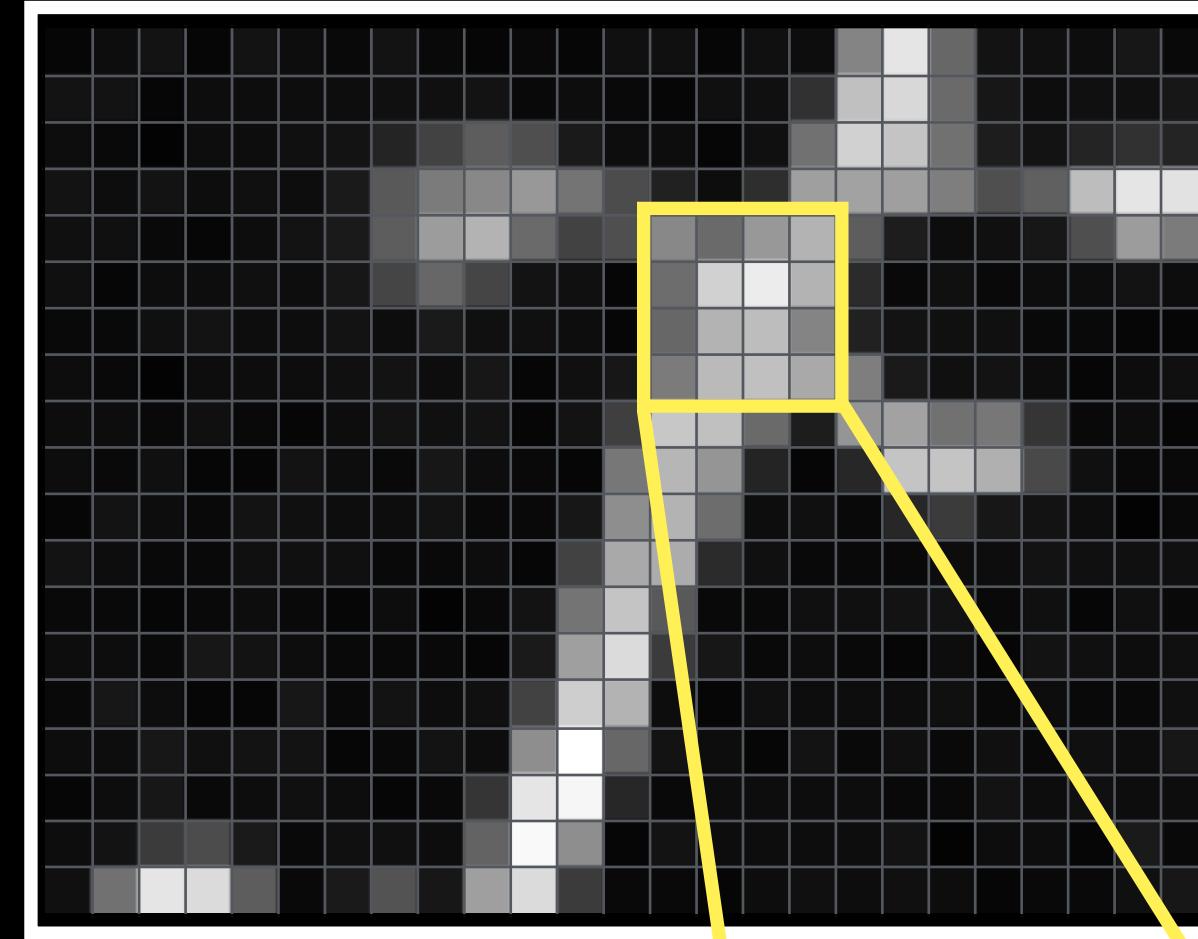




# Introduction to Digital Images



Pixel = Picture Element



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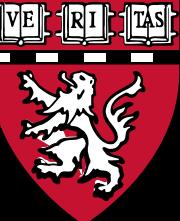
6	13	19	6	19	13	9	19	9	6	9	6	16	16	6	16	13	132	229	103	19	16	13	23	9	9
19	19	6	13	13	13	13	16	16	19	9	13	9	6	16	16	49	192	216	106	23	13	16	16	23	13
13	9	4	13	13	16	19	36	66	93	79	26	13	13	6	16	113	209	196	113	29	19	36	49	36	33
19	13	19	13	16	13	26	89	123	136	152	116	76	33	13	46	159	162	159	126	79	96	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

A digital image is a matrix of numbers!





# Introduction to Digital Images

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

+ 2 =

138	108	154	181
111	211	238	181
105	181	191	134
125	188	194	171

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

+

6	13	19	6
19	19	6	13
13	9	4	13
19	13	19	13

142	119	171	185
128	228	242	192
116	188	193	145
142	199	211	182

=

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

- 2 =

134	104	150	177
107	207	234	177
101	177	187	130
121	184	190	167

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

-

6	13	19	6
19	19	6	13
13	9	4	13
19	13	19	13

130	93	133	173
90	190	230	166
90	170	185	119
104	173	173	156

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

× 2 =

272	212	304	358
218	418	472	358
206	358	378	264
246	372	384	338

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

×

6	13	19	6
19	19	6	13
13	9	4	13
19	13	19	13

816	1378	2888	1074
2071	3971	1416	2327
1339	1611	756	1716
2337	2418	3648	2197

=

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

: 2 =

68	53	76	90
55	105	118	90
52	90	95	66
62	93	96	85

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

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6	13	19	6
19	19	6	13
13	9	4	13
19	13	19	13

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23	8	8	30
6	11	39	14
8	20	47	10
6	14	10	13





# Introduction to Digital Images

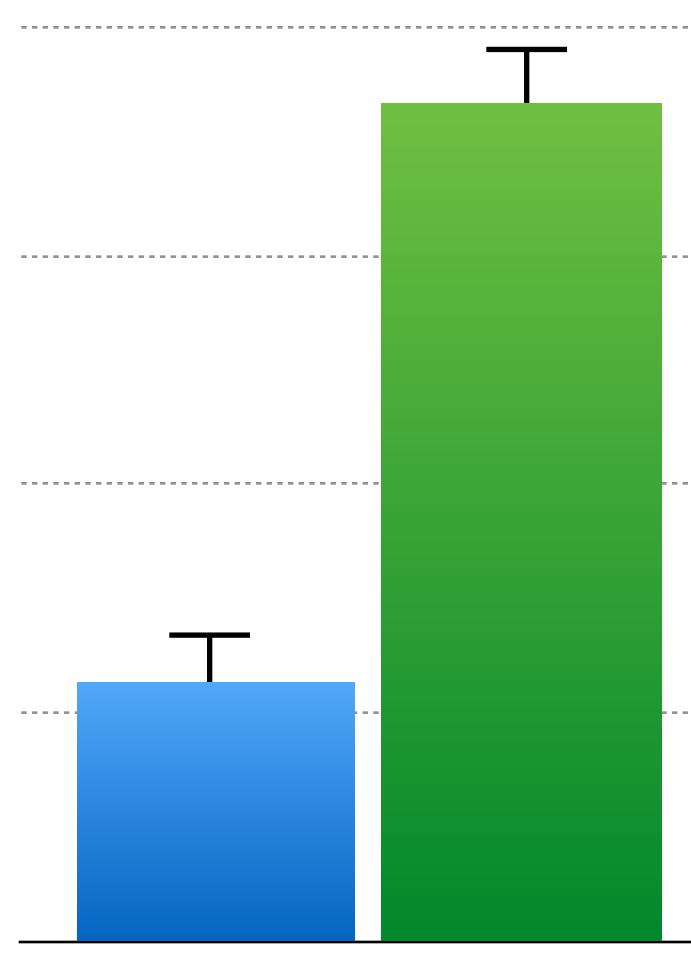


Images in publications and presentations should be used to **communicate** a finding...  
not **be** the finding

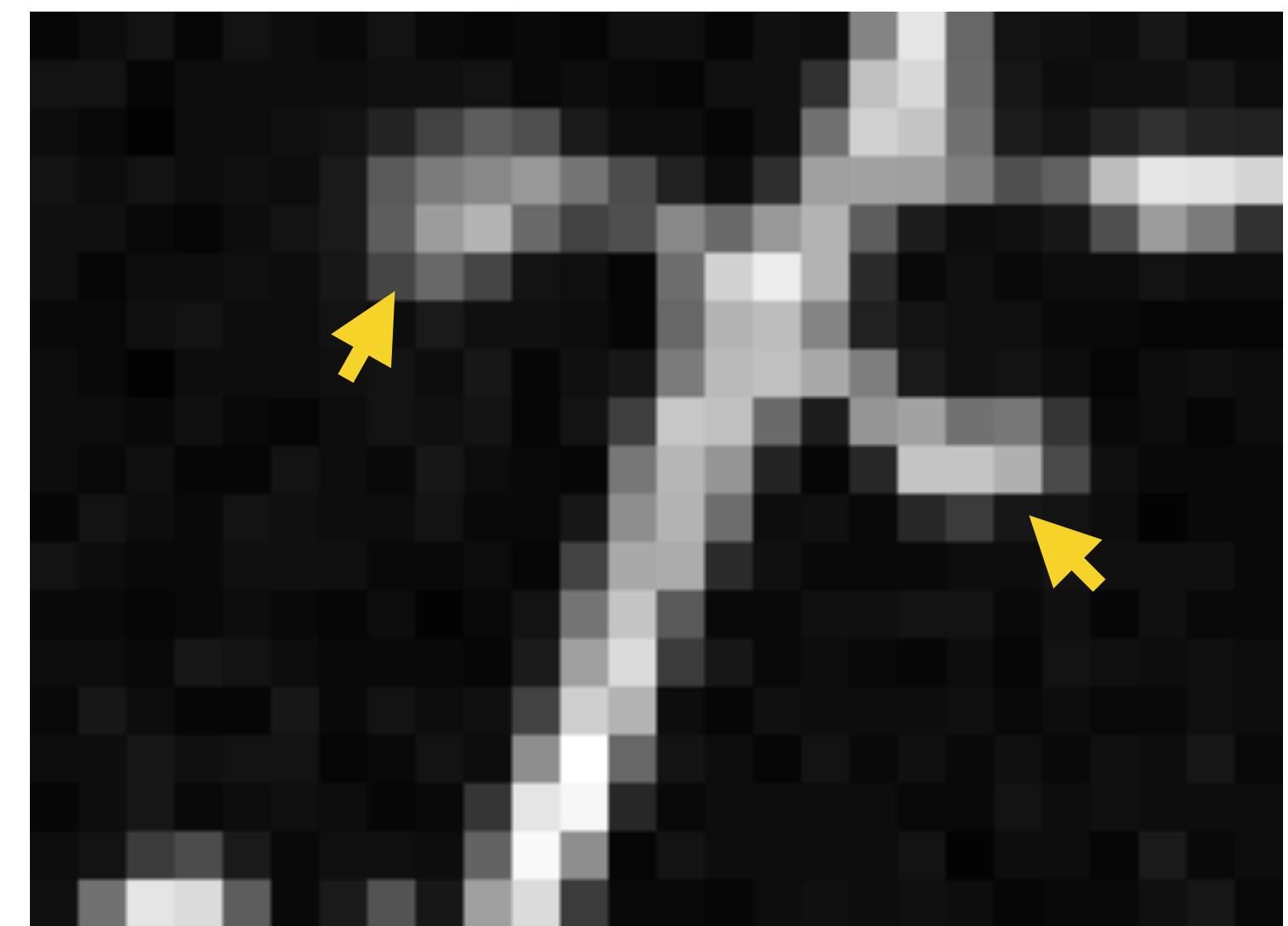
this is your **data**

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	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	6	26	159	219	59	23	9	13	9	6	13	6	19	16	13	16	13	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	13	23	9	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	6	9	9	16	23	9	9		

