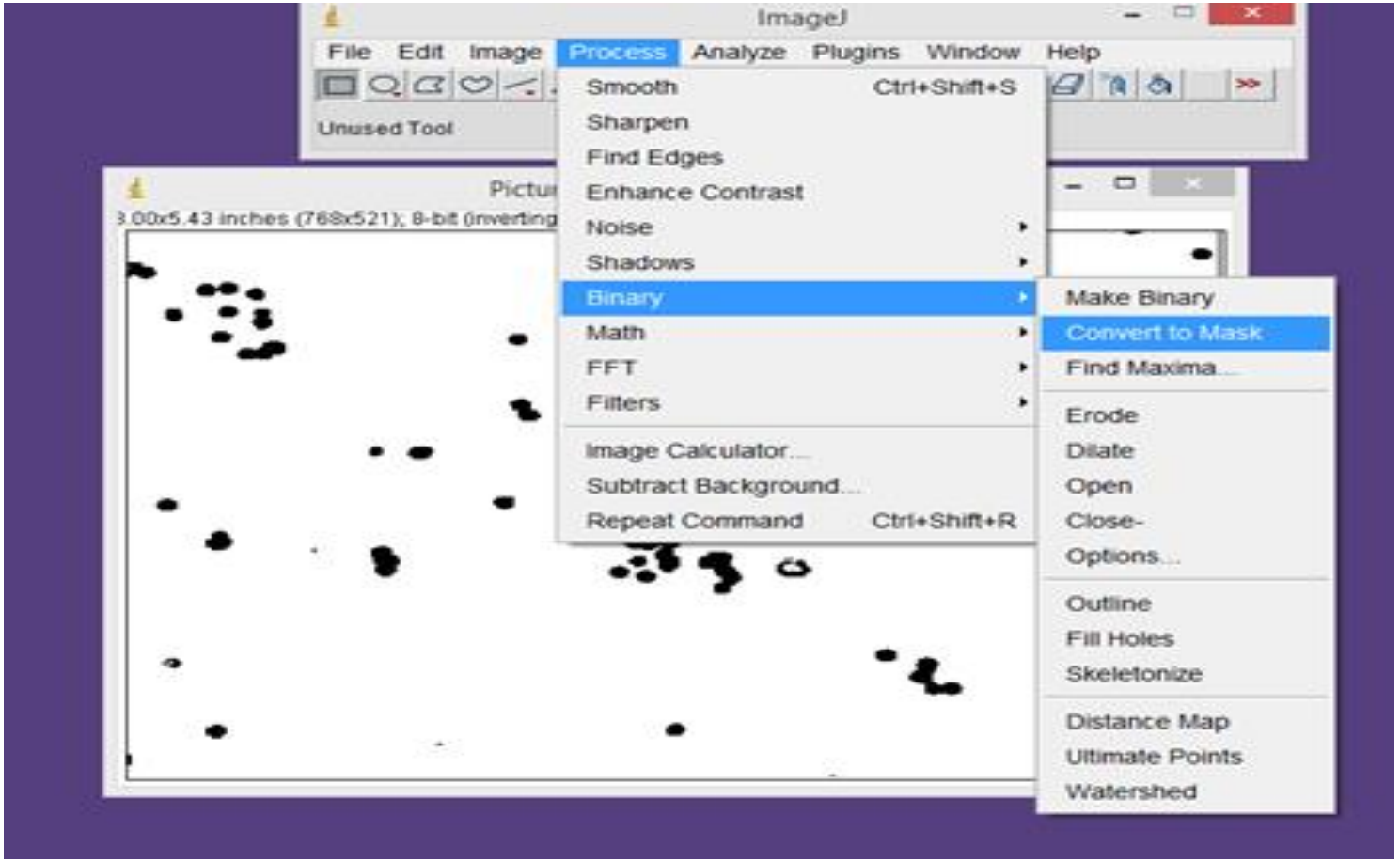
Click apply


ImageJ
File Edit Image Process Analyze Plugins Window Help
Picture for cell count.tif (75%)
