



Introduction to Bioimage Analysis using QuPath

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Image Analysis Collaboratory



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

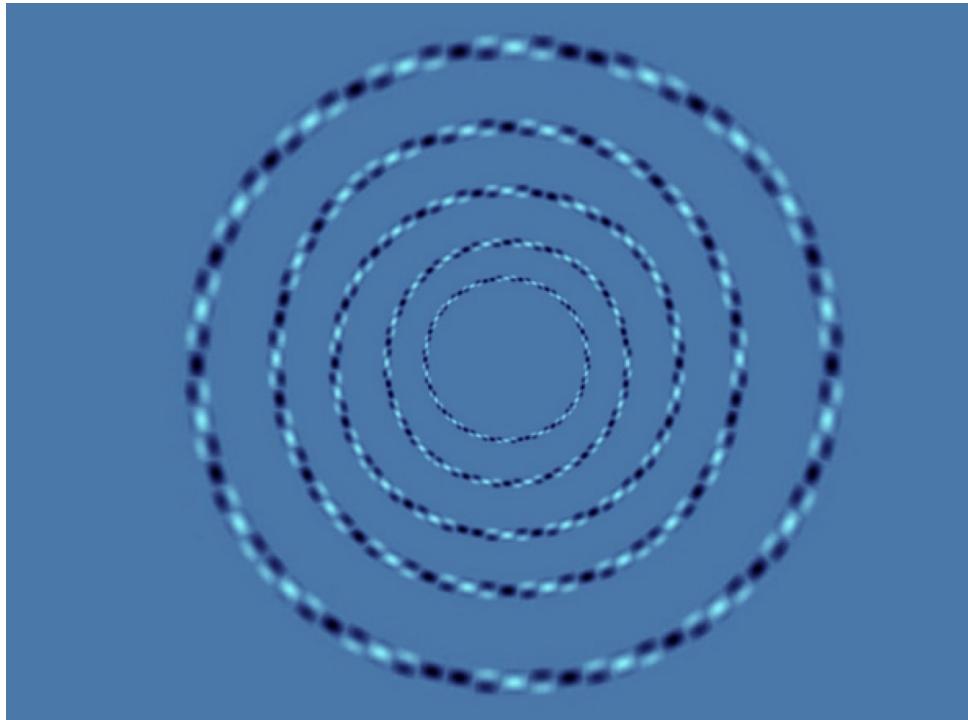
- Make pretty pictures (processing)
 - publications, talks, websites, ...
- Get numbers out of pictures (analysis)
 - cell sizes, vessel lengths, GFP expression level, ...
- Make experiment possible (automation)
 - whole-genome screen: millions of images
- Objectivity and Reproducibility
 - in science, 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