this is your **result**



this just helps to  
**communicate** the result

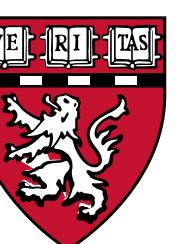




# Inspect Individual Pixel Values



<https://pyapp-kit.github.io/ndv>

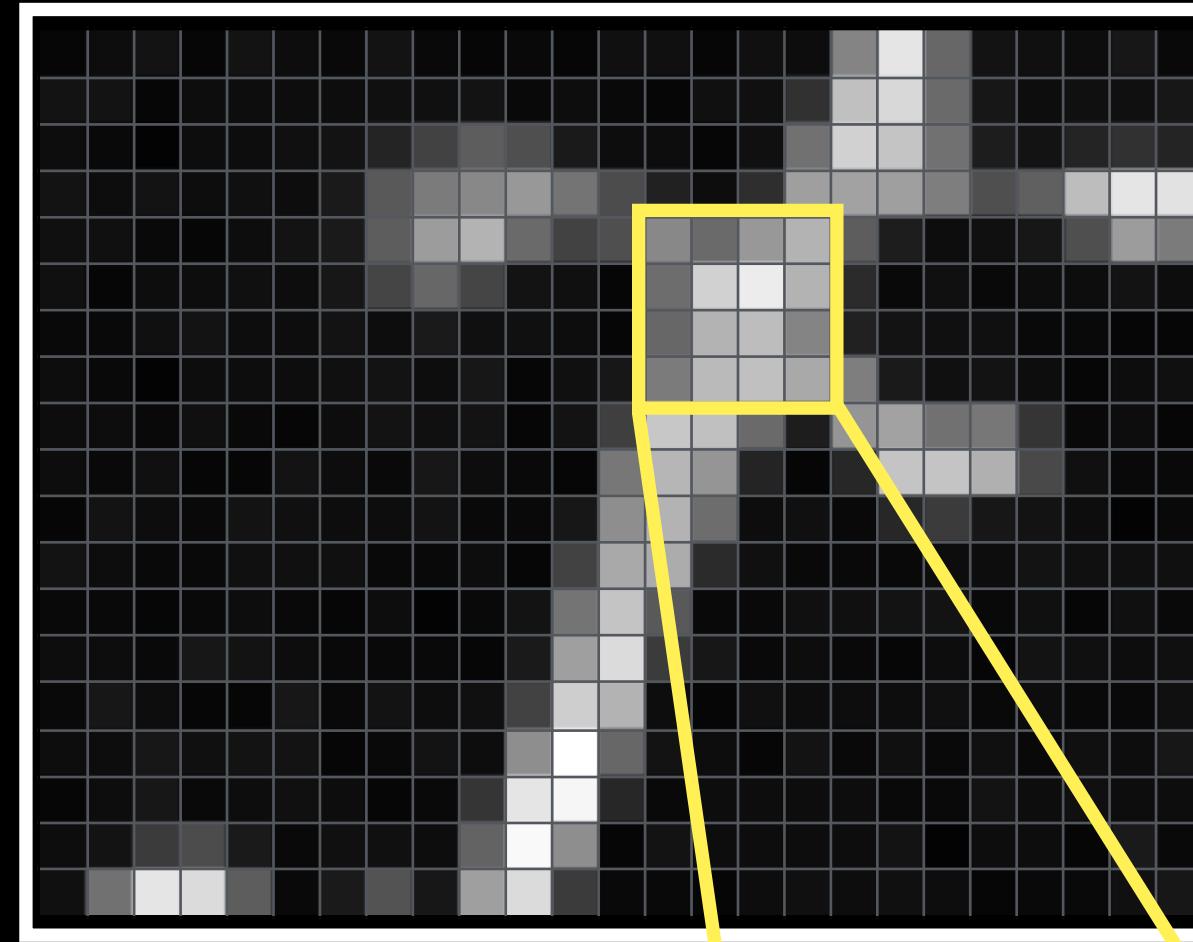




# Introduction to Digital Images



Pixel = Picture Element



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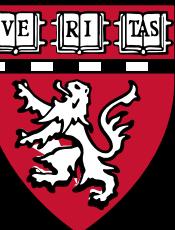
6	13	19	6	19	13	9	19	9	6	9	6	16	16	6	16	13	132	229	103	19	16	13	23	9	9	
19	19	6	13	13	13	13	16	16	16	19	9	13	9	6	16	16	49	192	216	106	23	13	16	16	23	13
13	9	4	13	13	16	19	36	66	93	79	26	13	13	6	16	113	209	196	113	29	19	36	49	36	33	
19	13	19	13	16	13	26	89	123	136	152	116	76	33	13	46	159	162	159	126	79	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

A digital image is a matrix of numbers!

Where do these numbers come from?





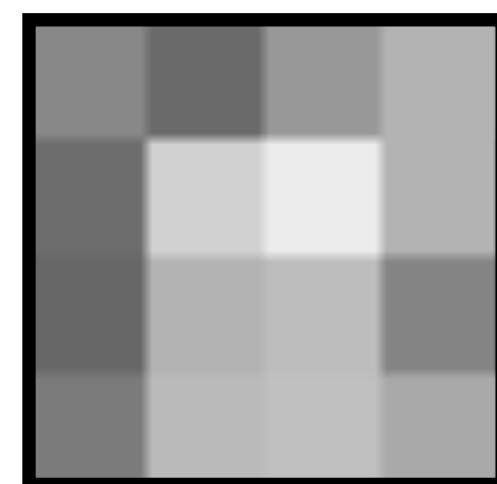
# Detectors in Fluorescence Microscopy



The detectors used in fluorescence microscopy are **monochromatic**.

Cameras or PMTs are **not able to distinguish between different wavelengths**(they just collect photons), you need **fluorescence filters** to separate your fluorophores.

The detector converts photons in digital numbers (linear relation).



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

Each pixel in the digital image has **one digital value** that **depends on** the **intensity** of the signal emitted by the **sample**.

**Digital Values = Pixel Intensity Value**

The **range** of possible **digital values** is defined by the **bit depth**.





# Detectors in Fluorescence Microscopy - Bit Depth



The **bit depth** defines the range of possible **digital values** that each pixel can have, usually **8, 12 or 16 bit**.

The **bit depth** is expressed in **grey values**.

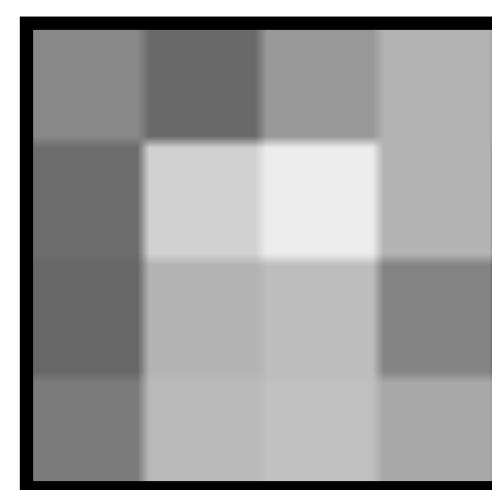
**bit depth of the image = bit depth of the detector**  
(Unless you change that during acquisition)

