Illustration reprinted from Pete Bankhead.

Introduction to Bioimage Analysis using QuPath

Antoine A. Ruzette & Simon F. Nørrelykke

Image Analysis Collaboratory

Get the course materials

https://hms-iac.github.io/qupath-workshop

One-stop resource for everything we'll cover today

Let’s start the download an example image

1. Browse to the workshop website >
2. Download the .vsi whole-slide image
3. Once done, unzip it
4. Save it
4. Right-click on the installer file > Open > Confirm Open

Workshop plan

1. Introduction to digital image analysis
2. Installing QuPath and your first project
3. GUI layout and toolbars
4. Introducing objects: annotations and detections
5. Saving, sharing and receiving QuPath projects
6. Nuclei detection and measurements (incl. StarDist)
7. Cell classification
8. Automating tissue annotations (pixel classifier)
9. Advance topic: scripting and workflows

Acknowledgments

- **Pete Bankhead** et al.
  - QuPath and its amazing documentation
- **Peter Sobolewski**
  - *Introduction to QuPath* workshop at the The Jackson Laboratory
- **Nina Kozlova**
  - Whole-slide image used in this workshop

Self-introductions

1. My **name** is *Antoine*
2. My **position** is as an *Associate in Systems Biology*
3. My **lab** is *the Image Analysis Collaboratory and the Megason Lab*
4. I have *confocal microscopy* **images** of *cancer tissues, embryos, …*
5. A **fun fact** about me is *I used to be a brewer*

Self-introductions

1. **Motivate** the use of algorithms in image analysis
2. **Introduce** some image-analysis nomenclature
3. **Learn** to use QuPath effectively and reproducibly

Reasons to learn image processing

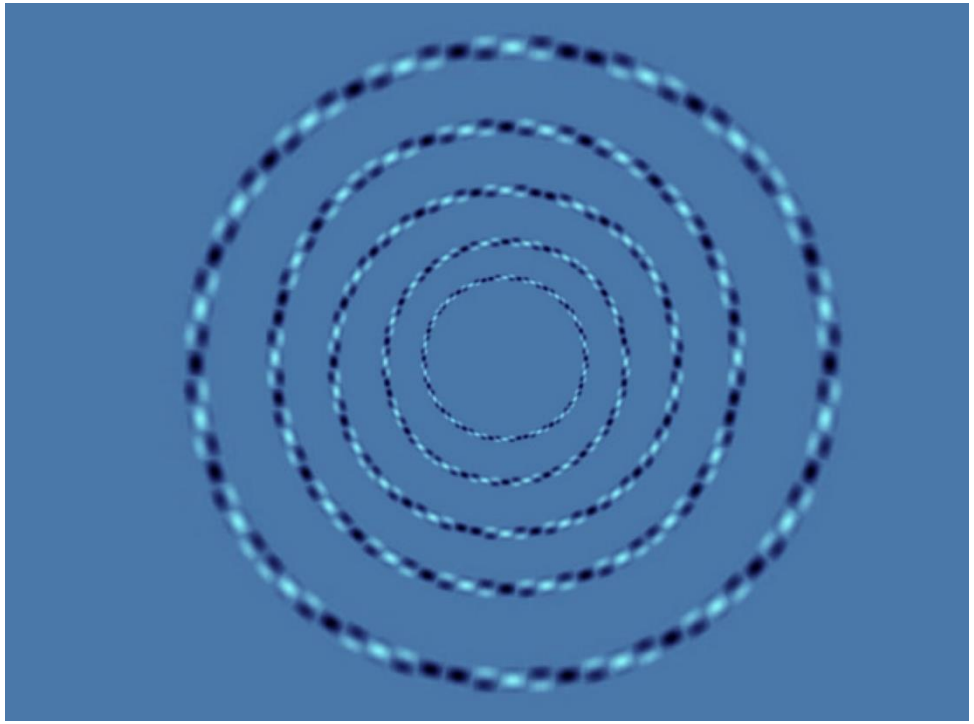
- Make pretty pictures (processing)
  - publications, talks, websites, …
- Get numbers out of pictures (analysis)
  - cell sizes, vessel lengths, GPF expression level, …
- Make experiment possible (automation)
  - whole-genome screen: millions of images
- Objectivity and Reproducibility
  - in science, it’s your duty!

Reasons **not** to learn image processing

none

Why should we analyze images with computers at all?