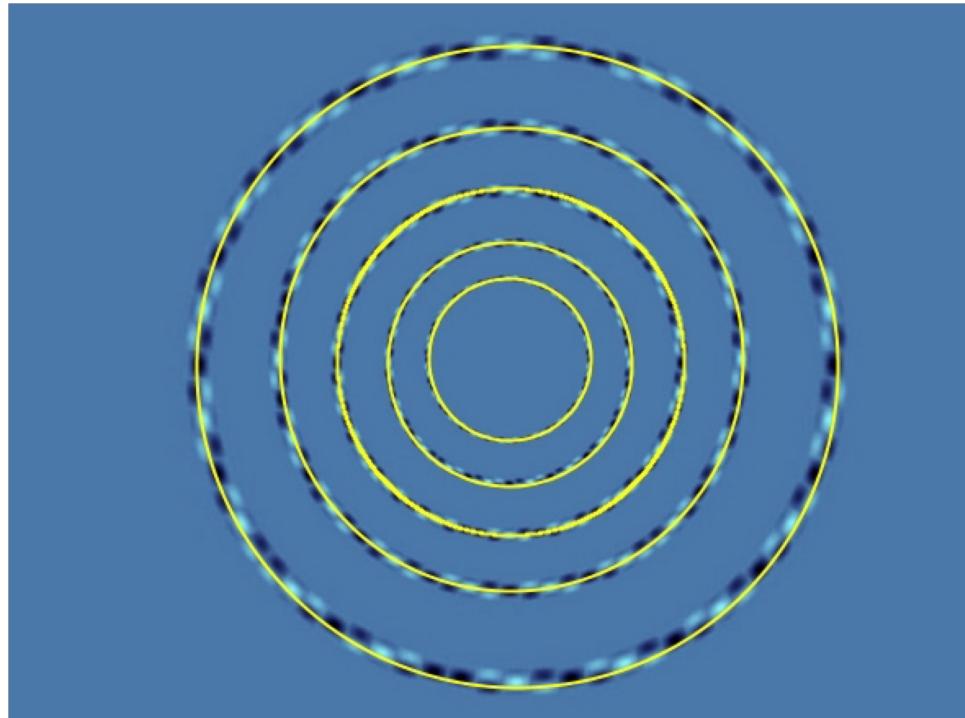


<https://www.moillusions.com/perfect-circles-optical-illusion/>

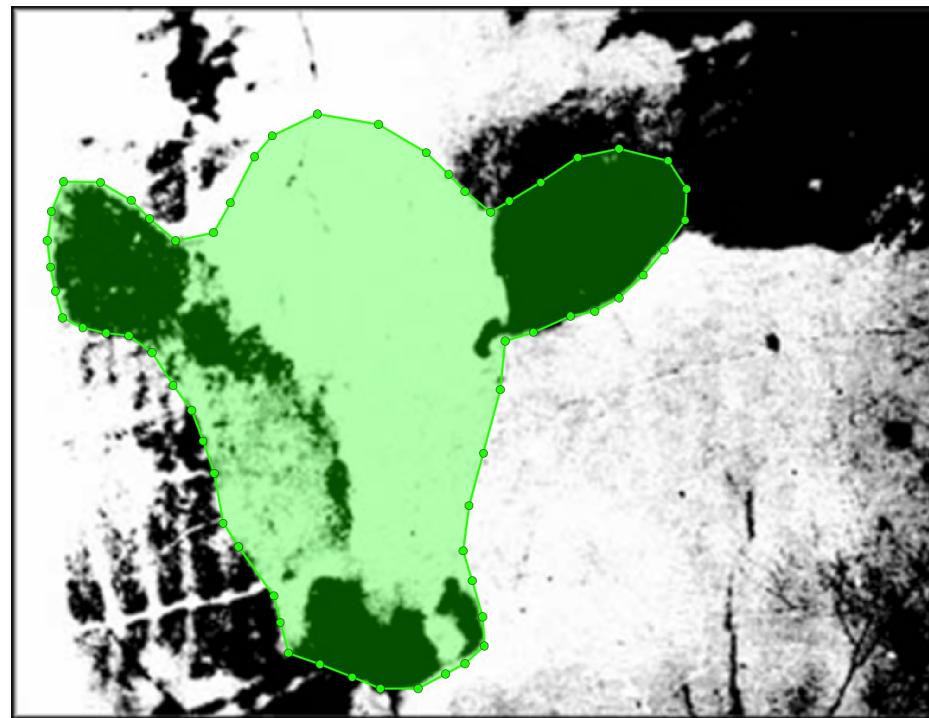


<http://www.brainbashers.com>

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

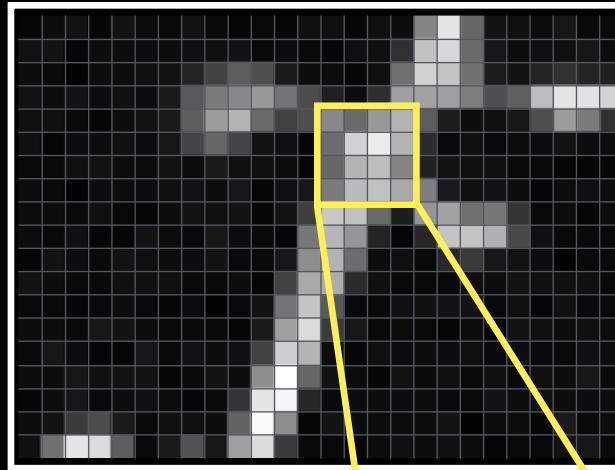


Think of this even ***before*** you acquire the images!

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

What is an image?

A digital image is a matrix of numbers!