**x bit = a range of  $2^X$  grey values**

**8 bit** image = **each pixel** can have  $2^8$  **grey values** = 256 grey values = **range 0-255**

**12 bit** image = **each pixel** can have  $2^{12}$  **grey values** = 4096 grey values = **range 0-4095**

**16 bit** image = **each pixel** can have  $2^{16}$  **grey values** = 65536 grey values = **range 0-65535**



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

**Digital Value = Pixel Intensity Value = Grey Value**





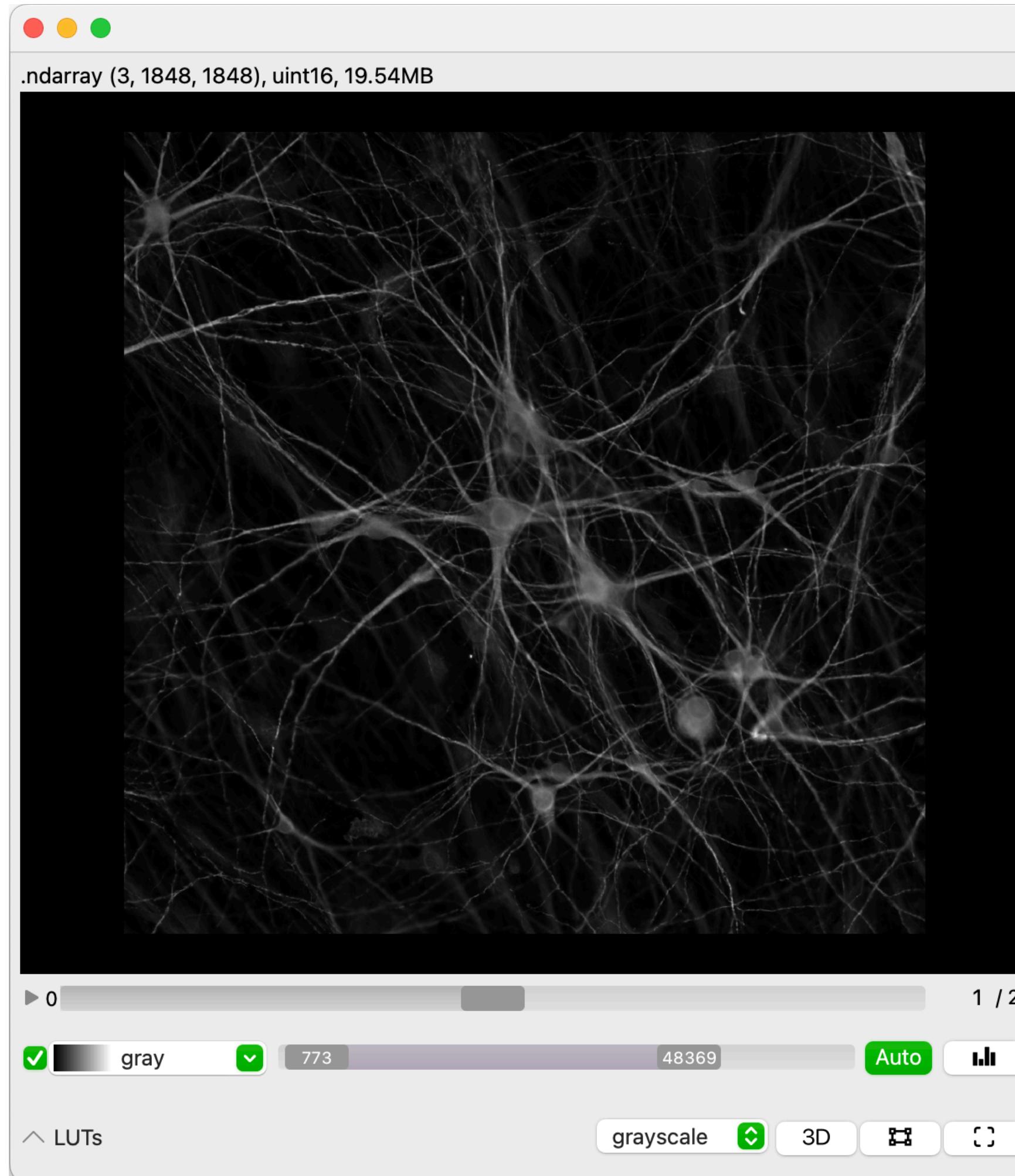
# Bit Depth



numpy

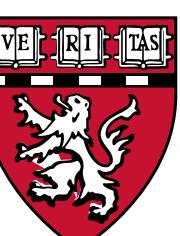


ndv



## numpy array

```
array([[ 1928,  1791,  1750, ... ,  1906,  1943,  1891],  
       [ 1822,  1726,  1775, ... ,  1795,  1921,  1862],  
       [ 1843,  1749,  1759, ... ,  1961,  1993,  1777],  
       ...,  
       [ 1737,  1696,  1800, ... ,  1660,  1726,  1751],  
       [ 1770,  1699,  1958, ... ,  1655,  1710,  1753],  
       [ 1669,  1717,  1650, ... ,  1729,  1661,  1829]],  
  
      [[ 3746,  4124,  4156, ... ,  2774,  2862,  2949],  
       [ 4215,  4514,  4481, ... ,  2833,  2785,  2985],  
       [ 4675,  4621,  4481, ... ,  2937,  2893,  2841],  
       ...,  
       [ 4115,  4092,  3715, ... ,  2891,  3136,  3226],  
       [ 3938,  3949,  3895, ... ,  2432,  2813,  2935],  
       [ 3978,  3873,  3930, ... ,  2176,  2495,  2791]],  
  
      [[ 5819,  5702,  5581, ... ,  12589,  12691,  12703],  
       [ 6646,  6056,  5764, ... ,  12388,  12902,  12488],  
       [ 6928,  6775,  6620, ... ,  12064,  12366,  12917],  
       ...,  
       [ 2362,  2293,  2346, ... ,  4860,  5033,  5450],  
       [ 2292,  2290,  2363, ... ,  4285,  4389,  4335],  
       [ 2161,  2237,  2395, ... ,  4359,  4072,  4156]]],  
shape=(3, 1848, 1848), dtype=uint16)
```



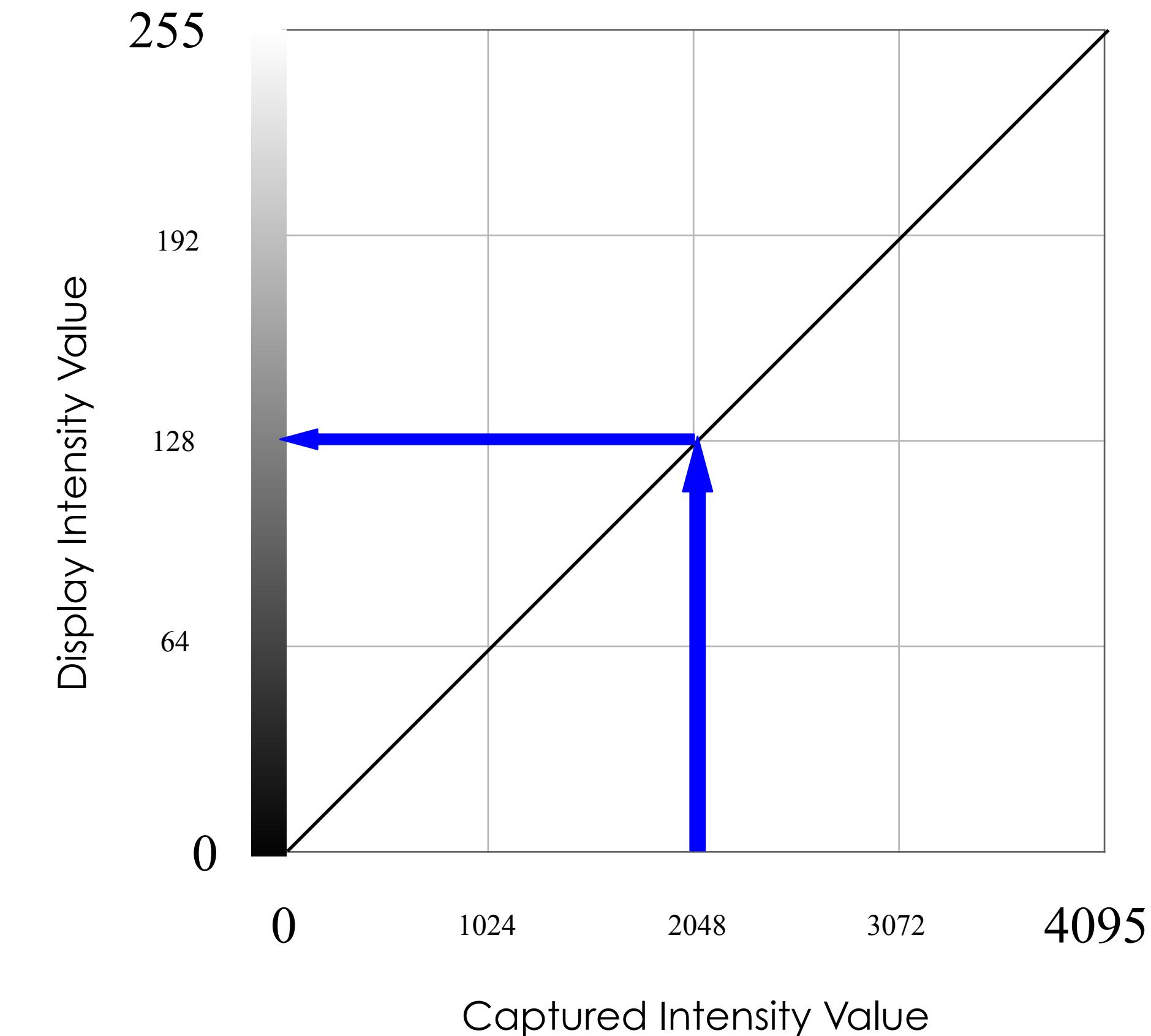
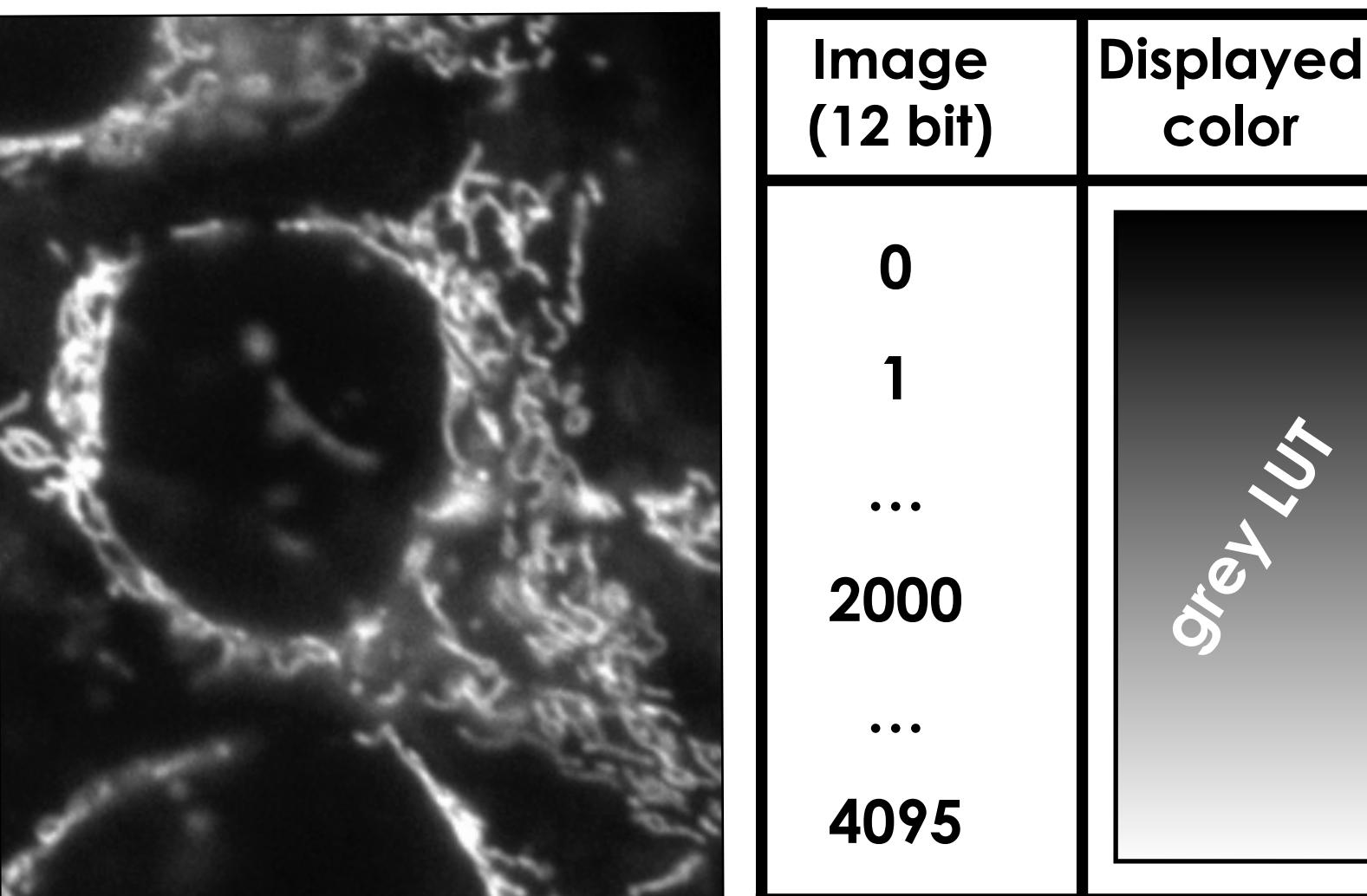
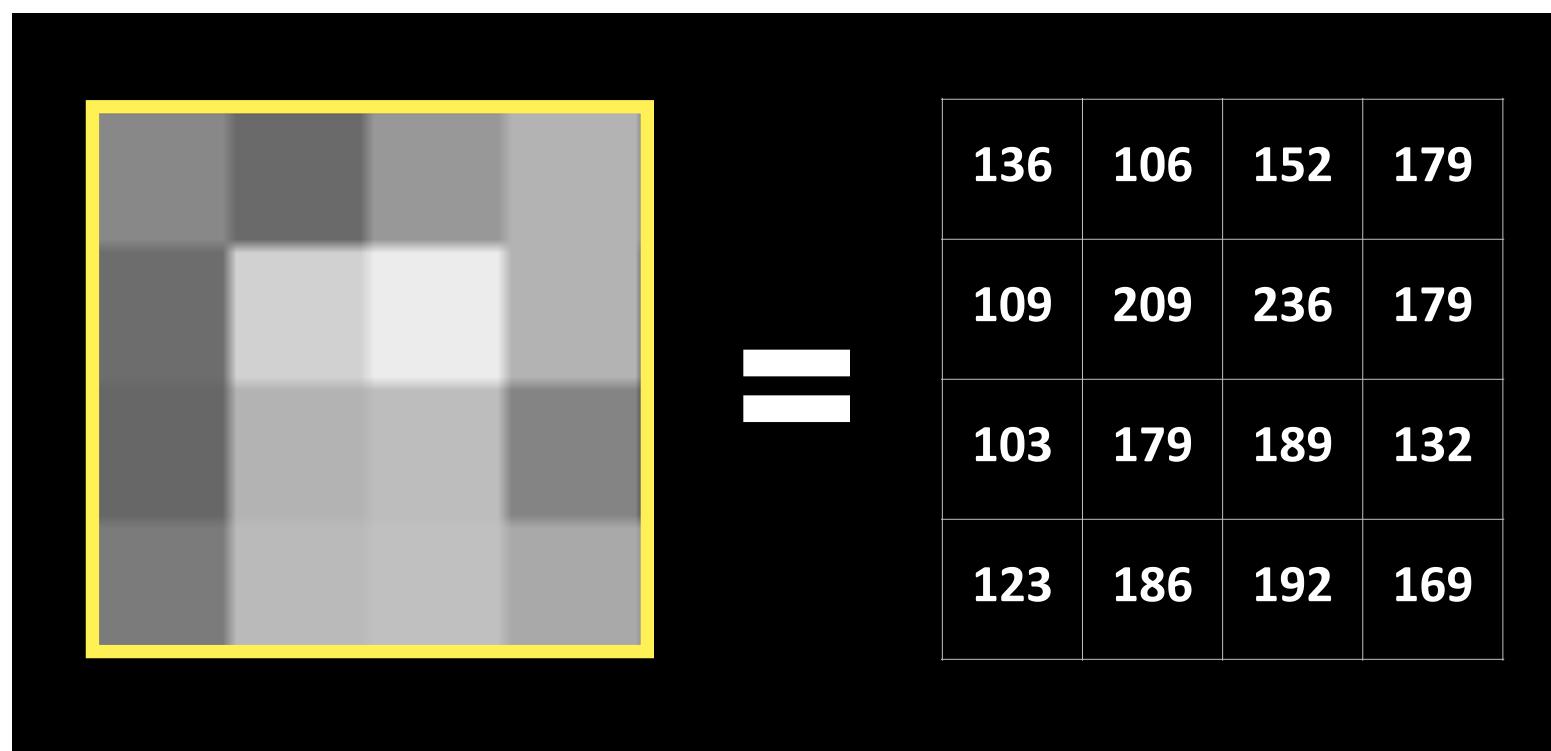


