



FIJI IS JUST IMAGE J

FIJI

OK, SO WHAT IS IMAGE J?

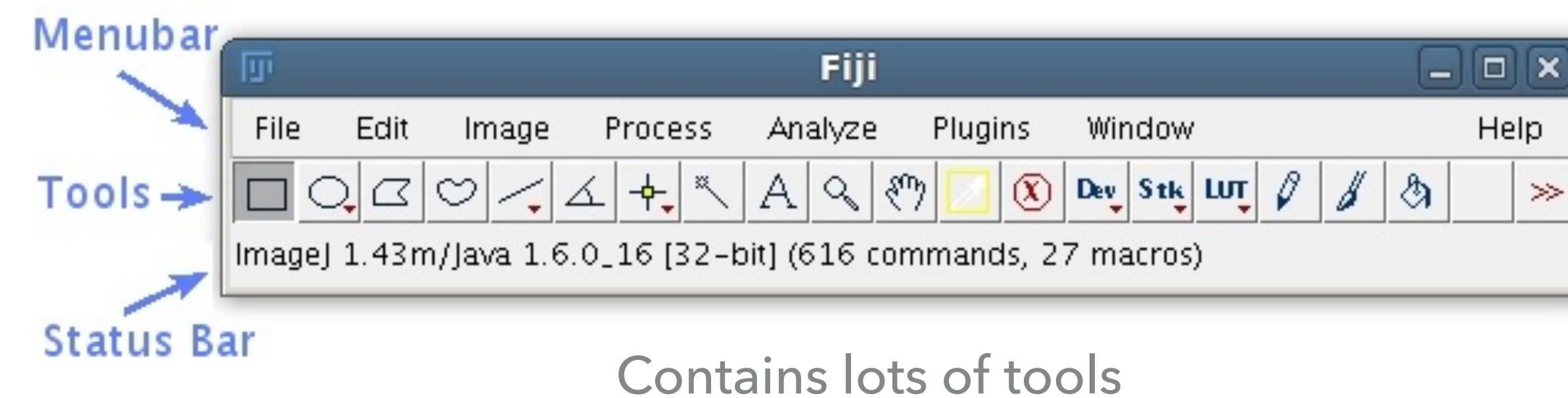
- ▶ ImageJ is an open source image processing program designed for scientific multidimensional images.
- ▶ Fiji is a "batteries-included" distribution of ImageJ
- ▶ bundles a lot of plugins which facilitate scientific image analysis.
- ▶ Lots of Scientists use ImageJ / Fiji

GETTING STARTED

FIJI INTERFACE

THE MAIN WINDOW

- But Most functionality is found in the **Menu Bar**



File

File Input / Output

Edit

Selection / ROI handling

Image

Visualization, Contrast enhancement, Color

Process

Image Filters

Analyze

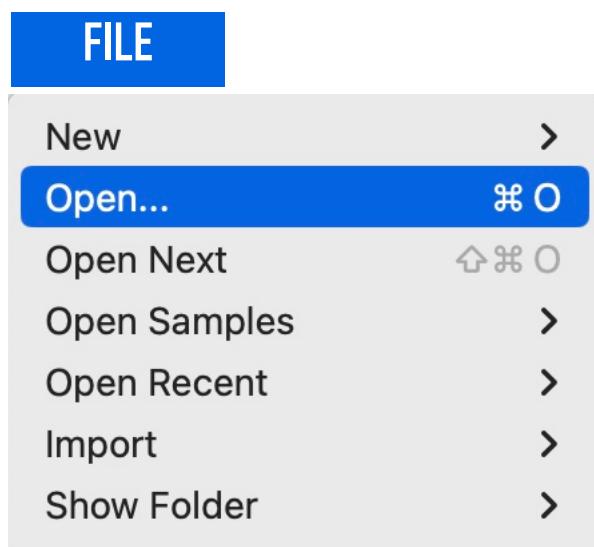
Statistics (histograms, regionprops)

Plugins

Add-ons

GETTING STARTED

OPEN AN IMAGE

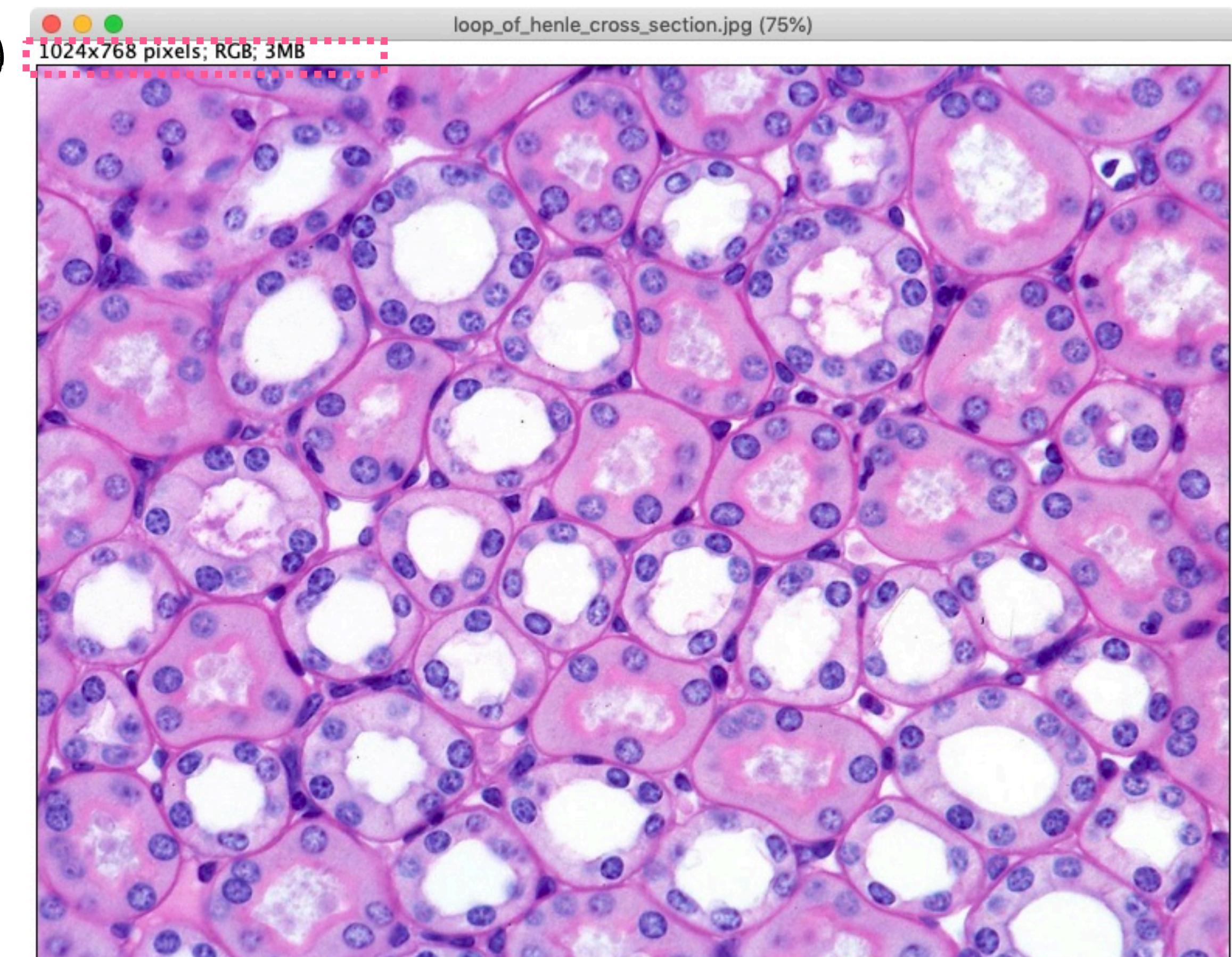


1

navigate to the Data 2 folder and open
'loop_of_henle_cross_section.jpg'

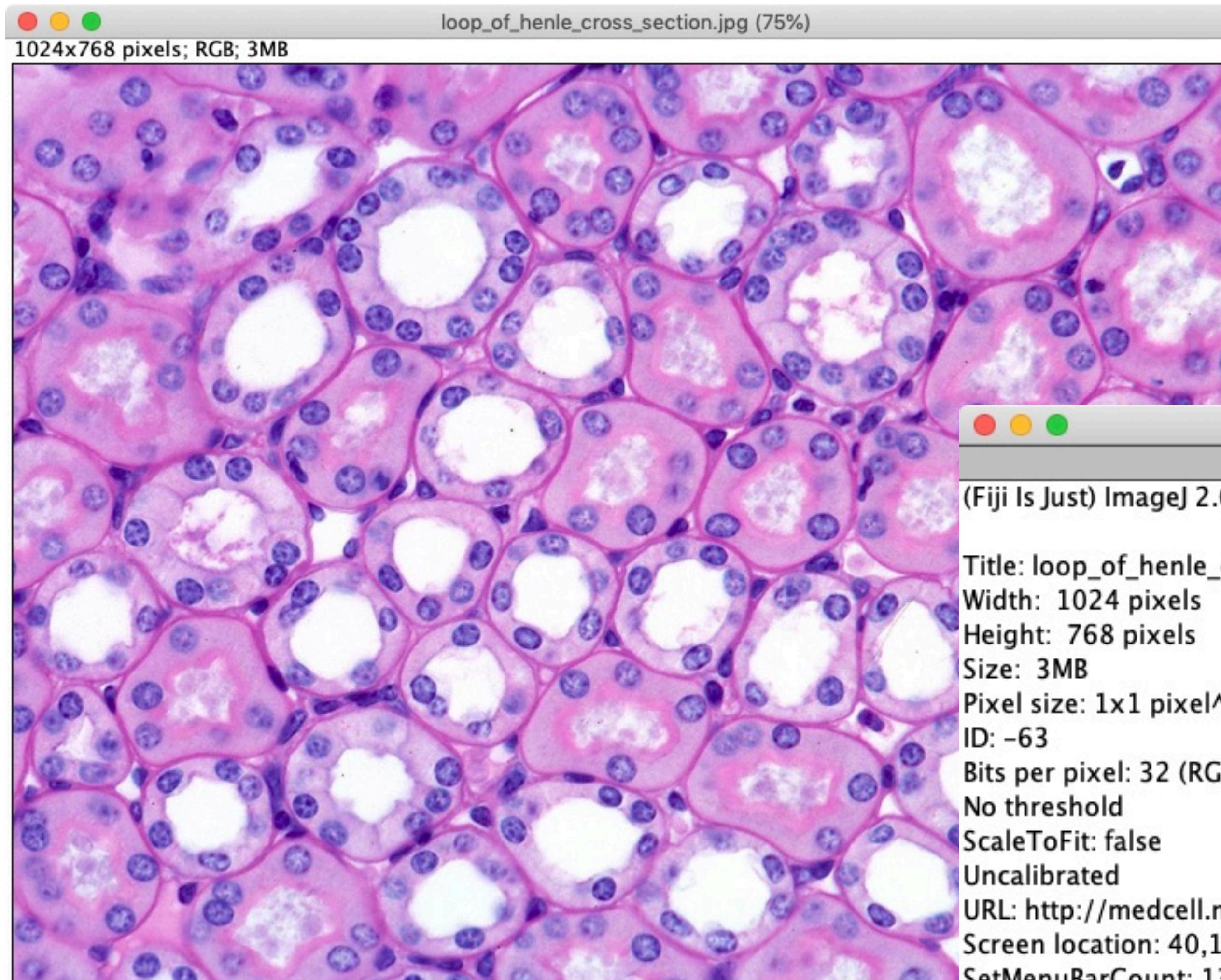
- ▶ **Inspect Image**
 - Width = 1024
 - Height = 768
 - RGB truecolor image
 - size = 3 megabytes

2



GETTING STARTED

GET INFO

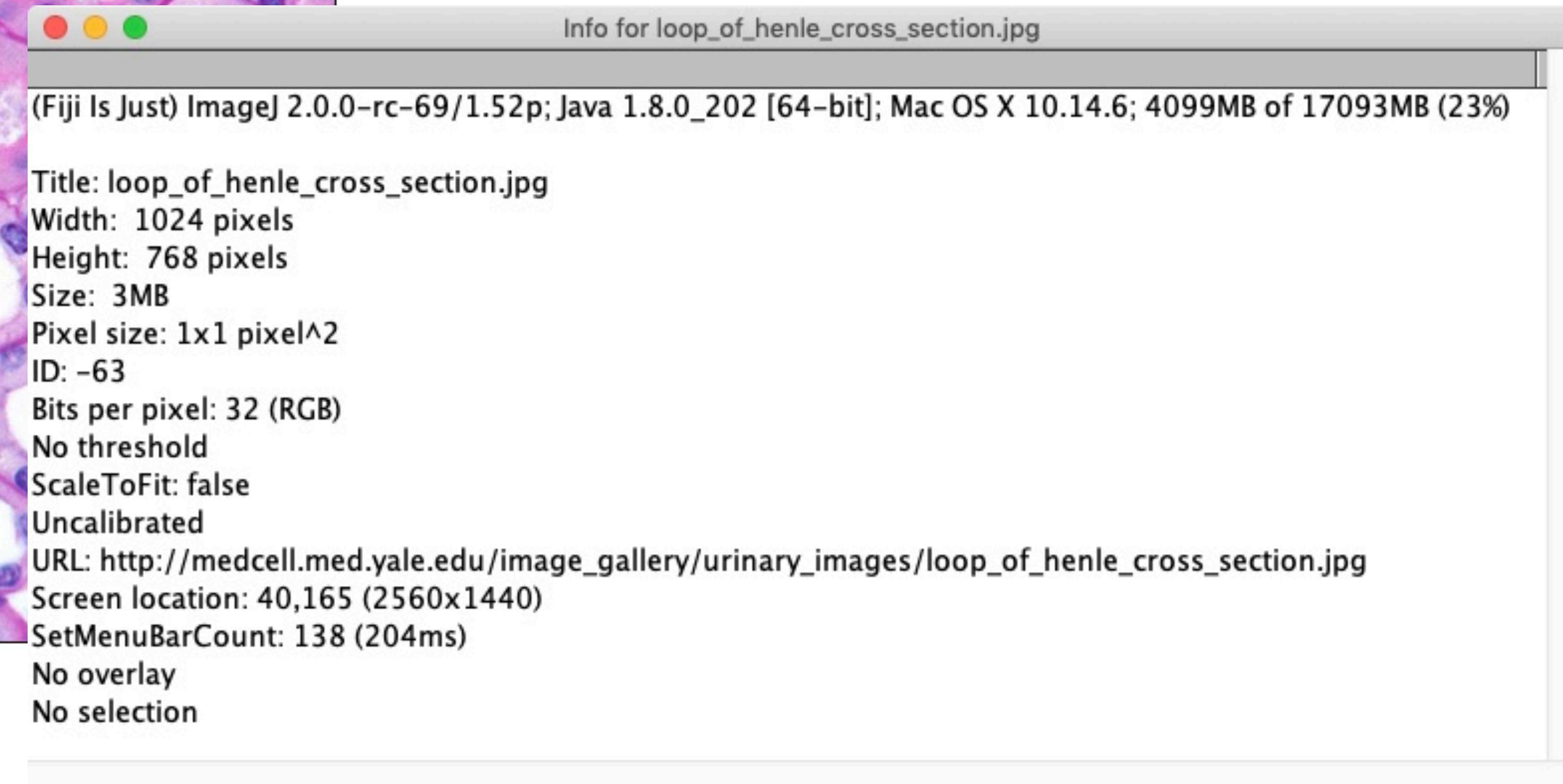


1

Type the letter 'i' or ctrl-i while hovering over image

2

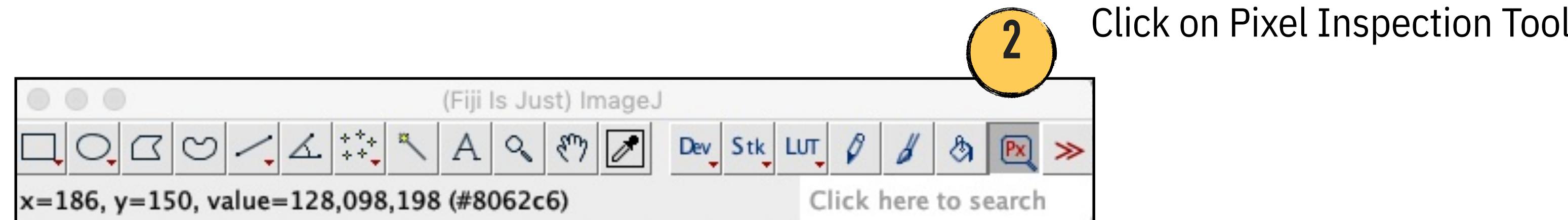
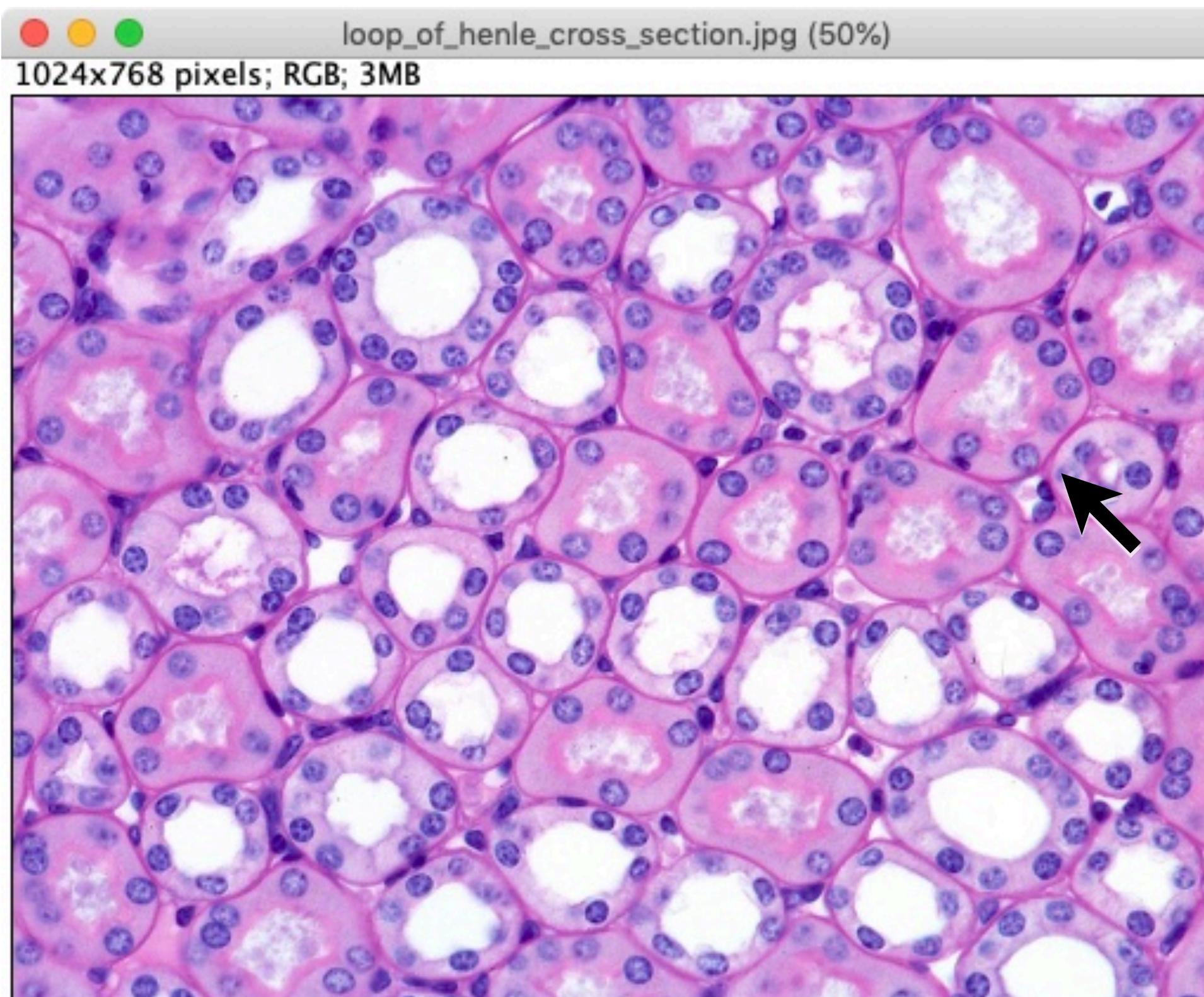
info window appears



GETTING STARTED

EXPLORE IMAGE

- 1 Move mouse around image and review info status bar in main window



- 2 Click on Pixel Inspection Tool

Prefs	197	198	199	200	201
112213,183,233212,182,232208,178,228206,186,235205,185,236206					
113209,183,231209,183,231205,179,227203,183,232203,183,234205					
114199,181,229198,180,228195,177,225197,177,226197,177,228199					
115193,181,229192,180,228188,176,224194,174,223193,173,224193					
116200,188,236197,185,233192,180,228198,178,227197,177,228196					
117207,193,244205,191,242199,185,236205,185,234203,183,234203					
118205,186,241205,186,241200,181,236202,182,231202,182,233204					

- 3 Review Pixel values as you drag the mouse around the image

IMAGE EXPLORATION

HISTOGRAMS AND CHANNELS

GETTING STARTED

SEARCH FUNCTION



1 type histogram

The Quick Search dialog is open, showing search results for "Histogram". The first result, "Histogram / Analyze/Histogram", is selected and highlighted with a yellow circle containing the number 2. The dialog includes sections for Commands, Ops, Script templates, and external links to ImageJ Wiki and Forum. To the right, detailed information about the selected command is displayed, including its menu path ("Analyze > Histogram"), shortcut ("meta H"), identifier ("legacy:ij.plugin.Histogram"), and location ("jars/ij-1.52p.jar").

Quick Search

Commands

Histogram / Analyze/Histogram

2D Histogram / Plugins/Analyze/2D

Color Histogram / Analyze/Color Hi

Color Histogram... / Help/About Plu

Ops

`image.histogram(image, numBins)`

Script templates

ImageJ Wiki

ImageJ Forum

Histogram

Menu path
Analyze > Histogram

Shortcut
meta H

Identifier
legacy:ij.plugin.Histogram

Location
jars/ij-1.52p.jar

Run

Help

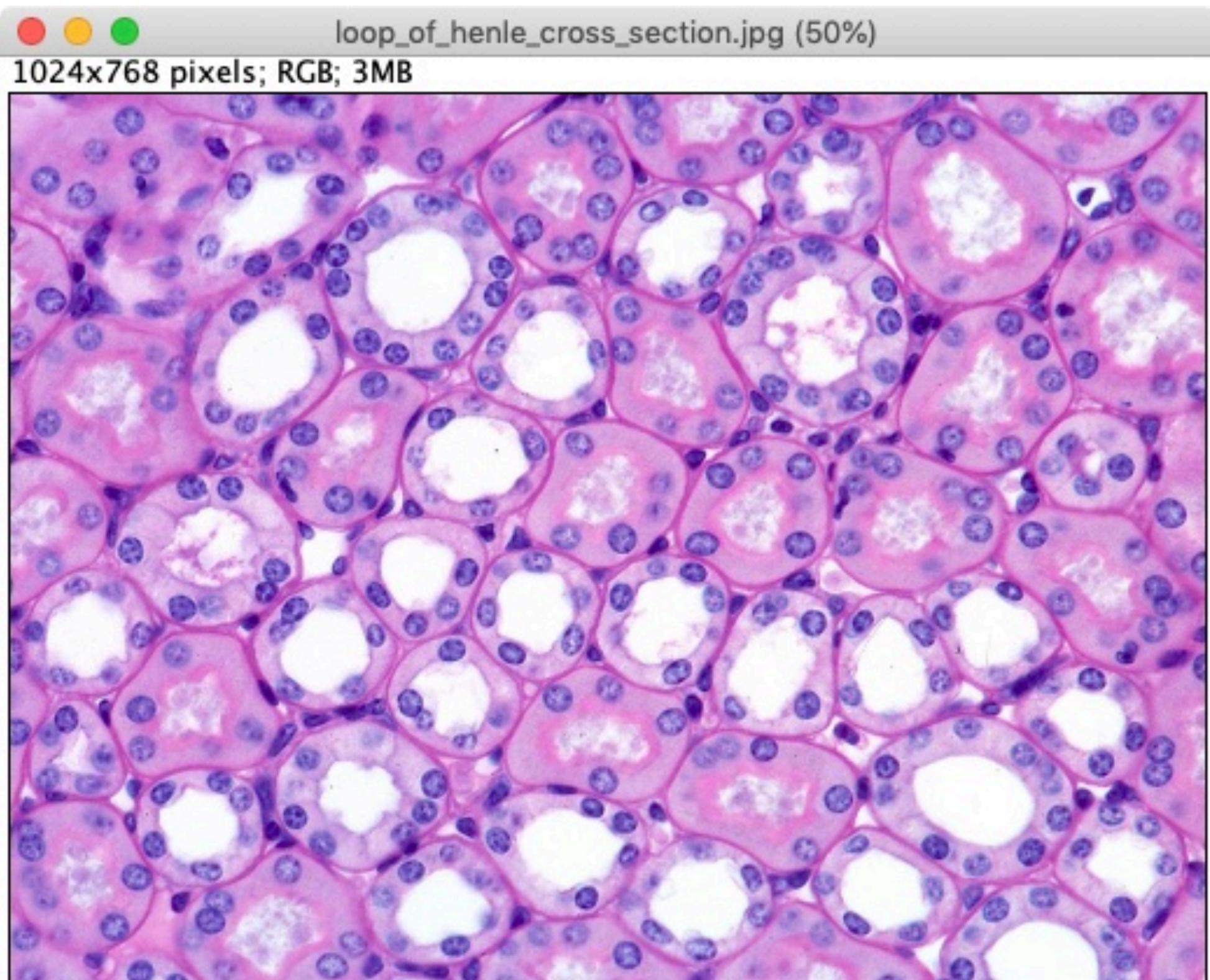
Source

2
Review menu locations
(under commands)

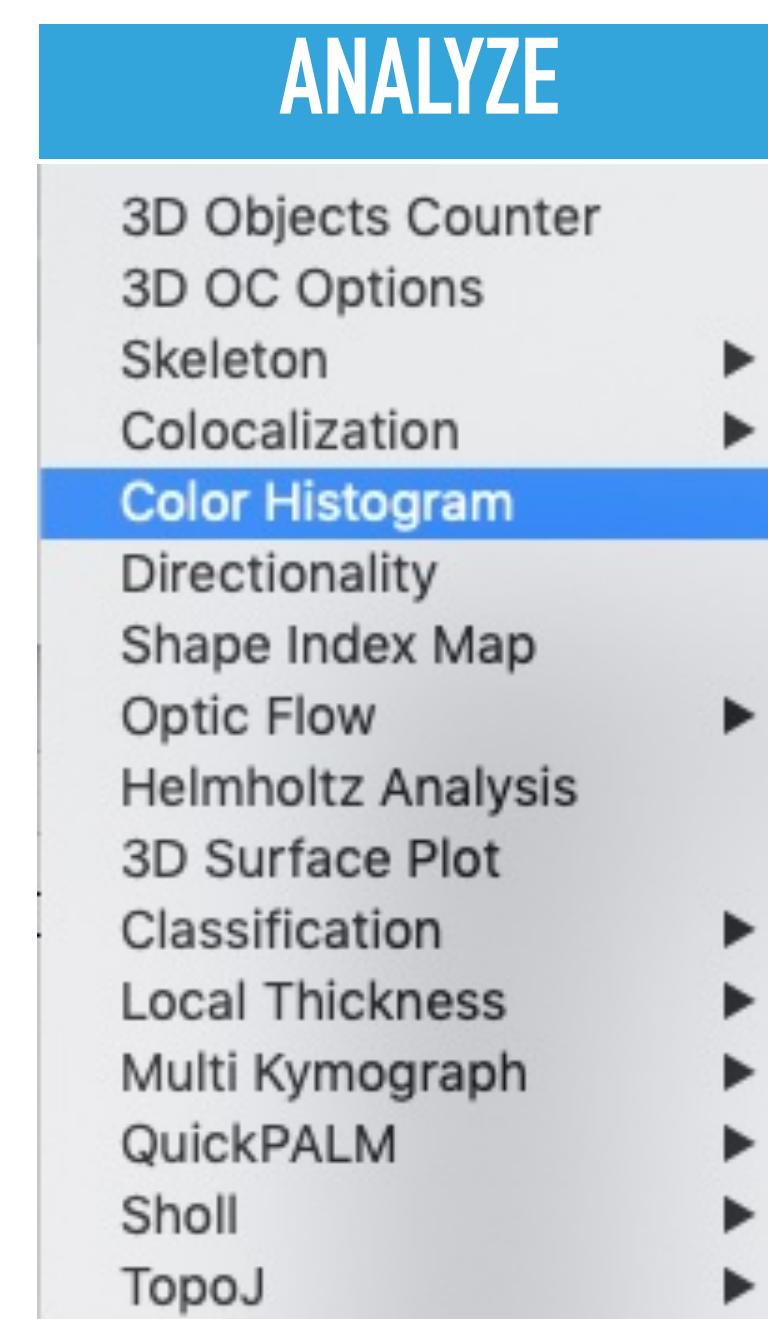
IMAGE EXPLORATION

COLOR HISTOGRAM

- 1 Click on main image to activate

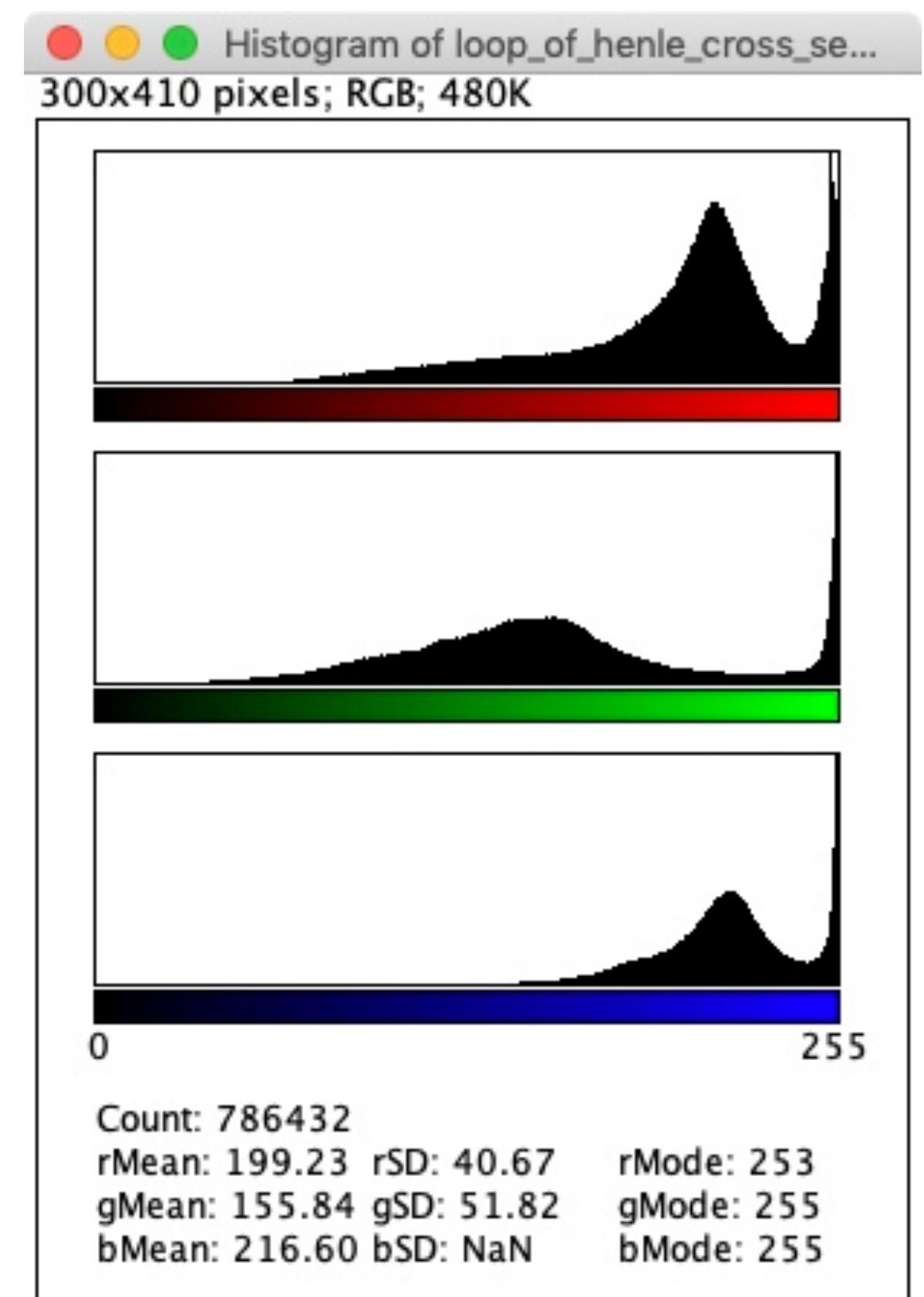


2



3

Enjoy histograms. Notice that there is one histogram per channel. Also notice that this is an **8-bit** image