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|----|-----|-----|-----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|-----|-----|-----|----|
| 6 | 13 | 19 | 6 | 19 | 13 | 9 | 19 | 9 | 6 | 16 | 16 | 6 | 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 | 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 | 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 | 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 | 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 | 9 | 9 | 13 | 6 | 66 | 169 | 172 | 43 | 16 | 9 | 9 | 9 | 13 | 13 | 19 | 16 | 16 | 9 | | |
| 9 | 9 | 6 | 9 | 13 | 9 | 6 | 13 | 4 | 9 | 19 | 116 | 196 | 89 | 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 | 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 | 9 | 13 | 16 | 13 | 6 | 9 | 53 | 229 | 246 | 39 | 9 | 13 | 13 | 9 | 9 | 19 | 13 | 16 | 13 | 13 | 13 | 13 |
| 13 | 19 | 59 | 76 | 26 | 9 | 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 | 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 |

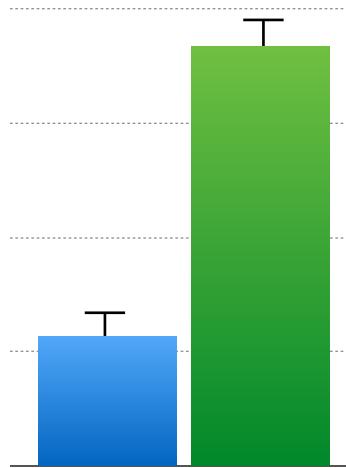
Pixel = Picture Element

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

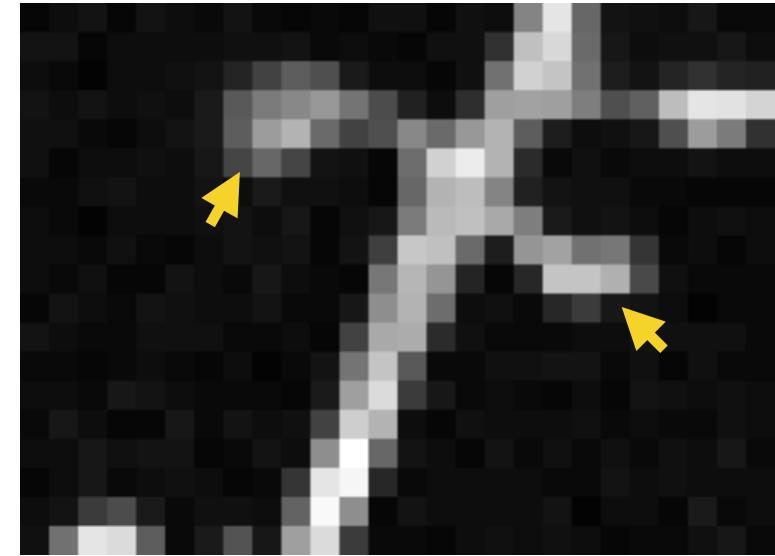
this is your **data**

| | | | | | | | | | | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|-----|-----|-----|-----|----|
| 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 | 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 | 158 | 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 | 9 | 9 | 19 | 13 | 16 | 13 | 13 | 13 | 13 | |
| 13 | 19 | 59 | 76 | 26 | 9 | 16 | 16 | 13 | 99 | 249 | 142 | 6 | 19 | 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