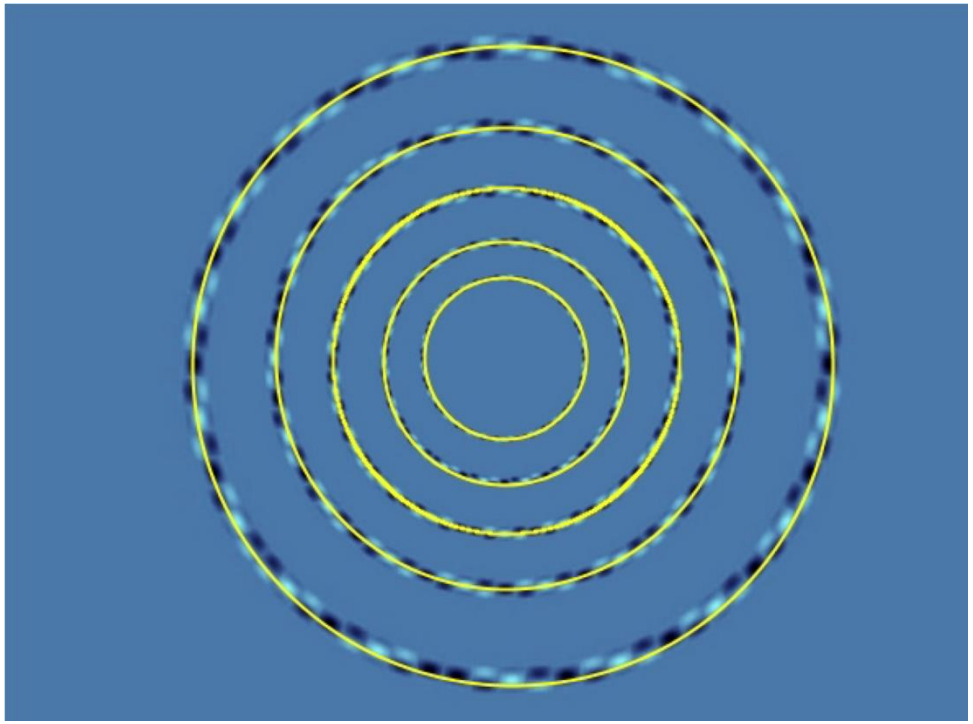
Color perception and pattern recognition is individual – science less so



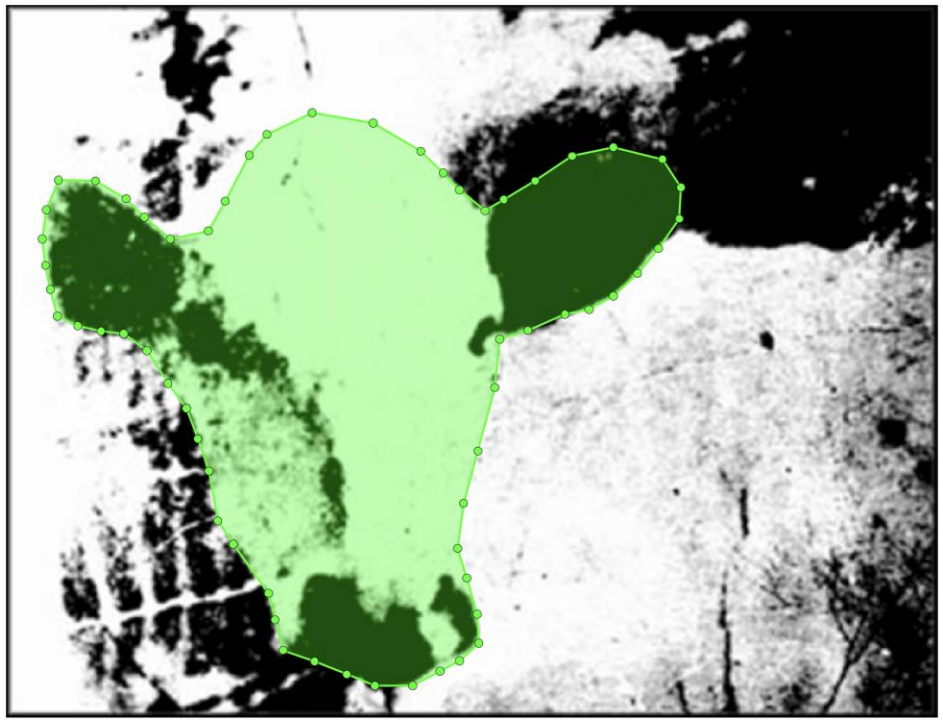
https://www.moillusions.com/perfect-circles-optical-illusion/

http://www.brainbashers.com

Color perception and pattern recognition is individual – science less so



https://www.moillusions.com/perfect-circles-optical-illusion/



http://www.brainbashers.com

In other words,

“Each human brain is a very complex neural network trained on different data – predictions will vary”

Antoine

A typical image analysis workflow

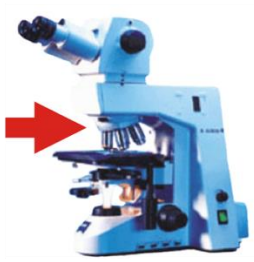
- There are typically *five* steps in an image analysis
- Often a good idea to structure work along these lines before starting

Think of this even ***before*** you acquire the images!