3.00x5.43 inches (768x521); 8-bit (inverting LUT); 391K

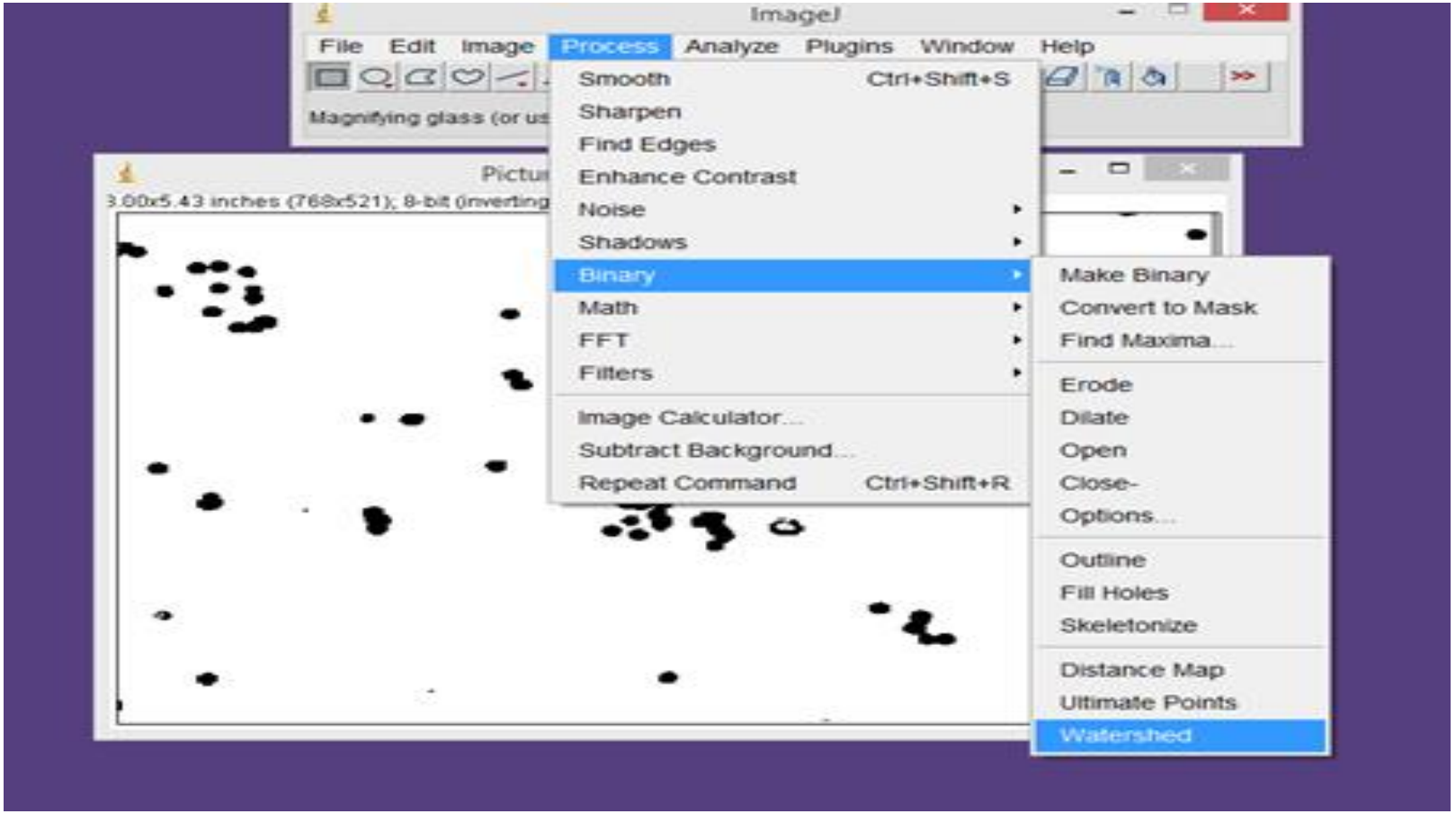
To fill holes in cells choose process then binary then fill holes