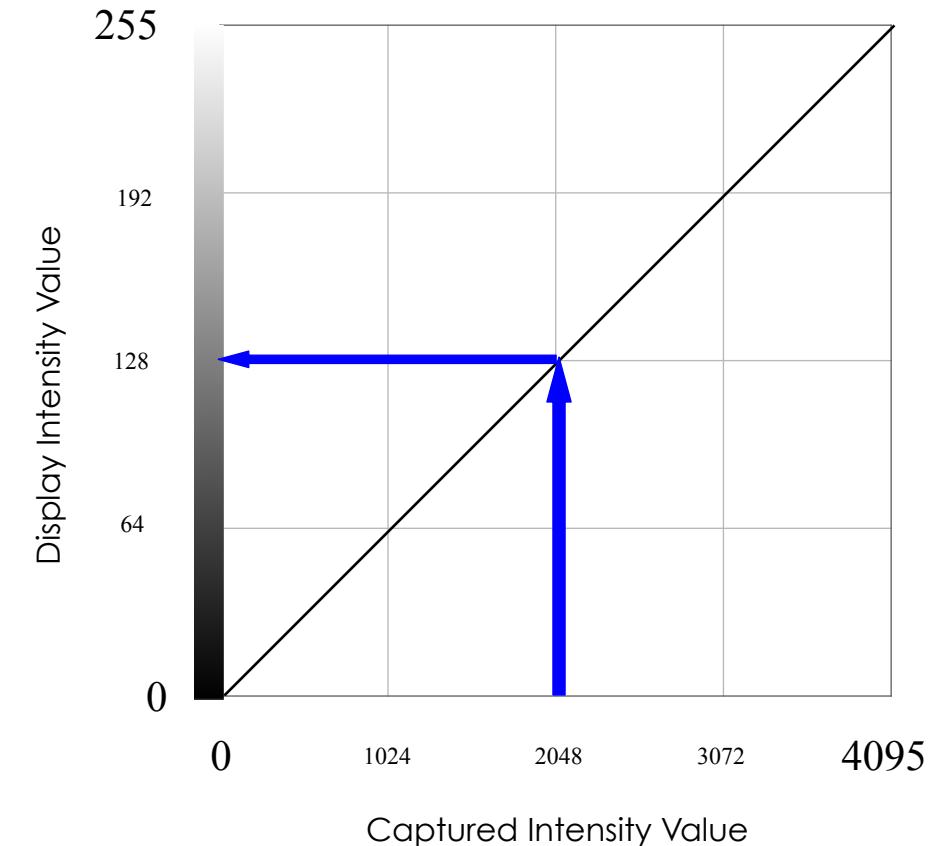
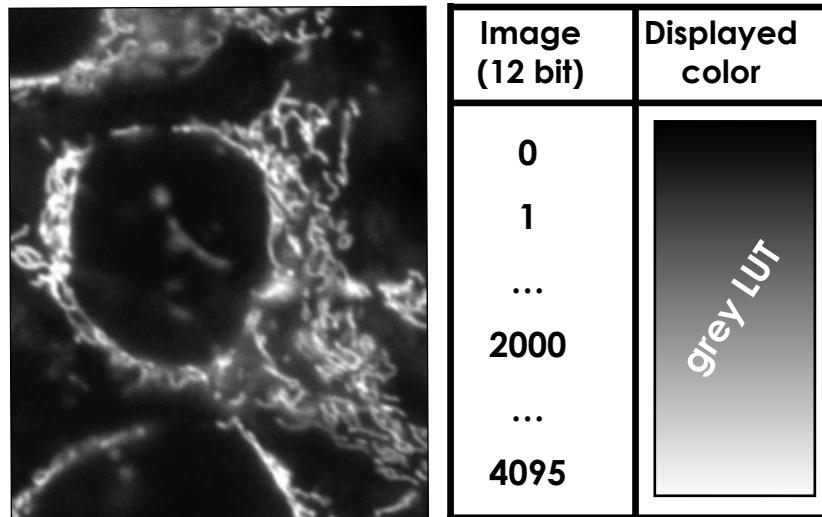
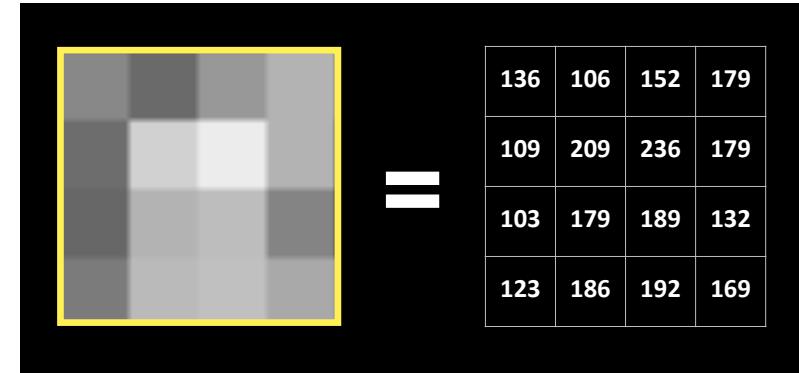


Display your images

Mapping Image Intensity to Monitor Intensity (LookUp Tables)