otherwise image analysis may become only a *post-mortem* on your experiment

Image processing vs analysis

Image Formation
object in → image out



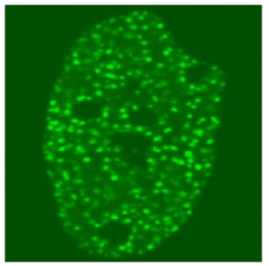


Image Processing
image in → image out

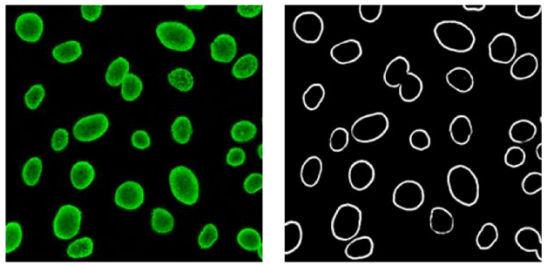
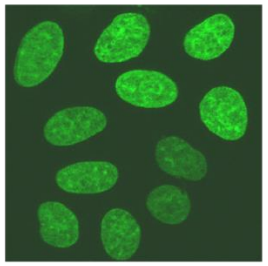


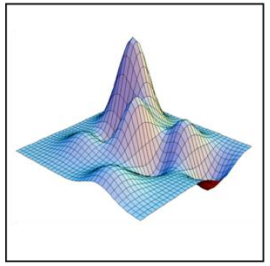
Image Analysis
image in → features out



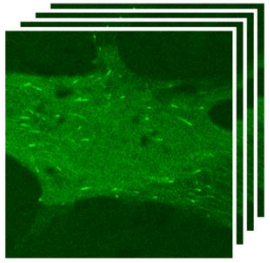
Obj	Area	Perim
1	324.2	98.5
2	406.7	140.3
3	487.1	159.2
4	226.3	67.8
5	531.8	187.6
6	649.5	203.1
7	582.6	196.4
8	498.0	162.9
9	543.2	195.1

Computer Graphics
numbers in → image out

X	Y	I
-3.54	-2.32	0.50
-2.78	-1.90	0.12
-1.15	0.42	3.09
0.45	1.65	5.89
1.83	2.18	7.72
2.98	3.33	2.07
4.21	3.96	-4.58
5.62	4.54	-11.45
7.16	5.02	-3.63

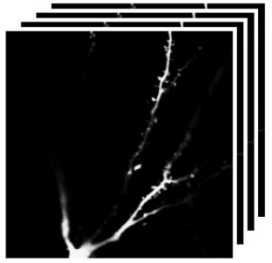


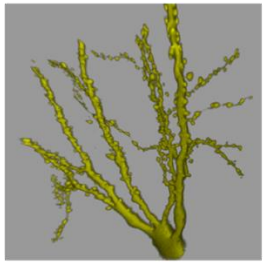
Computer Vision
image in → interpretation out



The series shows microtubule growth in a live neuron. The average speed of the distal ends is comparable in the cell body, dendrites, axons, and growth cones.

Visualization
image in → representation out





What is an image?

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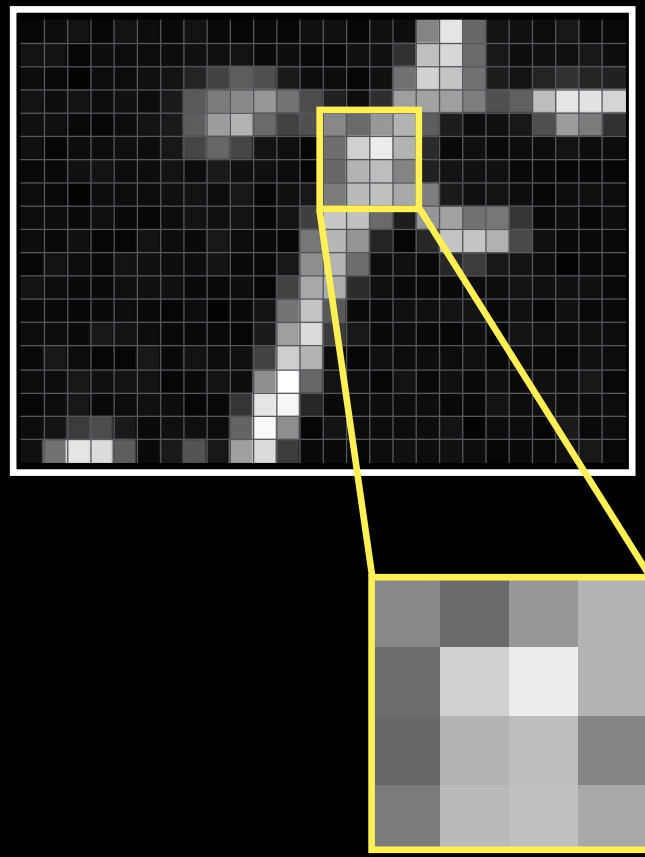
A digital image is a matrix of numbers!