# Display your Images

## Mapping Image Intensity to Monitor Intensity (**LookUp Tables**)

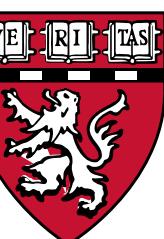
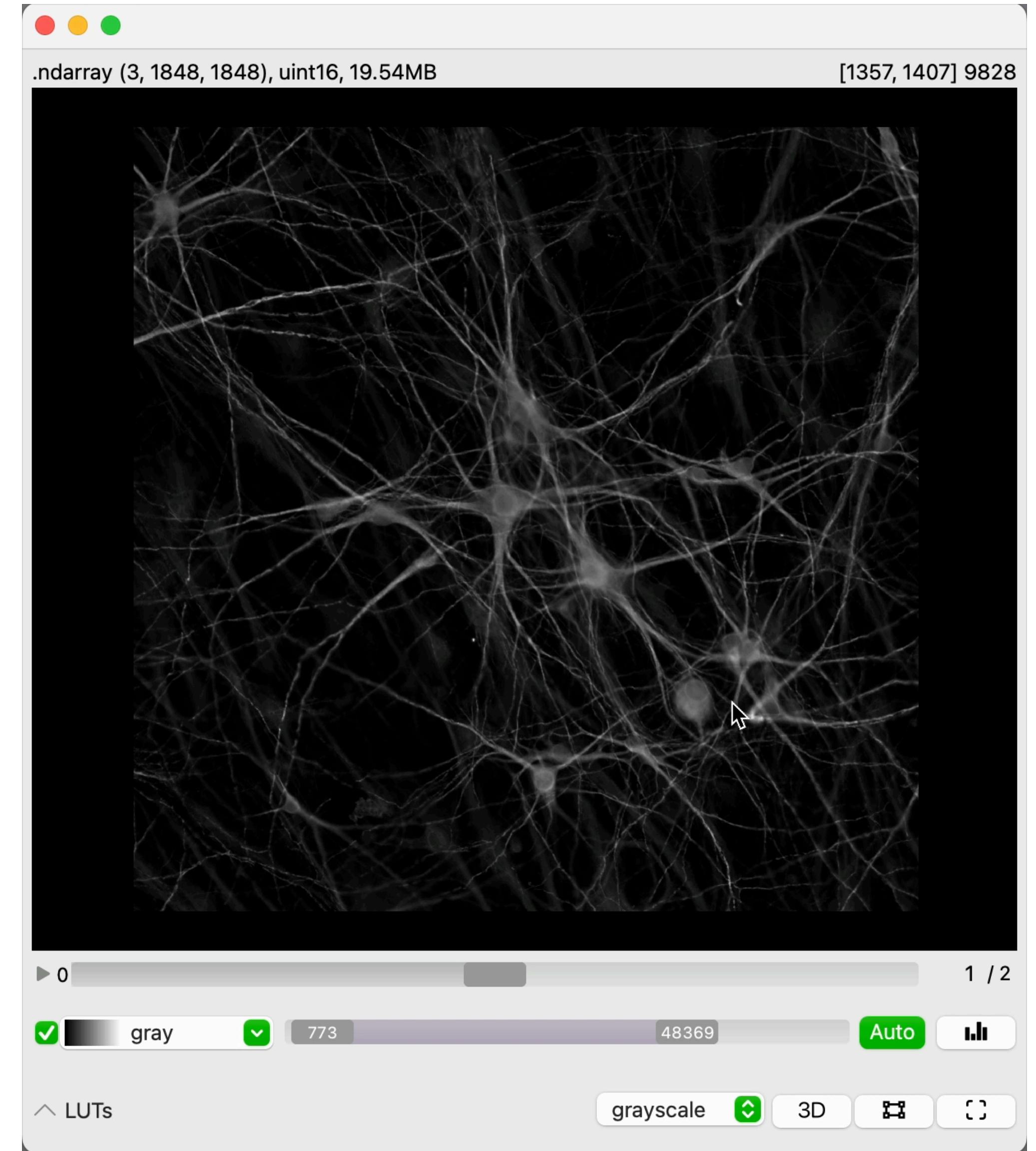
**LUT** = how the grey values are displayed