LUT = how the grey values are displayed

LUTs do not change the pixel values

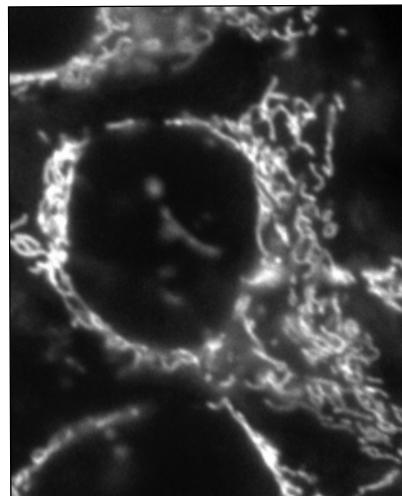


Images and Colors

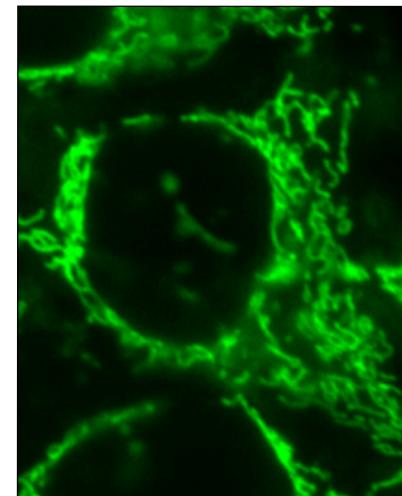
Lookup Tables (LUTs)

LUT = how the grey values are displayed

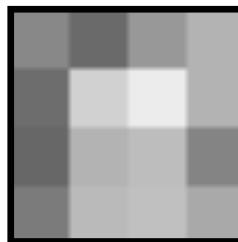
LUTs do not change the pixel values



| Image (8 bit) | Displayed color |
|------------------|--------------------|
| 0 | |
| 1 | |
| ... | |
| 100 | |
| ... | |
| 255 | grey LUT |

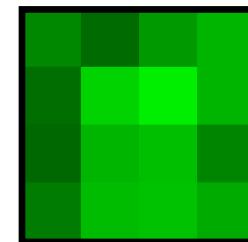


| Image (8 bit) | Displayed color |
|------------------|--------------------|
| 0 | |
| 1 | |
| ... | |
| 100 | |
| ... | |
| 255 | green LUT |



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



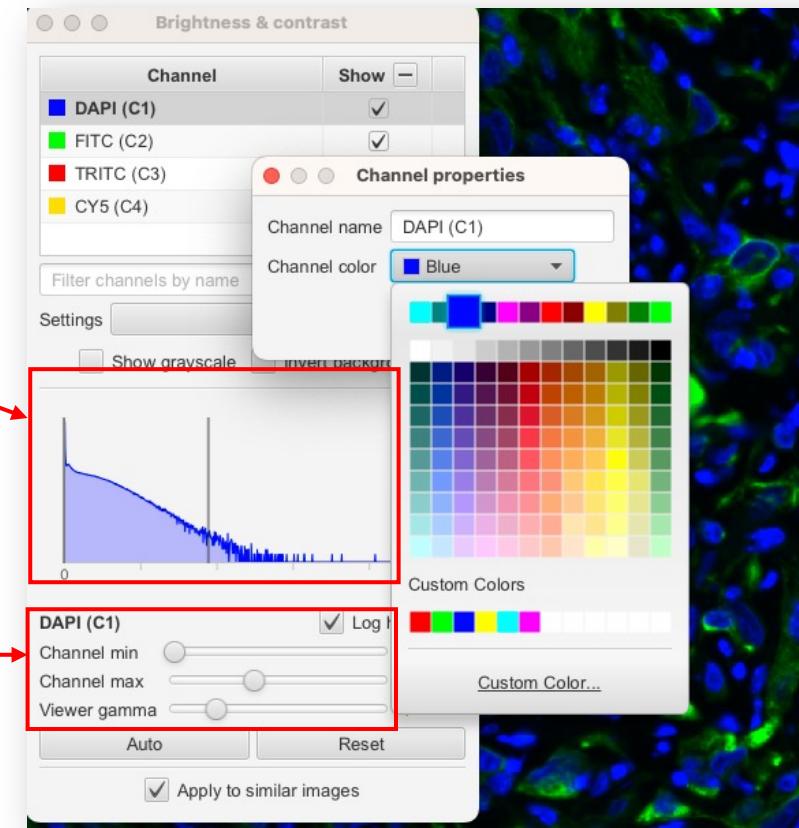
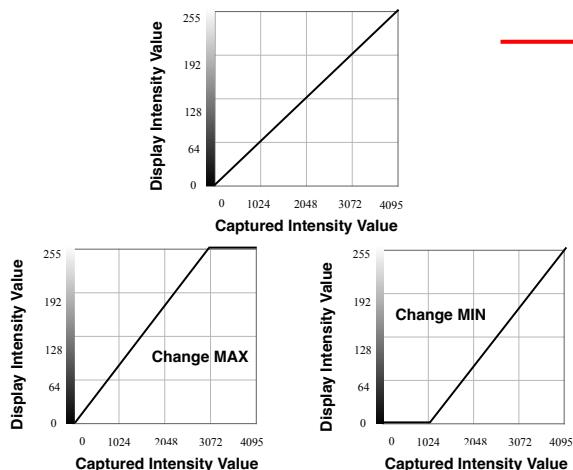
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| | | | |
|-----|-----|-----|-----|
| 136 | 106 | 152 | 179 |
| 109 | 209 | 236 | 179 |
| 103 | 179 | 189 | 132 |
| 123 | 186 | 192 | 169 |