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6	13	19	6	19	13	9	19	9	6	9	6	16	16	6	16	13	132	229	103	19	16	13	23	9	9
19	19	6	13	13	13	13	16	16	19	9	13	9	6	16	16	49	192	216	106	23	13	16	16	23	13
13	9	4	13	13	16	19	36	66	93	79	26	13	13	6	16	113	209	196	113	29	19	36	49	36	33
19	13	19	13	16	13	26	89	123	136	152	116	76	33	13	46	159	162	159	126	79	96	189	229	226	212
16	16	9	6	13	19	26	93	156	179	106	66	79	136	106	152	179	93	29	13	16	23	79	156	123	49
16	6	13	13	16	13	23	69	103	69	19	16	6	109	209	236	179	43	9	16	9	13	13	19	13	13
9	9	16	19	13	13	19	13	26	16	16	13	6	103	179	189	132	33	19	16	16	9	9	6	6	6
13	9	4	13	13	13	16	19	13	23	6	16	23	123	186	192	169	126	26	16	19	13	6	13	16	13
13	13	9	16	9	6	13	19	16	19	6	19	63	199	192	106	29	149	162	113	119	53	9	13	6	13
13	9	16	6	6	19	13	9	23	13	9	6	119	182	149	36	6	39	196	196	176	73	16	9	9	9
6	19	13	9	19	16	13	13	19	9	9	23	142	179	109	13	16	9	39	59	23	19	13	4	9	9
19	13	9	9	16	16	16	9	9	13	6	66	169	172	43	16	9	9	9	13	13	19	16	16	16	9
9	9	6	9	13	9	6	13	4	9	19	116	196	89	9	9	16	16	19	19	9	16	6	16	9	9
13	13	9	23	19	13	9	9	9	6	26	159	219	59	23	9	13	9	6	13	6	19	16	13	16	13
9	23	13	6	6	23	9	19	13	16	66	206	179	13	6	16	13	13	13	16	9	13	9	9	16	13
13	13	23	16	19	19	6	9	19	13	142	255	103	19	13	6	19	9	16	9	16	9	16	13	23	9
6	13	23	9	13	16	13	6	9	53	229	246	39	9	13	13	13	13	9	9	19	13	16	13	13	13
13	19	59	76	26	9	16	16	13	99	249	142	6	19	13	13	13	13	19	4	13	13	6	26	9	13
16	113	229	219	93	9	26	83	23	159	219	59	9	9	6	13	16	13	16	13	6	9	9	16	23	9

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136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

Pixel = Picture Element

CITE
Core for Imaging Technology & Education

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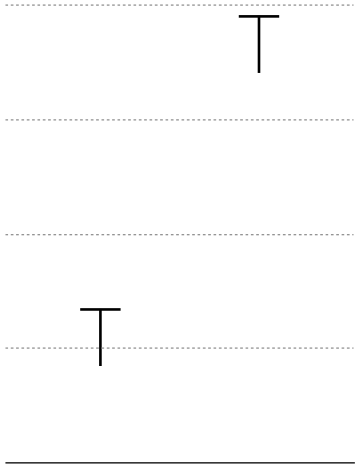
Images in publications and presentations
should be used to ***communicate*** a finding...
not ***be*** the finding