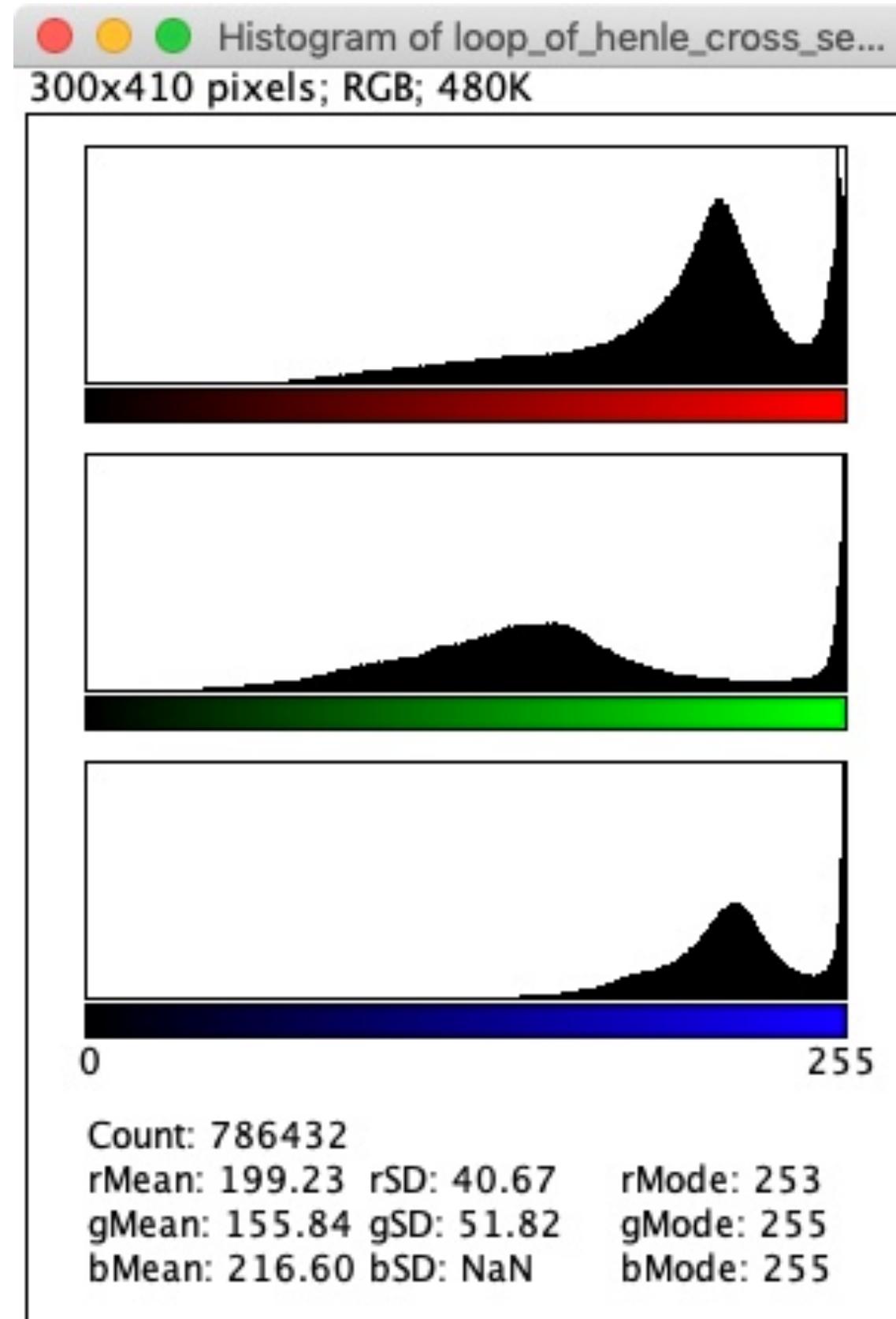


4

Click on List to bring up the intensity values

IMAGE EXPLORATION

COLOR HISTOGRAM



1

Click on “Copy”

2

In MATLAB, hover over the workspace and paste. The Import window should come up. Rename the column variables I, R, G, B

A	B	C	D
T			
I	R	G	B
Number	Number	Number	Number
0	0	52	7479
1	0	68	767
2	0	146	834
3	0	191	858
4	0	189	964
5	0	145	1038
6	0	153	1073
	



3

Click on Import Selection. You should now have a table in MATLAB with the table data from Fiji.

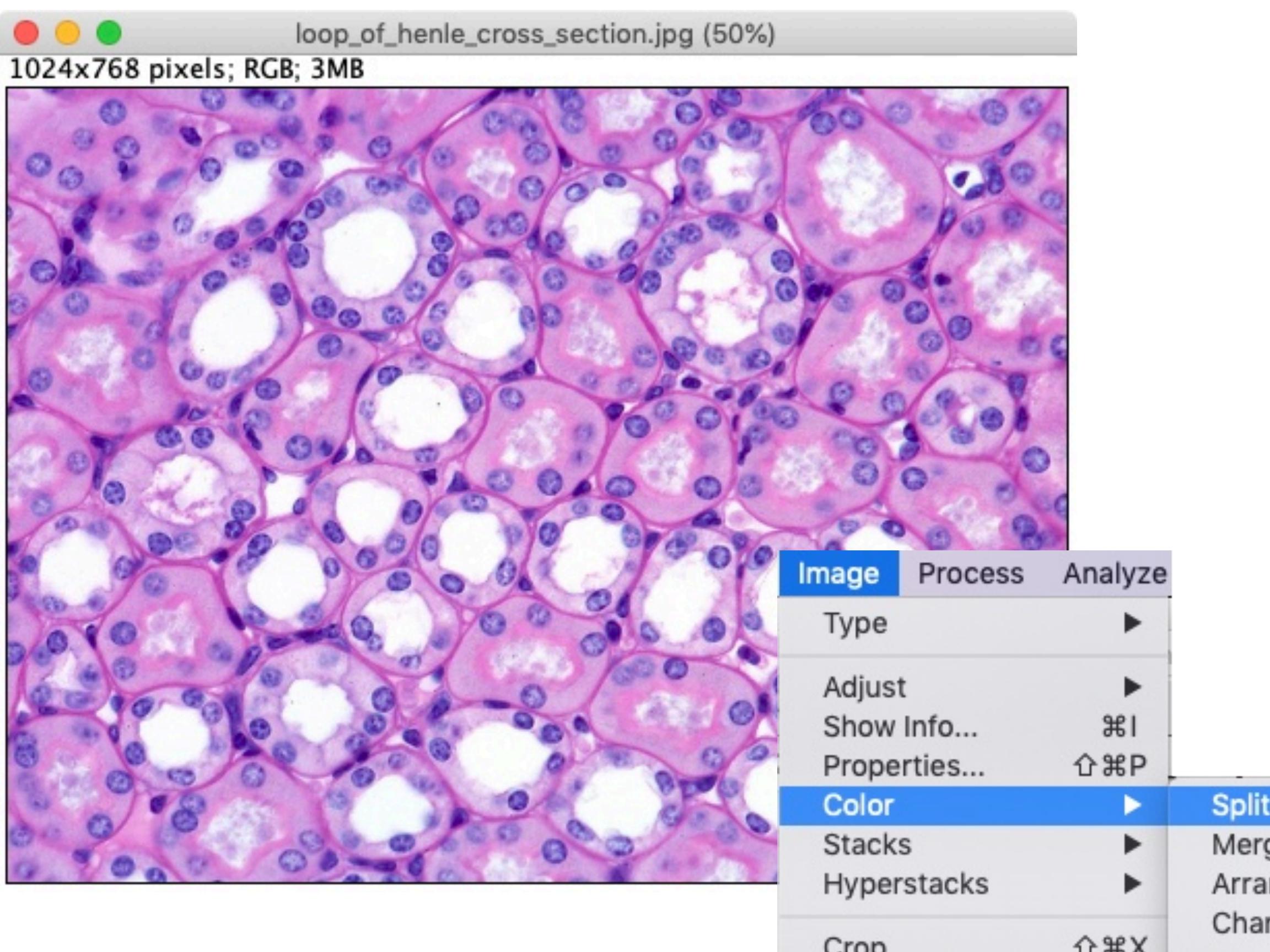
Name	Value	Size	Class
T	256x4 table	256x4	table

Notice that there are 256 rows in the table.

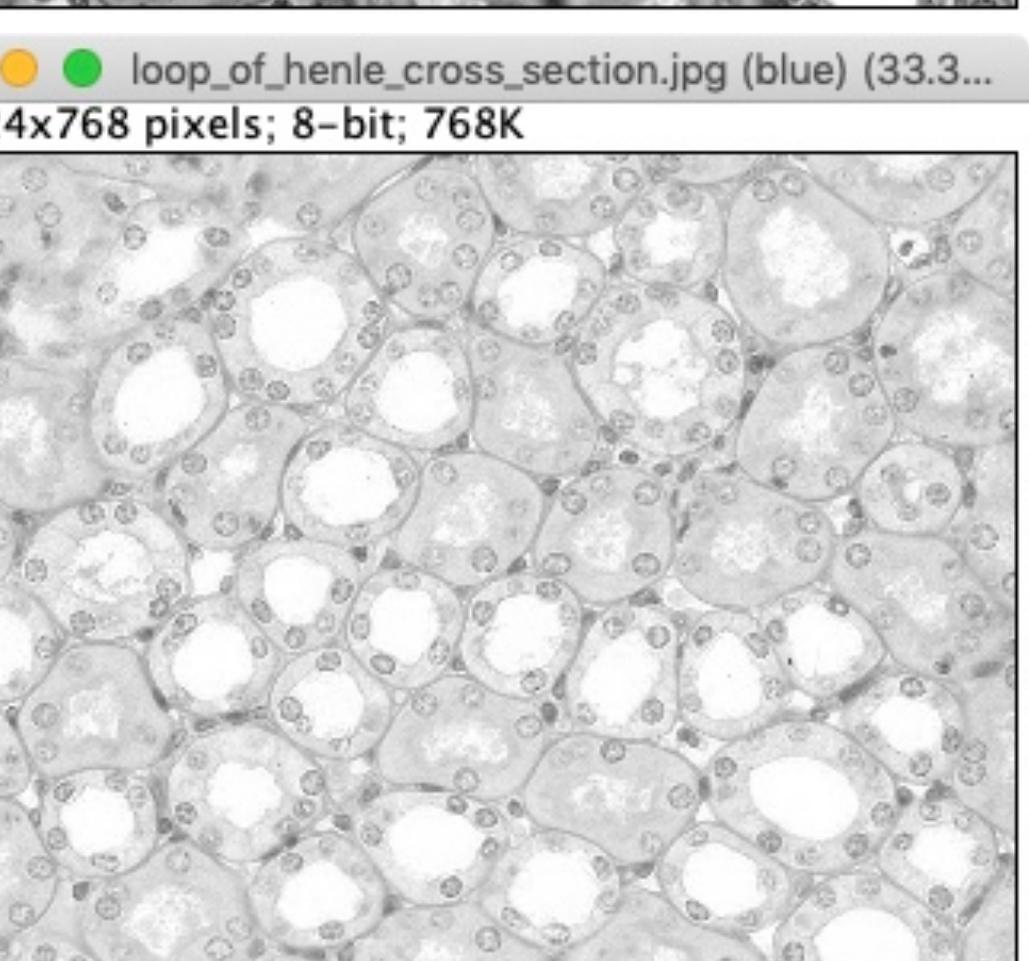
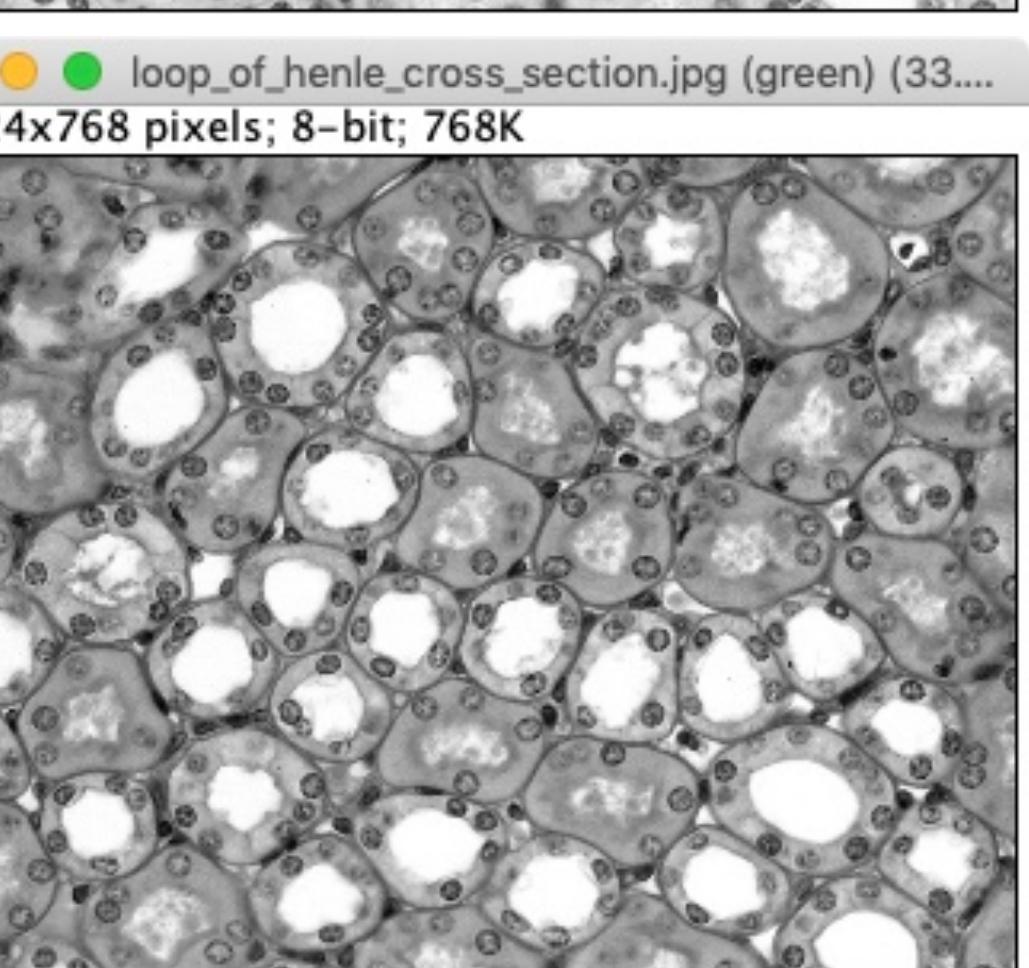
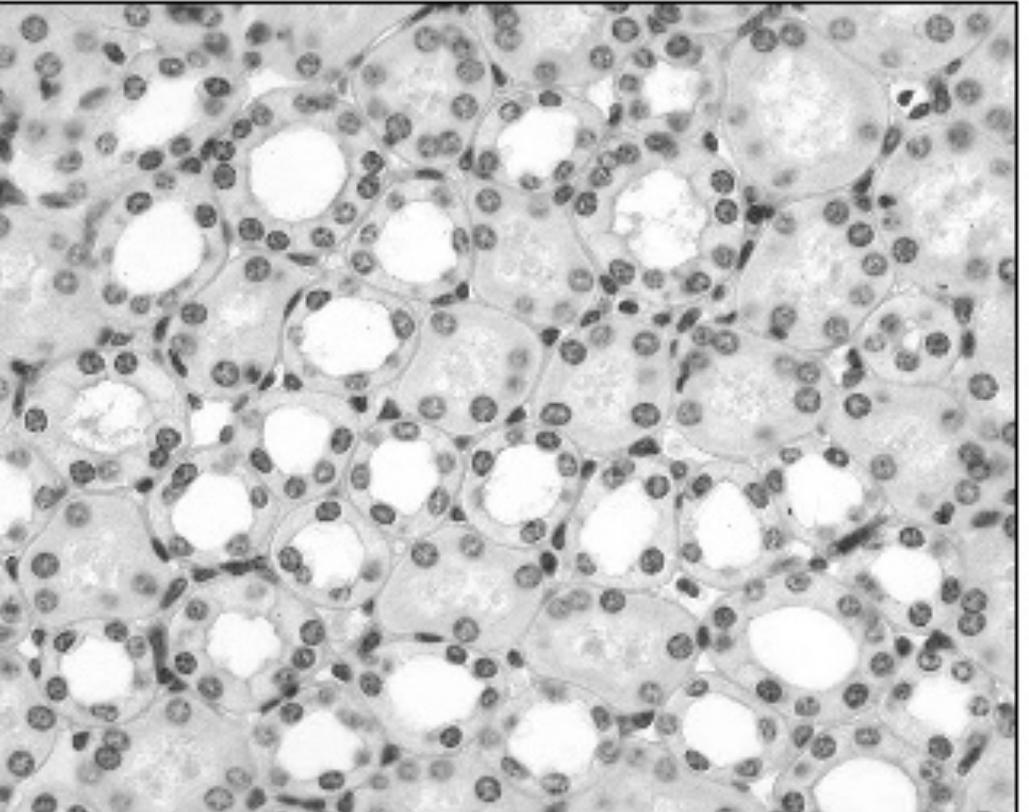
IMAGE EXPLORATION

SPLIT CHANNELS

1 Click on image to activate



loop_of_henle_cross_section.jpg (red) (33.3%)
1024x768 pixels; 8-bit; 768K



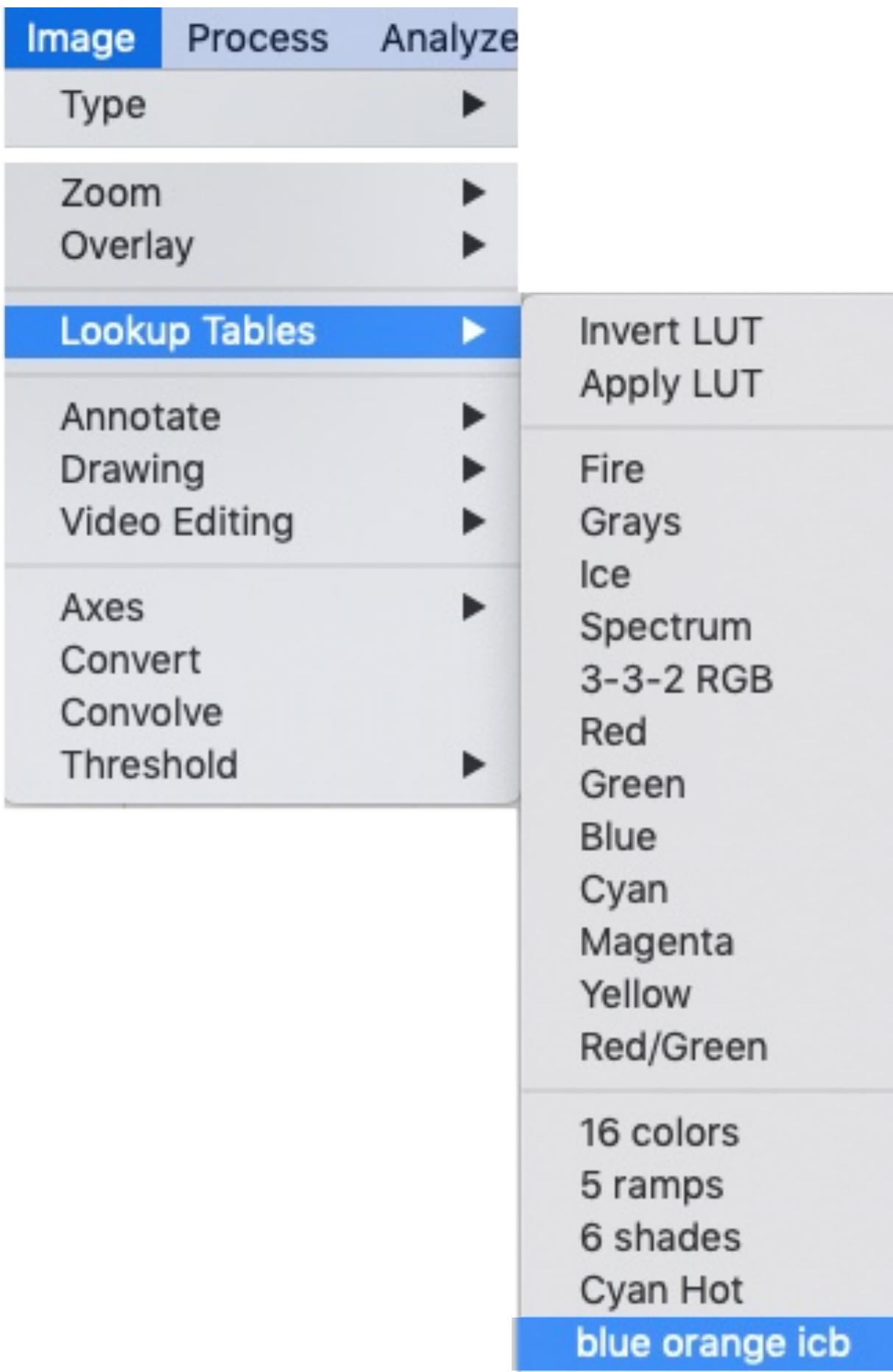
2

3

IMAGE EXPLORATION

APPLY LUT

1 Select Green Channel



3 Enjoy cool looking image

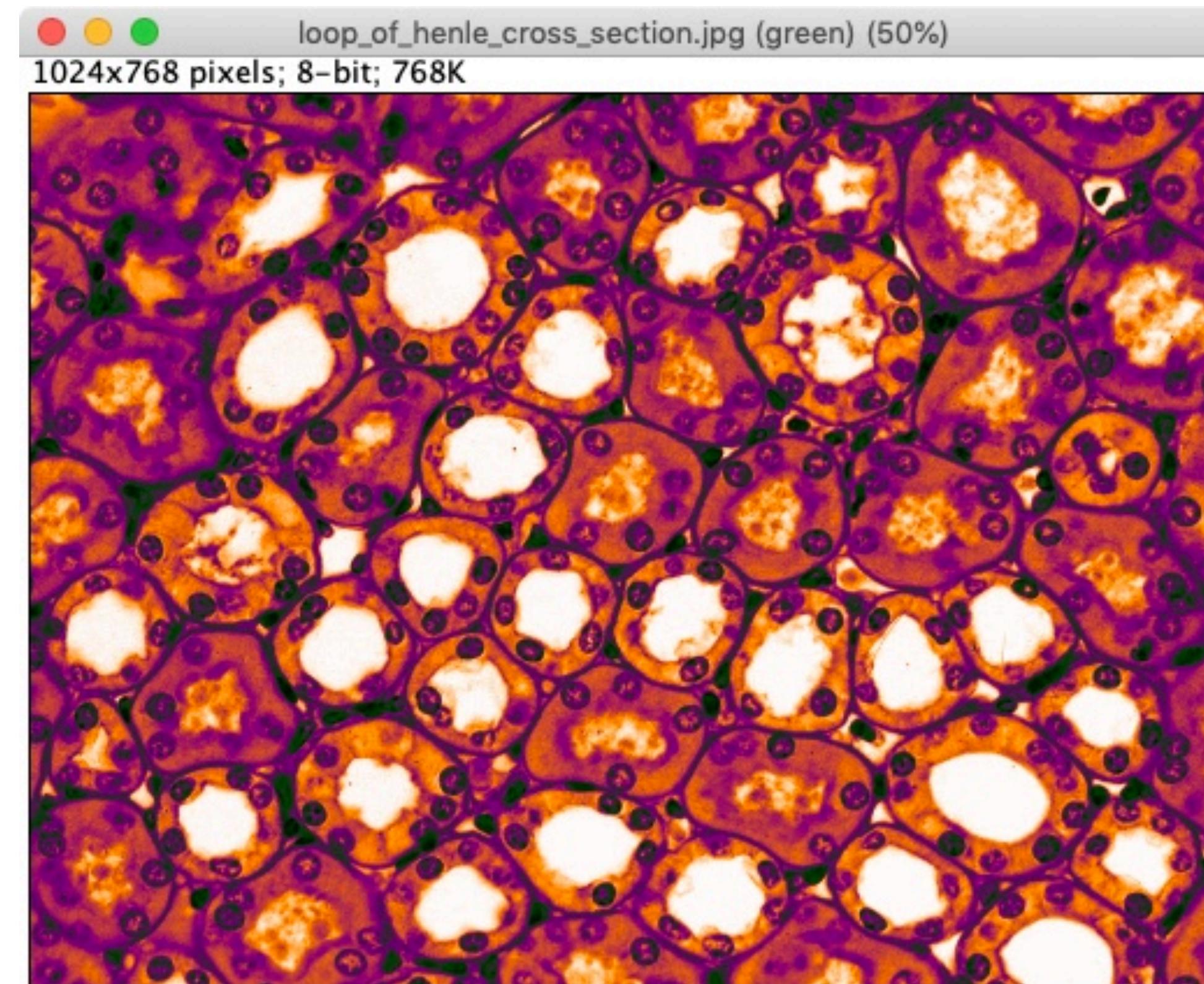
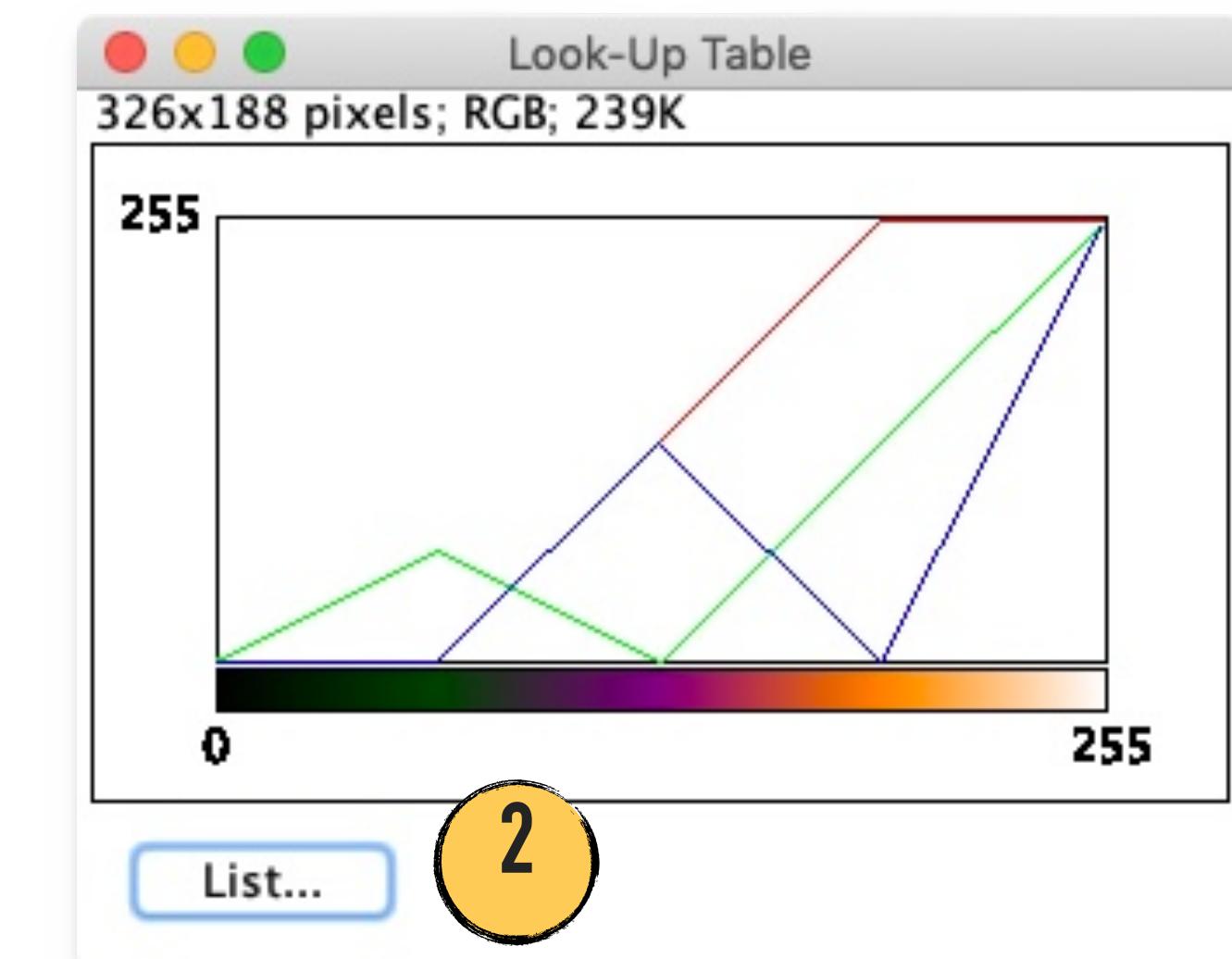
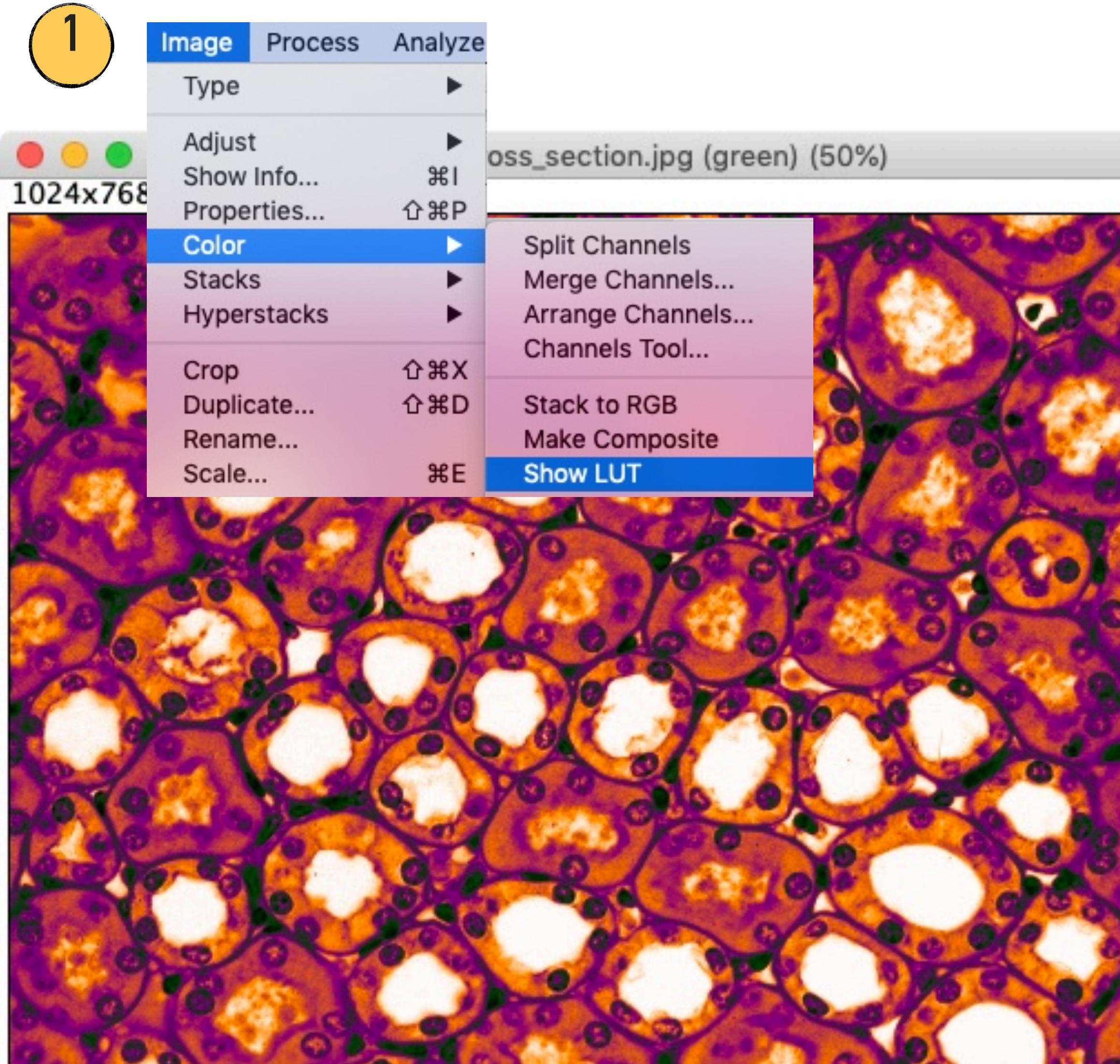