LUTs do not change the pixel values





# Display your Images - Brightness & Contrast



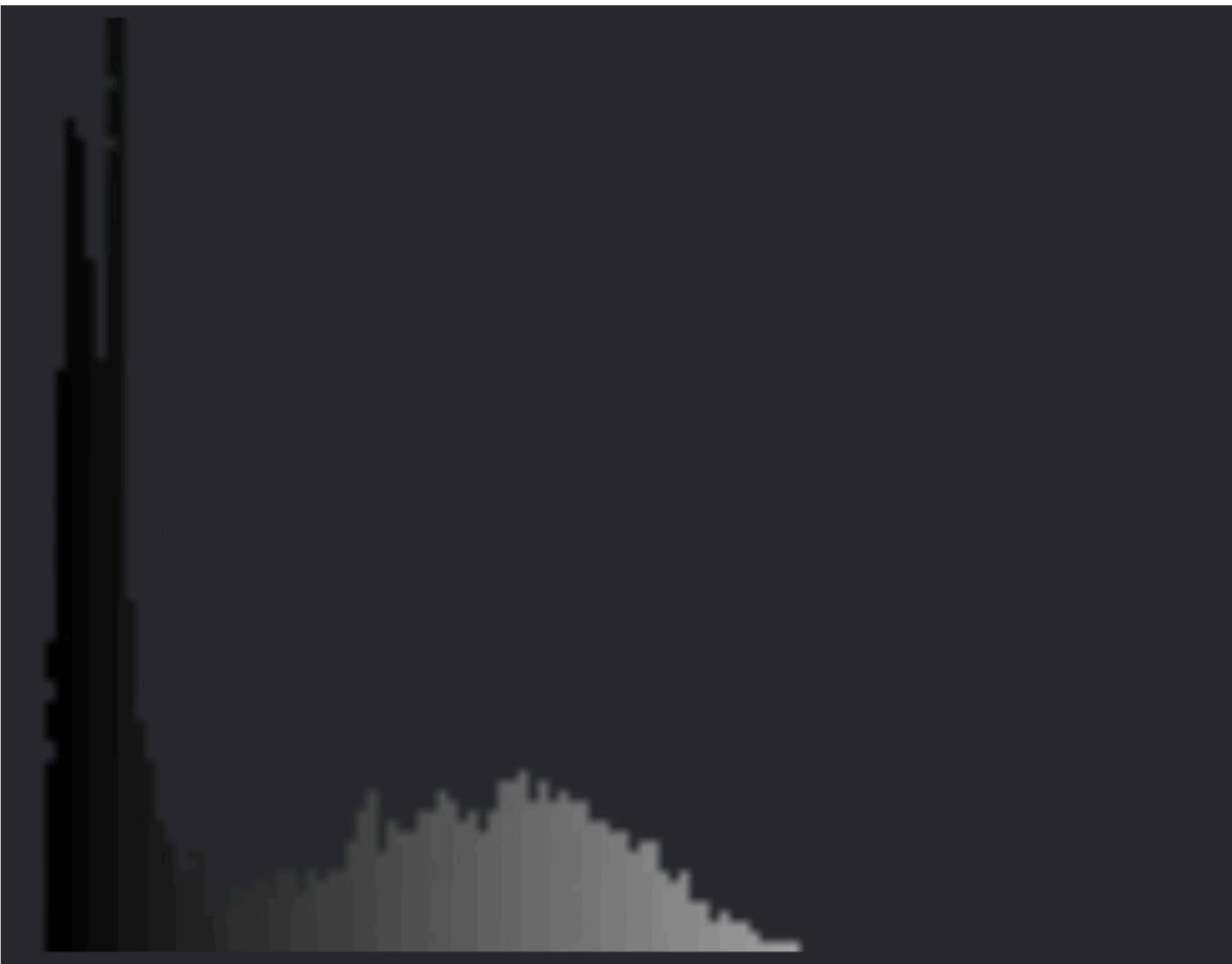


# Histogram

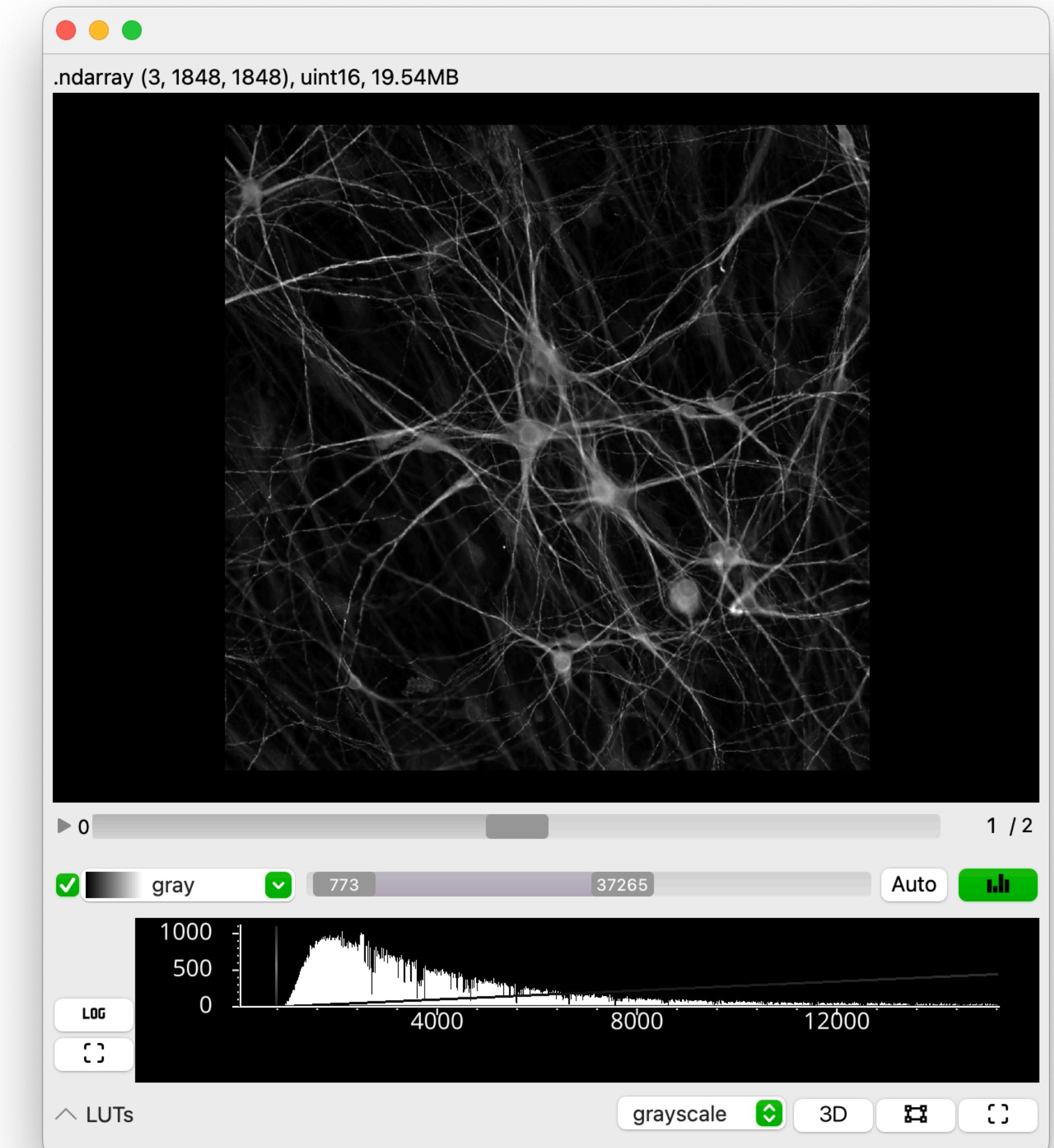


jaehyuk-lee: <https://jaehyuk-lee.com/animated-image-histogram/>

Pixel Count



Pixel Values

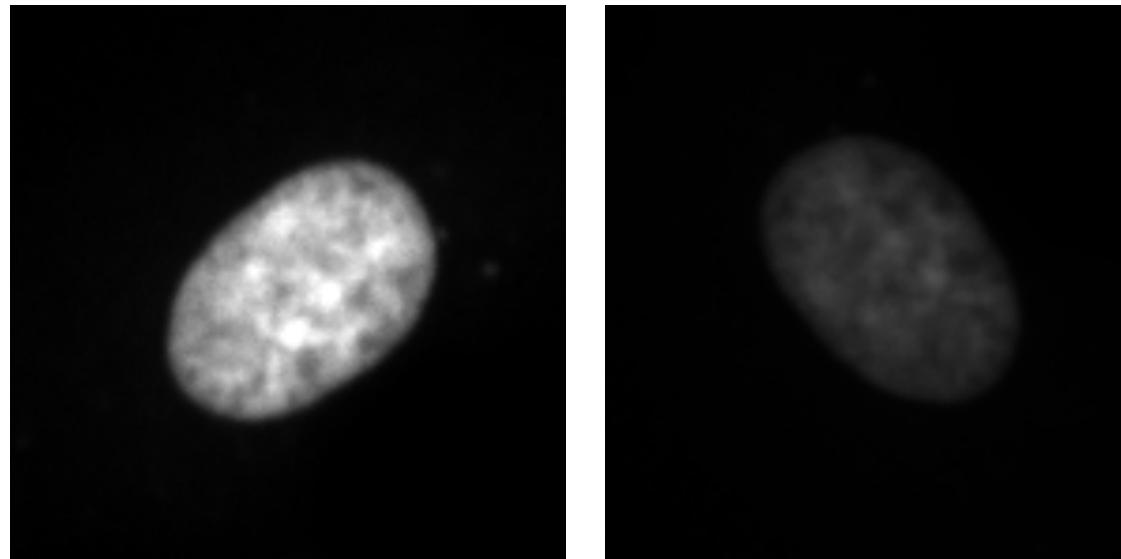




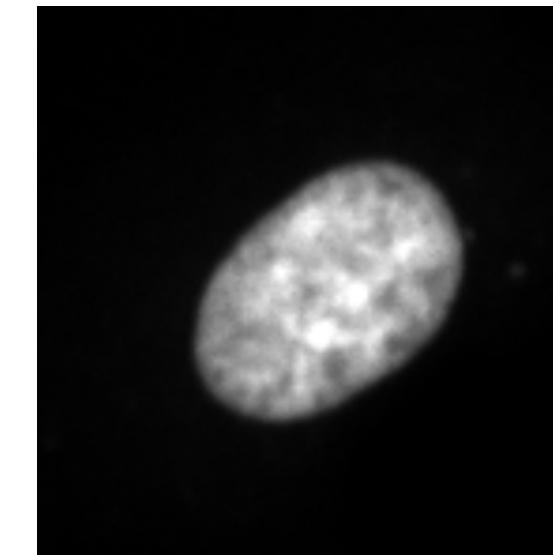
# Display your Images - Brightness & Contrast



Which image has more fluorescence?