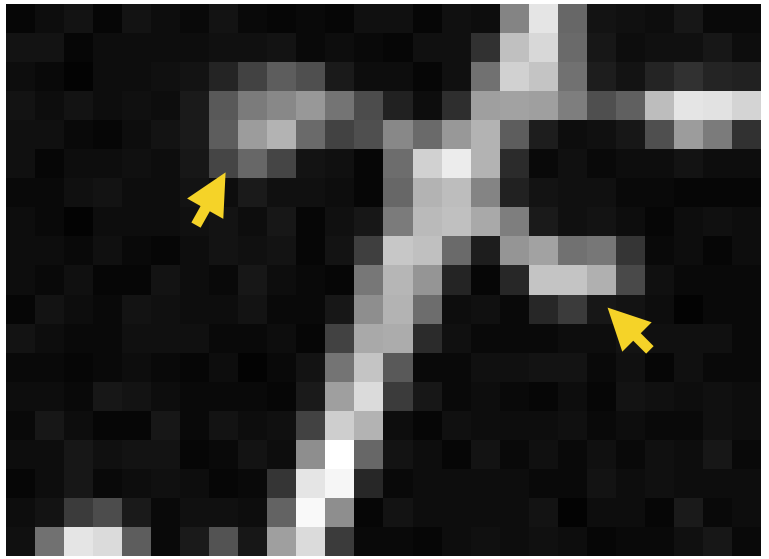
this is your **data**

6	13	19	6	19	13	9	19	9	6	9	6	16	16	6	16	13	132	229	103	19	16	13	23	9	9
19	19	6	13	13	13	13	16	16	19	9	13	9	6	16	16	49	192	216	106	23	13	16	16	23	13
13	9	4	13	13	16	19	36	66	93	79	26	13	13	6	16	113	209	196	113	29	19	36	49	36	33
19	13	19	13	16	13	26	89	123	136	152	116	76	33	13	46	159	162	159	126	79	96	189	229	226	212
16	16	9	6	13	19	26	93	156	179	106	66	79	136	106	152	179	93	29	13	16	23	79	156	123	49
16	6	13	13	16	13	23	69	103	69	19	16	6	109	209	236	179	43	9	16	9	13	13	19	13	13
9	9	16	19	13	13	19	13	26	16	16	13	6	103	179	189	132	33	19	16	16	9	9	6	6	6
13	9	4	13	13	13	16	19	13	23	6	16	23	123	186	192	169	126	26	16	19	13	6	13	16	13
13	13	9	16	9	6	13	19	16	19	6	19	63	199	192	106	29	149	162	113	119	53	9	13	6	13
13	9	16	6	6	19	13	9	23	13	9	6	119	182	149	36	6	39	196	196	176	73	16	9	9	9
6	19	13	9	19	16	13	13	19	9	9	23	142	179	109	13	16	9	39	59	23	19	13	4	9	9
19	13	9	9	16	16	16	9	9	13	6	66	169	172	43	16	9	9	9	13	13	19	16	16	16	9
9	9	6	9	13	9	6	13	4	9	19	116	196	89	9	9	16	16	19	19	9	16	6	16	9	9
13	13	9	23	19	13	9	9	9	6	26	159	219	59	23	9	13	9	6	13	6	19	16	13	16	13
9	23	13	6	6	23	9	19	13	16	66	206	179	13	6	16	13	13	13	16	9	13	9	9	16	13
13	13	23	16	19	19	6	9	19	13	142	255	103	19	13	6	19	9	16	9	16	9	16	13	23	9
6	13	23	9	13	16	13	6	9	53	229	246	39	9	13	13	13	13	9	9	19	13	16	13	13	13
13	19	59	76	26	9	16	16	13	99	249	142	6	19	13	13	13	13	19	4	13	13	6	26	9	13
16	113	229	219	93	9	26	83	23	159	219	59	9	9	6	13	16	13	16	13	6	9	9	16	23	9

this is your **result**



this just helps to
communicate the result

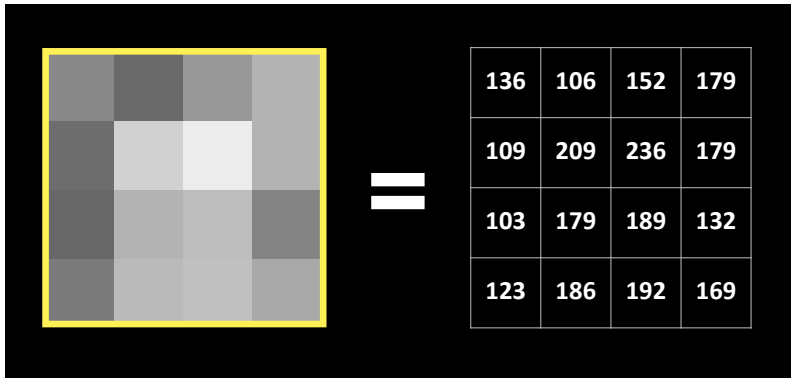


Display your images

Mapping Image Intensity to Monitor Intensity (LookUp Tables)