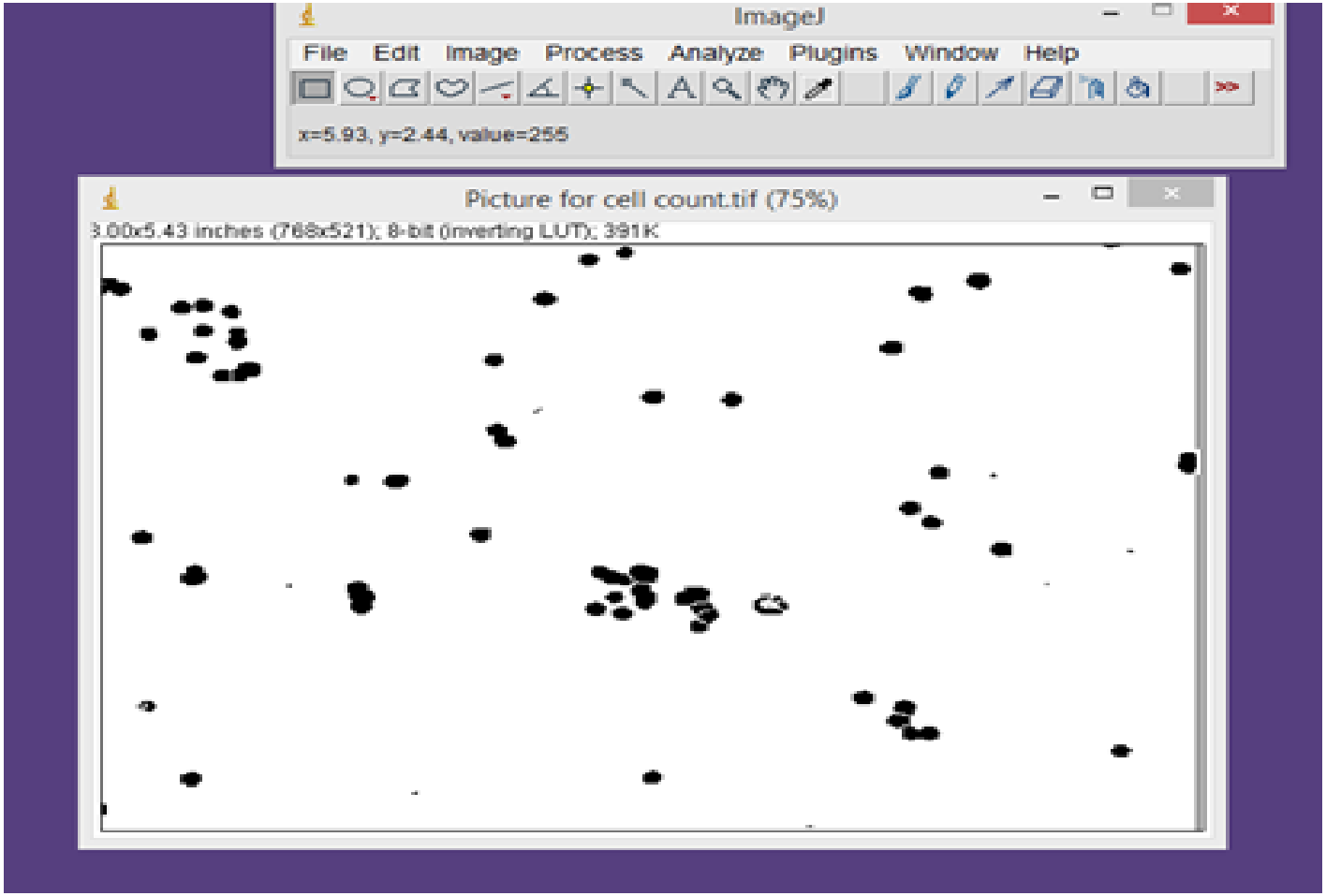
For much processing process…binary ….convert to mask



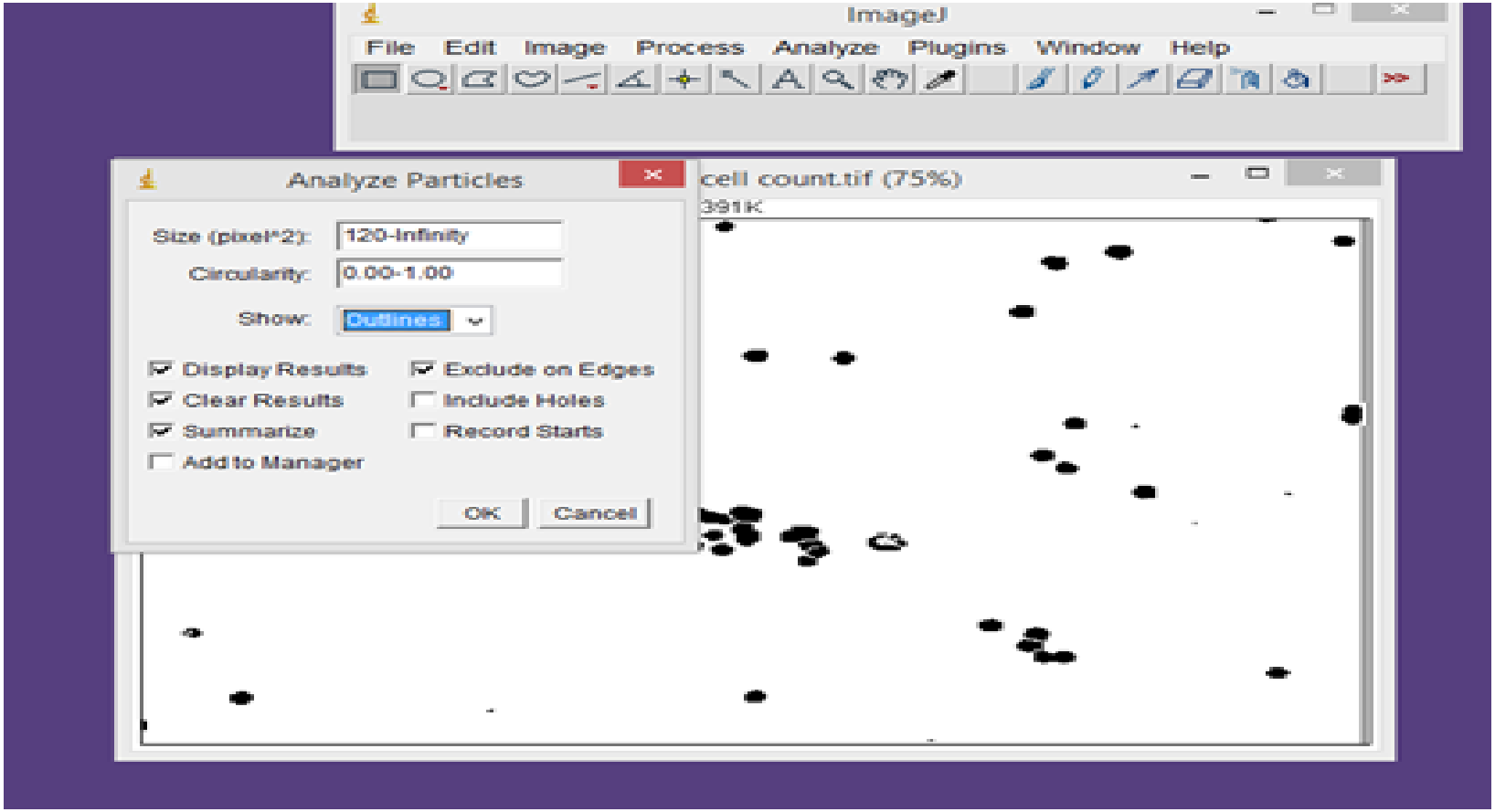