IMAGE EXPLORATION

SHOW LUT



The LUT is an array,
just like in MATLAB

3

Index	Red	Green	Blue
0	1	2	1
1	1	2	1
2	1	3	1
3	1	4	1
4	1	5	1
5	1	6	1
6	1	7	1
7	1	8	1
8	1	9	1
9	1	10	1
10	1	11	1
11	1	12	1
12	1	13	1
13	1	14	1
14	1	15	1
15	1	16	1
16	1	17	1
17	1	18	1
18	1	19	1
19	1	20	1
20	1	21	1
21	1	22	1
22	1	23	1
23	1	24	1
24	1	25	1
25	1	26	1
26	1	27	1
27	1	28	1
28	1	29	1
29	1	30	1
30	1	31	1
31	1	32	1
32	1	33	1
33	1	34	1
34	1	35	1

SHOW ALL THE LUTs

1

Select menu item

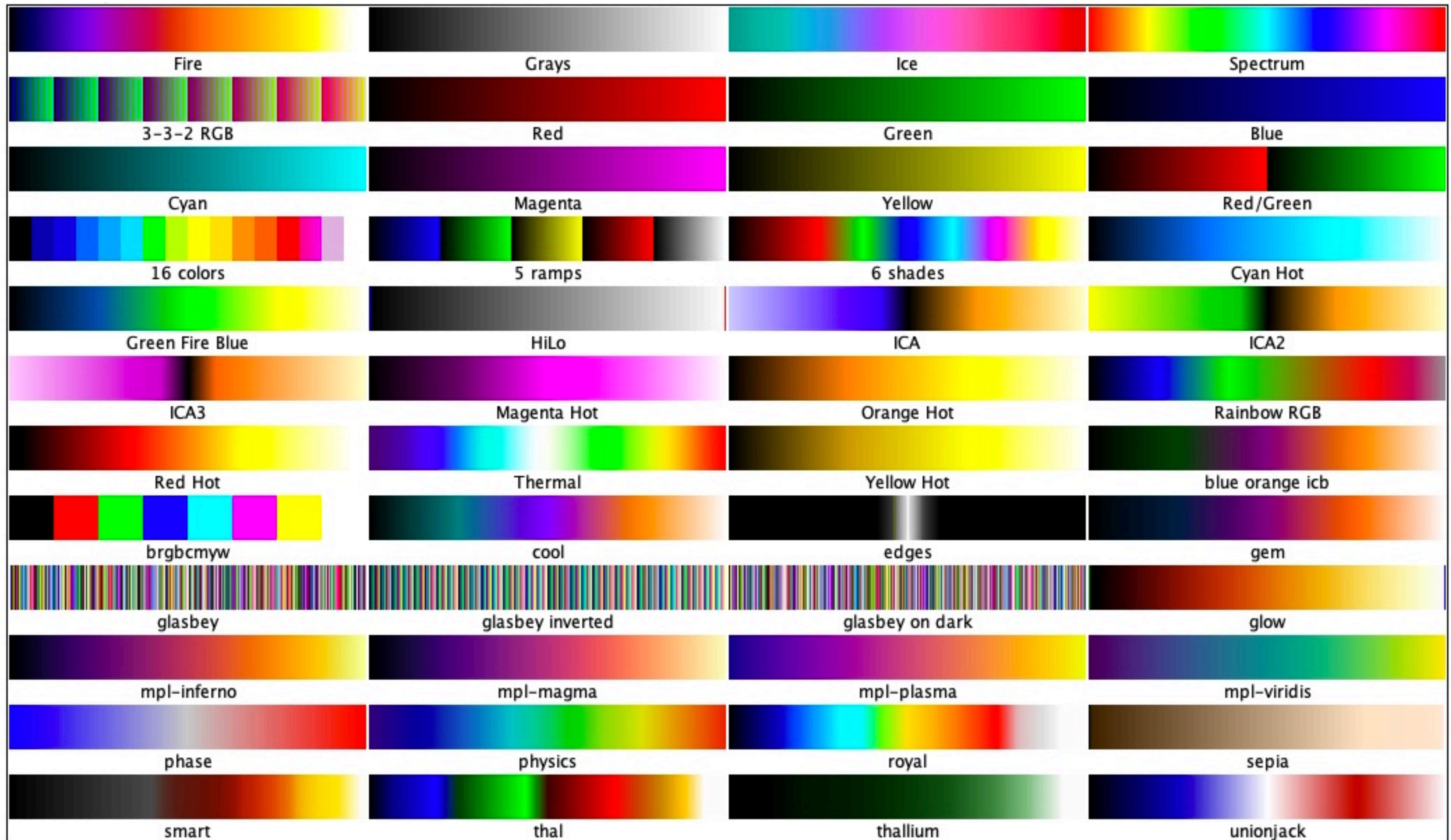
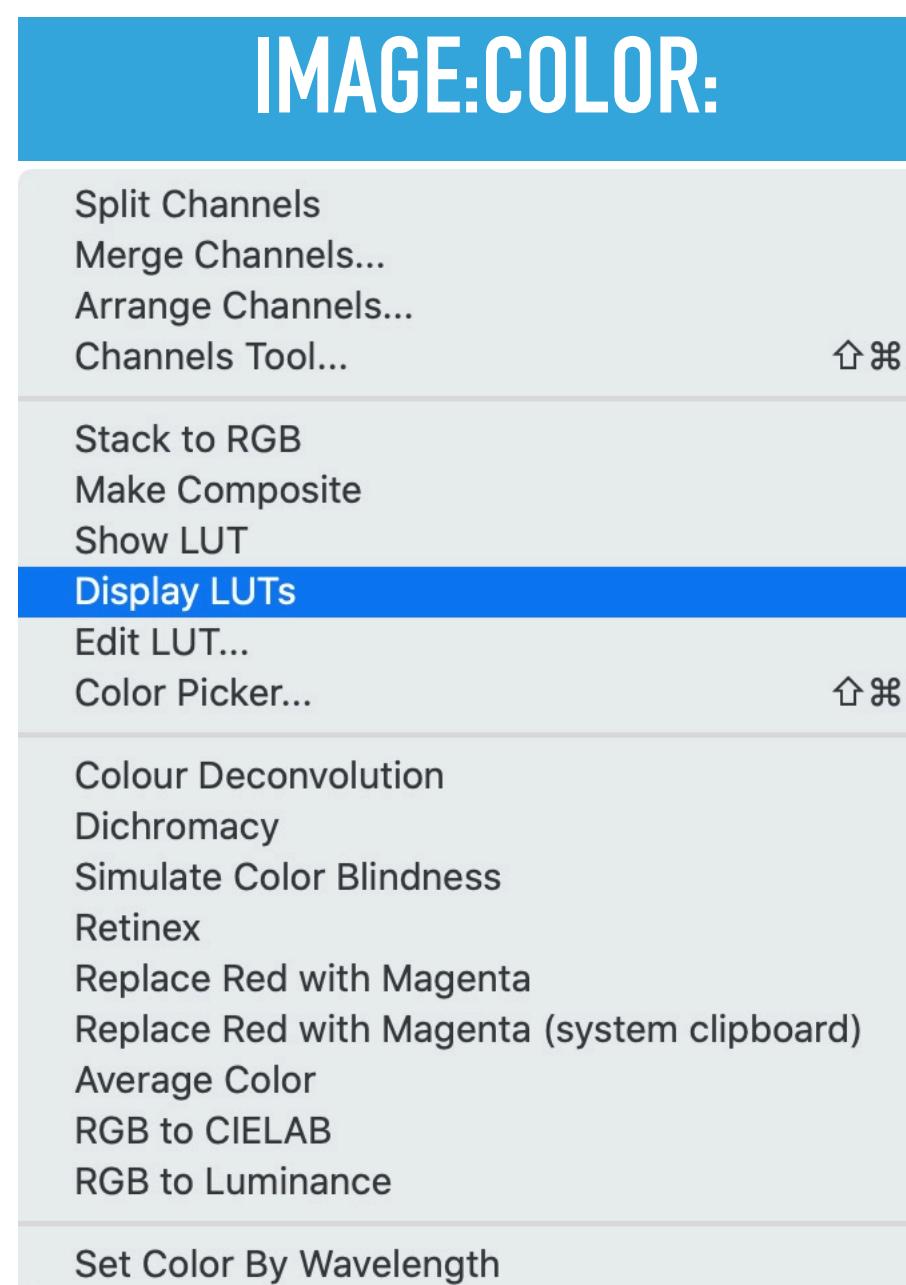
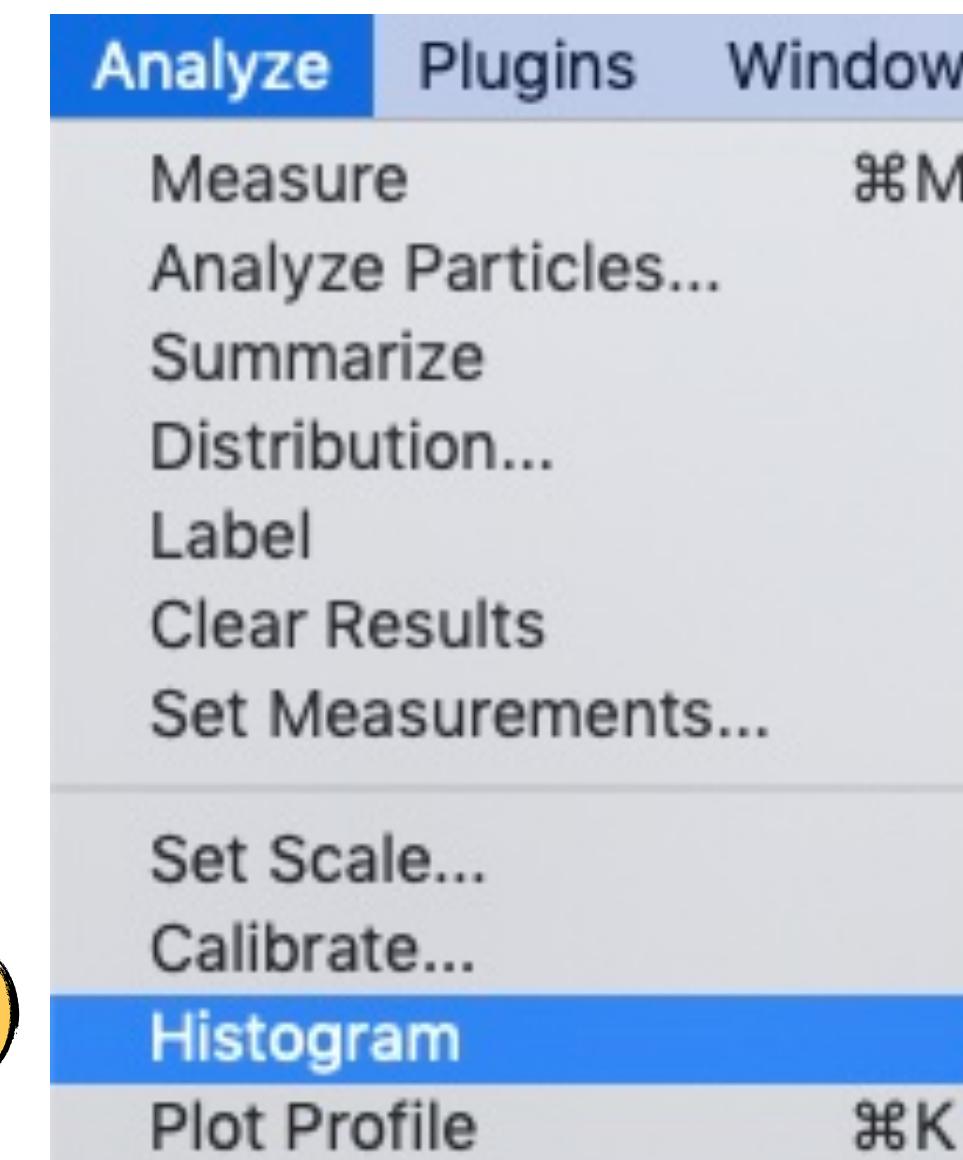
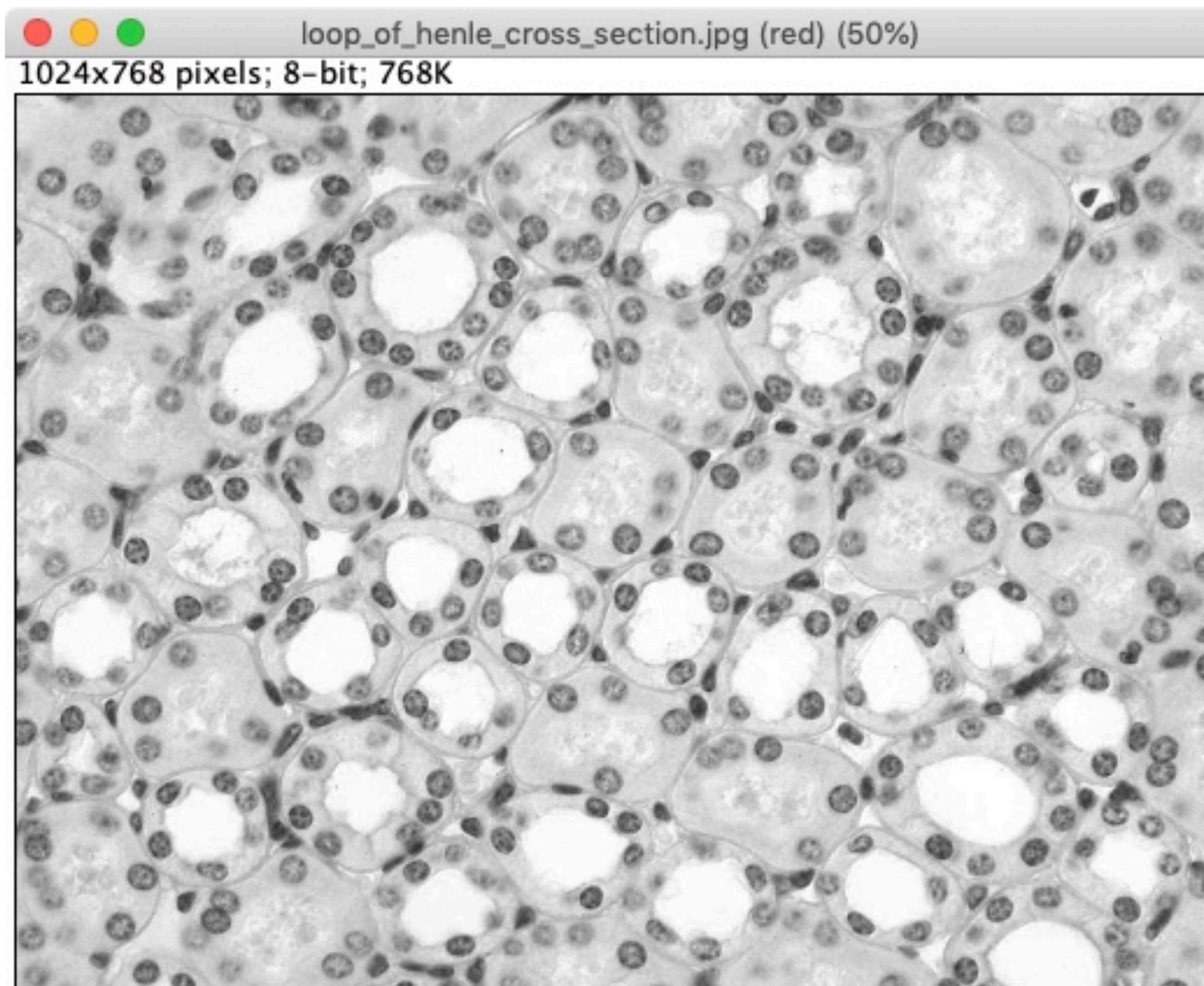


IMAGE EXPLORATION

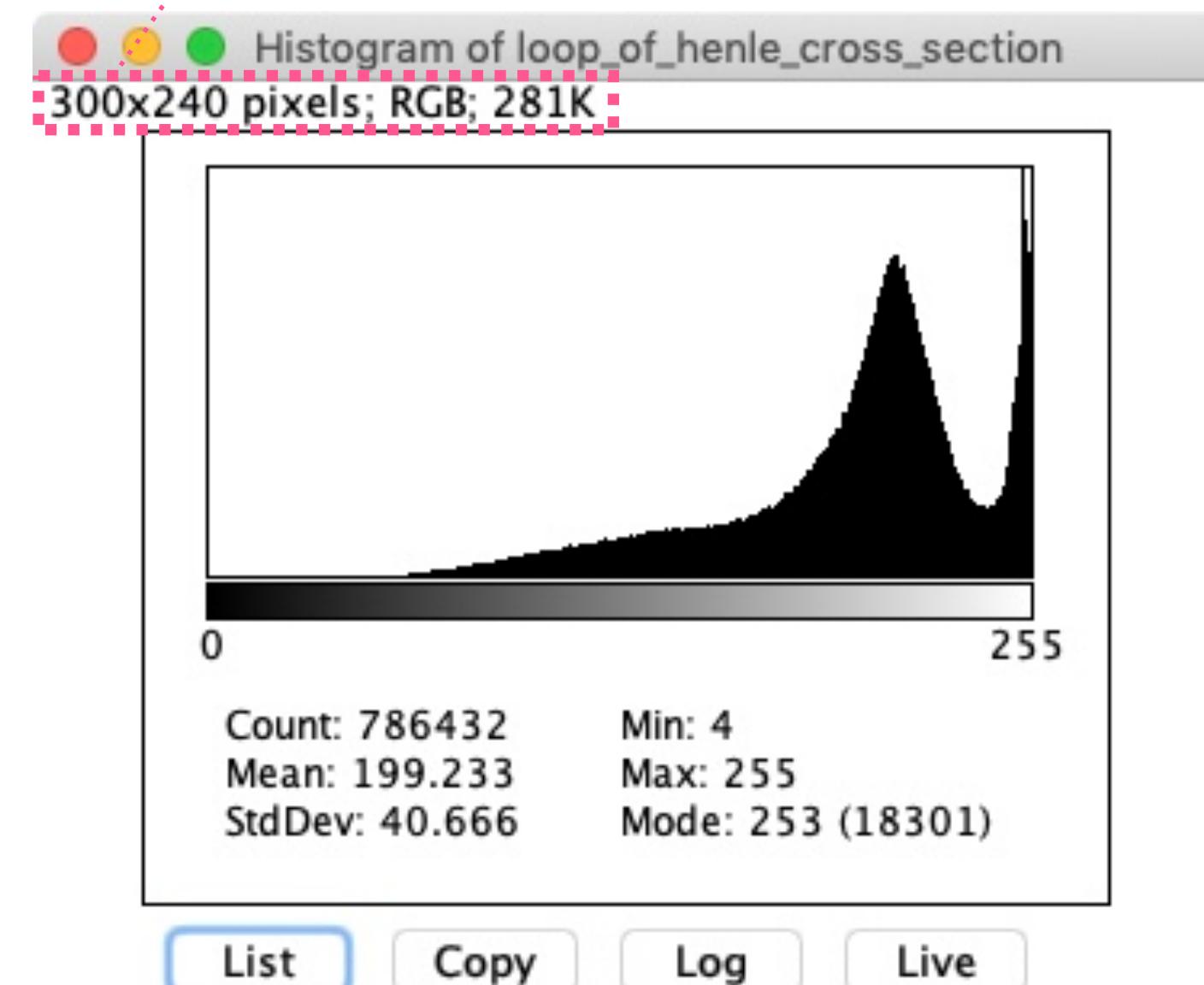
CHANNEL HISTOGRAM

1 Select Channel



2

Careful, image information listed here refers to the window containing the histogram, not the original image



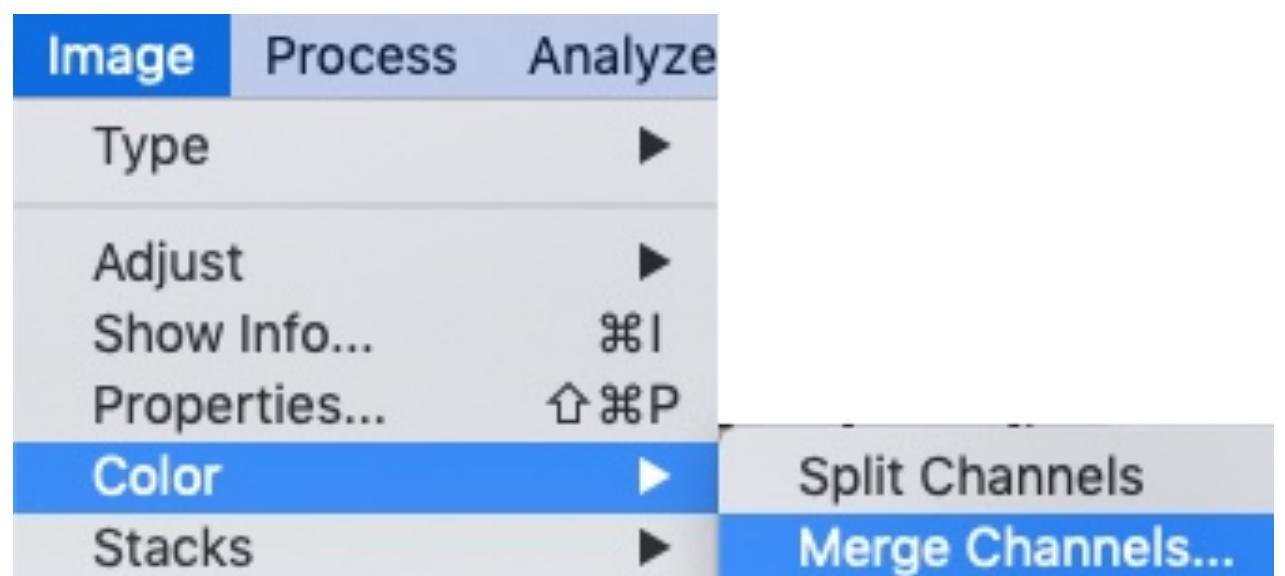
3

Histogram of channel appears

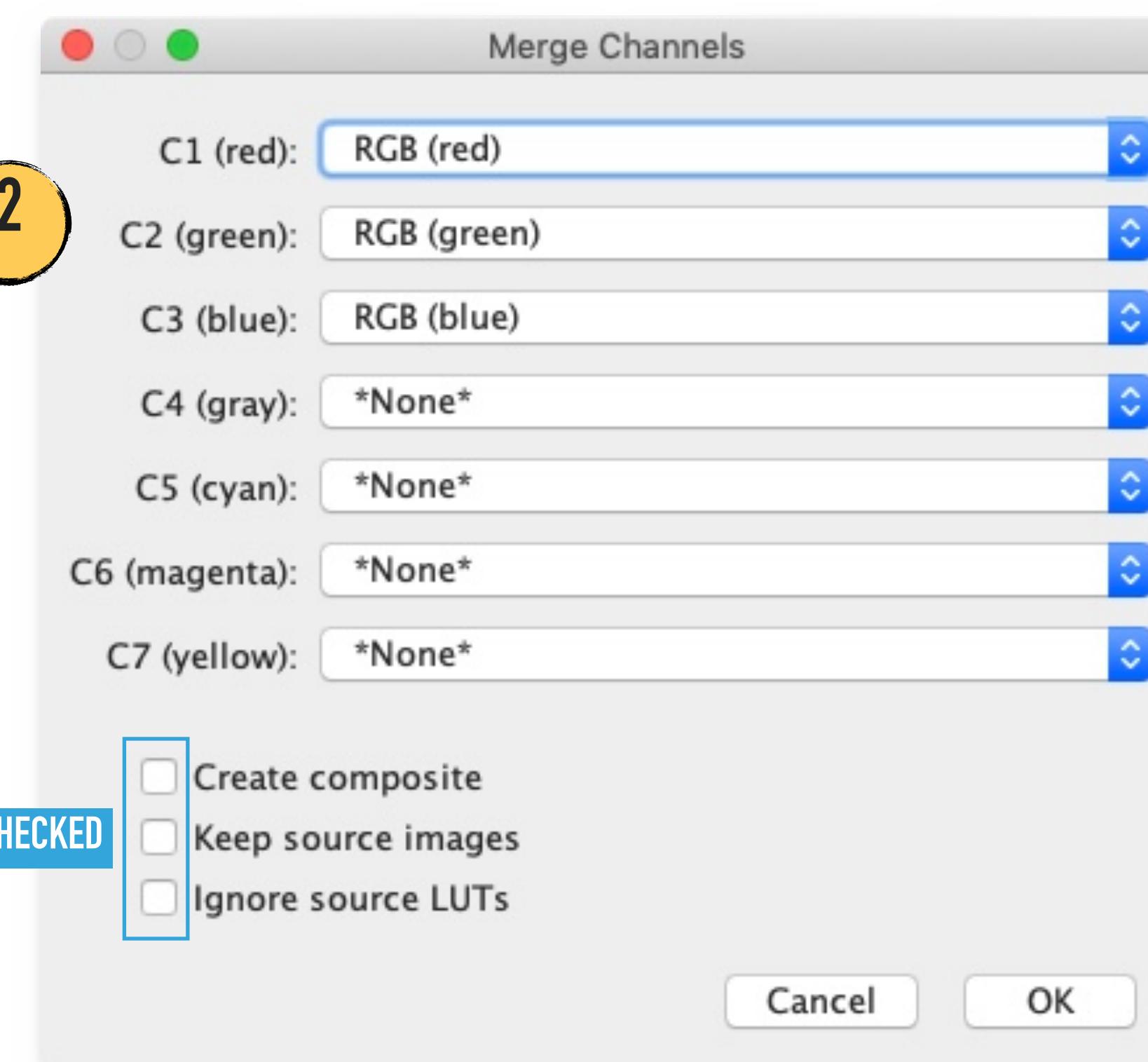
IMAGE EXPLORATION

REBUILD RGB

1



2



3

RGB image reappears (note name change)