LUT = how the grey values are displayed

LUTs do not change the pixel values



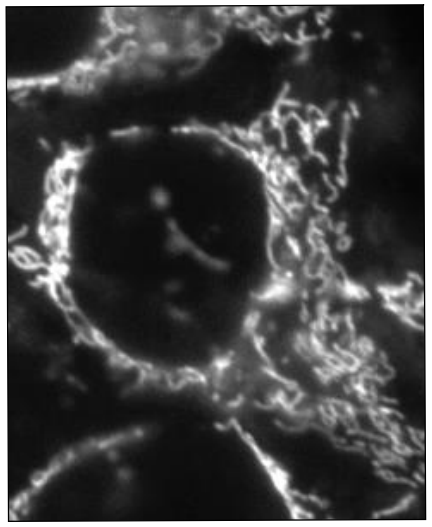
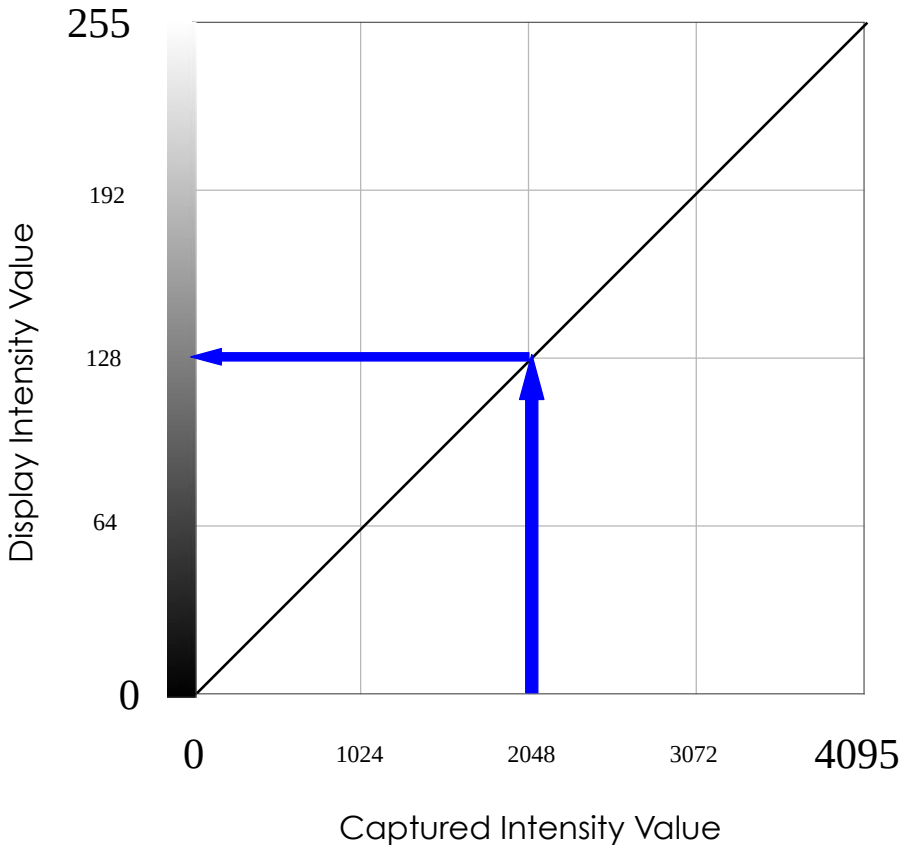


Image (12 bit)	Displayed color
0	grey LUT
1	
…	
2000	
…	
4095	



Images and Colors

Lookup Tables (LUTs)

LUT = how the grey values are displayed

LUTs do not change the pixel values

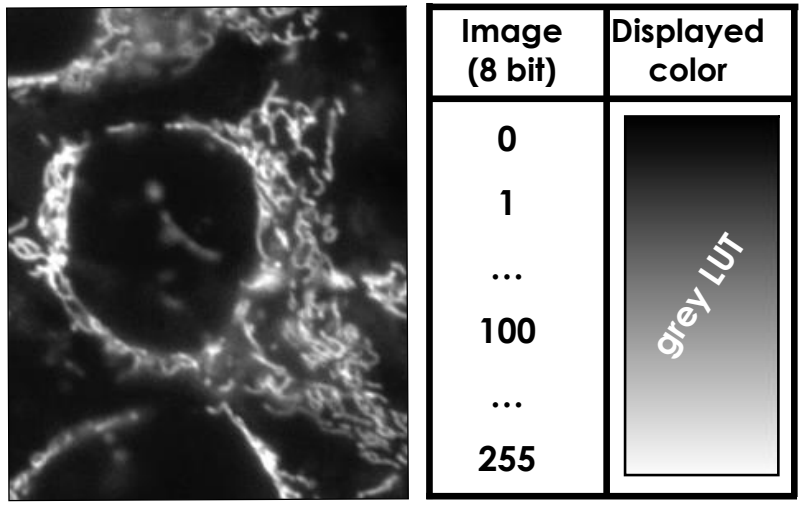
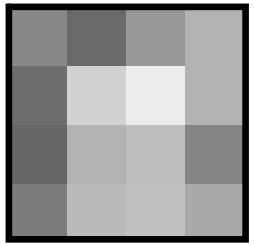


Image (8 bit)	Displayed color
0	grey LUT
1	
…	
100	
…	
255	



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136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

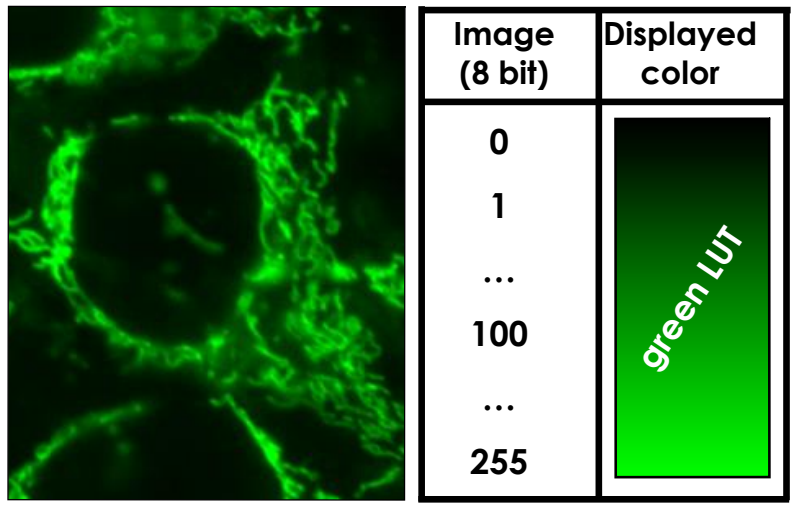
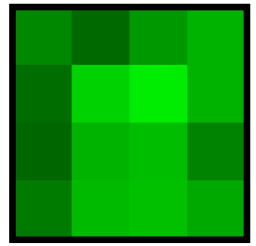