Mean:	<b>4803</b>	<b>4803</b>
Display range:	188- <b>16828</b>	188- <b>45514</b>



Mean:	<b>4803</b>	<b>4803</b>
Display range:	188- <b>16828</b>	188- <b>16828</b>

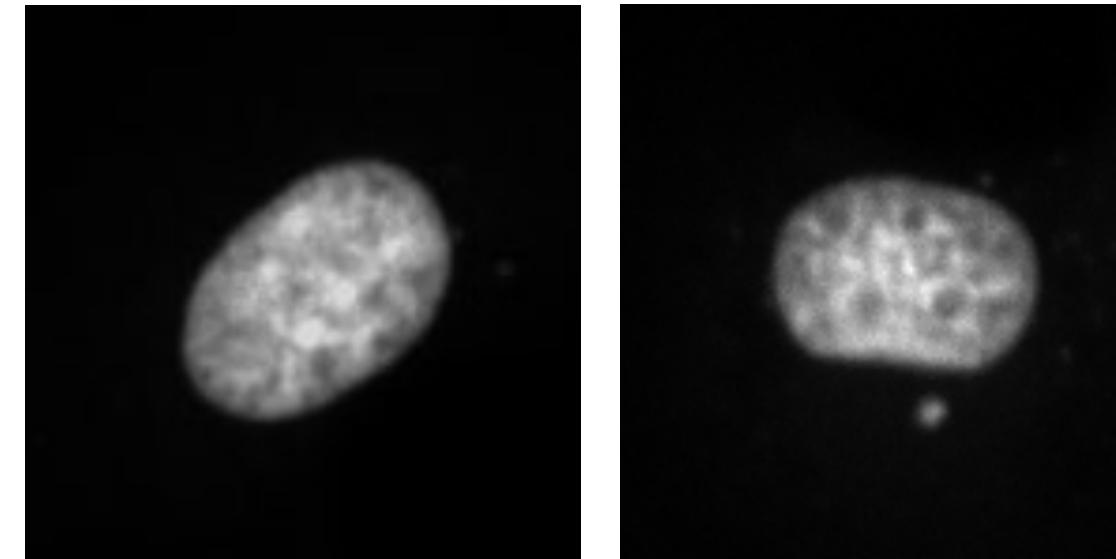




# Display your Images - Brightness & Contrast



Which image has more fluorescence?

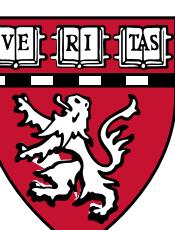


Mean:	<b>4803</b>	<b>2074</b>
Display range:	188- <b>19540</b>	112- <b>7768</b>

Do NOT trust your eyes,  
rely on numbers!



Mean:	<b>4803</b>	<b>2074</b>
Display range:	188- <b>19540</b>	188- <b>19540</b>





# Images and Colors - Lookup Tables (LUTs)

*LUT = how the grey values are displayed*

*LUTs do not change the pixel values*

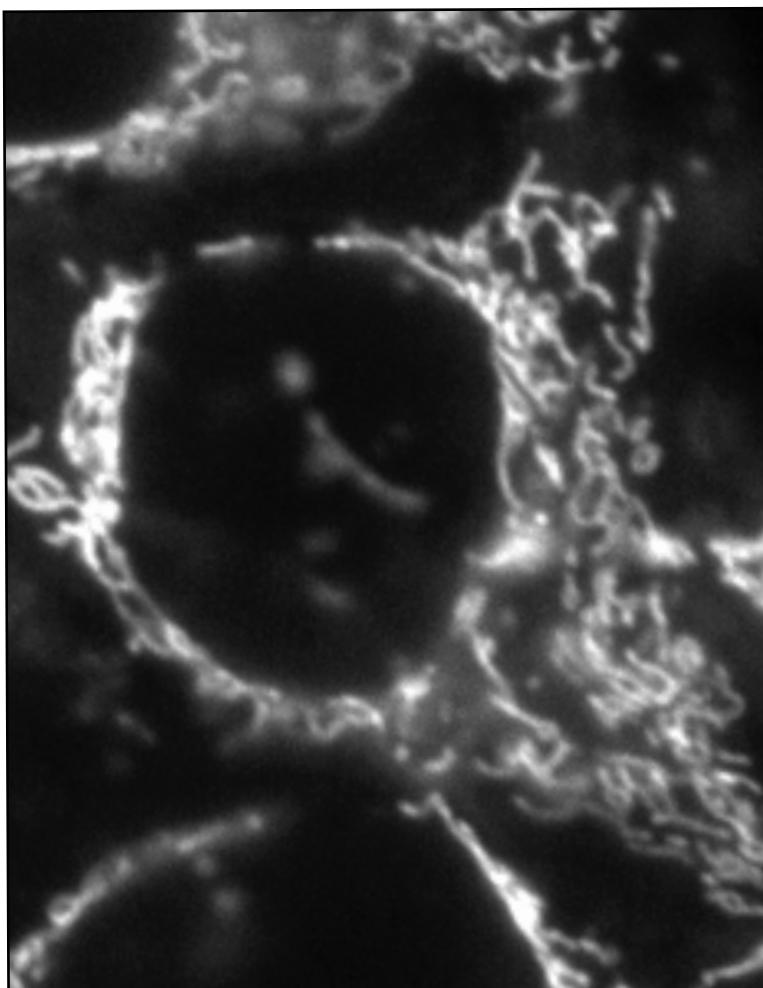


Image (8 bit)	Displayed color
0	
1	
...	
100	
...	
255	

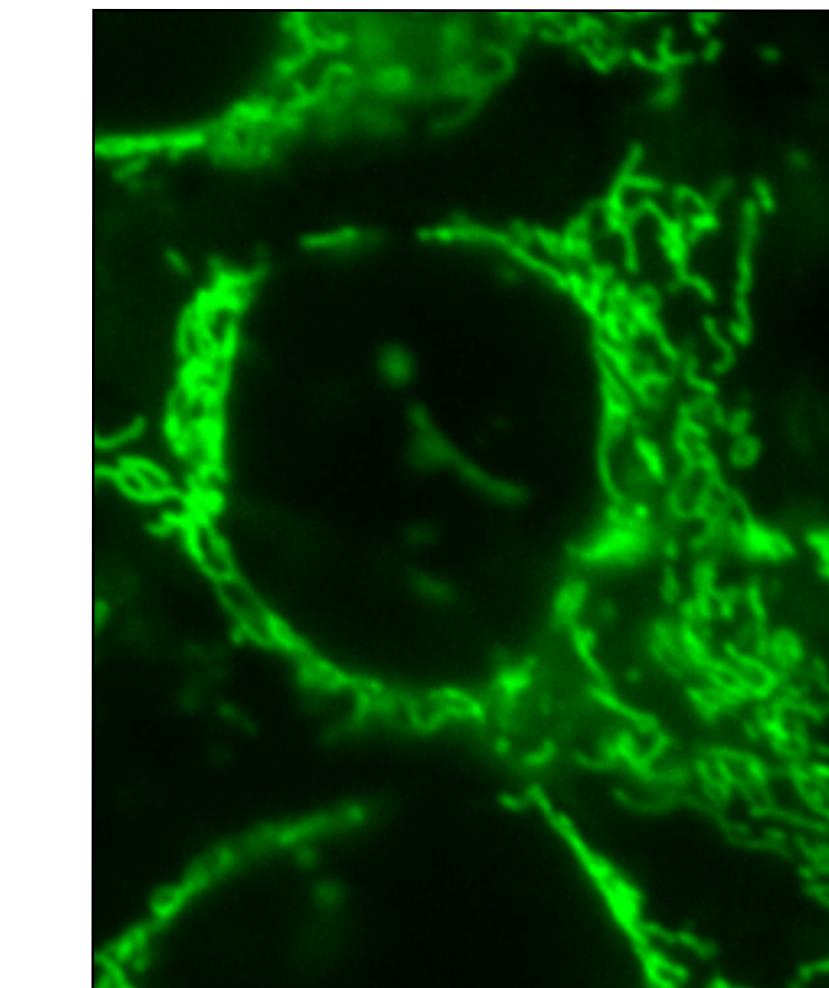
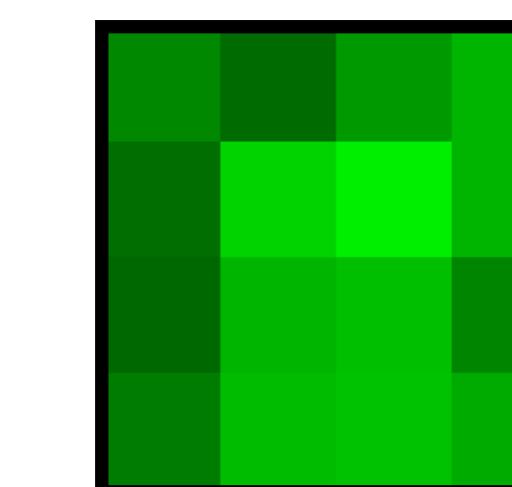