Display images: color, brightness & contrast

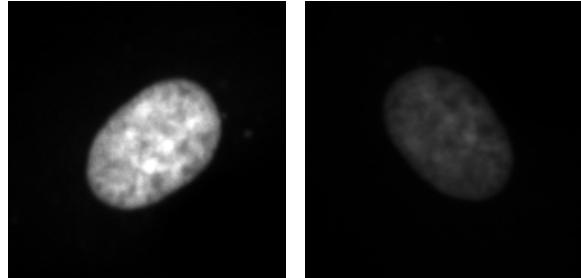
- If you are imaging a blue fluorophore, you are not forced to display it in blue!
- Pixel histogram represents the distribution of pixel values in the image
- LUT range

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

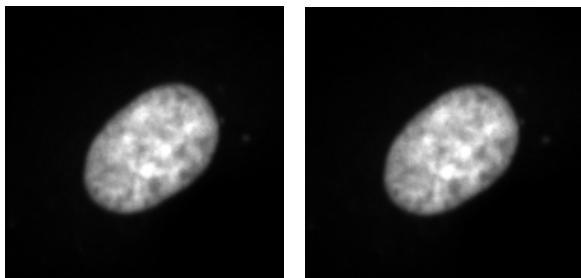


Display a file: Brightness & Contrast

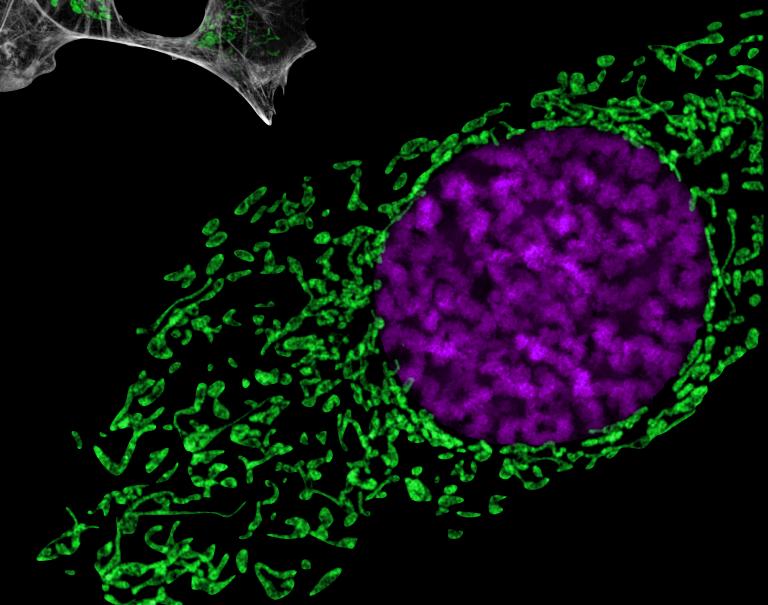
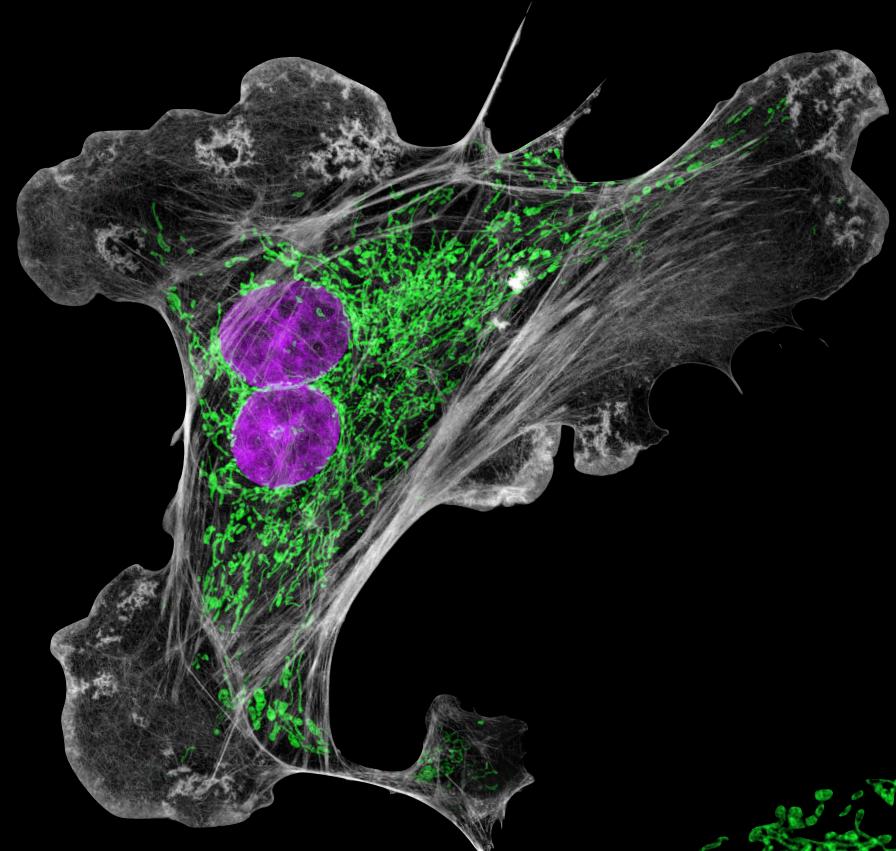
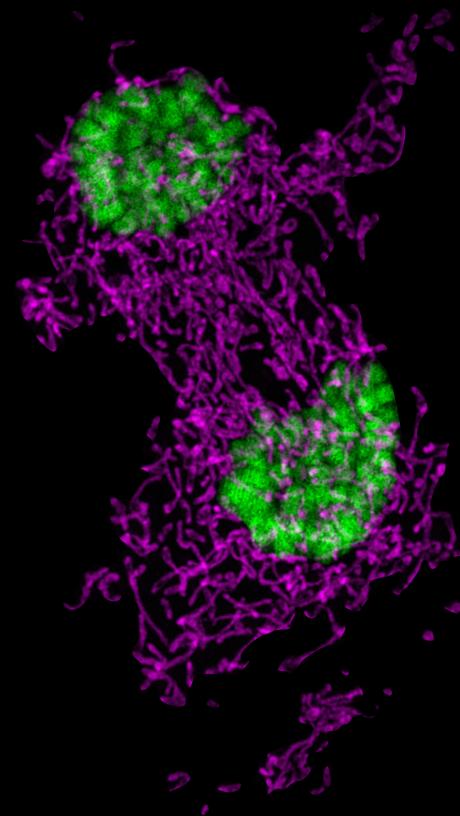