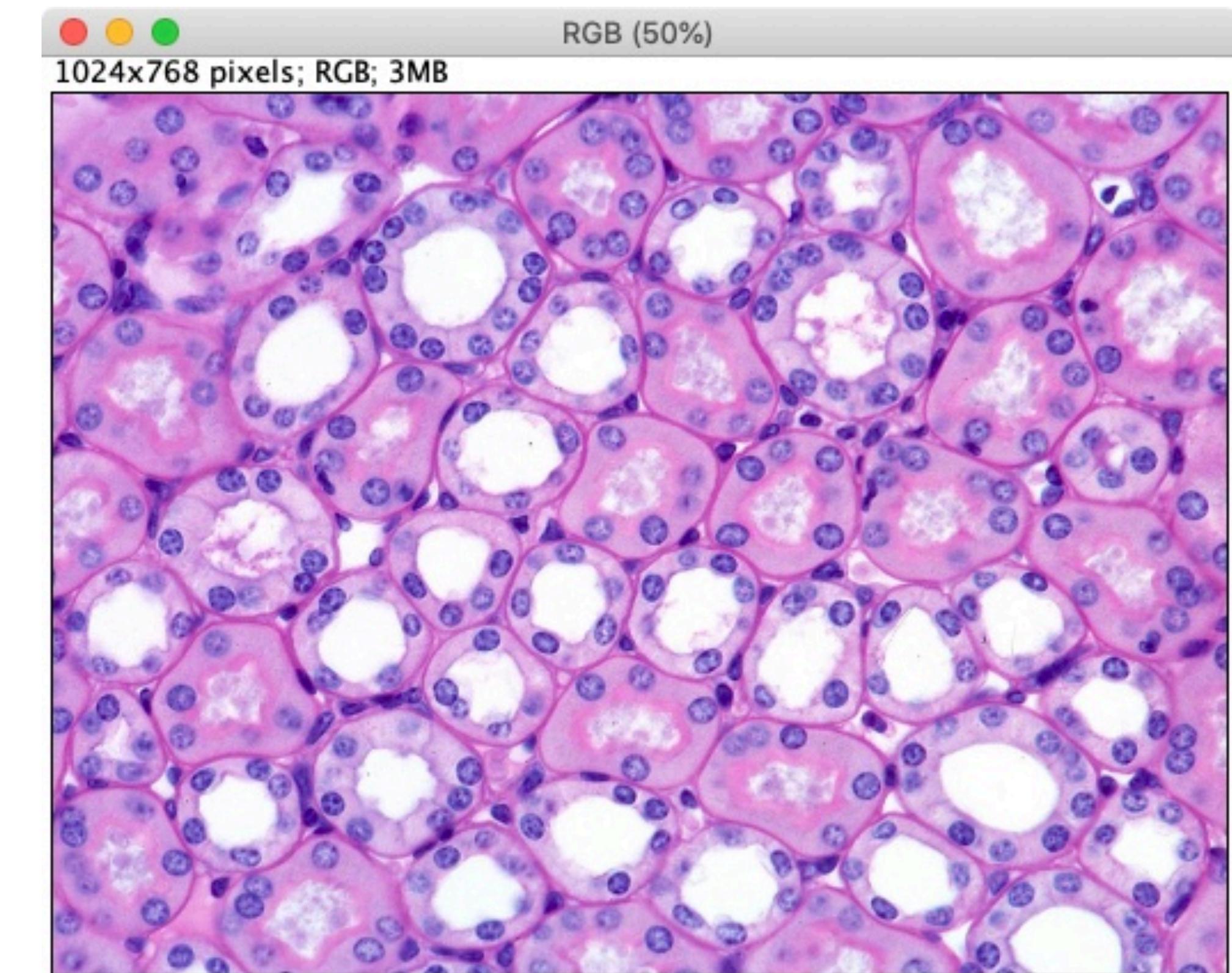
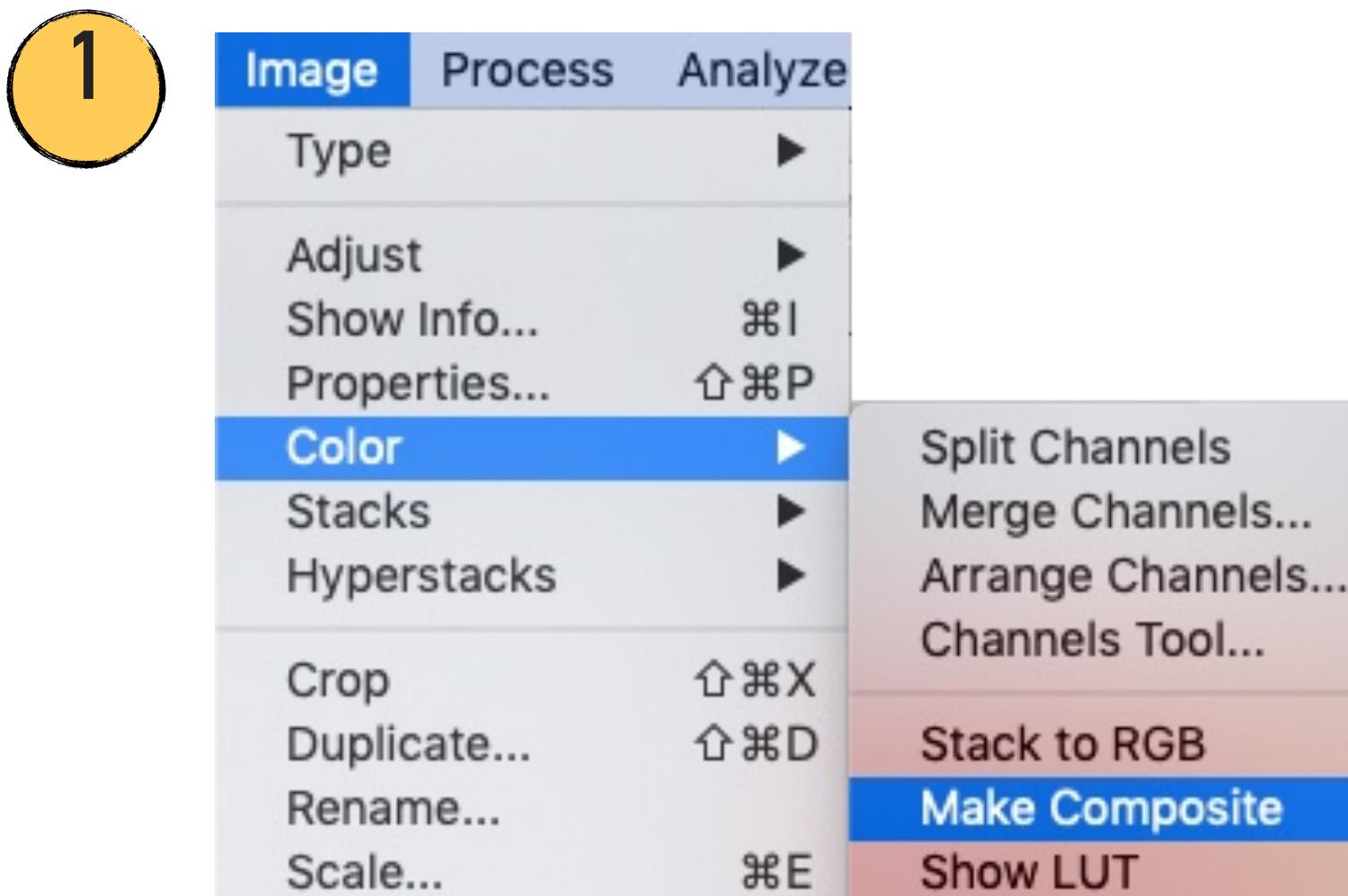


IMAGE PROCESSING

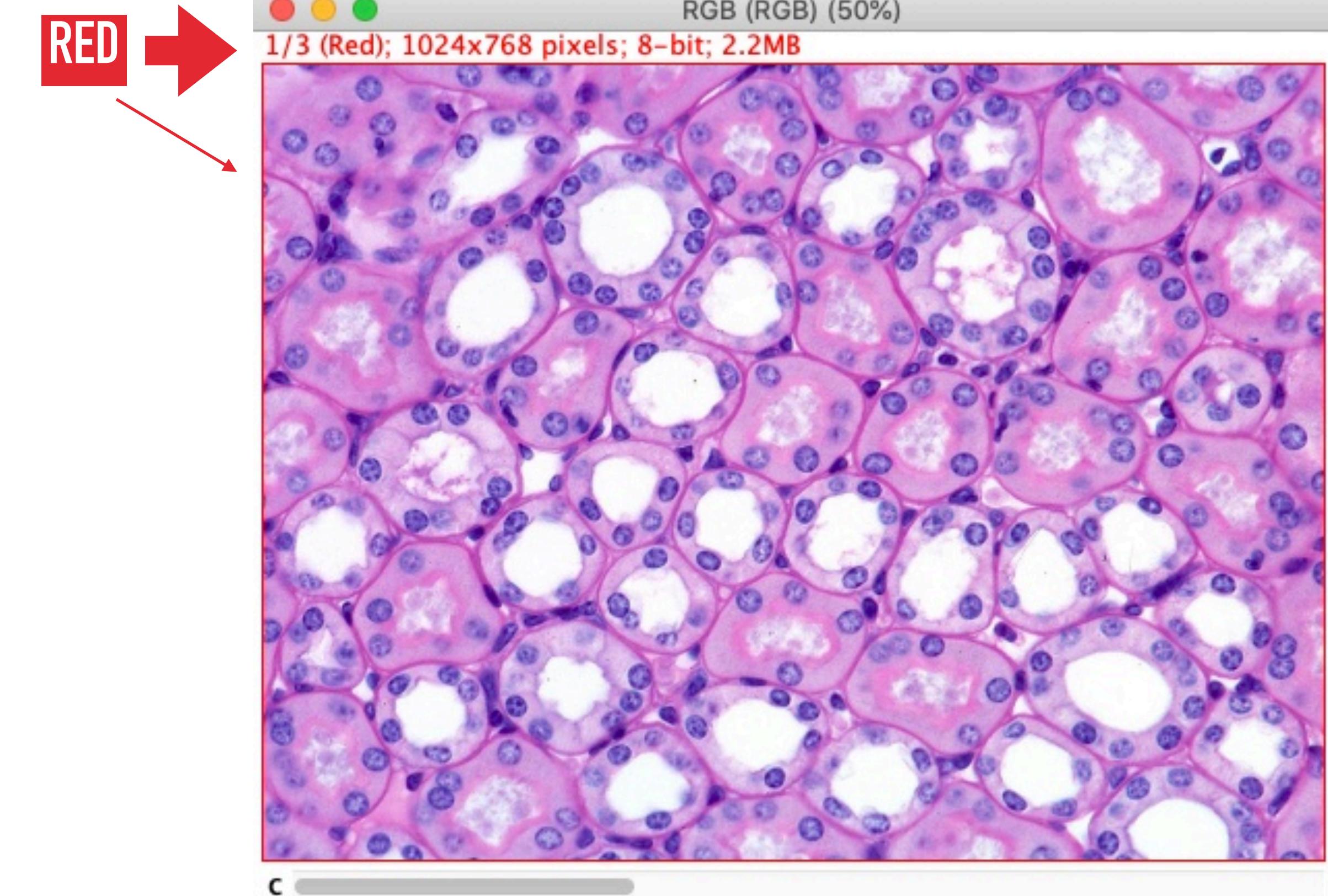
COLOR MODIFICATION

COLOR MODIFICATION

MAKE COMPOSITE



2 Image looks the same, but...



- ▶ Notice that info color and outline changes when slider moved
- ▶ Notice Info says $\frac{1}{3}$ (as in Channel 1 out of 3 channels)

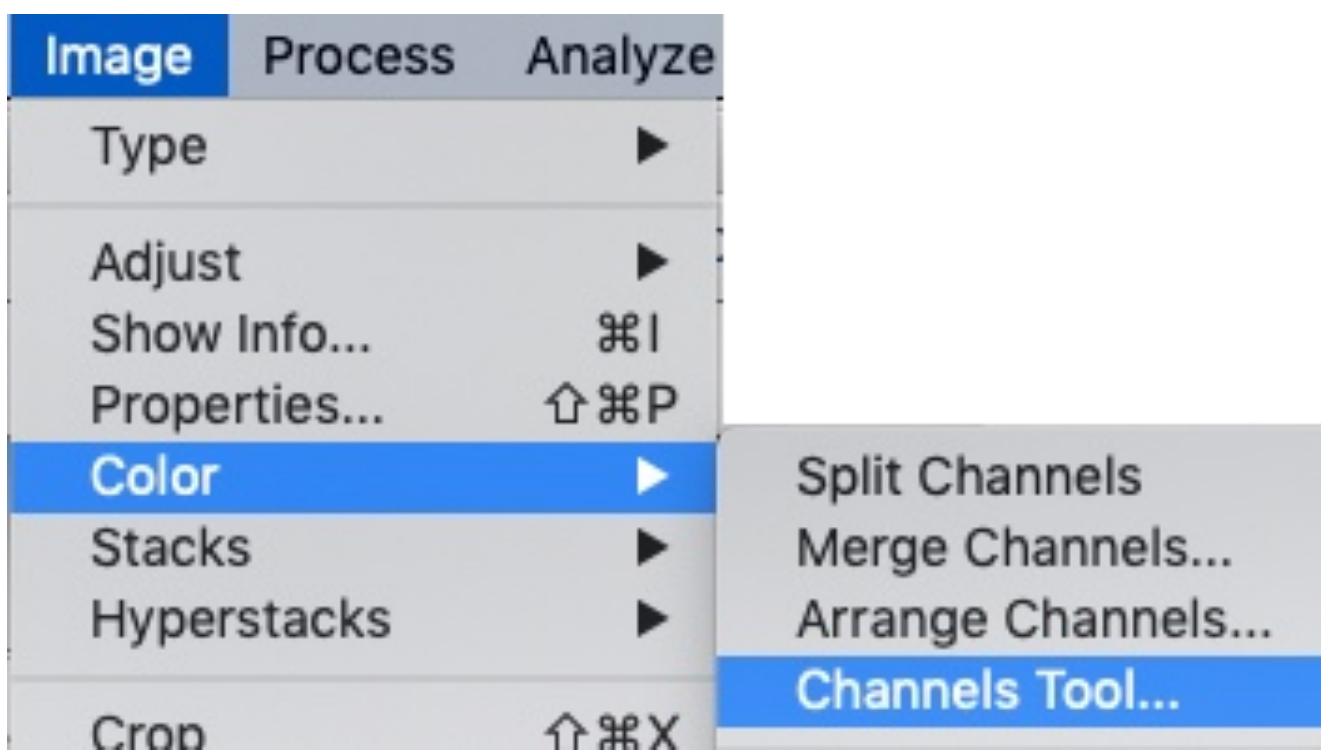
3

HYPERSTACK VIEWER

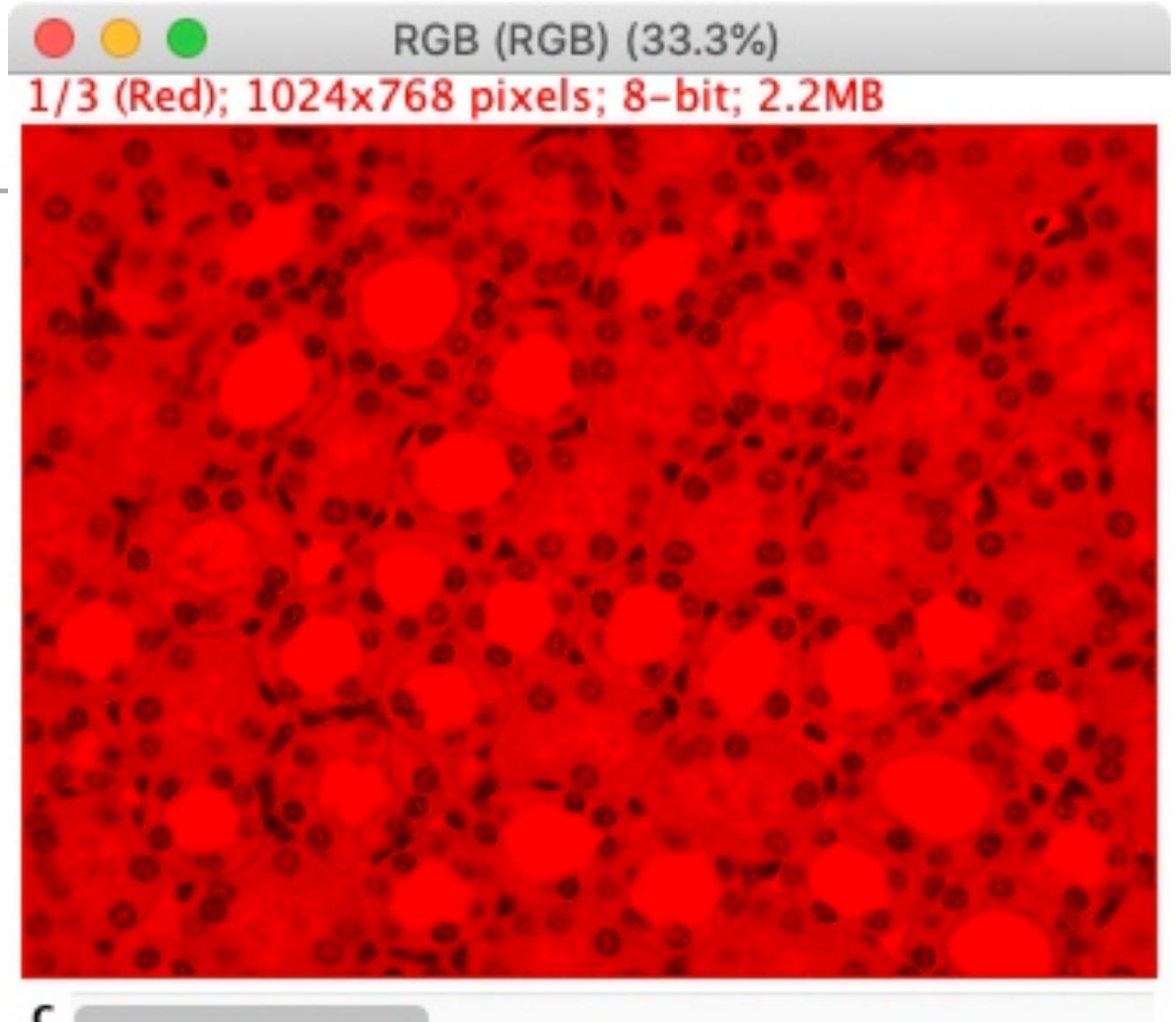
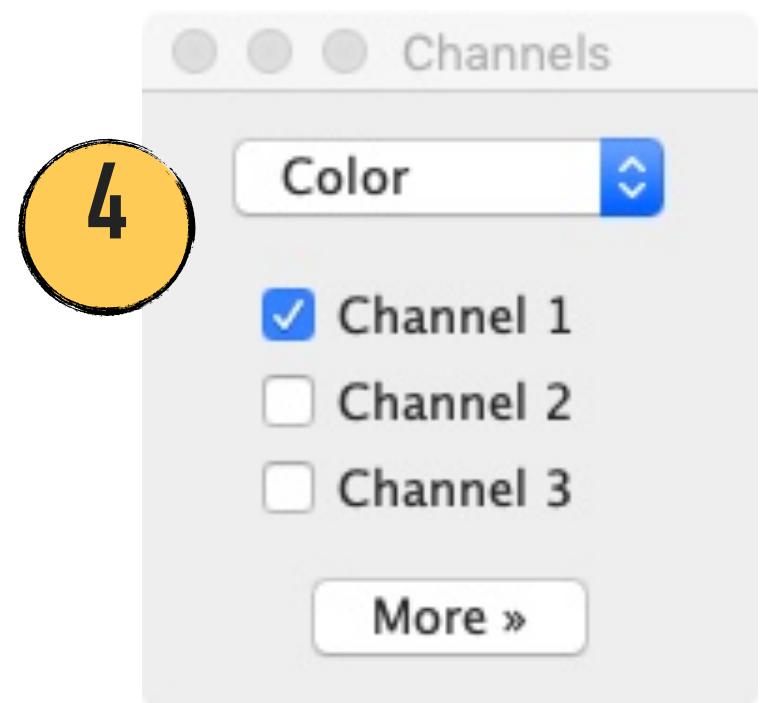
COLOR MODIFICATION

CHANNEL TOOL

1



4

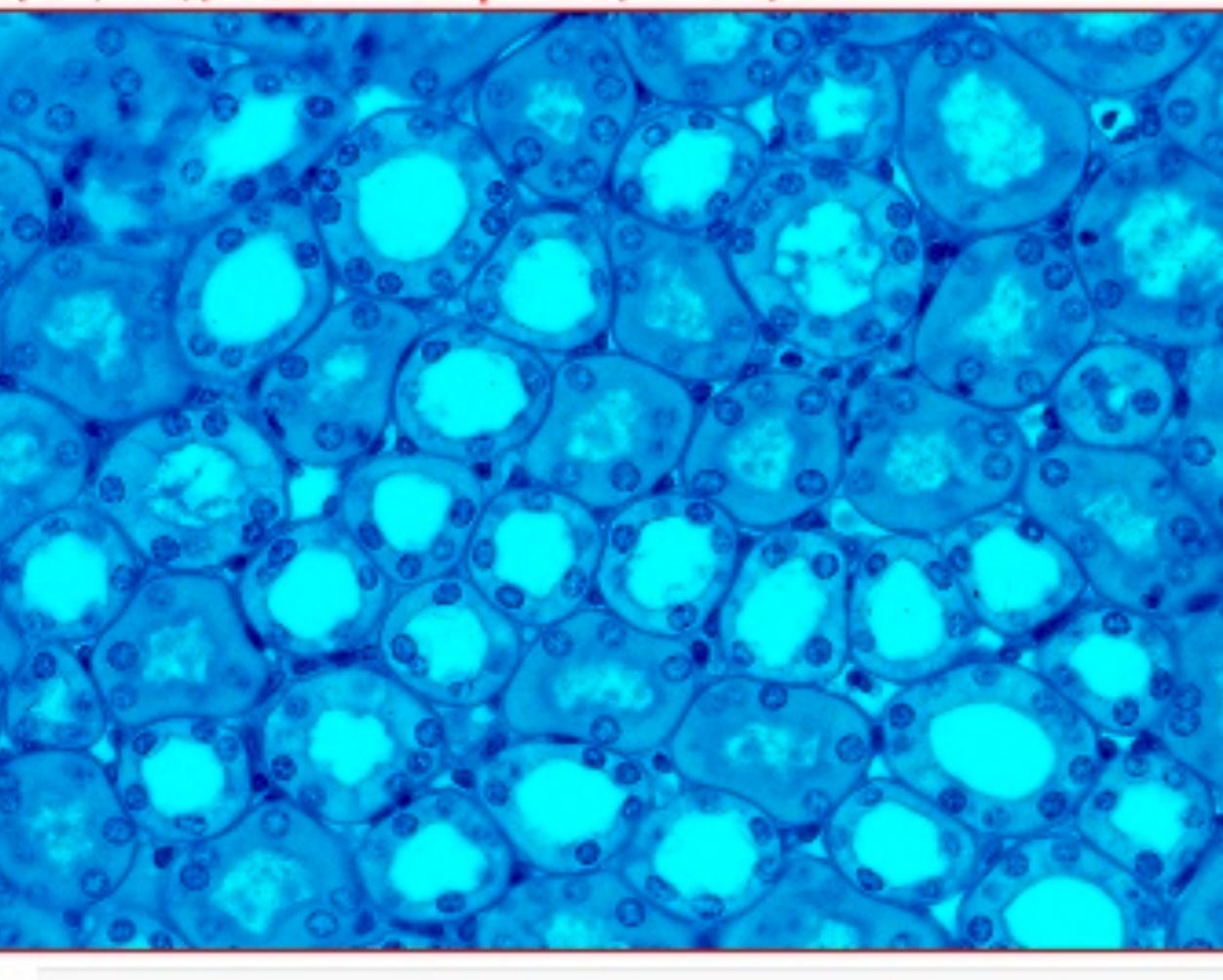


red channel...

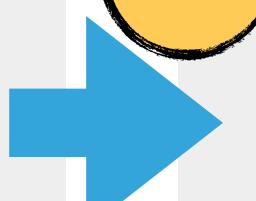
3

Image without red channel...

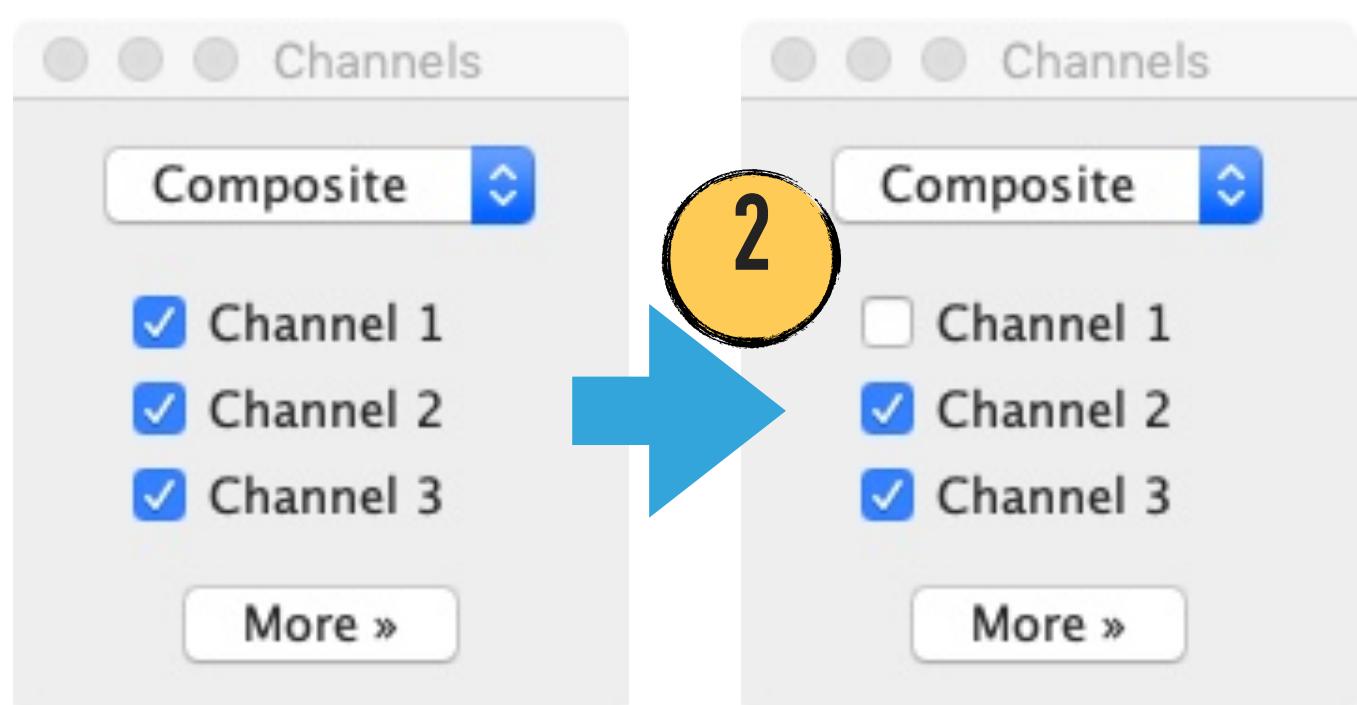
RGB (RGB) (33.3%)
1/3 (Red); 1024x768 pixels; 8-bit; 2.2MB



Blue and green make cyan



2



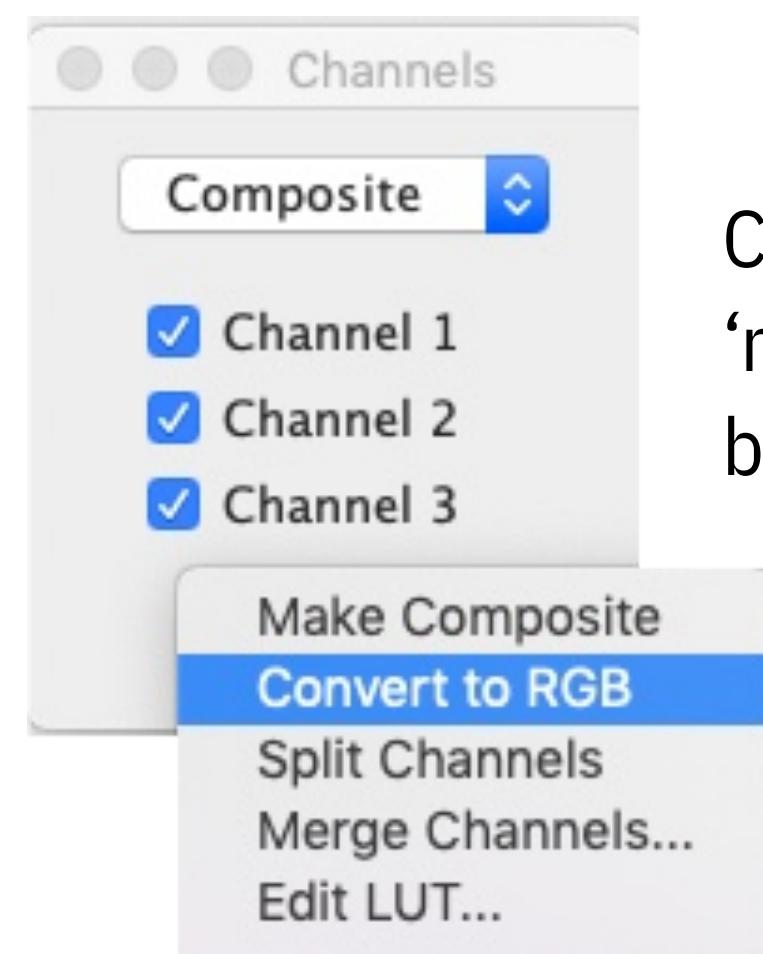
EXPLORE...

Turn off the green channel and notice how it is a critical component of the image even though the image doesn't look very green

COLOR MODIFICATION

MAGENTA

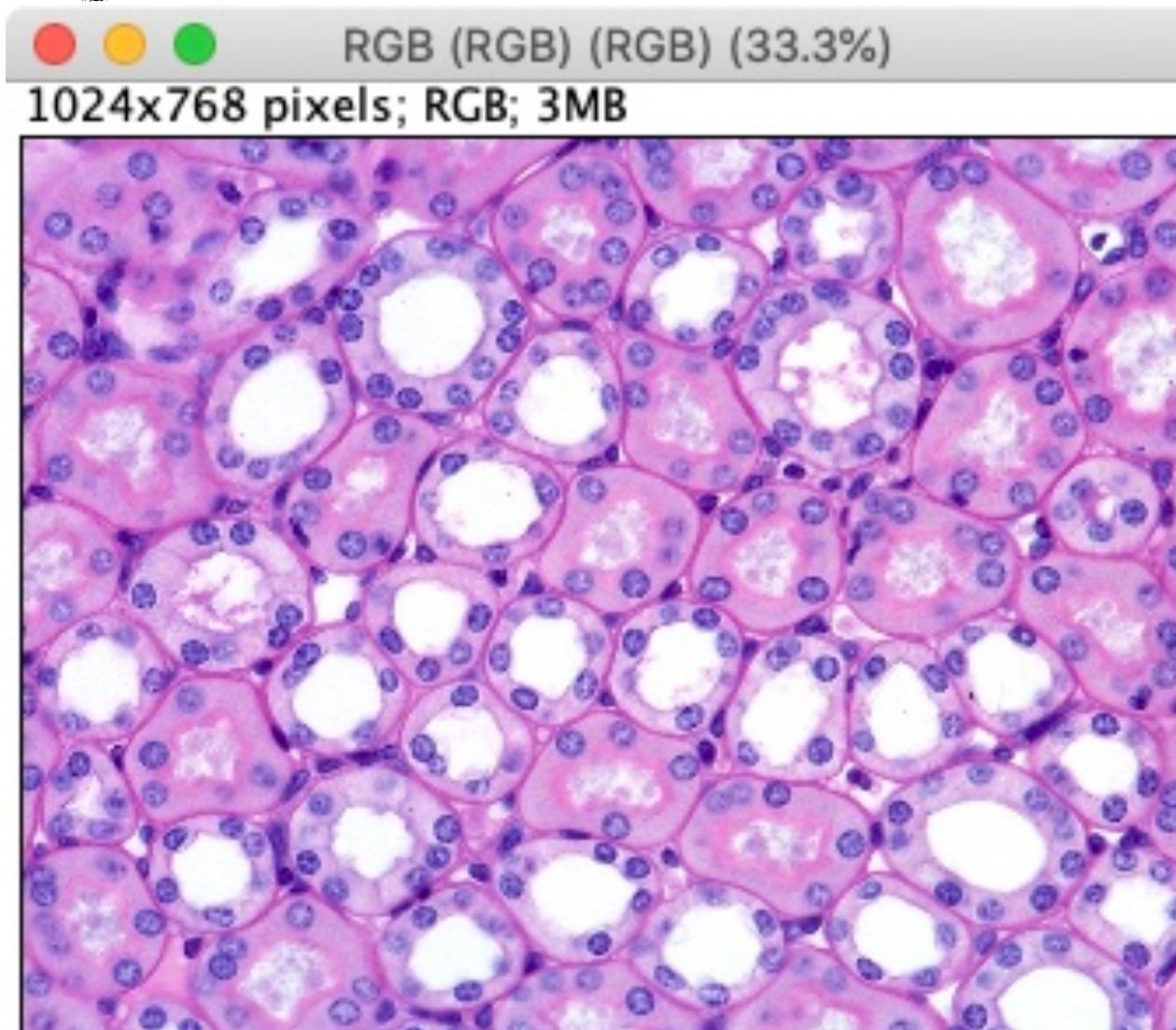
1



Click on
'more...' button

2

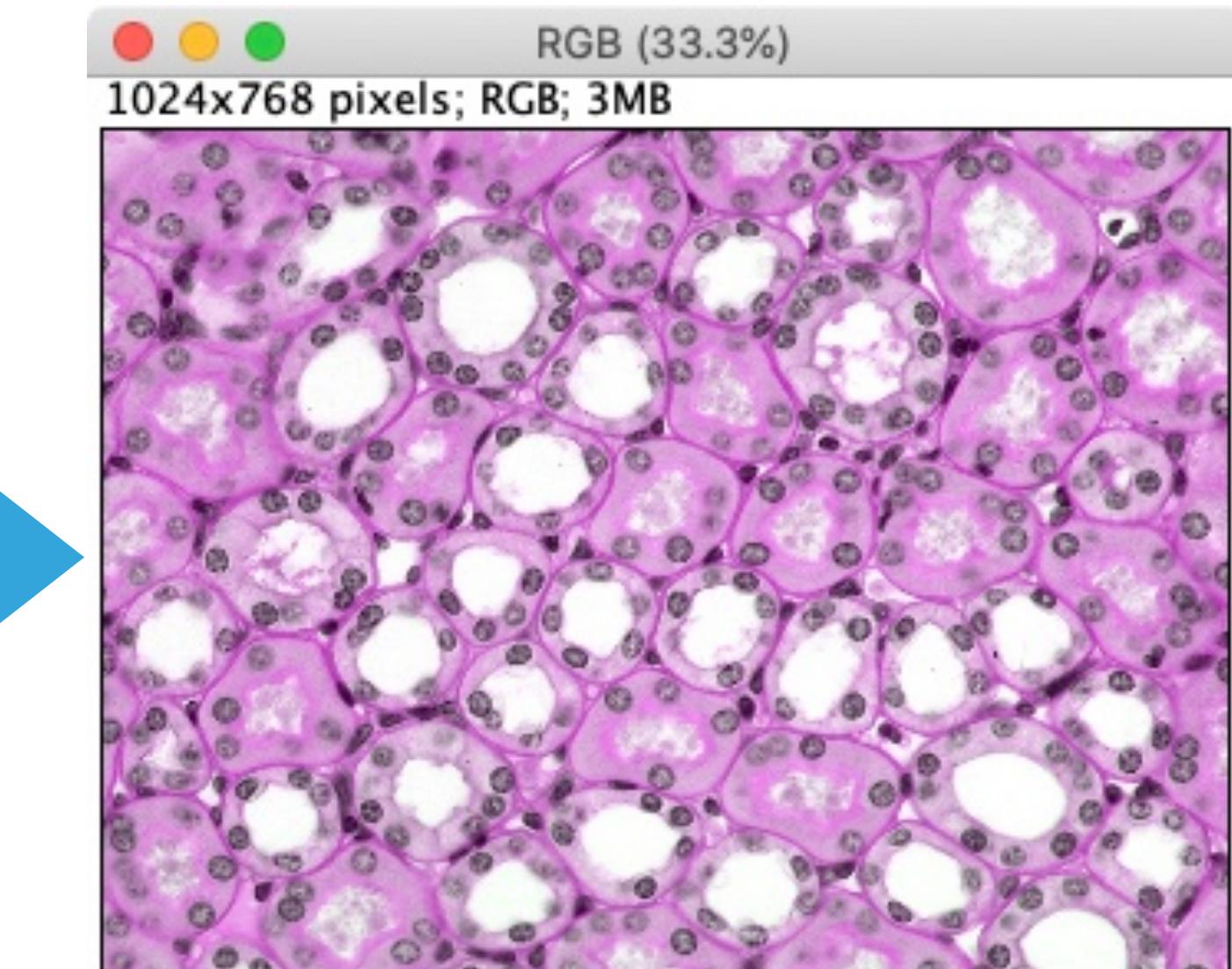
Activate RGB image



3

IMAGE:COLOR:

- Split Channels
- Merge Channels...
- Arrange Channels...
- Channels Tool... ⇧⌘Z
- Stack to RGB
- Make Composite
- Show LUT
- Display LUTs
- Edit LUT...
- Color Picker... ⇧⌘K
- Colour Deconvolution
- Dichromacy
- Simulate Color Blindness
- Retinex
- Replace Red with Magenta**
- Replace Red with Magenta (system clipboard)
- Average Color
- RGB to CIELAB
- RGB to Luminance
- Set Color By Wavelength

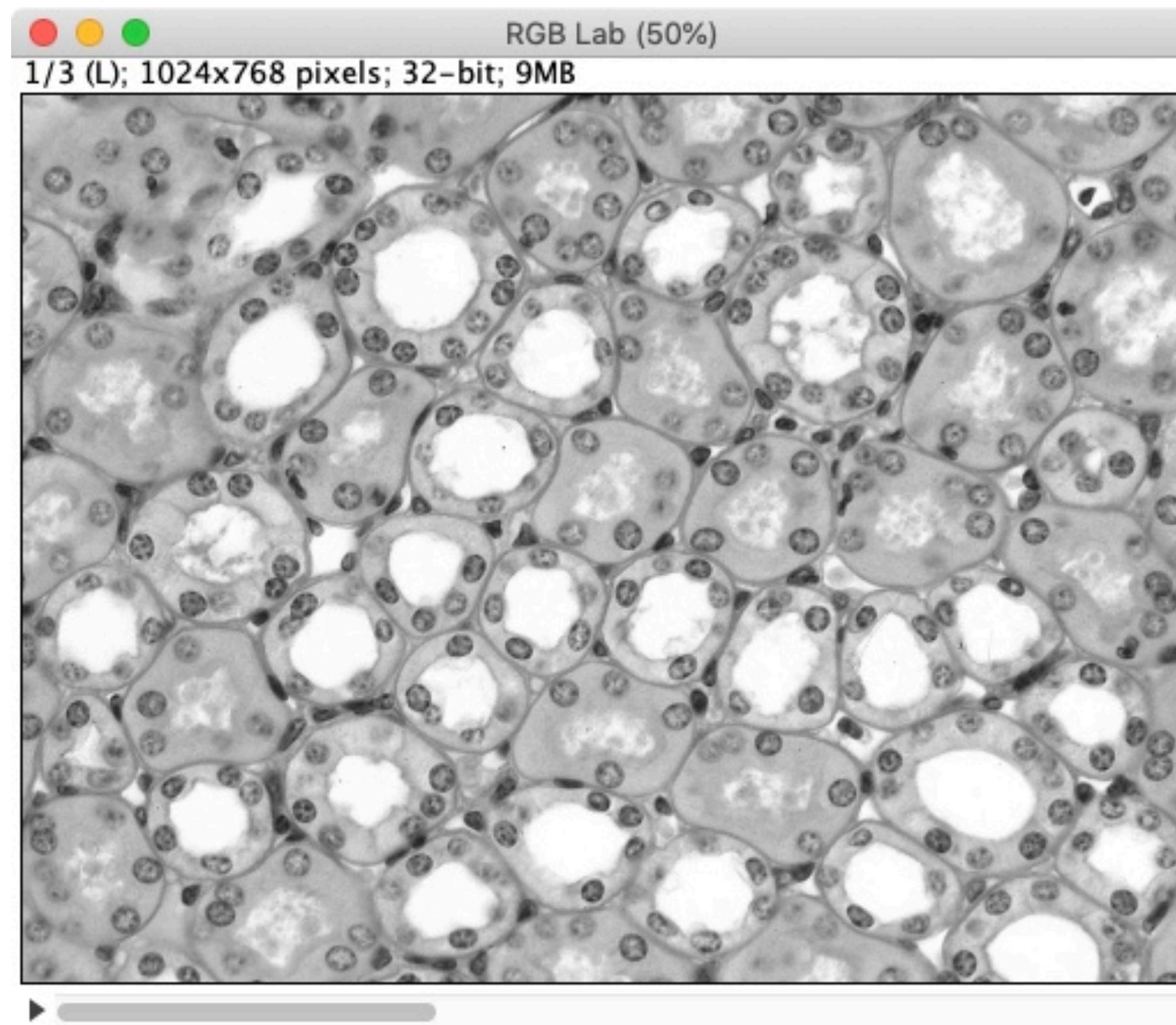


COLOR MODIFICATION

CIELAB AND LUMINANCE

1

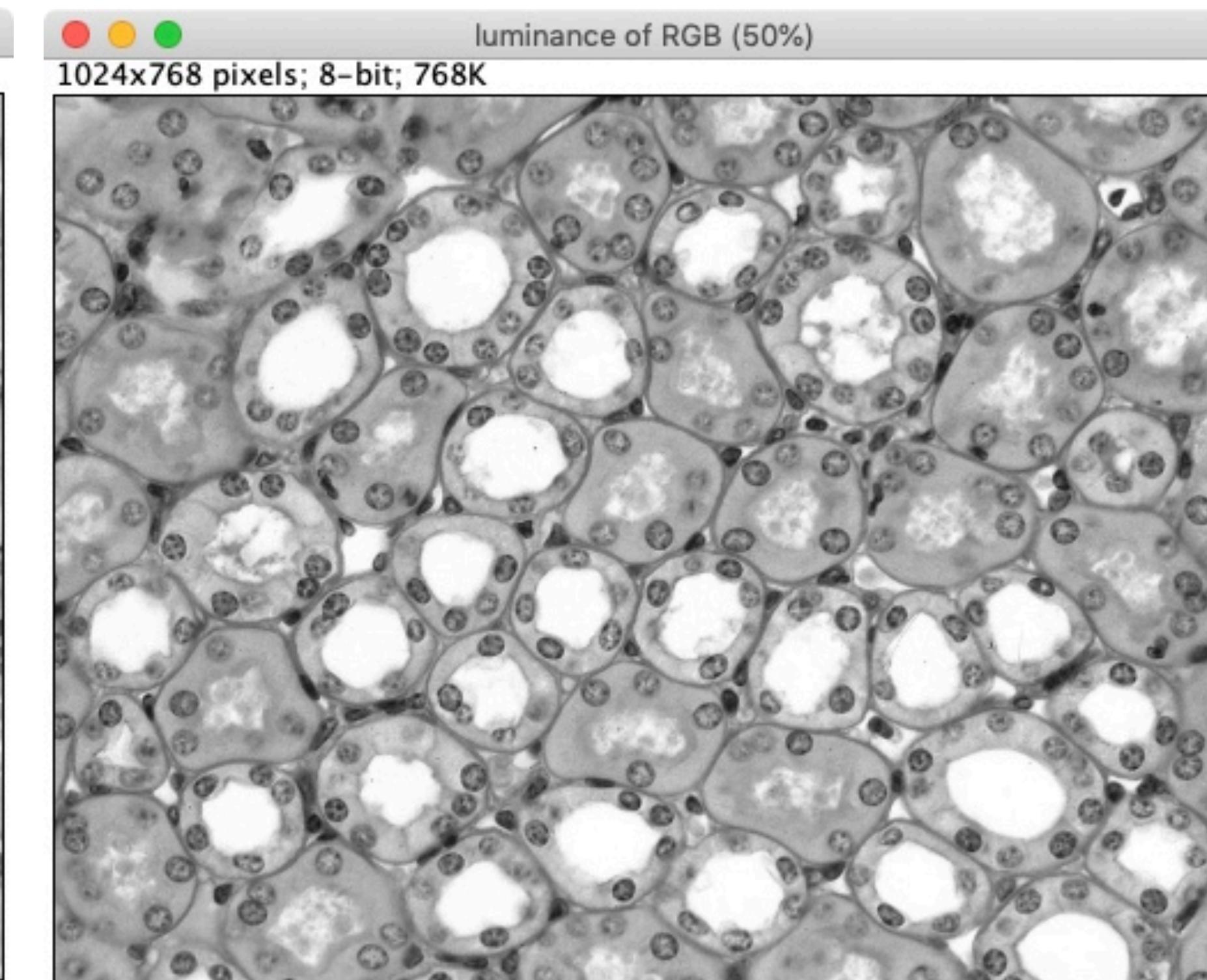
Image:Color:RGB to CIELAB



A composite image with an L, *a, and *b channel

2

Image:Color:RGB to Luminance



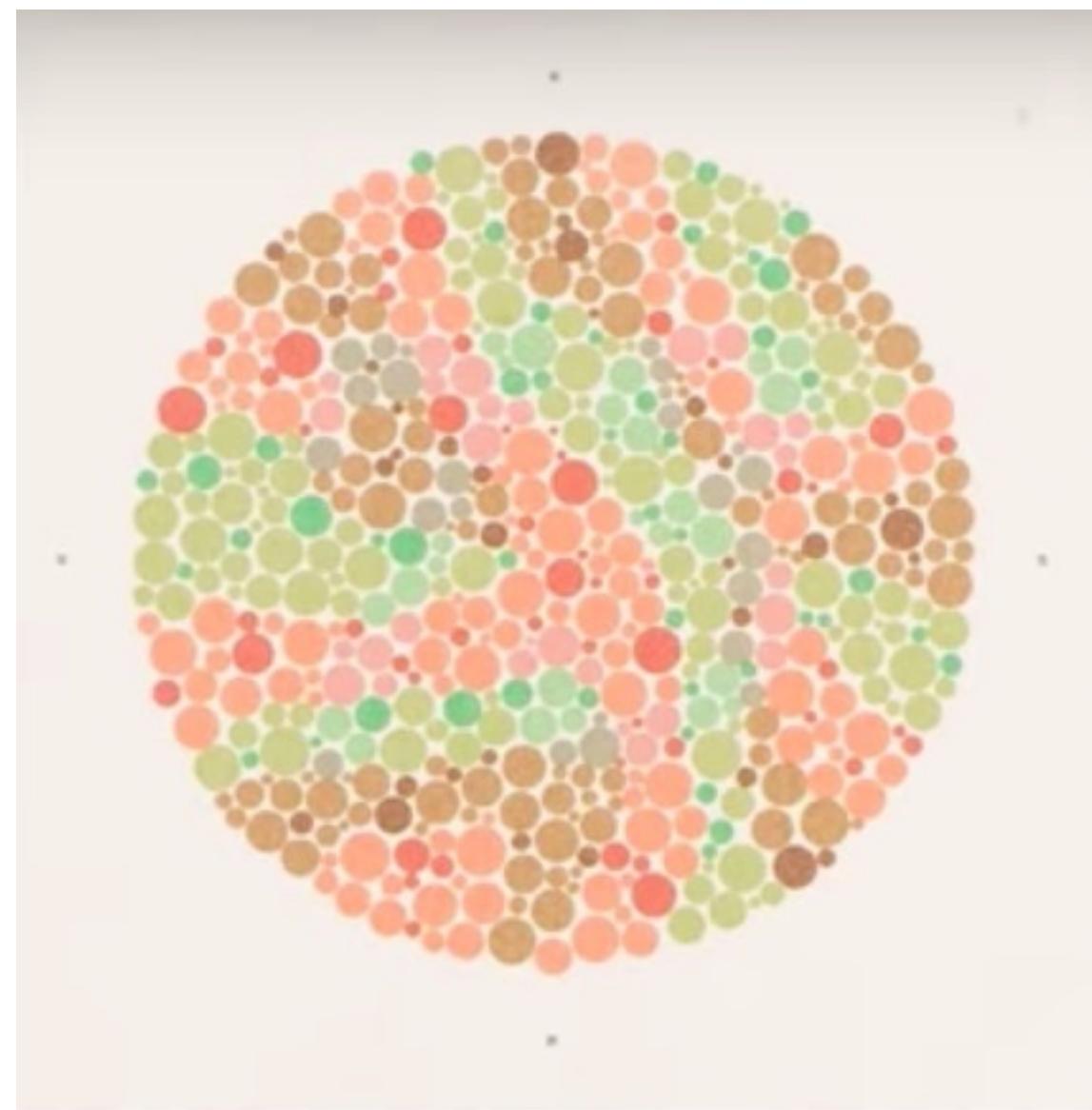
A grayscale image

COLOR MODIFICATION

COLOR BLIND TEST

1

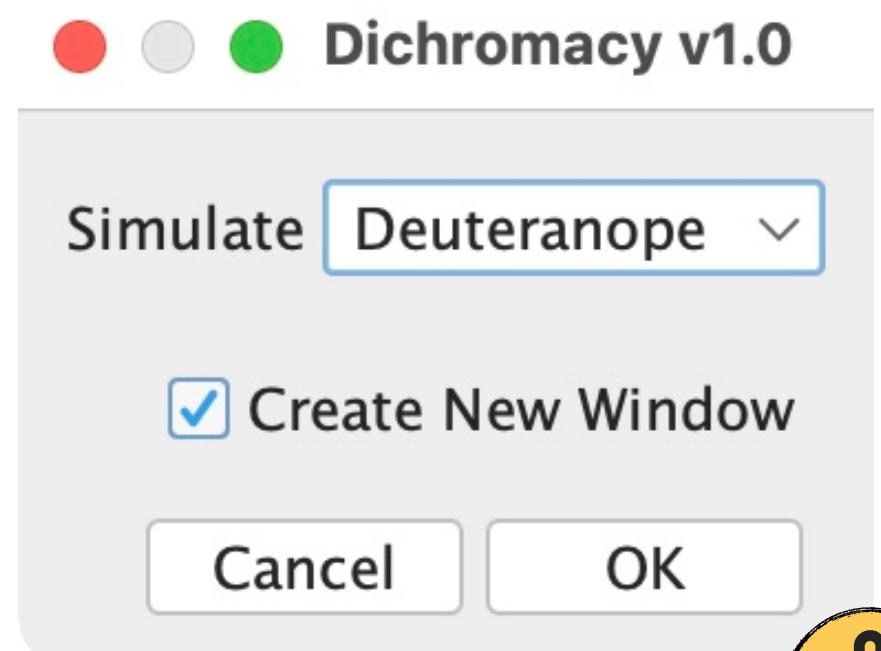
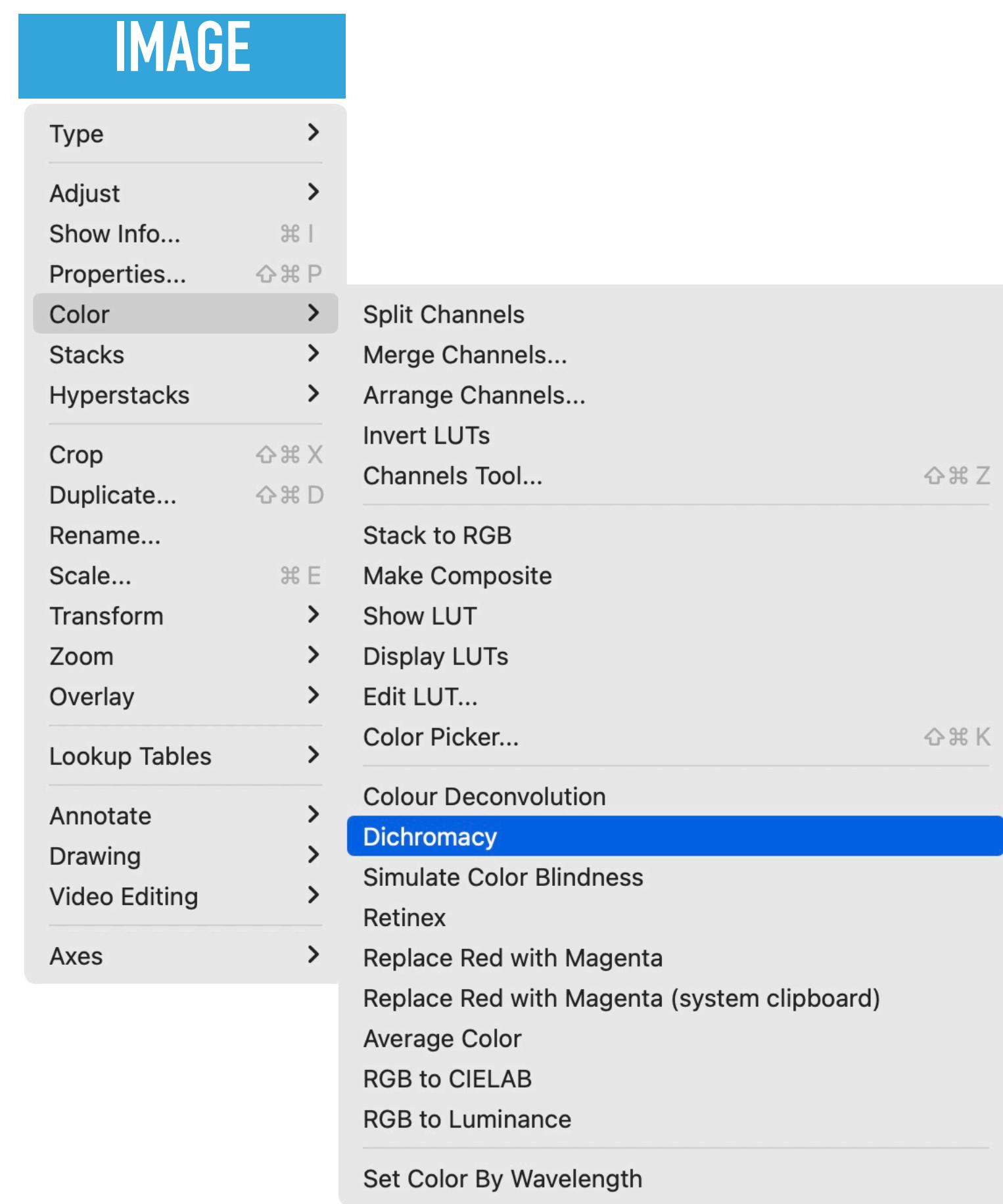
Navigate to the MATLAB Unit 2 data folder and open the “Colorblind-Test.png” image



What number do you see? Are you sure you're not colorblind?

2

Select the Dichromacy submenu item from the Image:Color Menu



3

Try Protanope and Deutanope. Now can you see the number?

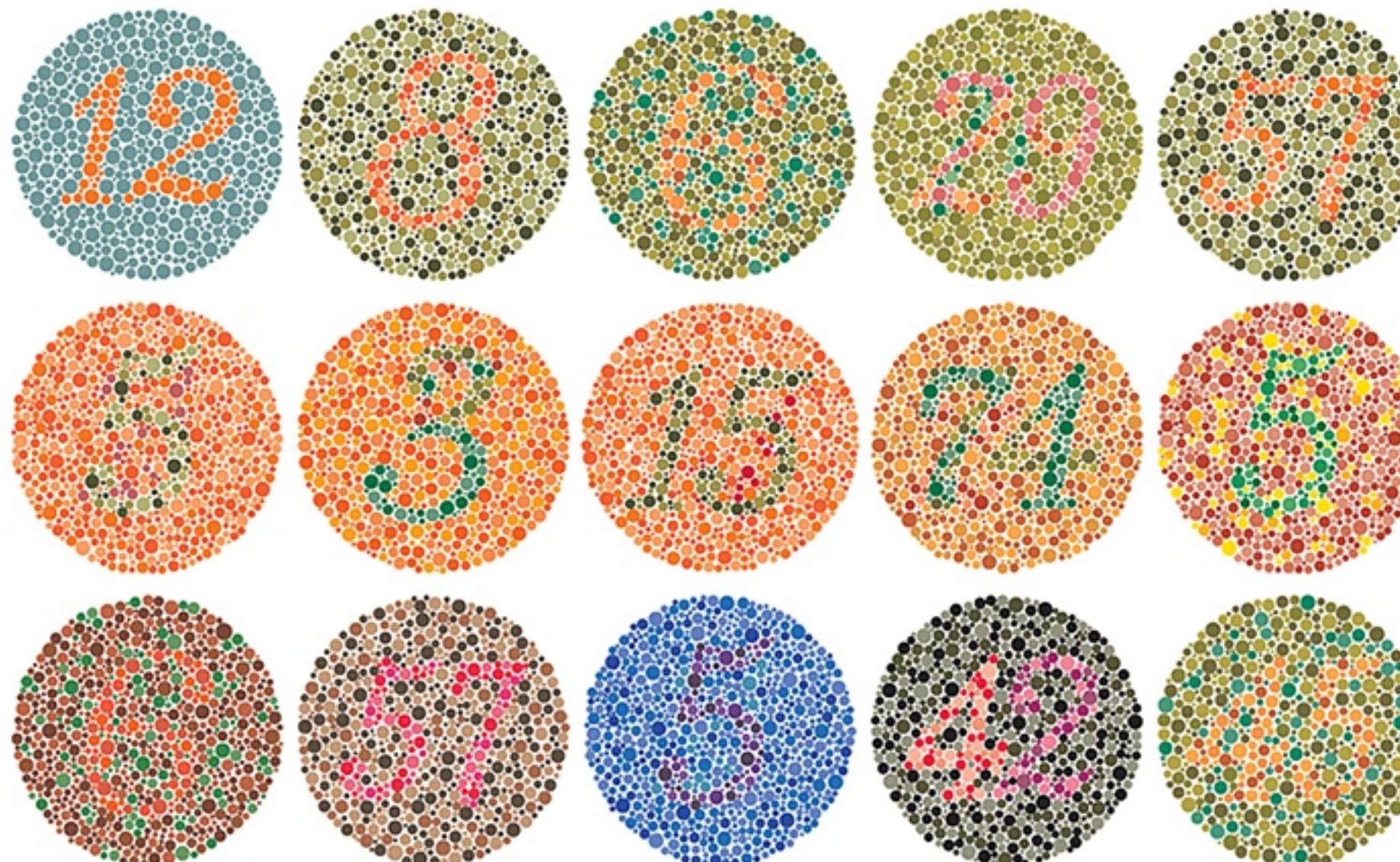
With deutanopia, you should see a "73".

With protanopia, you might see a crooked "23" due to the ambiguity that comes with working with little colored circles.

COLOR MODIFICATION

COLOR BLIND TEST 2

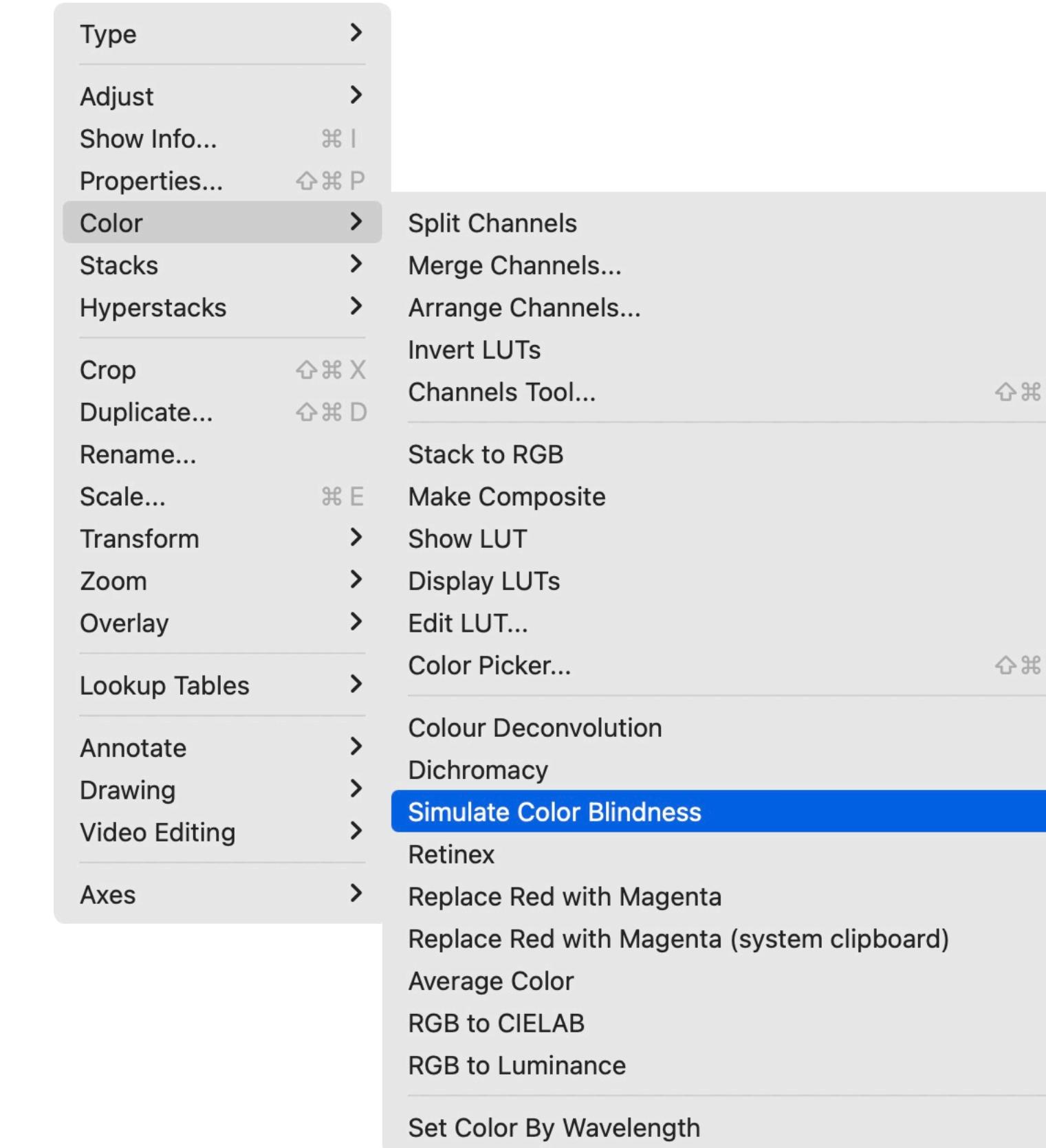
- 1 Open the actual colorblind test image, "Color_Deficiency_Ishihara_Test.jpg"



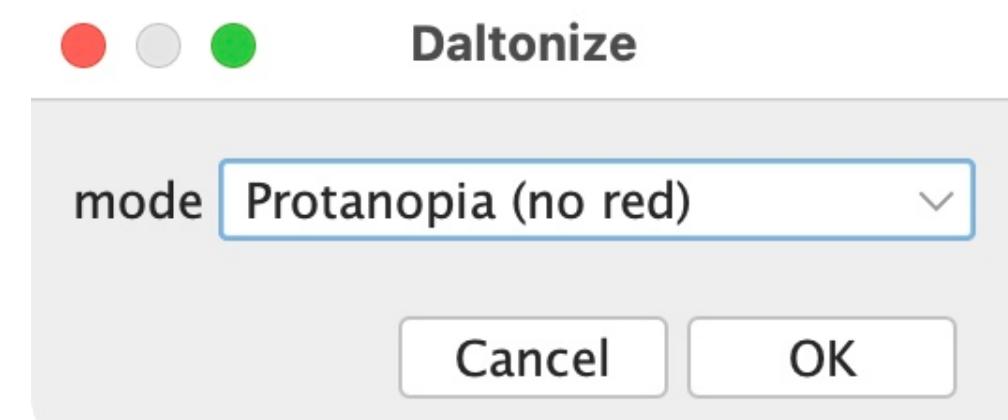
What number do you see? Are you sure you're not colorblind?