Image (8 bit)	Displayed color
0	
1	
...	
100	
...	
255	



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



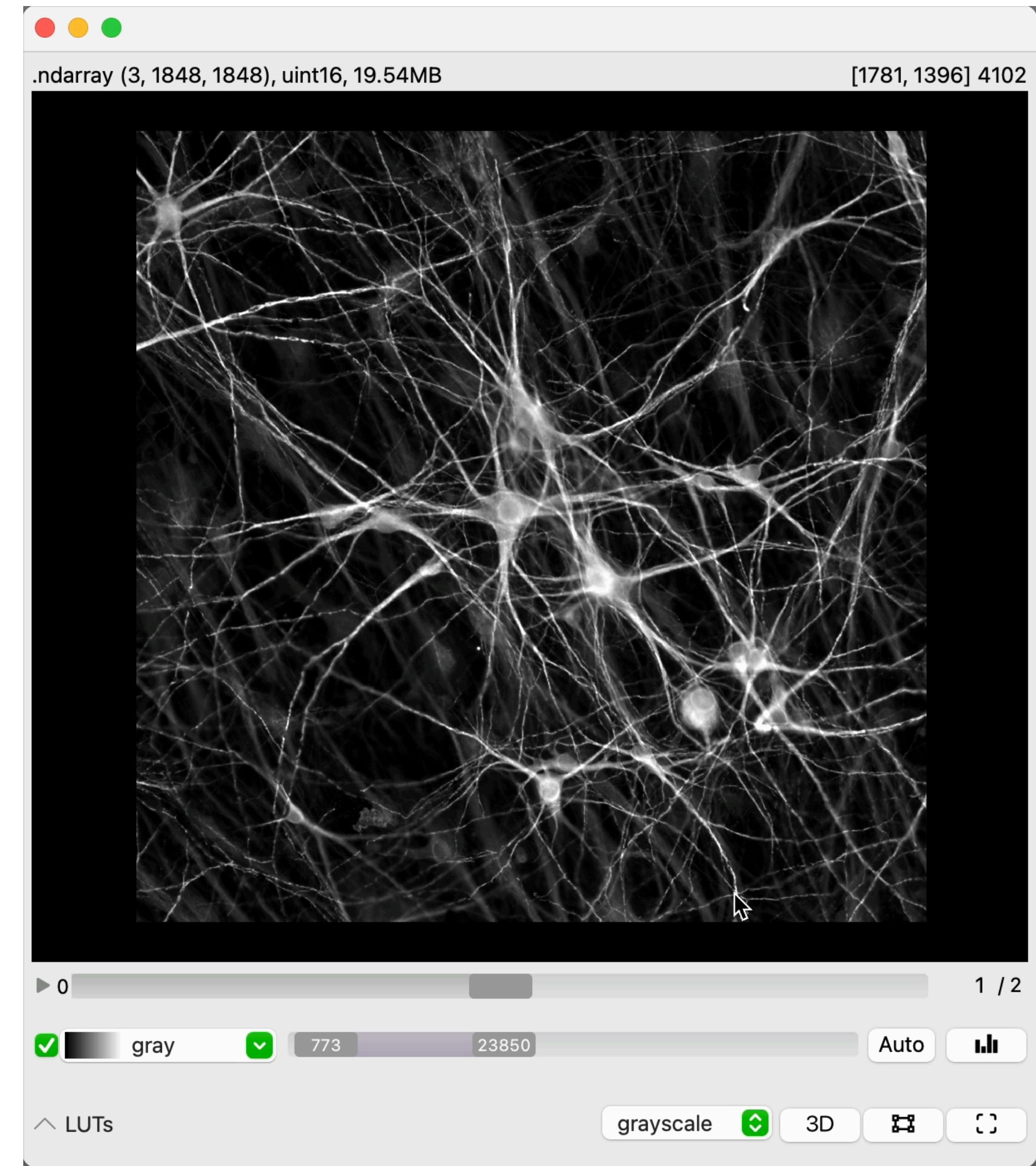
=

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





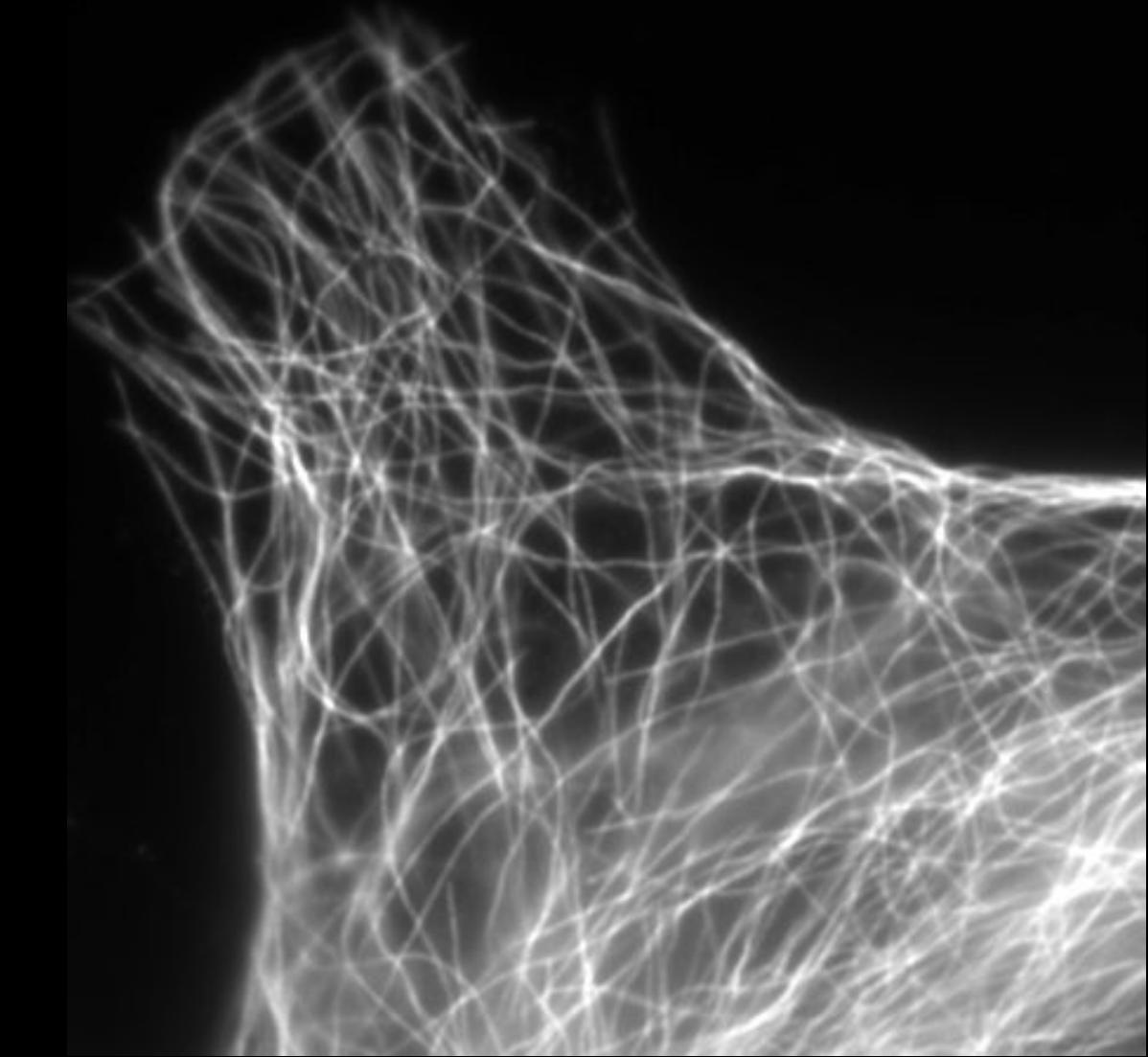
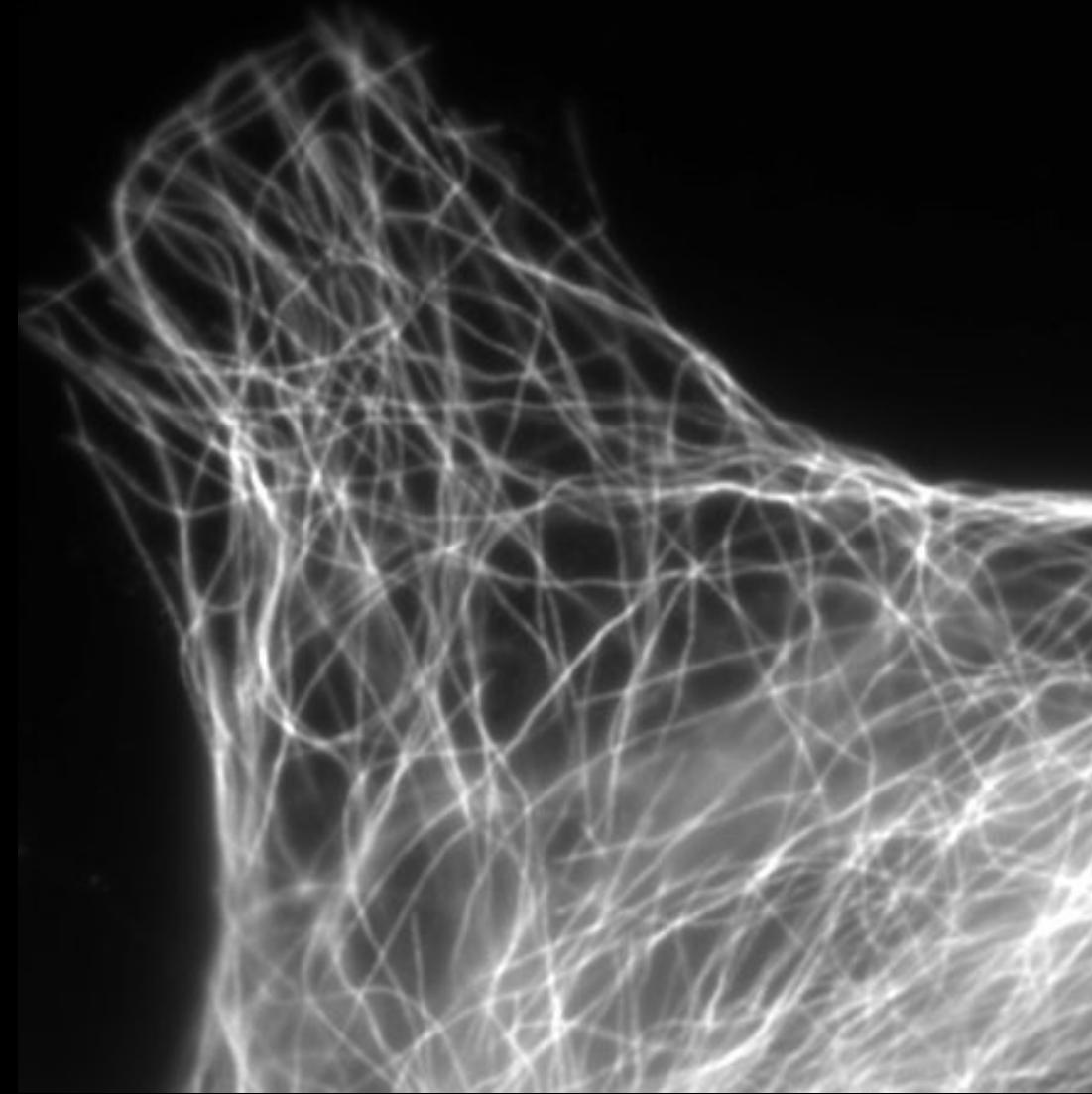
# Images and Colors - Lookup Tables (LUTs)





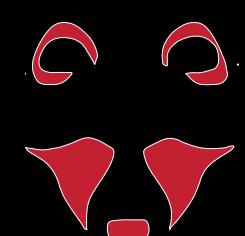
# Images and Colors - Choose the right LUT

Which is brighter?