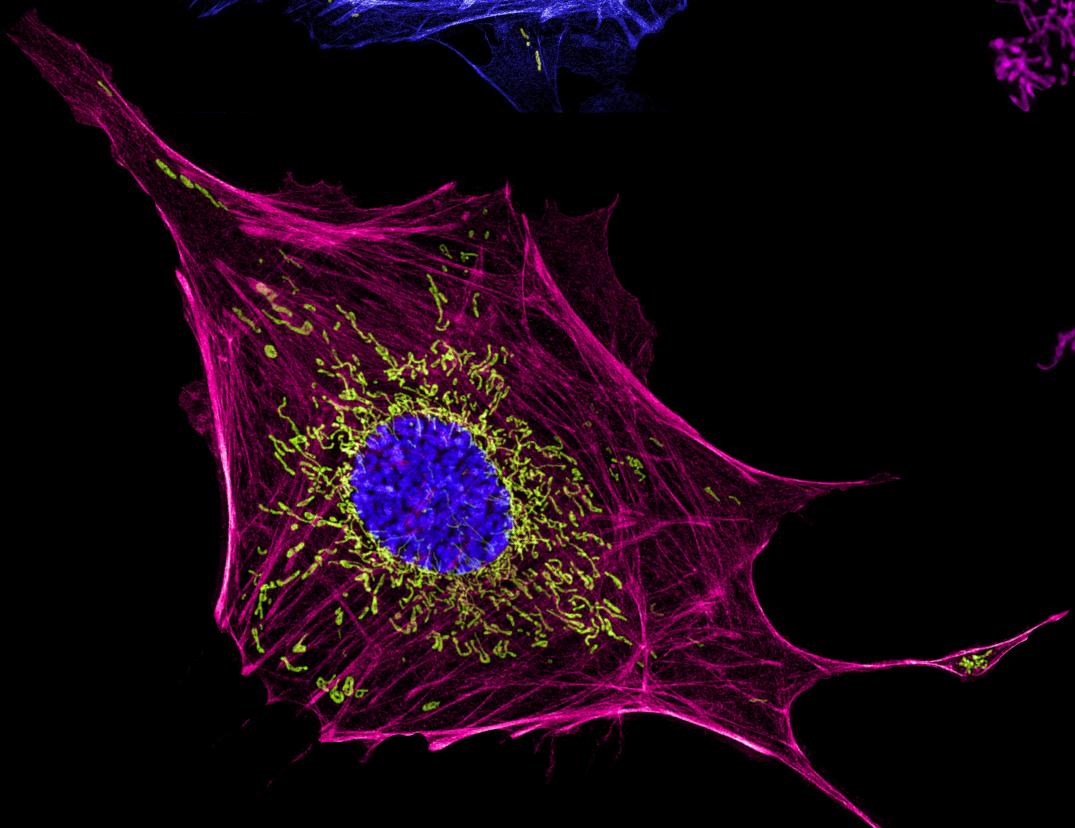
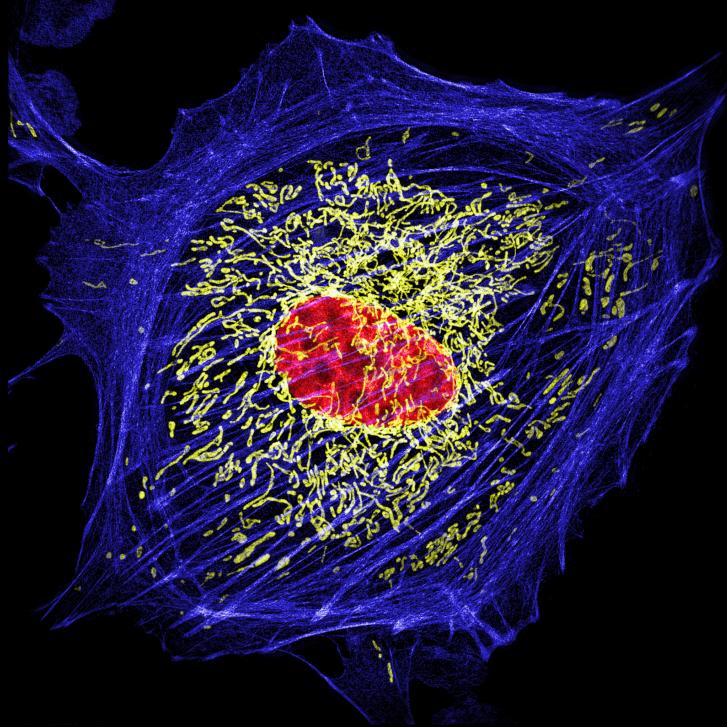
Which image has more fluorescence?



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



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



Talley Lambert