- 2 Select the Simulate Color Blindness submenu item

IMAGE



- 3



try the options

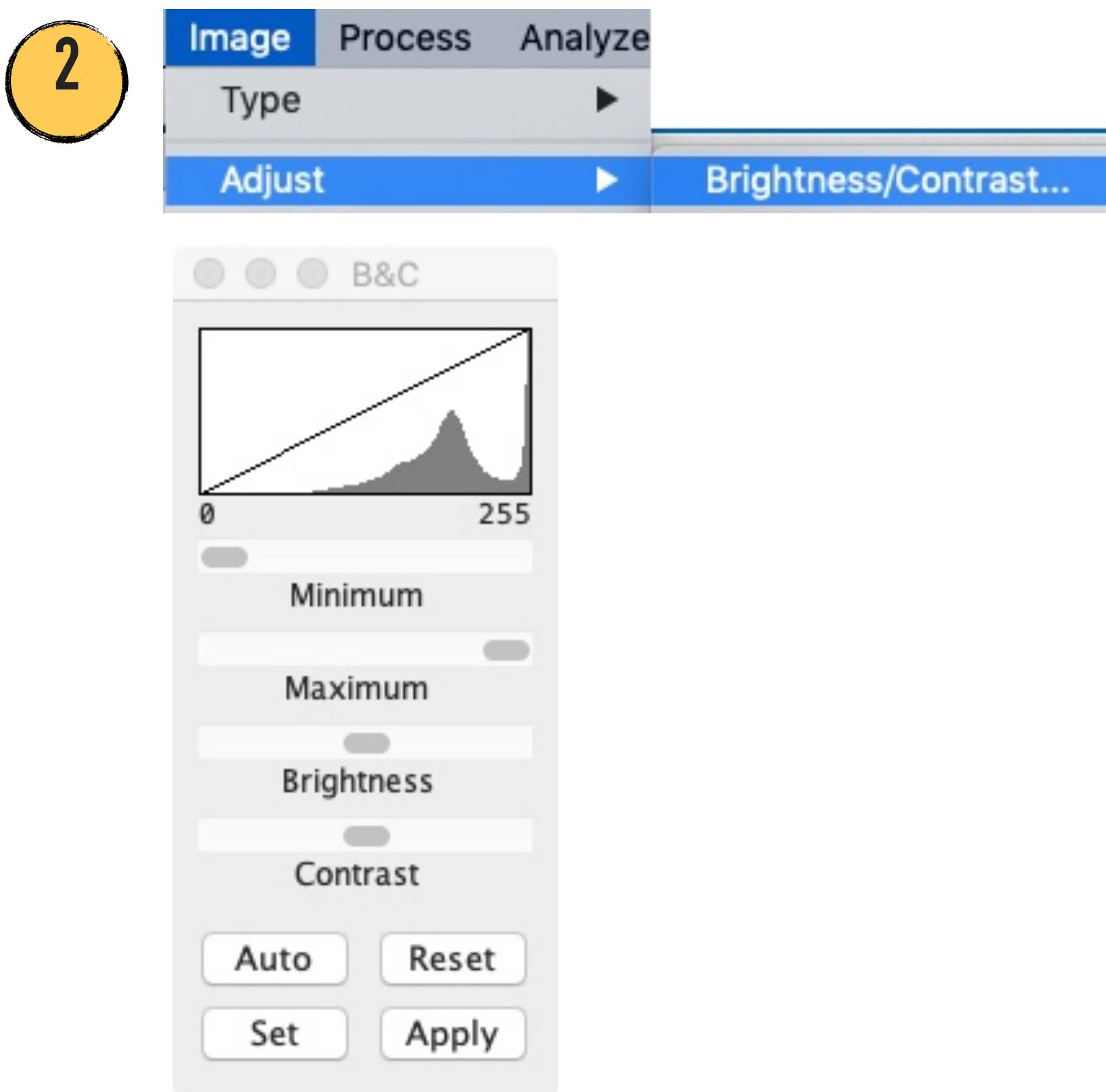
IMAGE PROCESSING

COLOR ENHANCEMENT

COLOR ENHANCEMENT

BRIGHTNESS, CONTRAST, COLOR BALANCE

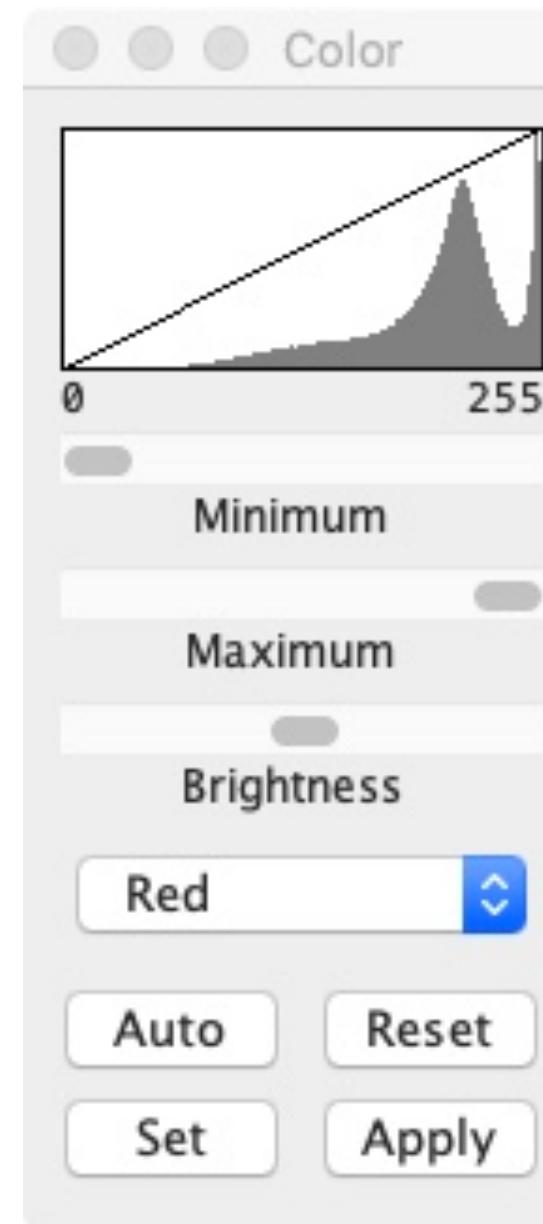
- 1 Select or reopen RGB image



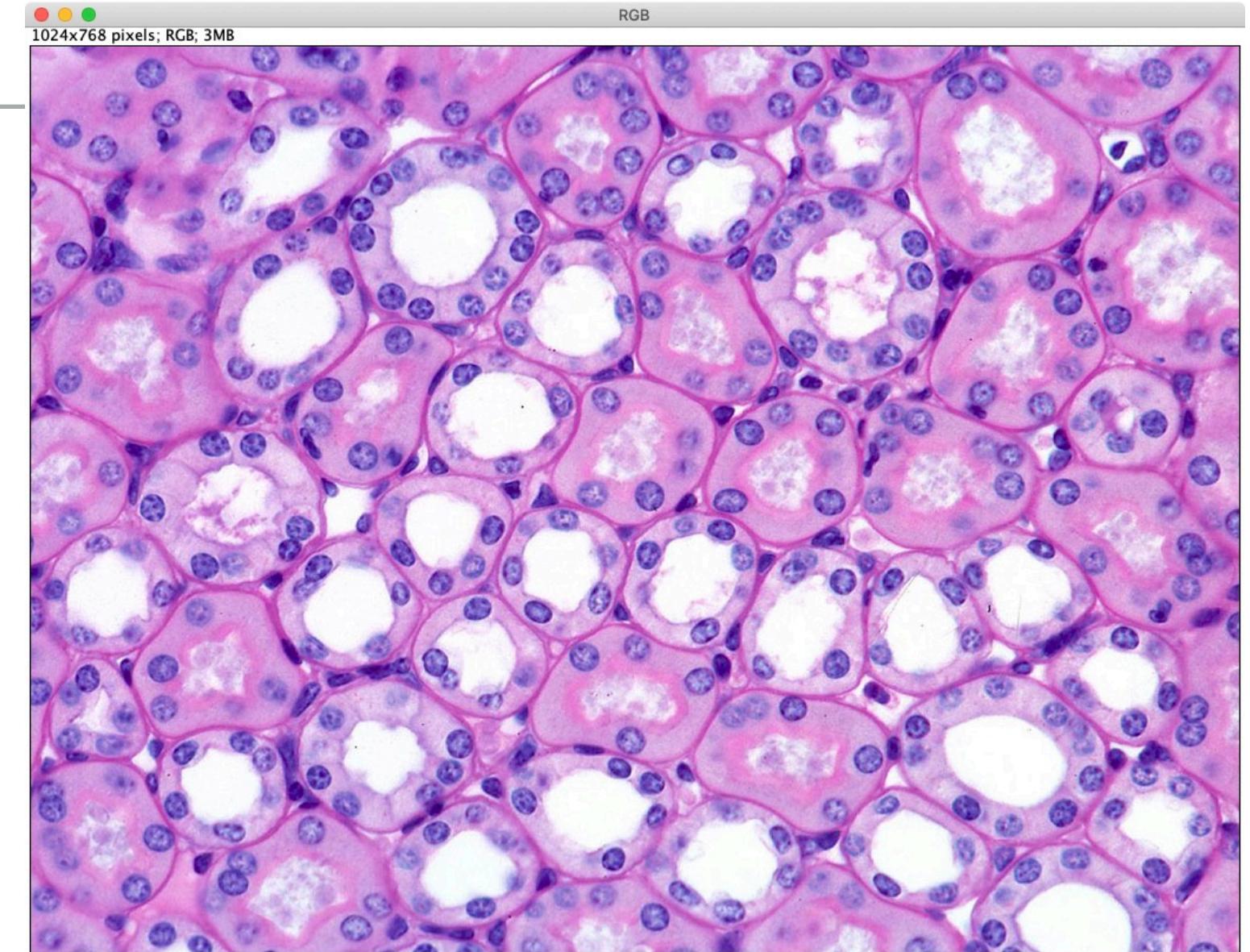
Adjusts all channels
equally in an RGB
image

- 2
- 3

Image : Adjust : Color Balance



Can adjust
Channels
separately

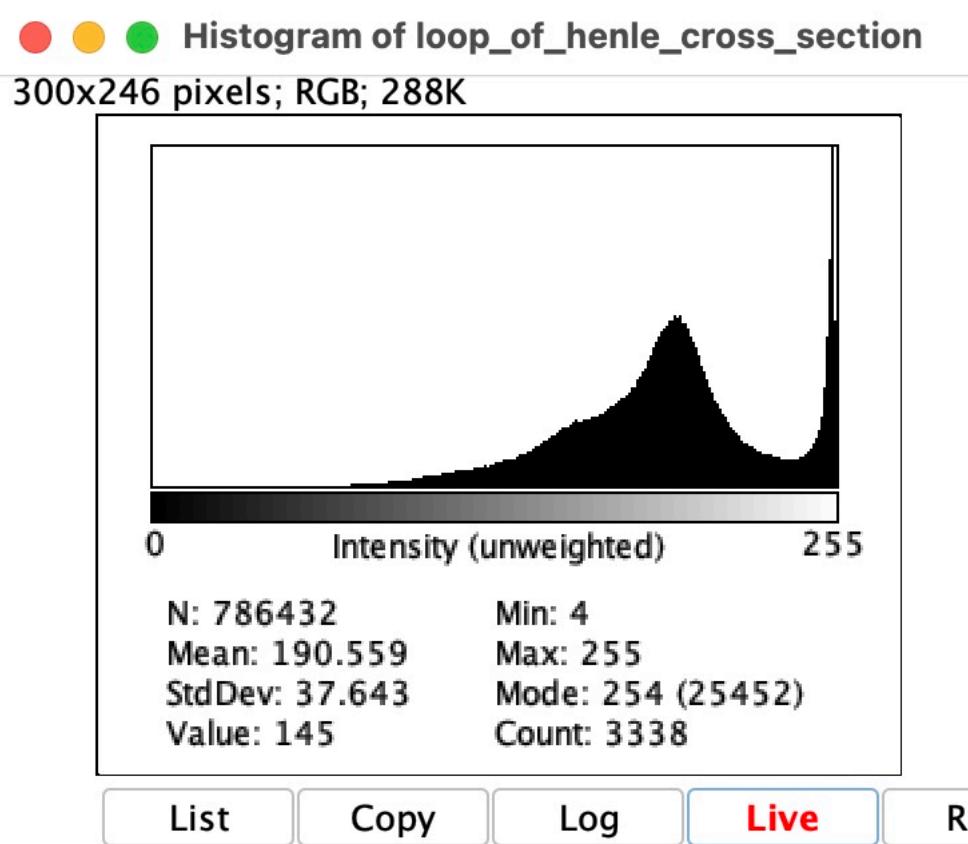


COLOR ENHANCEMENT

GAMMA

1 Select RGB image

2 Live Histogram



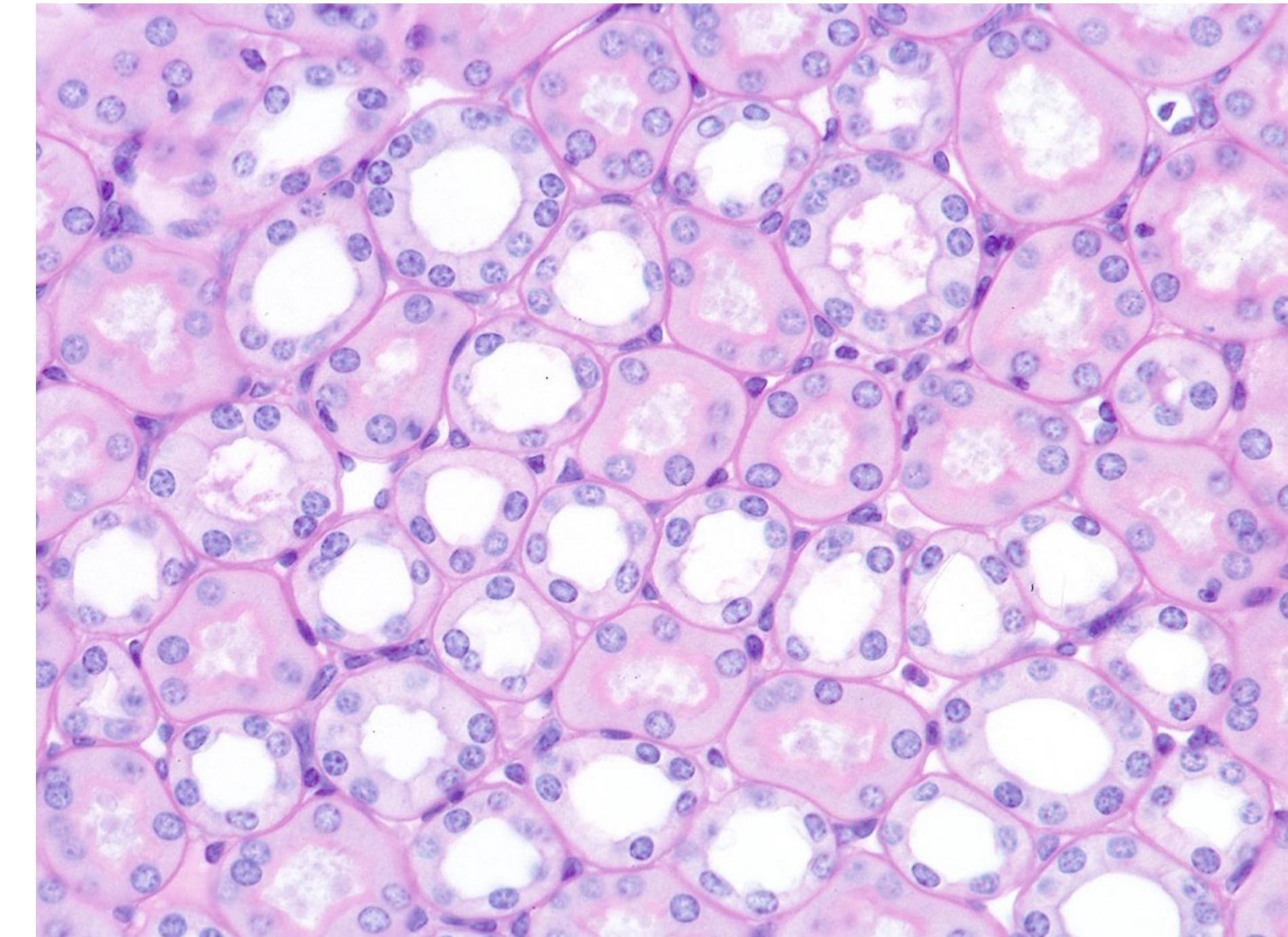
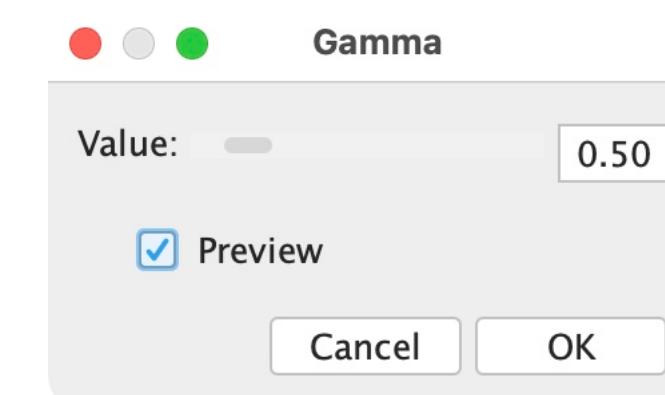
3

PROCESS

- Smooth
 - Sharpen
 - Find Edges
 - Find Maxima...
 - Enhance Contrast...
 - Noise >
 - Shadows >
 - Binary >
 - Math >**
 - FFT >
 - Filters >
 - Batch >
 - Image Calculator...
 - Subtract Background...
 - Repeat Command ⌘ R
 - Calculator Plus
 - Morphology >
 - Image Expression Parser
 - Image Expression Parser (Macro)
 - Multiple Image Processor
 - Enhance Local Contrast (CLAHE)
- Add...
 - Subtract...
 - Multiply...
 - Divide...
 - AND...
 - OR...
 - XOR...
 - Min...
 - Max...
 - Gamma...**
 - Set...
 - Log
 - Exp
 - Square
 - Square Root
 - Reciprocal
 - NaN Background
 - Abs
 - Macro...

4

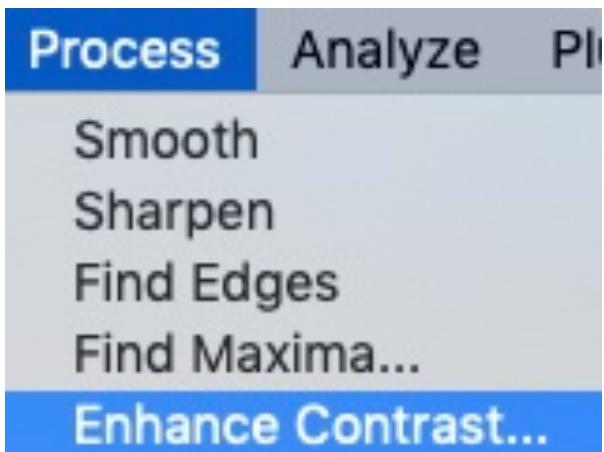
Check Preview, adjust value, keep eye on histogram



COLOR ENHANCEMENT

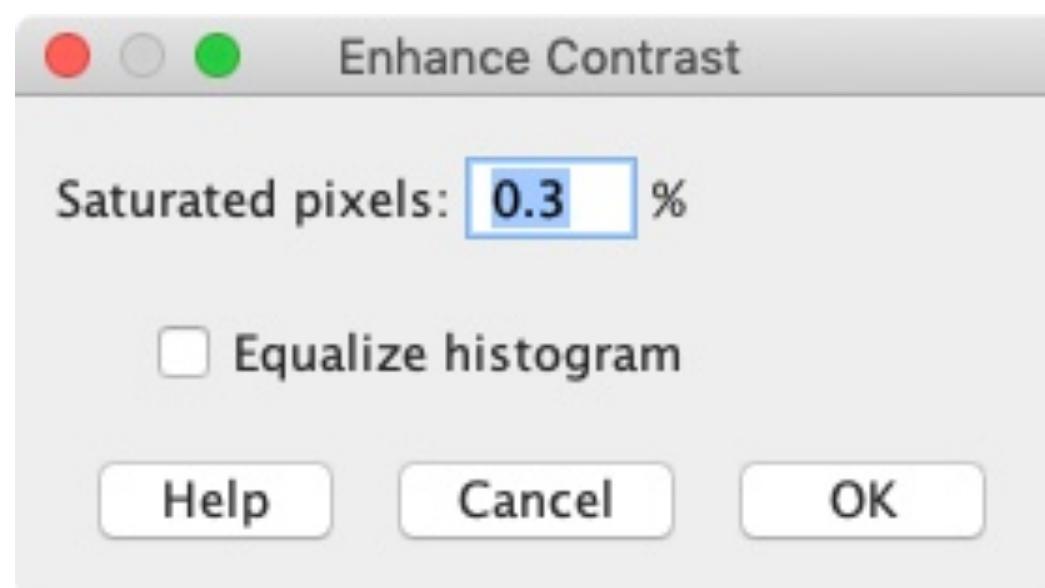
FILTERING

1



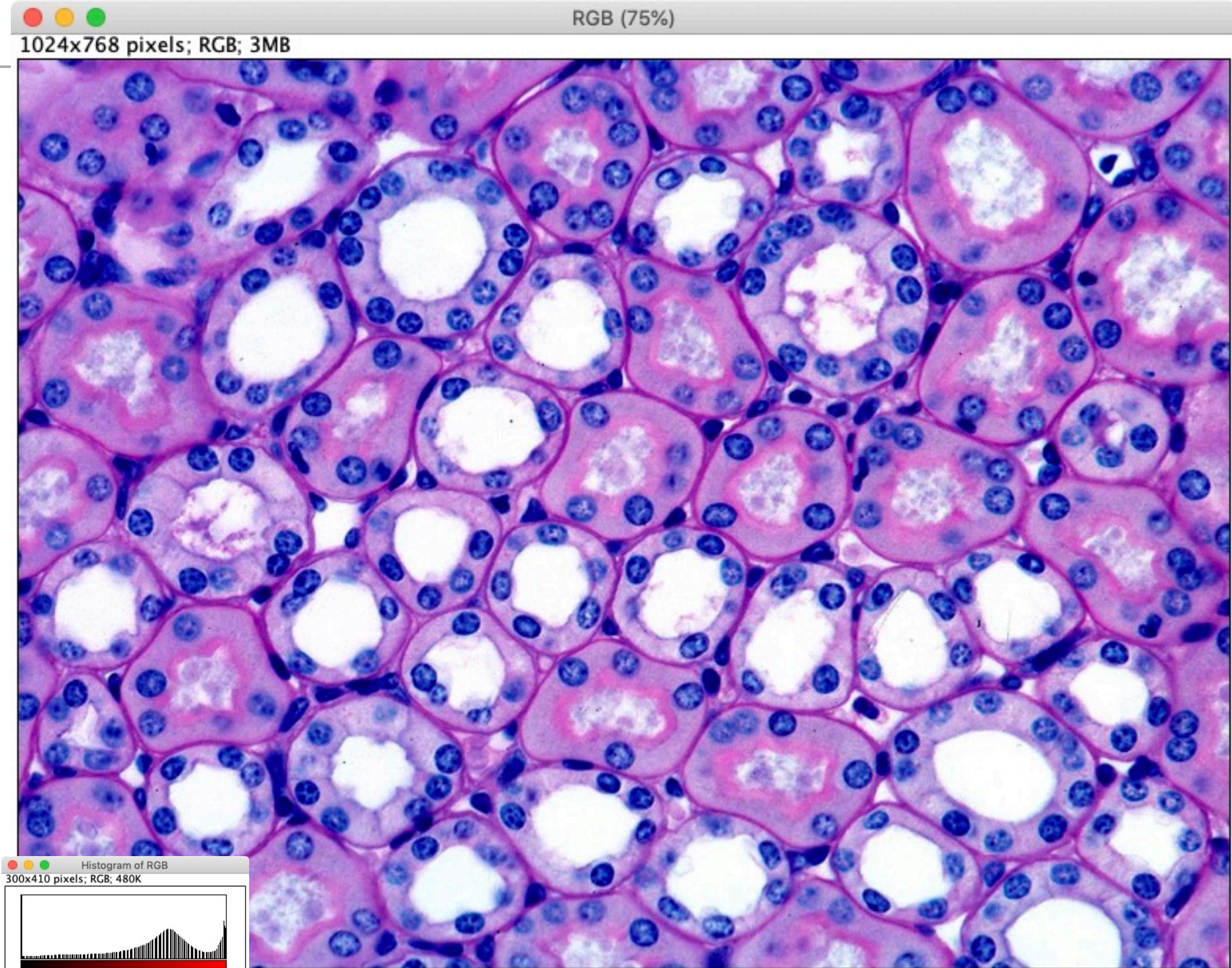
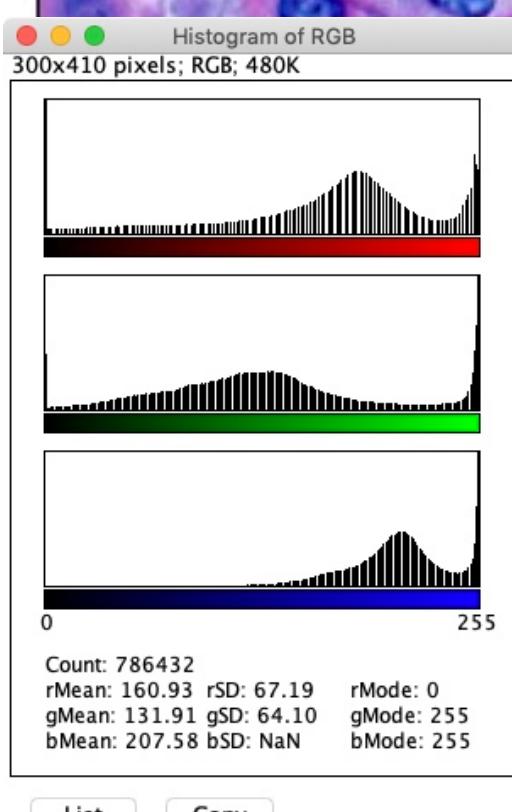
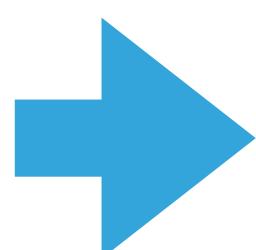
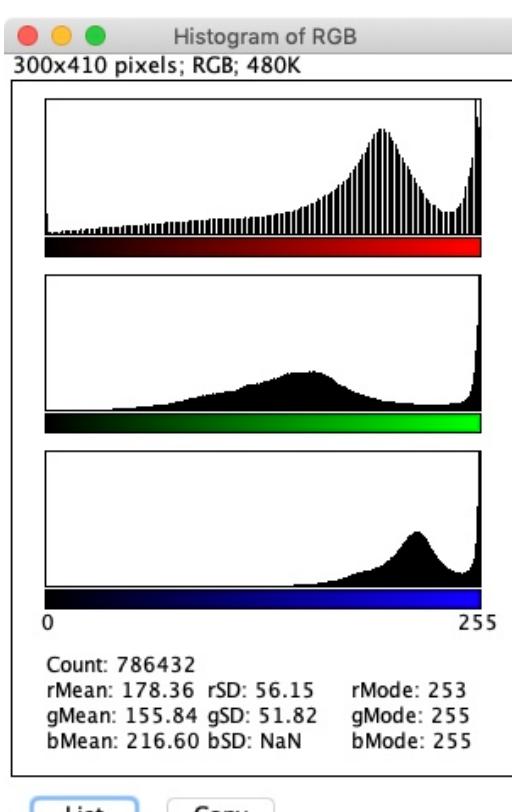
2