To separate the connected cells process ----binary ….watershed



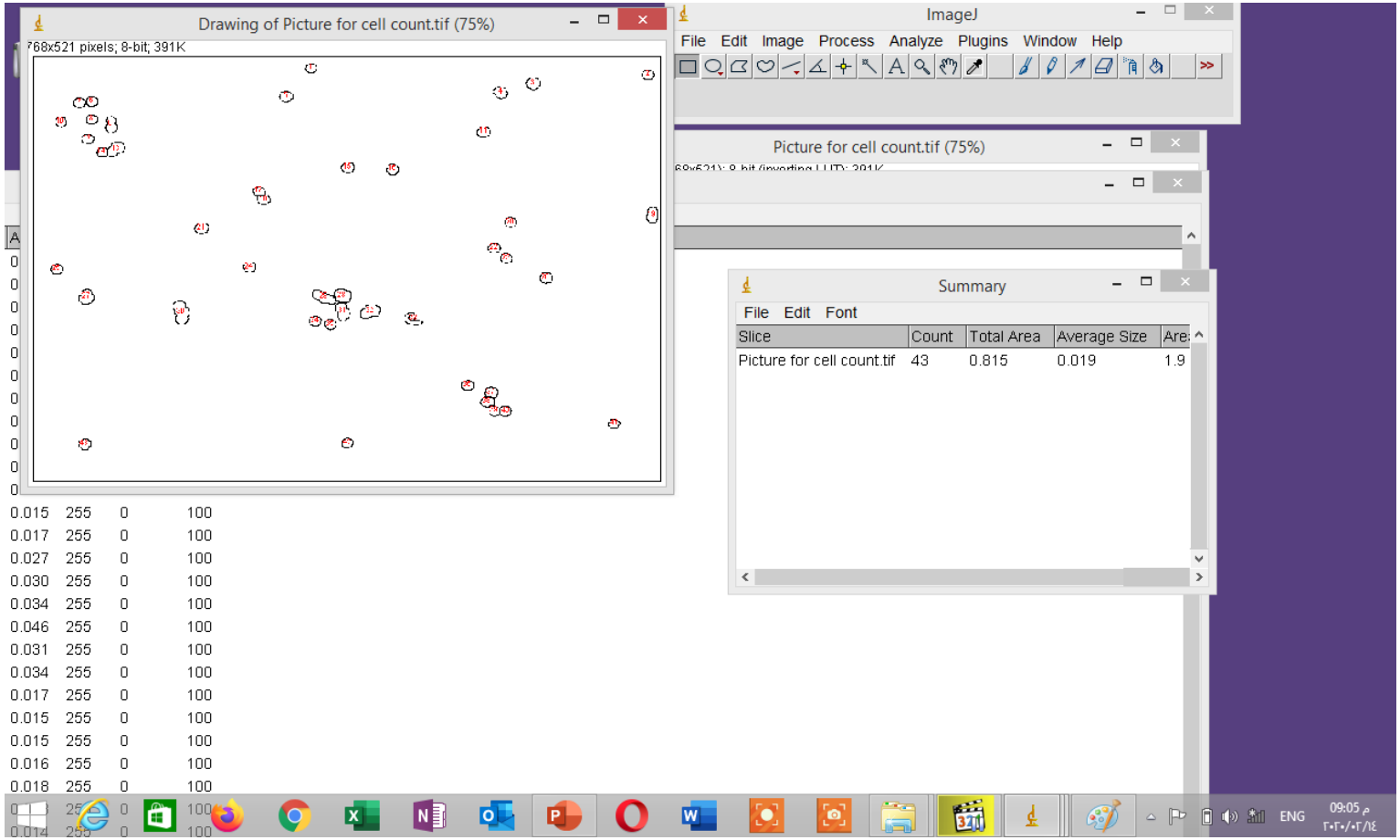
You will get a line between connected cells



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



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




THANK
YOU!