Image (8 bit)	Displayed color
0	green LUT
1	
…	
100	
…	
255	



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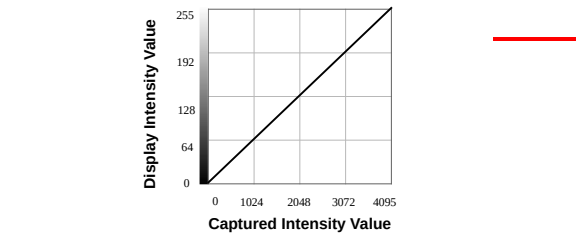
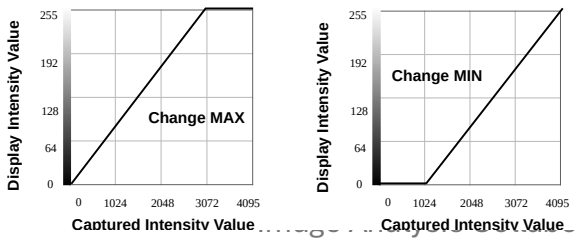
136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

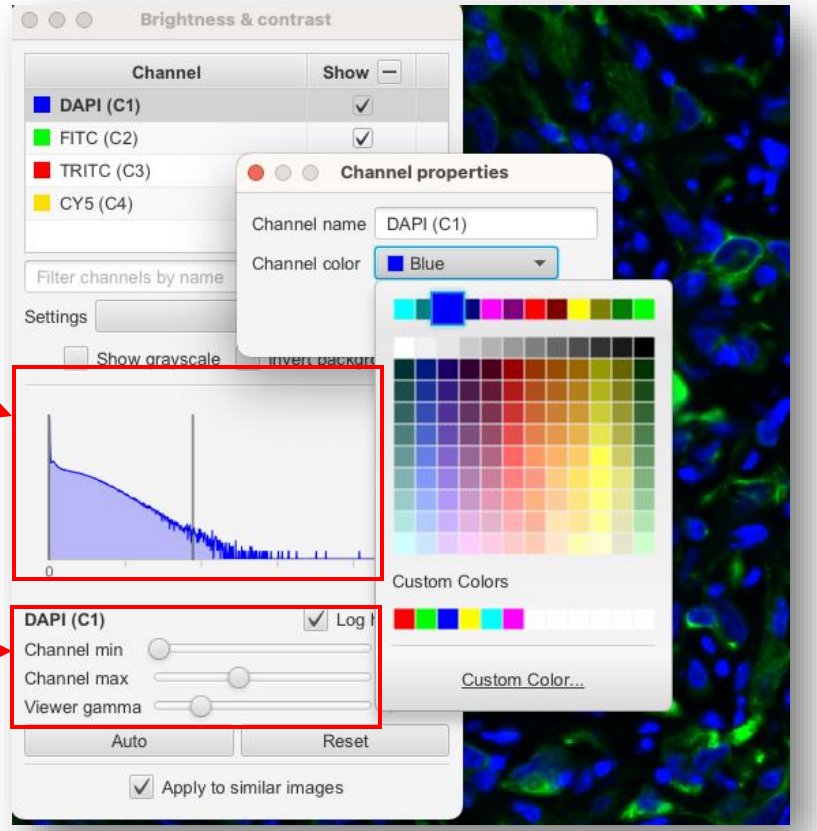
Display images: color, brightness & contrast

- If you are imaging a blue fluorophore, you are not forced to display it in blue!
- Pixel histogram represents the distribution of pixel values in the image
- LUT range

You are NOT changing the pixels values, you are just changing how the image is displayed (unless you click on the "Apply" button).