imadjust equivalent



3

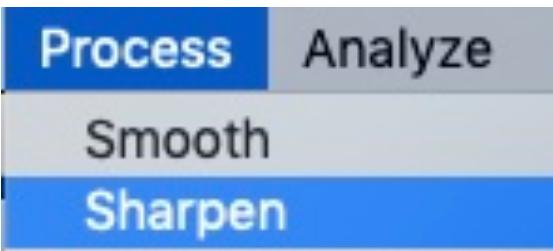
Histograms saturated



COLOR ENHANCEMENT

FILTERING

1

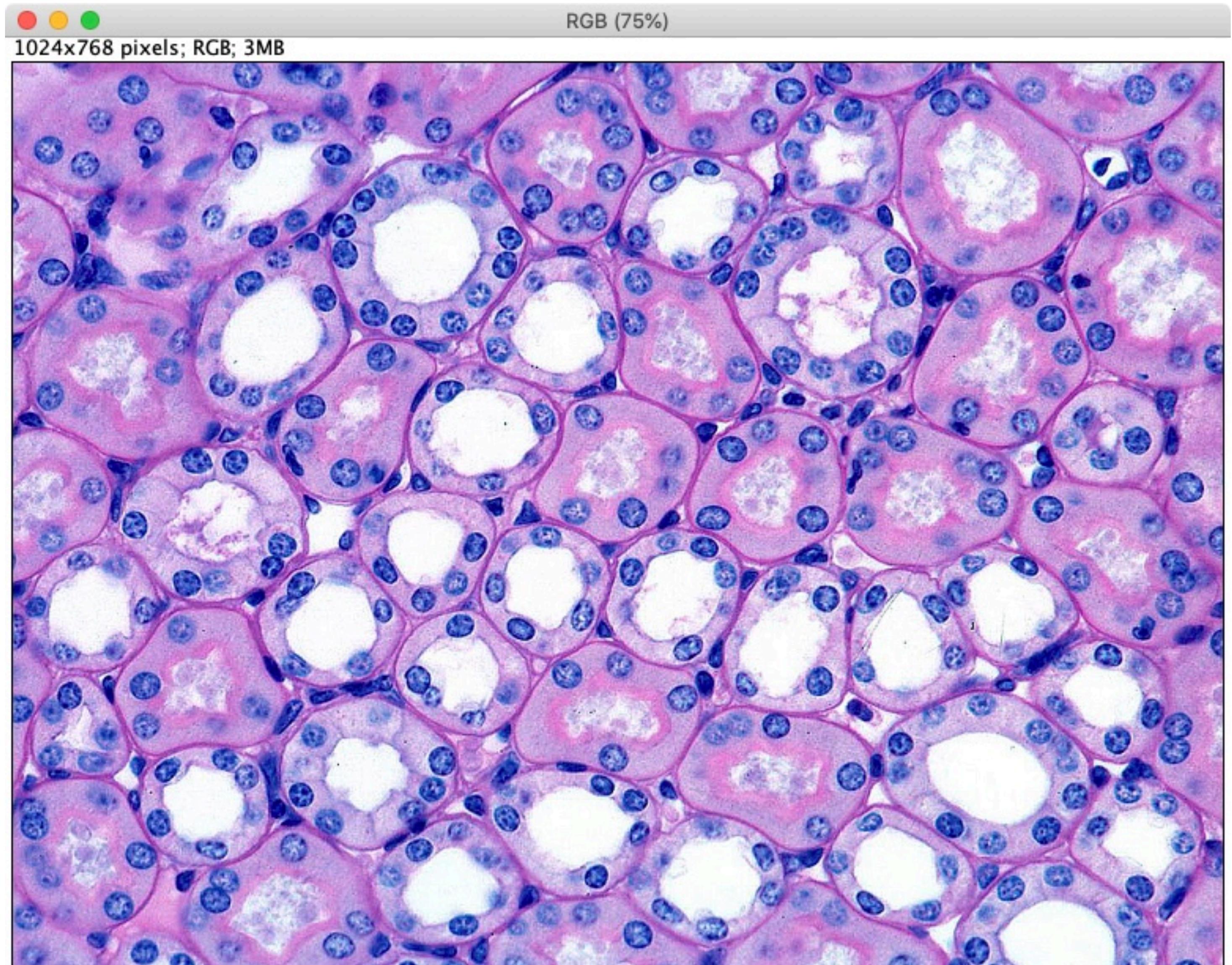


2

Image Sharpened

3

Undo to compare



COLOR ENHANCEMENT

FILTERS

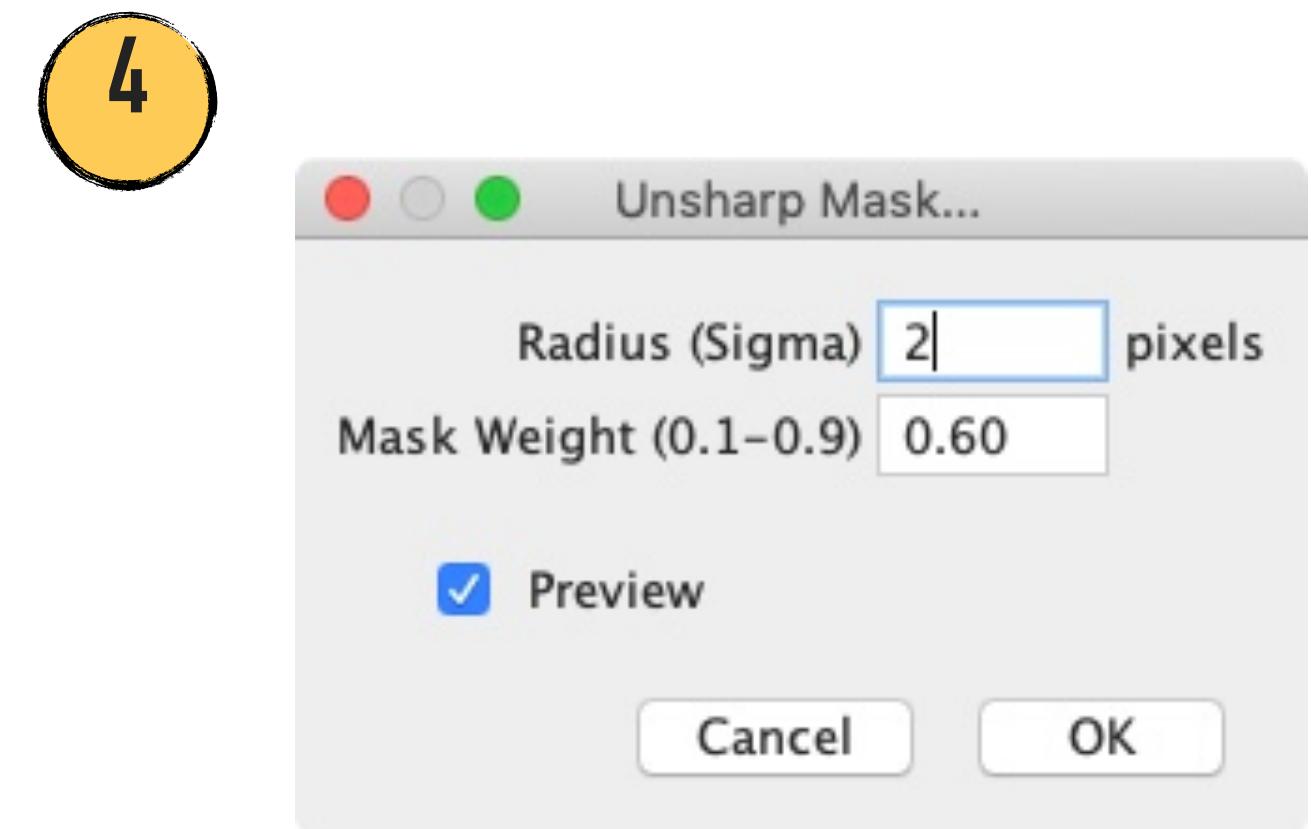
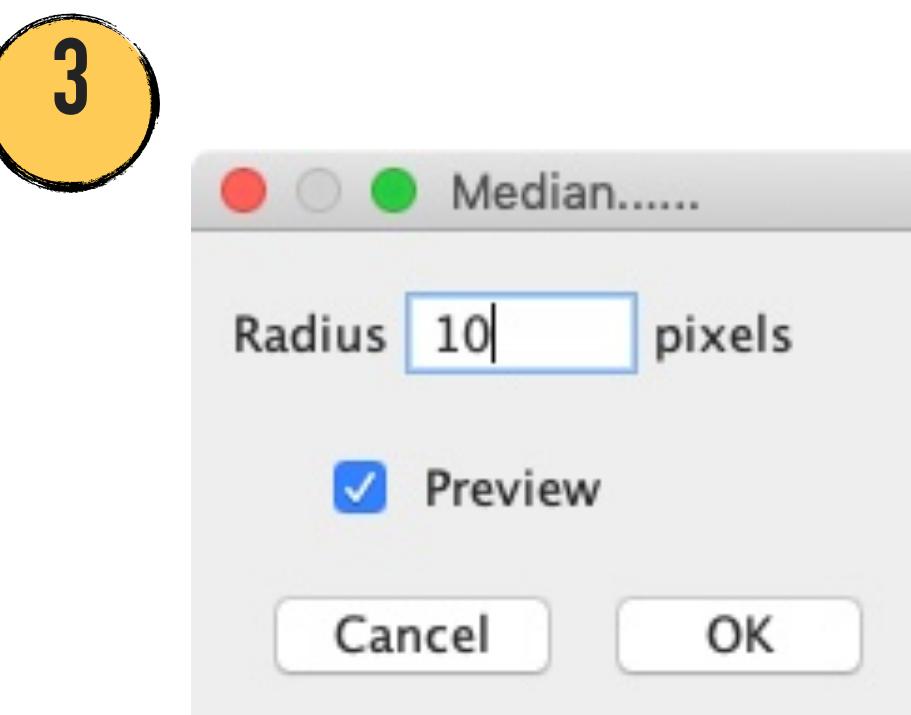
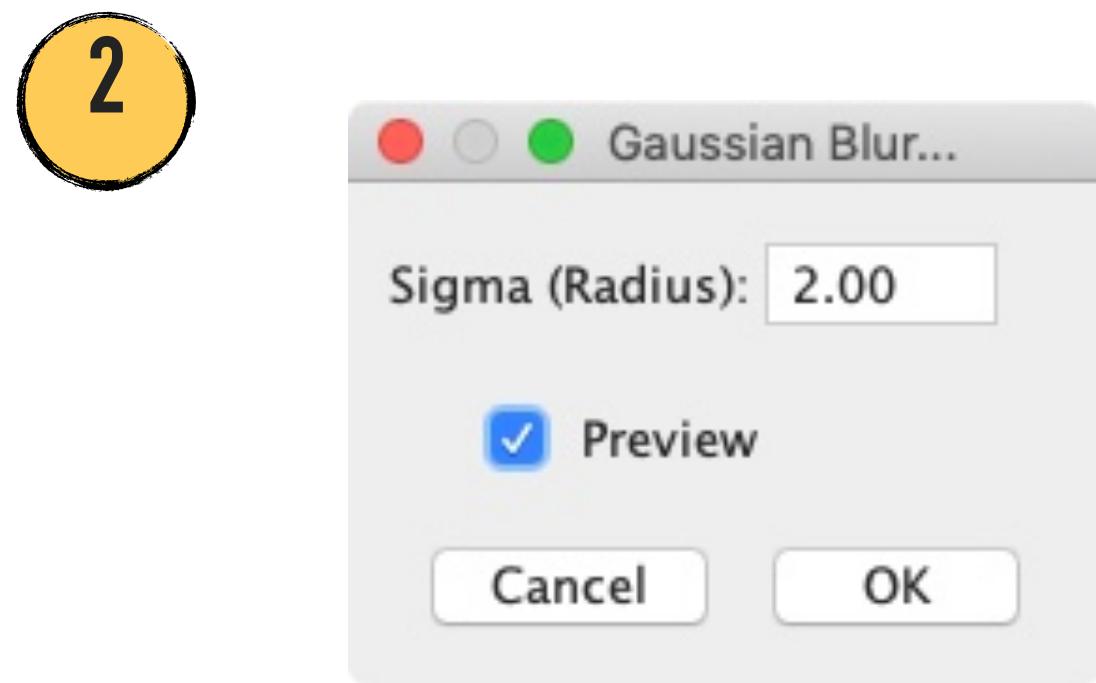
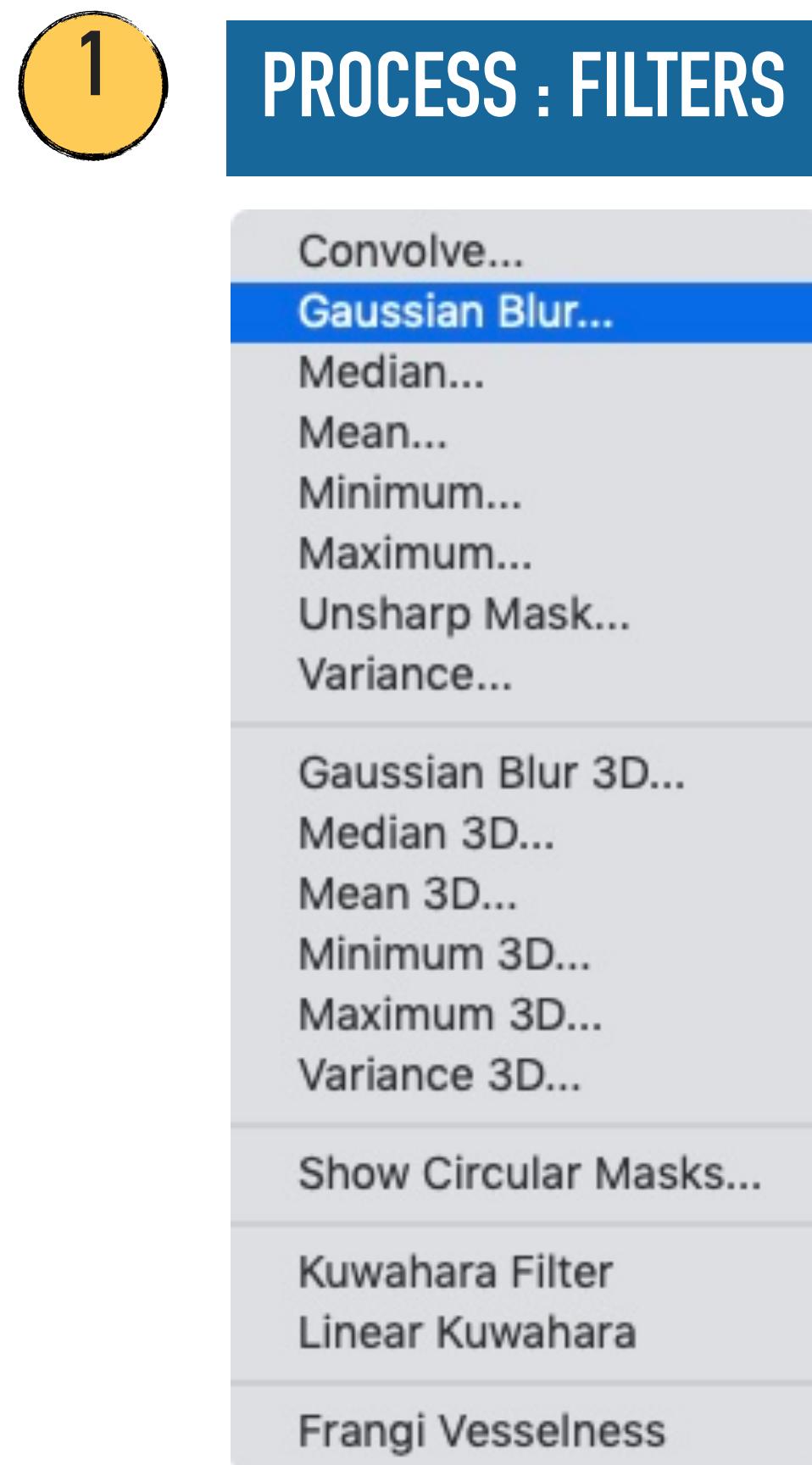


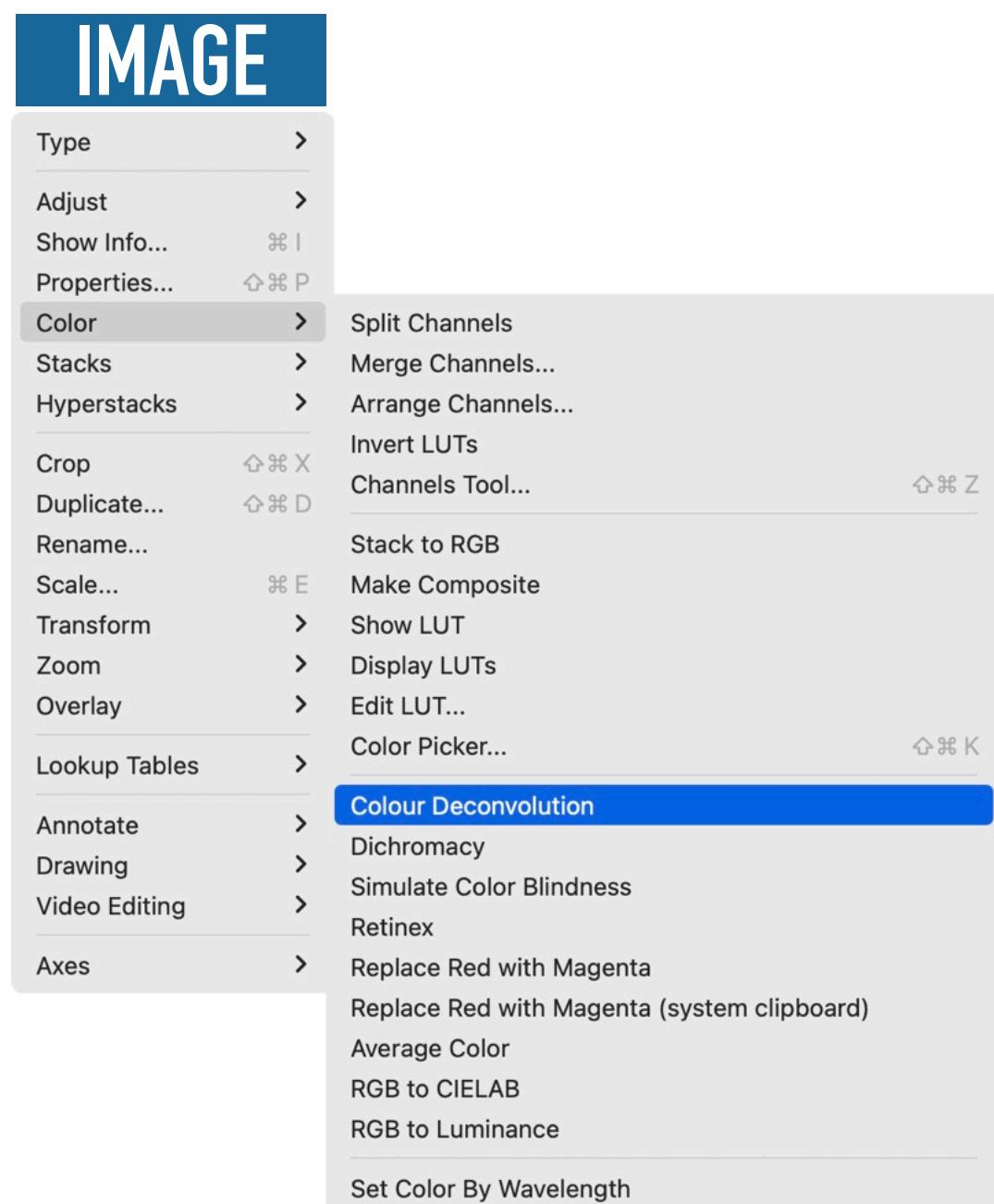
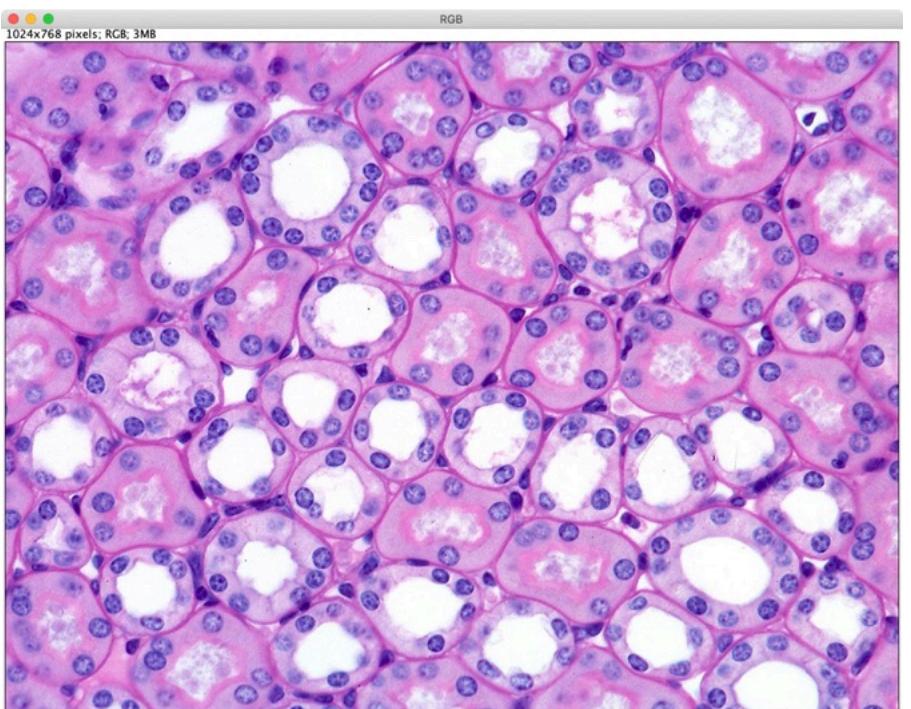
IMAGE PROCESSING

COLOR SEGMENTATION

COLOR SEGMENTATION

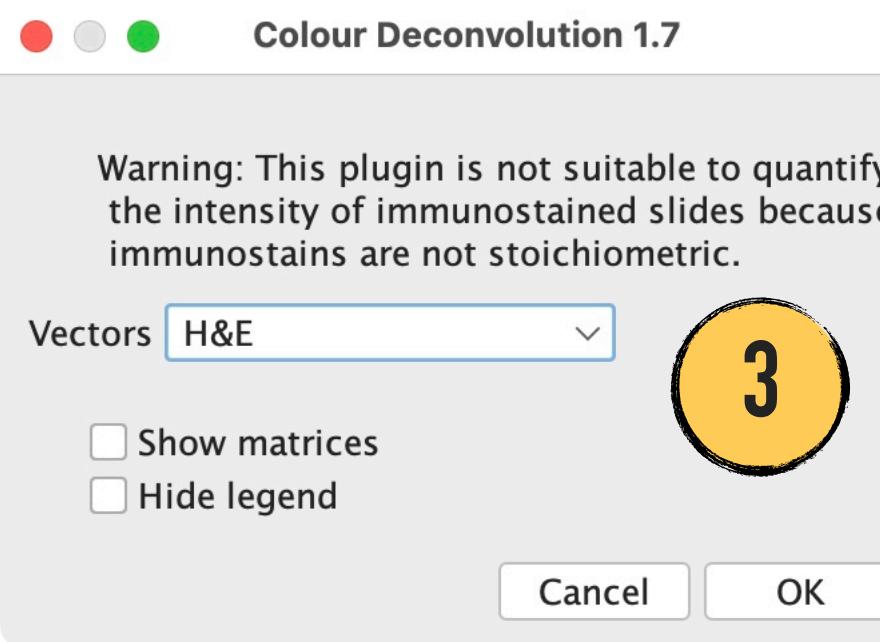
COLOR DECONVOLUTION

- 1 Select or reopen RGB image

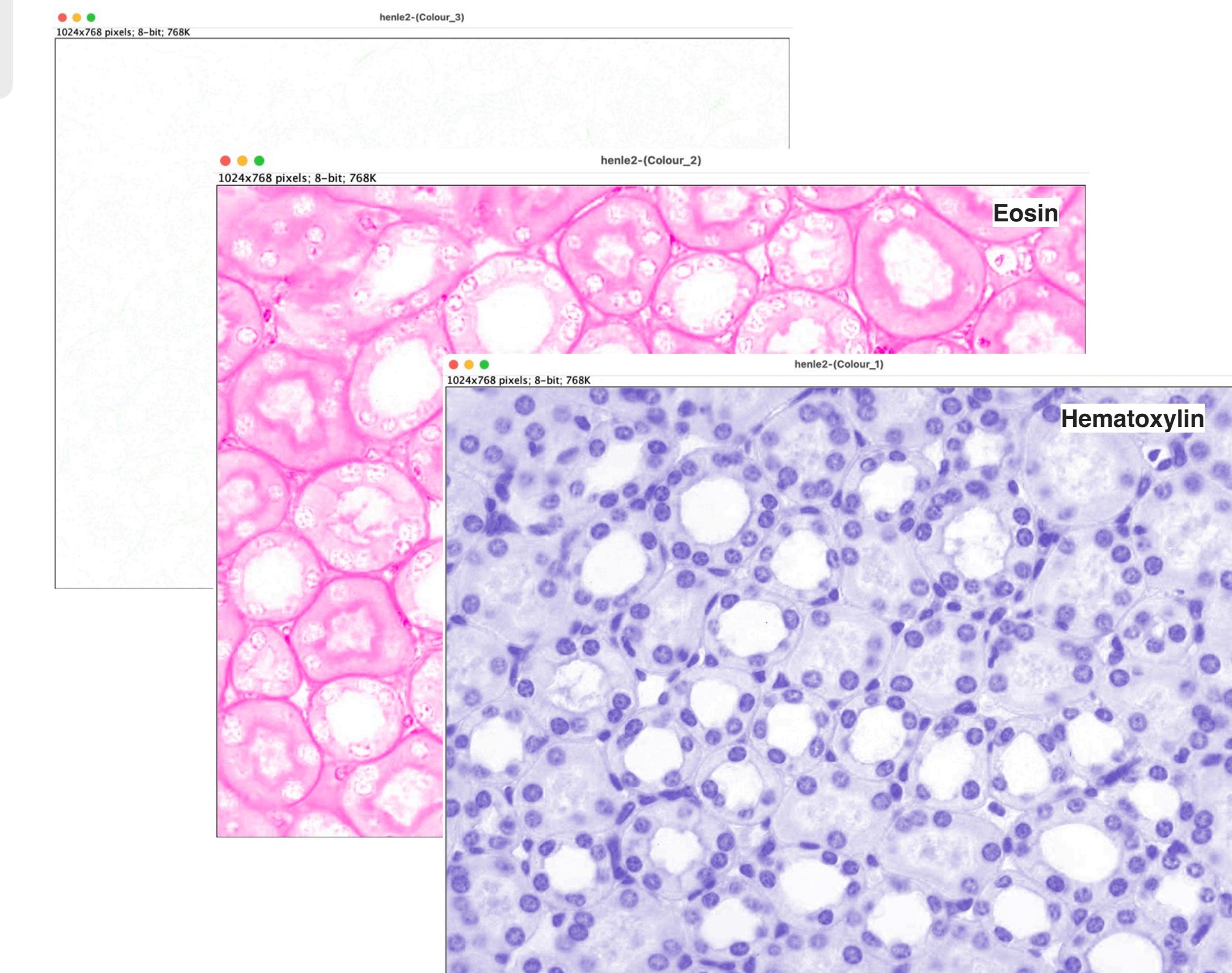


- 2 Color Deconvolution

Separates colors based on algorithm. Designed for color stained images



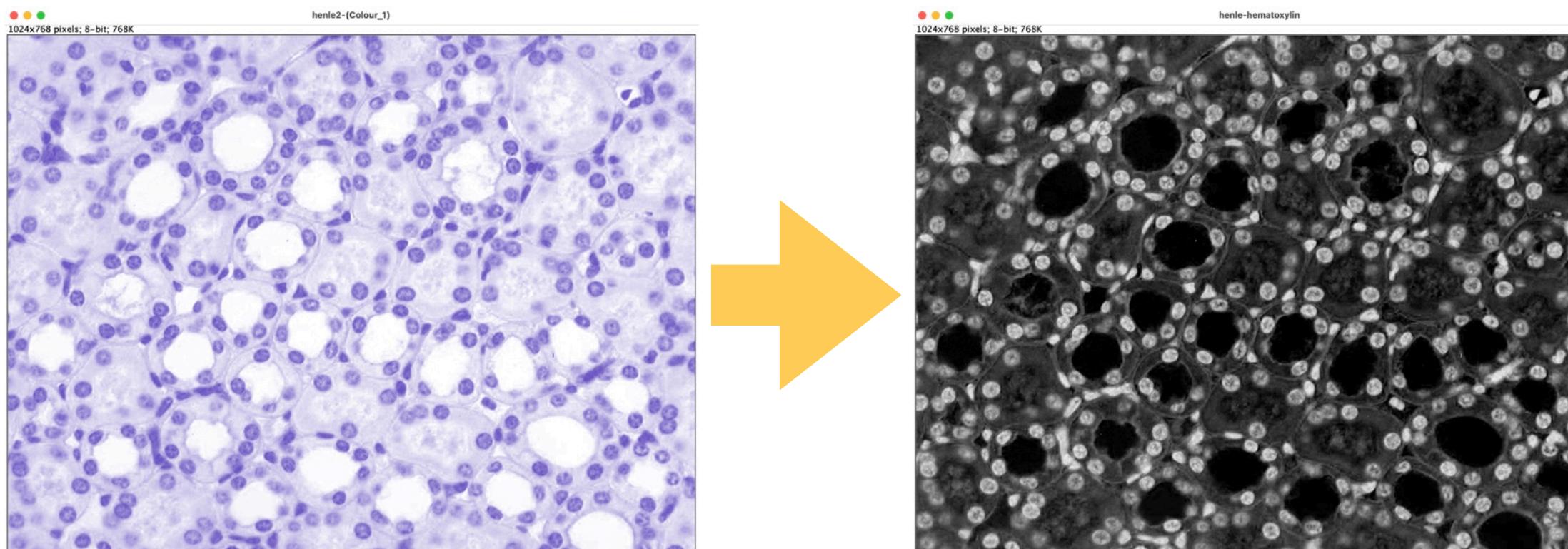
You get 3 new images of the separated stains



COLOR SEGMENTATION

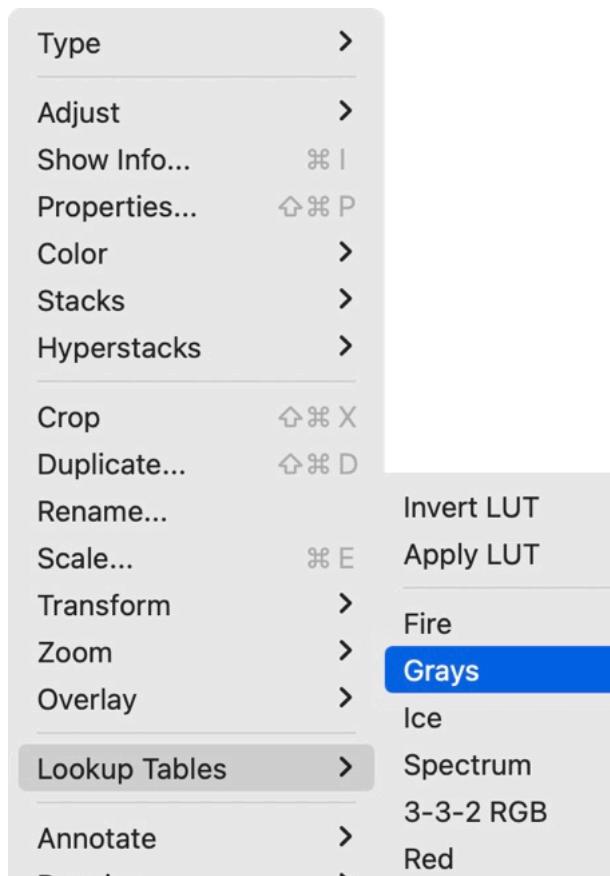
PREPROCESS IMAGE

1 Select hematoxylin channel



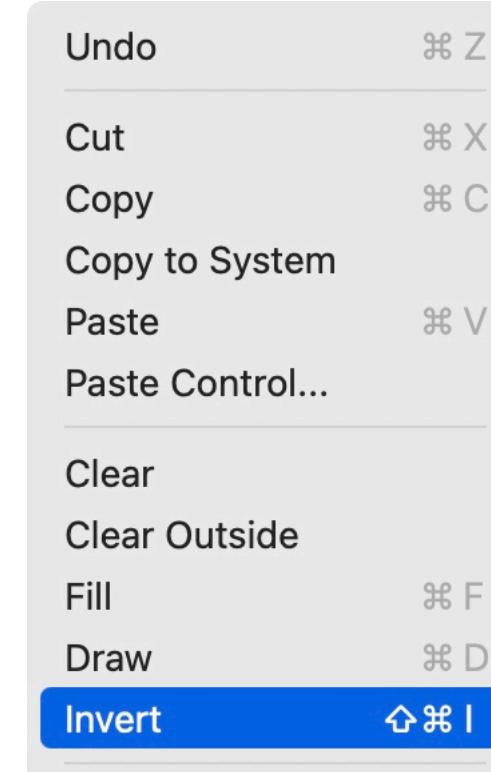
2 Change LUT to grays

IMAGE



3 Get complement -
Edit: Invert

EDIT

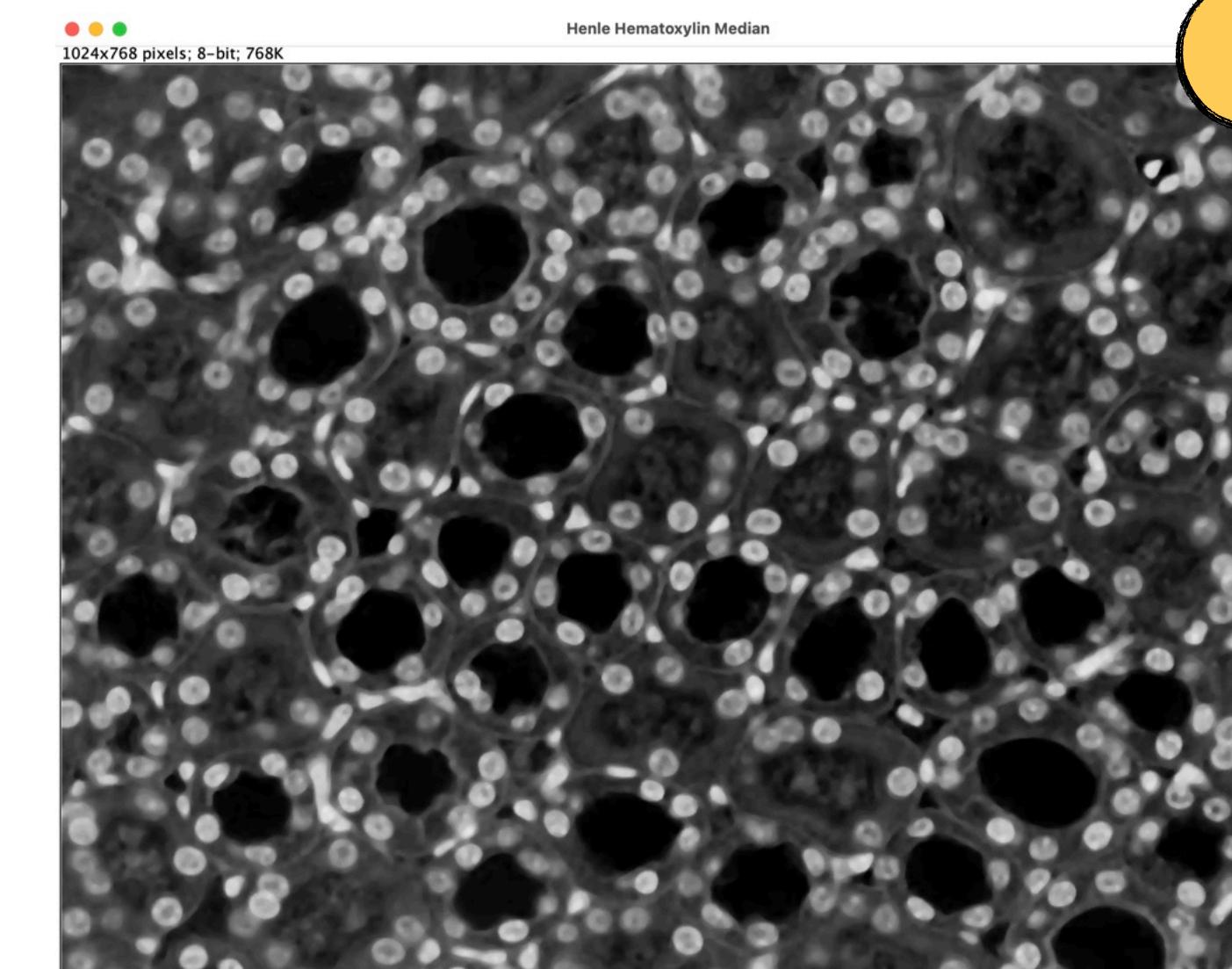
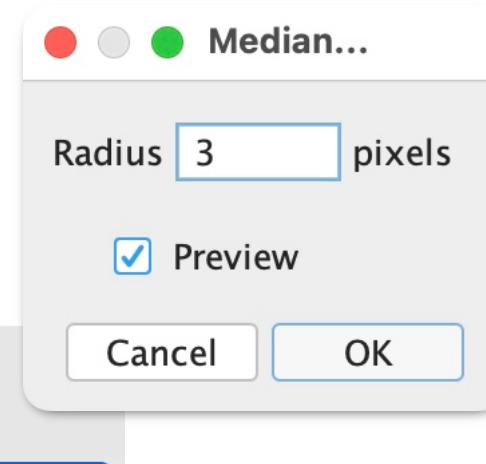


EDIT

Smooth
Sharpen
Find Edges
Find Maxima...
Enhance Contrast...
Noise
Shadows
Binary
Math
FFT
Filters
Batch
Image Calculator...
Subtract Background...
Repeat Command

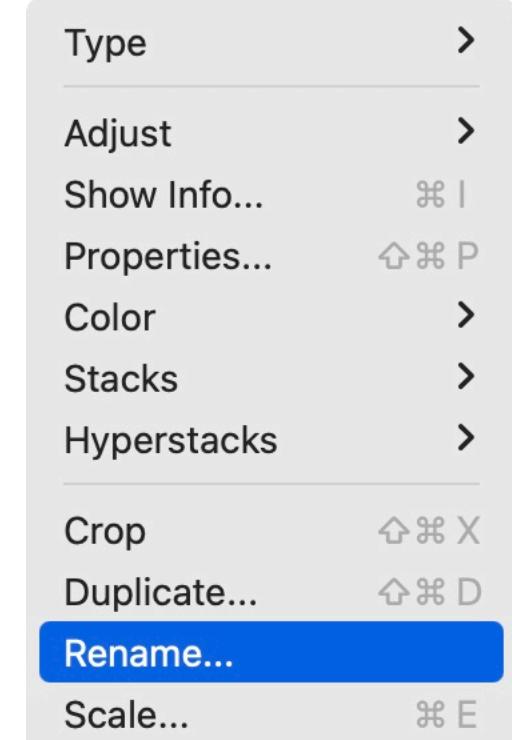
4

Filter Image:
Median Filter



5 Rename Image

IMAGE

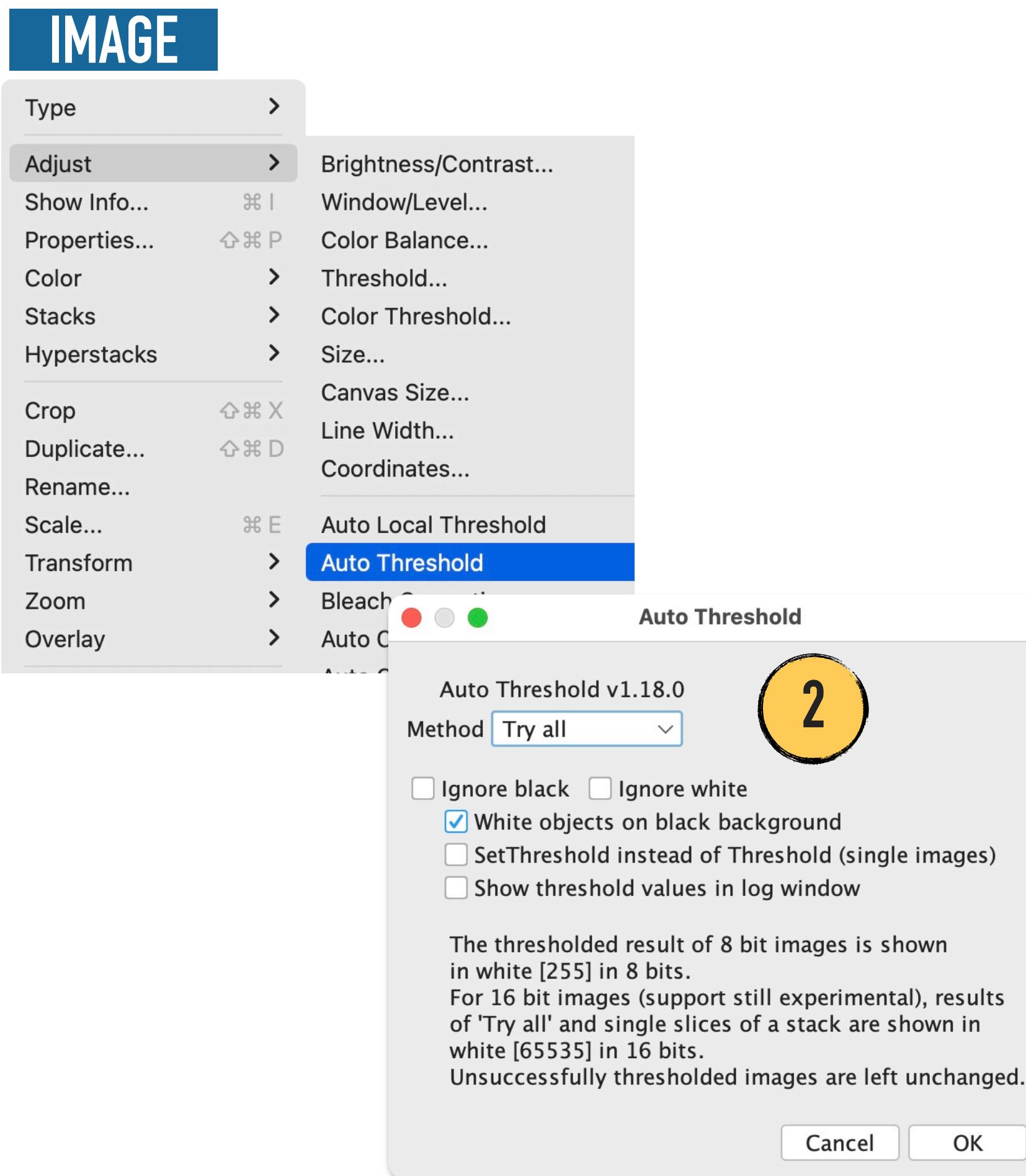


Henle Hematoxylin Median

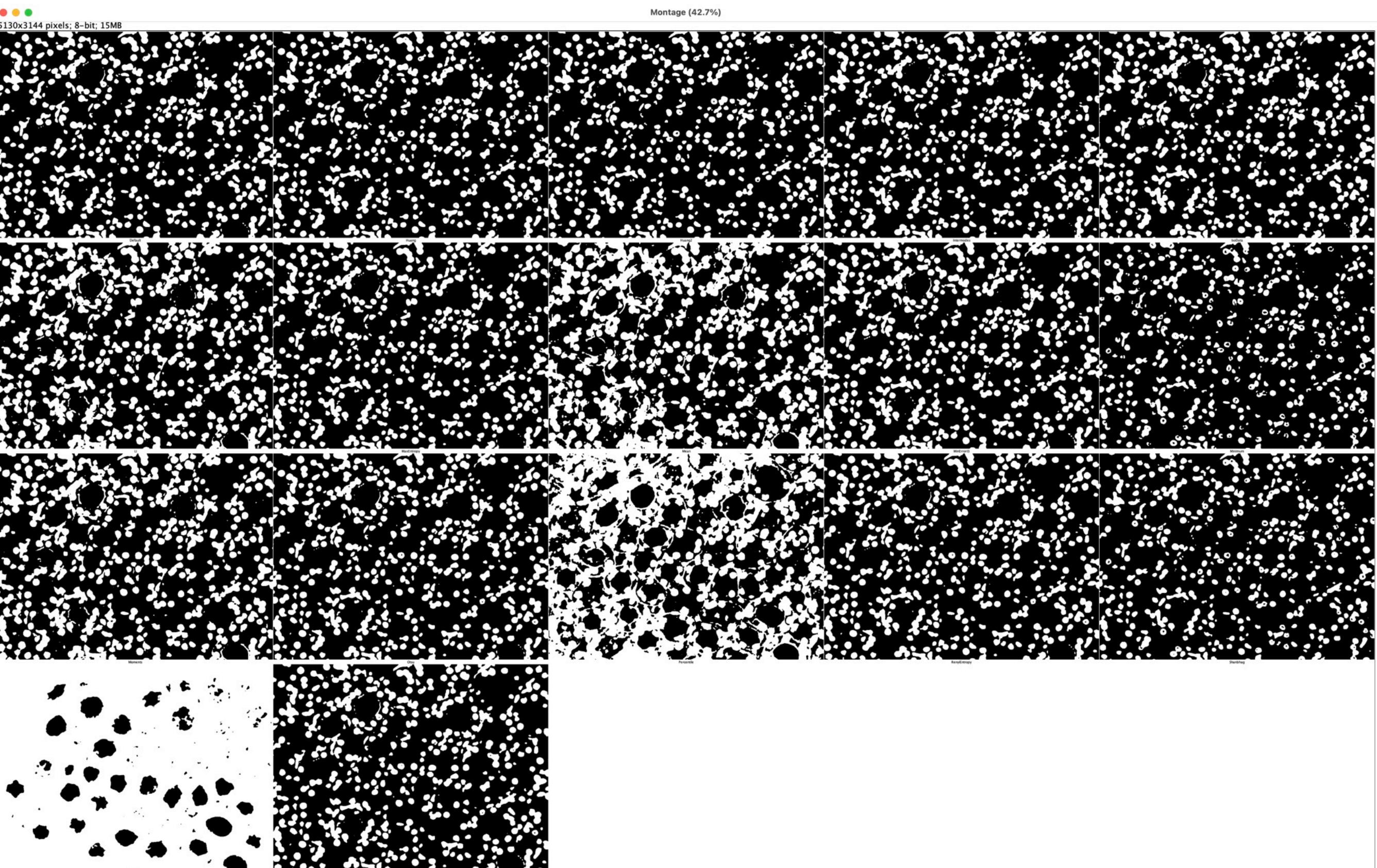
COLOR SEGMENTATION

THRESHOLD IMAGE

1 Threshold Henle Hematoxylin
Median image



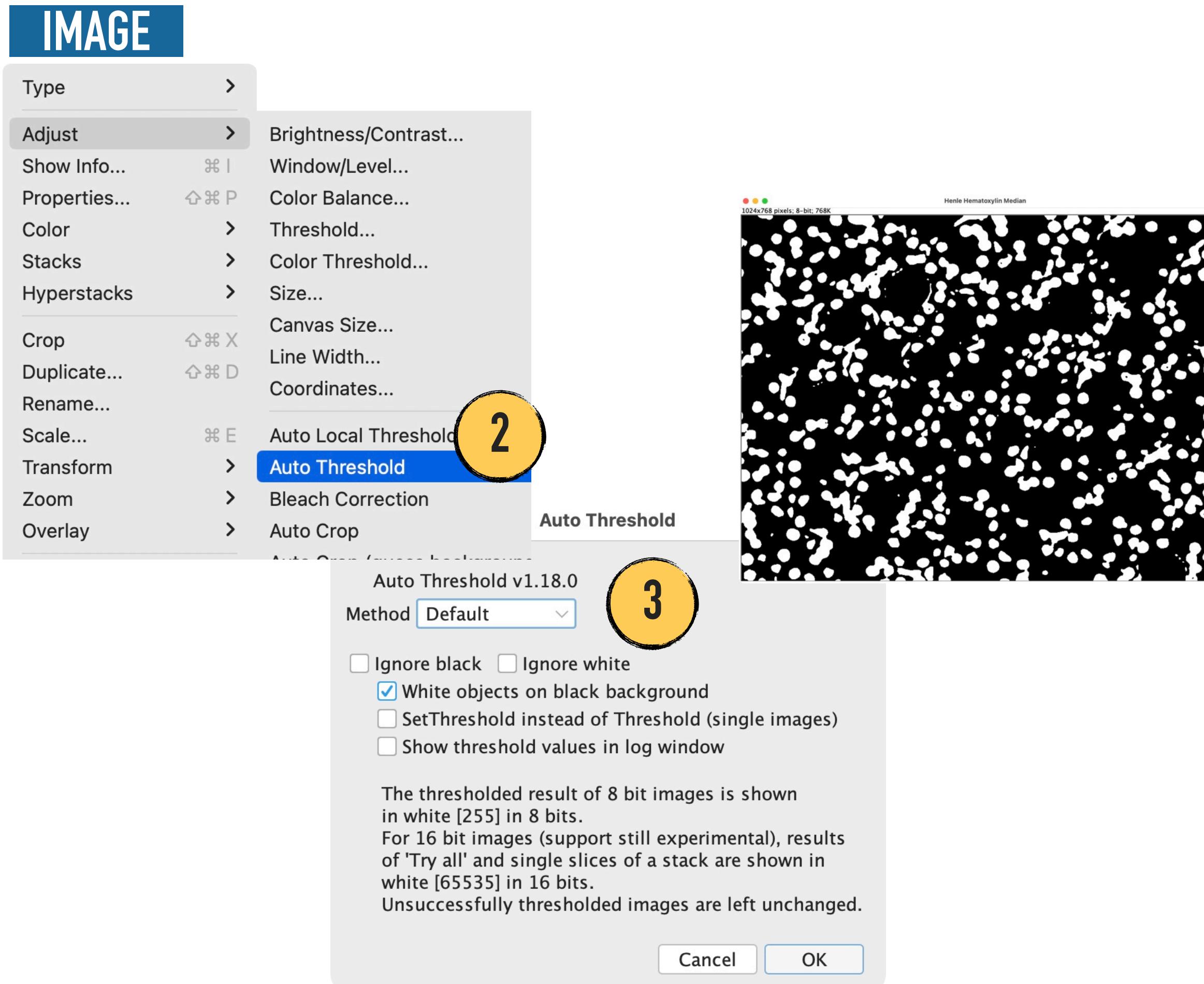
2 Threshold result - lots of different methods



COLOR SEGMENTATION

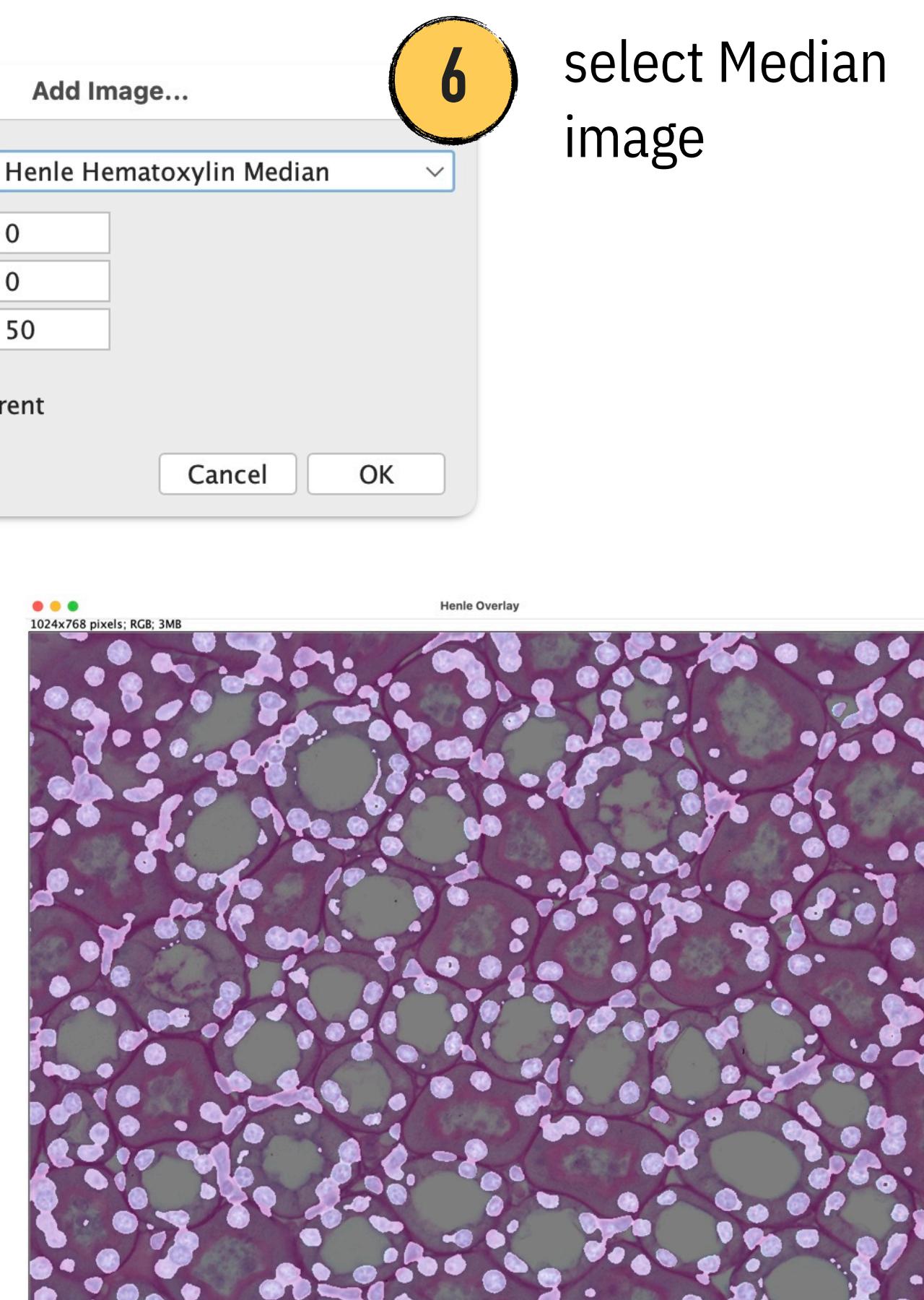
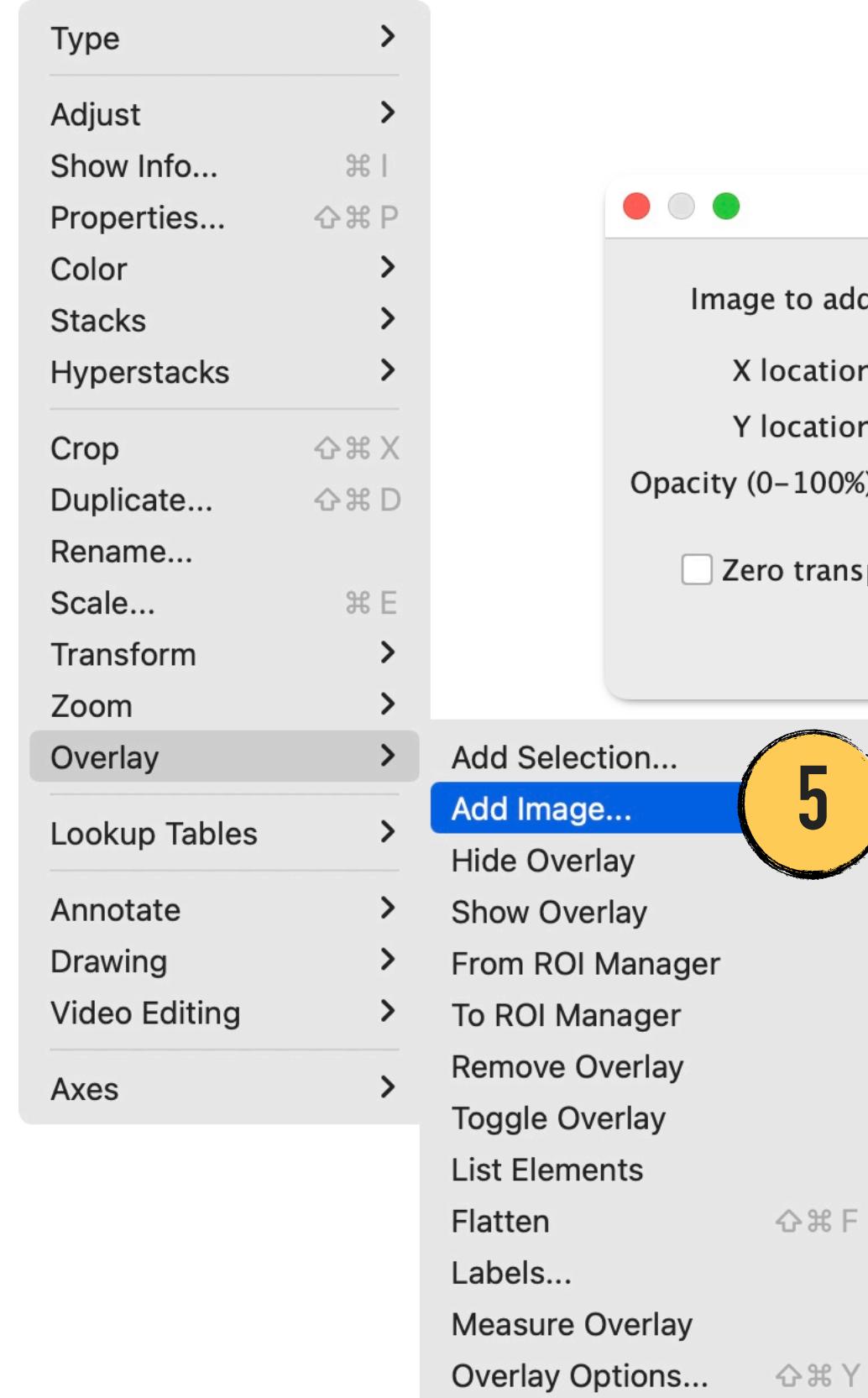
THRESHOLD IMAGE - DEFAULT METHOD

1 Select Median Image



4 Overlay. Select original image.
Duplicate. Name as Henle Overlay.

IMAGE



5 select Median image

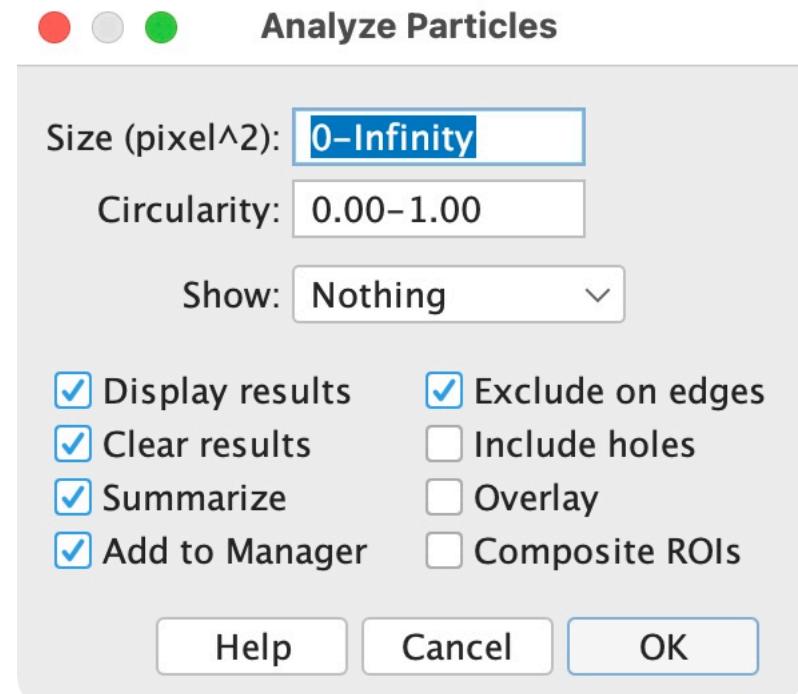
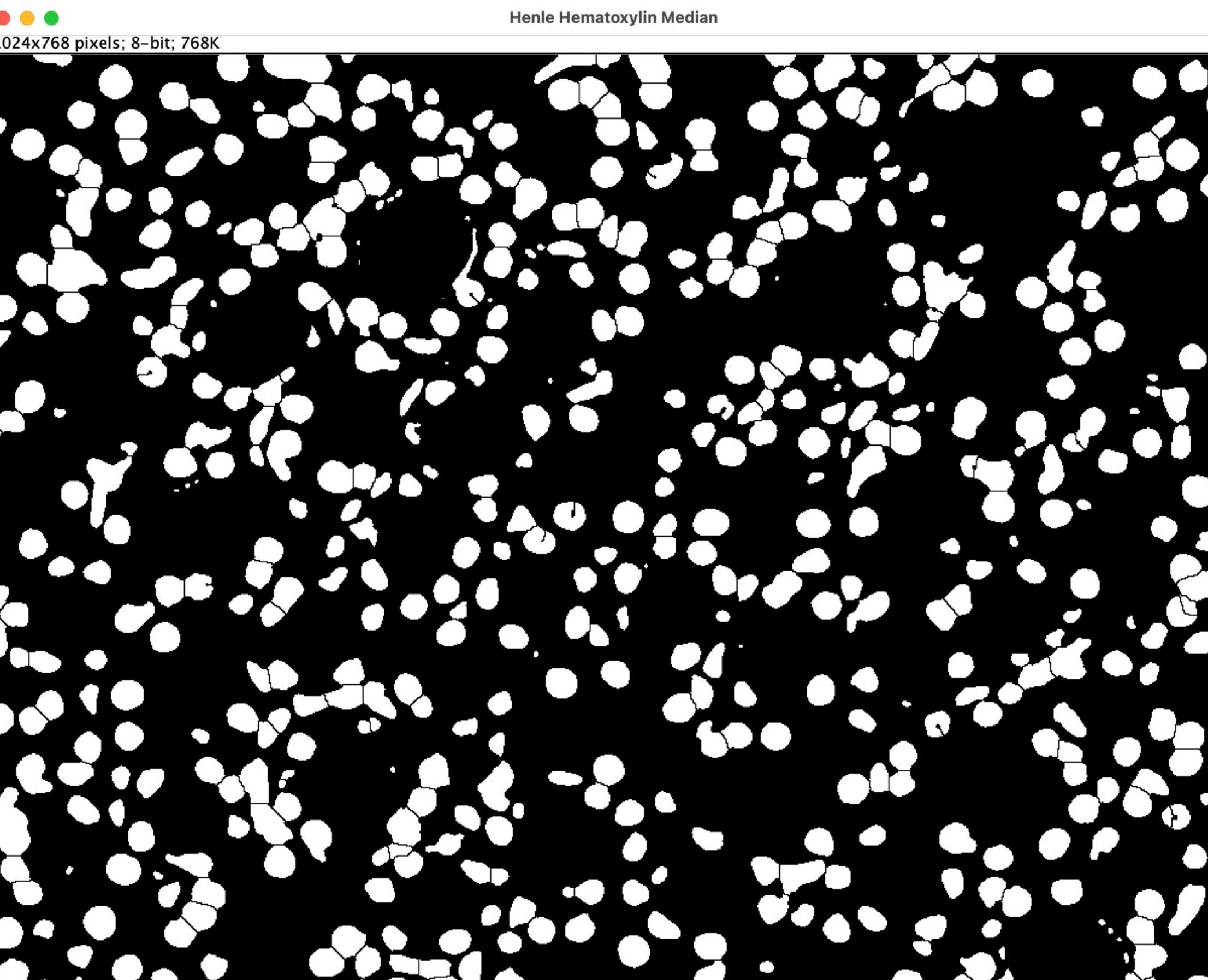
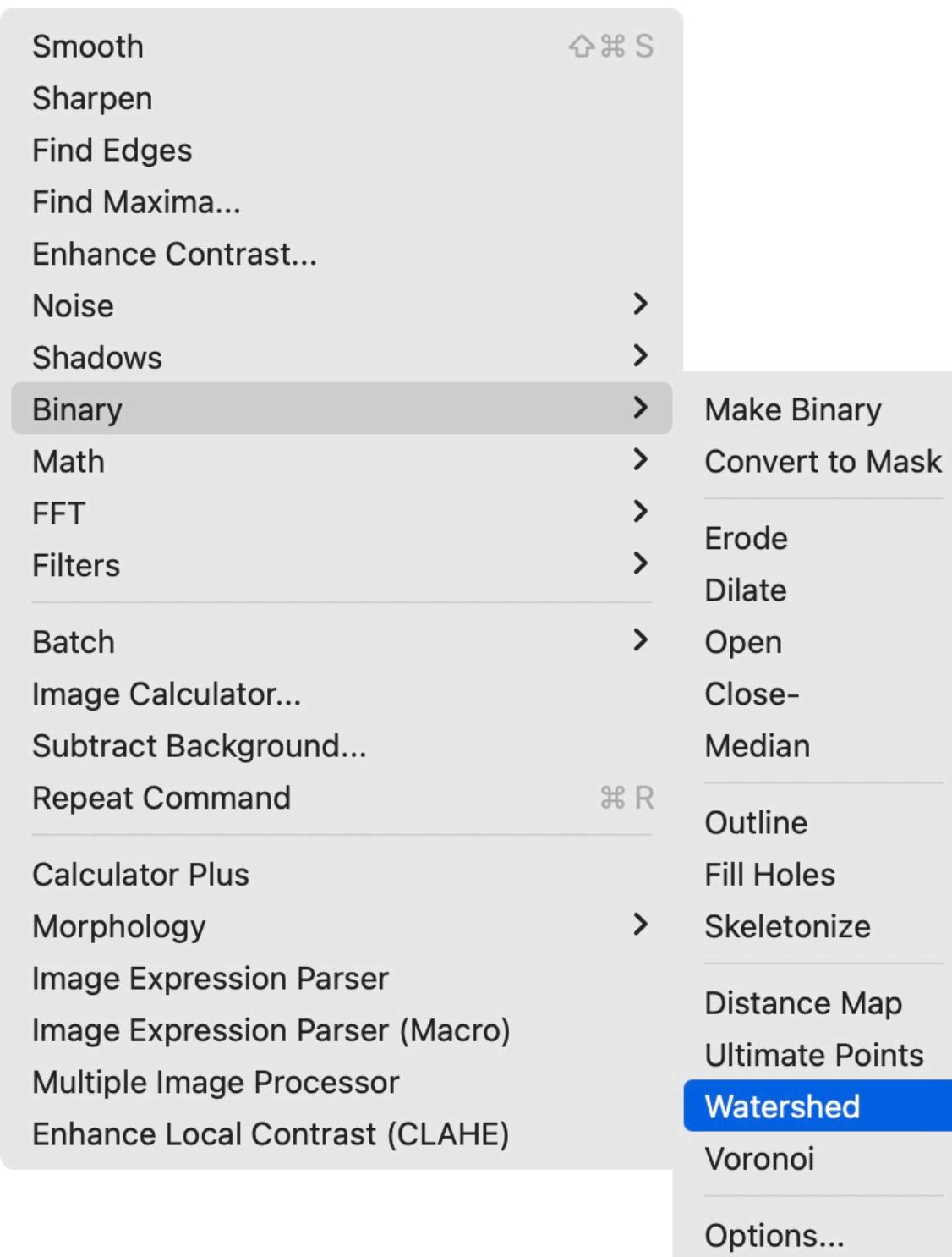
COLOR SEGMENTATION

WATERSHED TRANSFORMATION

1

Select Median Image

PROCESS



2

Remove noise:
Process:Binary:Open

COLOR SEGMENTATION REGION PROPERTIES

1

Select Median Image

ANALYZE