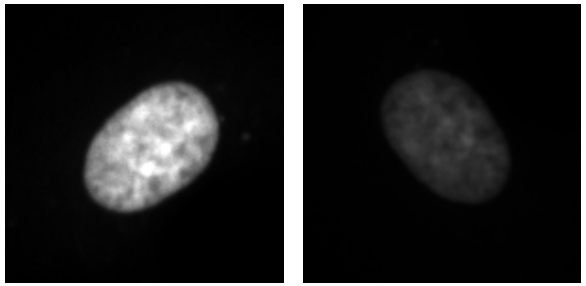




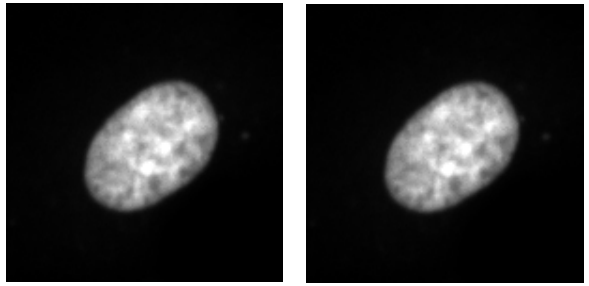


Display a file: Brightness & Contrast

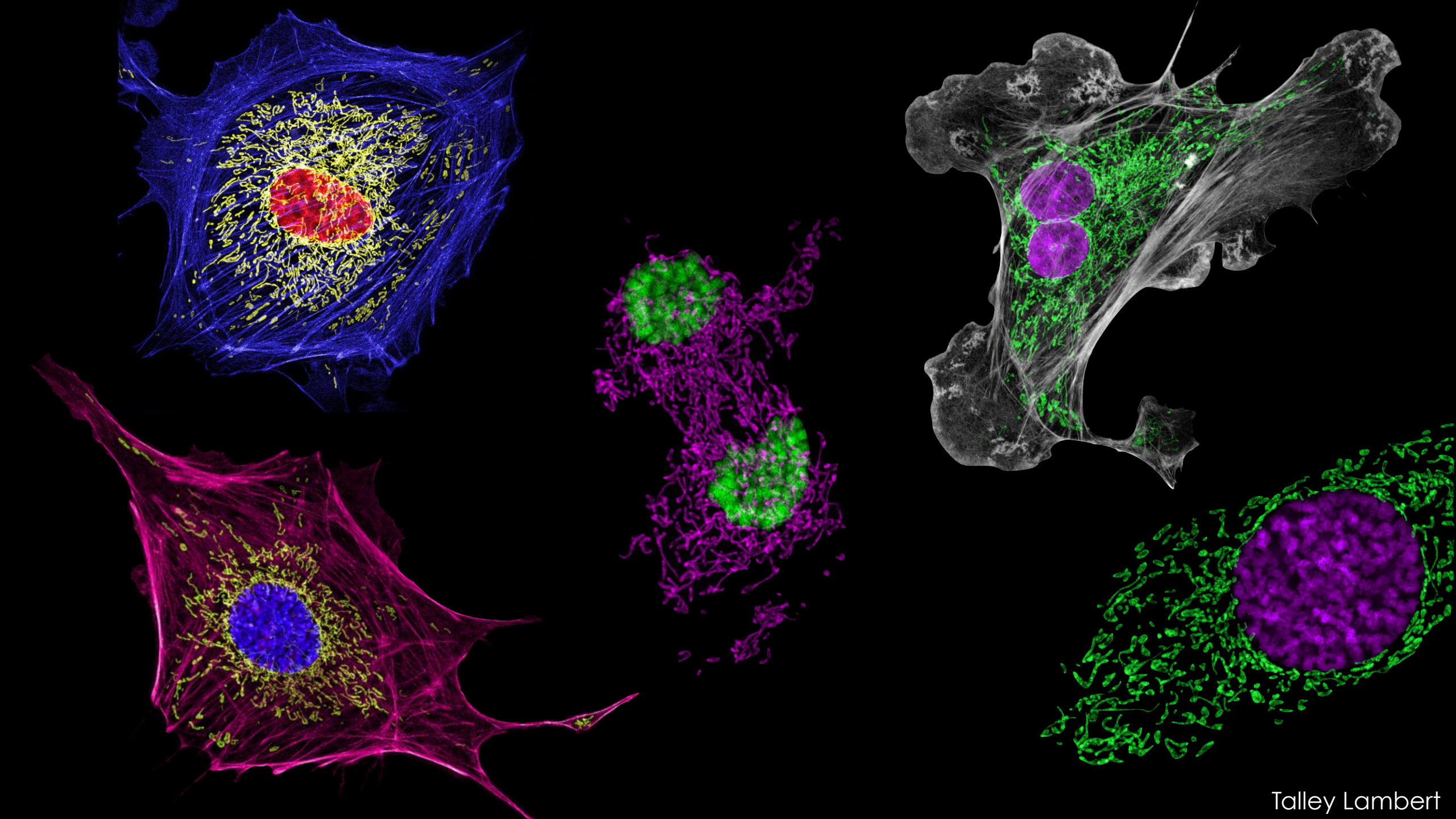
Which image has more fluorescence?



Mean:	**4803**	**4803**
Display range:	188-**16828**	188-**45514**



Mean:	**4803**	**4803**
Display range:	188-**16828**	188-**16828**



Talley Lambert

Save the downloaded example image (cont.)

1. Browse to the workshop website >
2. Download the .vsi whole-slide image (~2-5 min)
3. Create a folder named ***qupath_workshop*** (outside of your *downloads* folder)
4. Once the download is finished, unzip
5. Save the unzipped folder in the newly created ***qupath_workshop*** folder