The human eye evaluates intensity best in grayscale

If you are imaging for example a blue fluorophore, you are **NOT FORCED** to display it in blue!

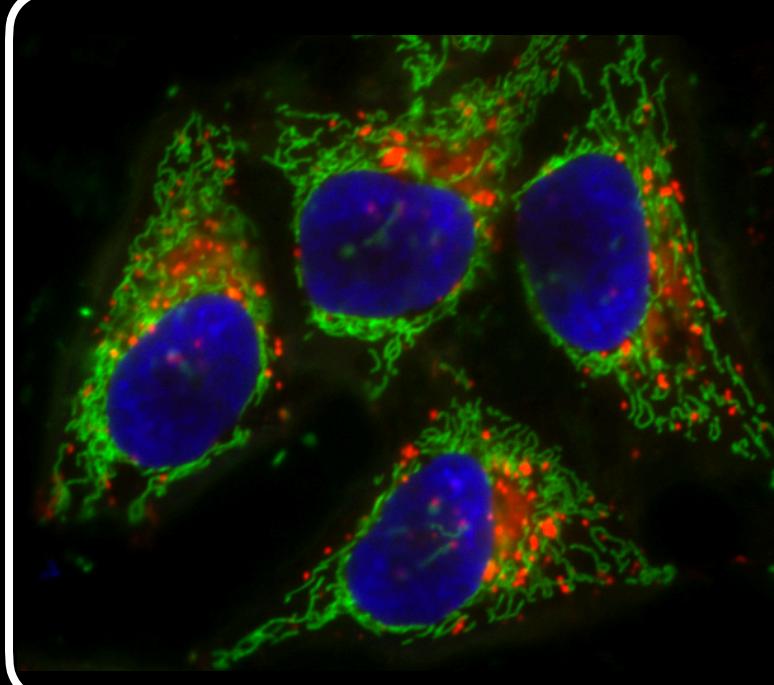




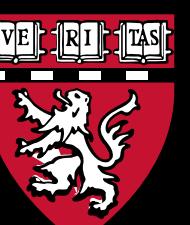
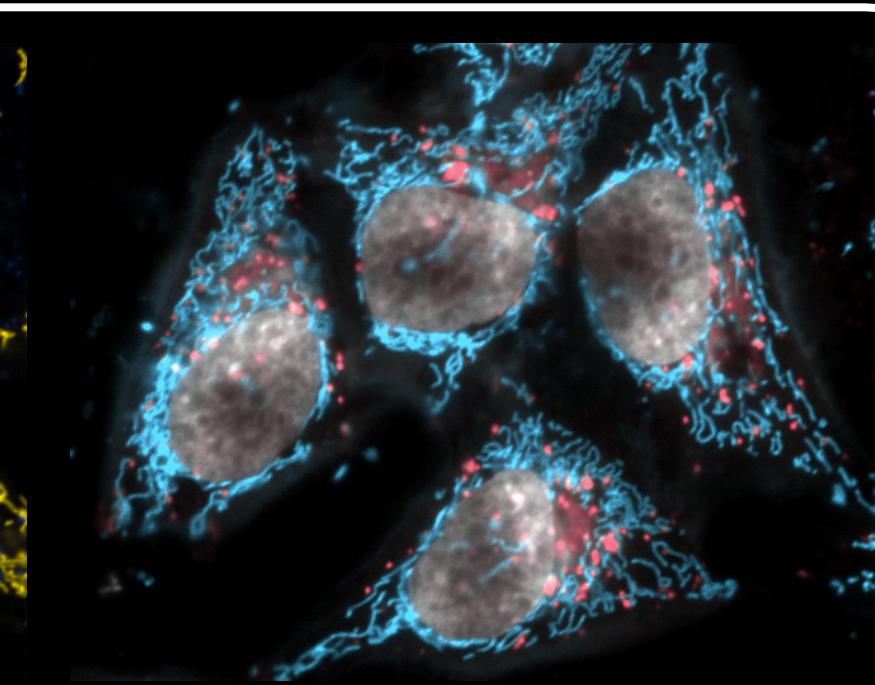
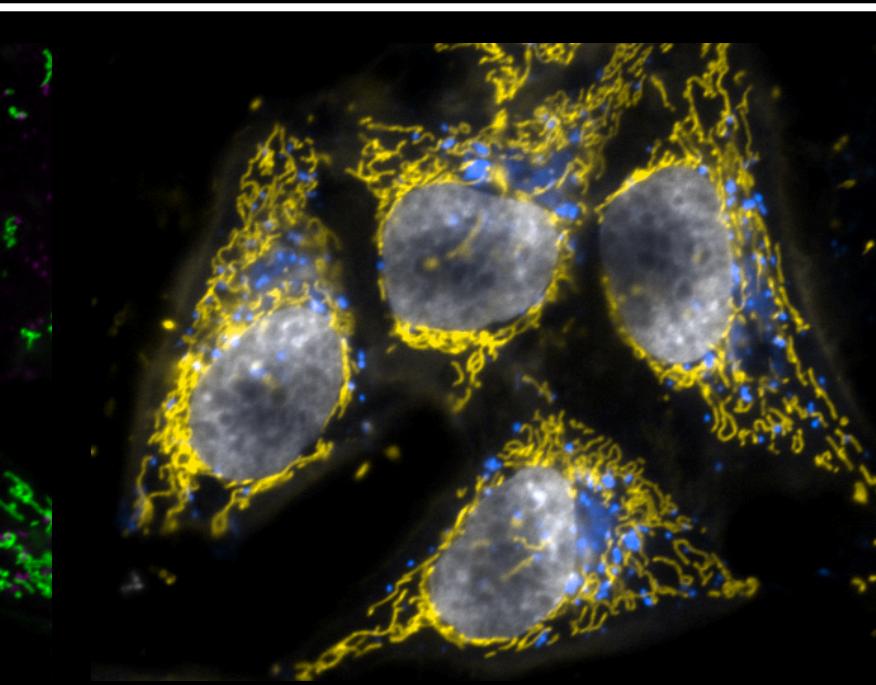
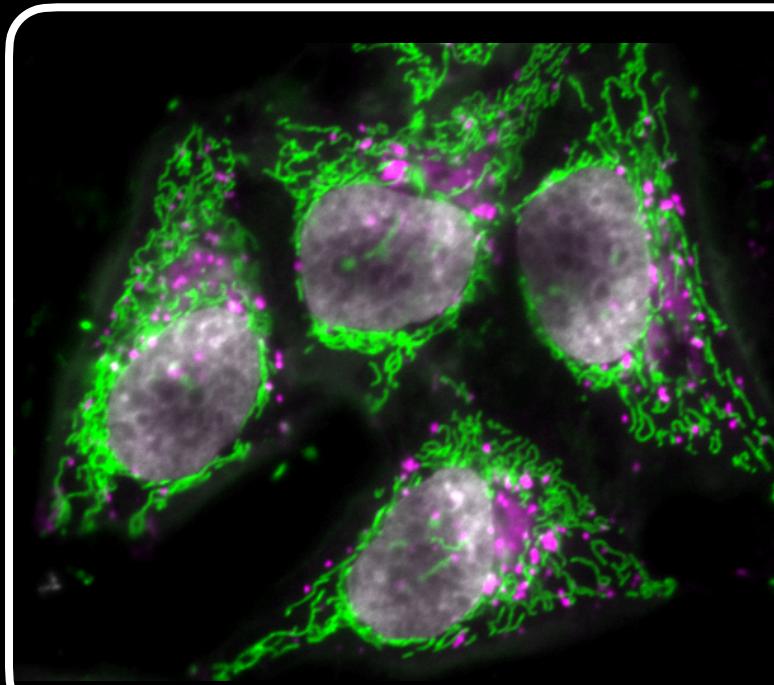
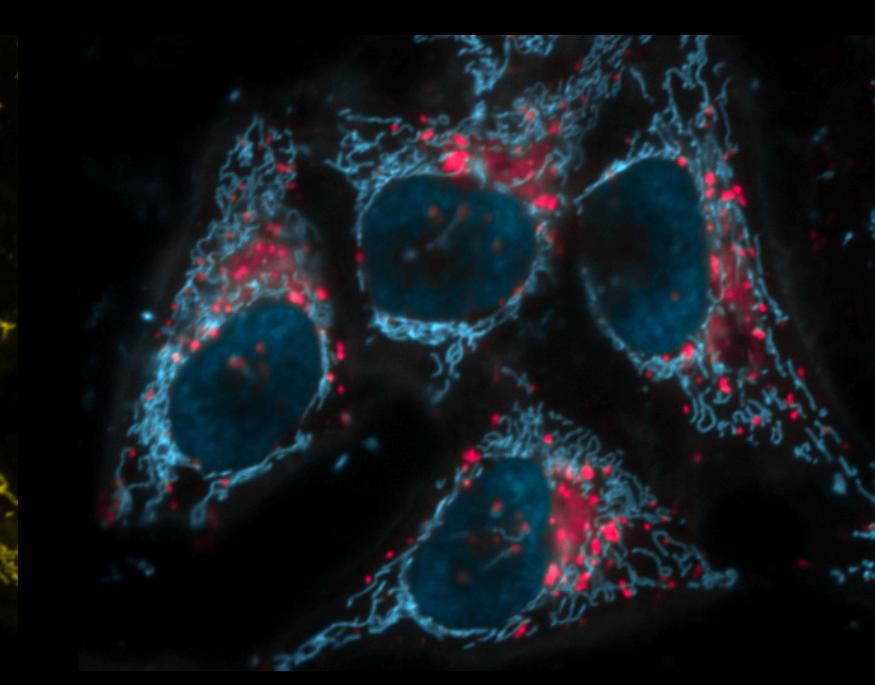
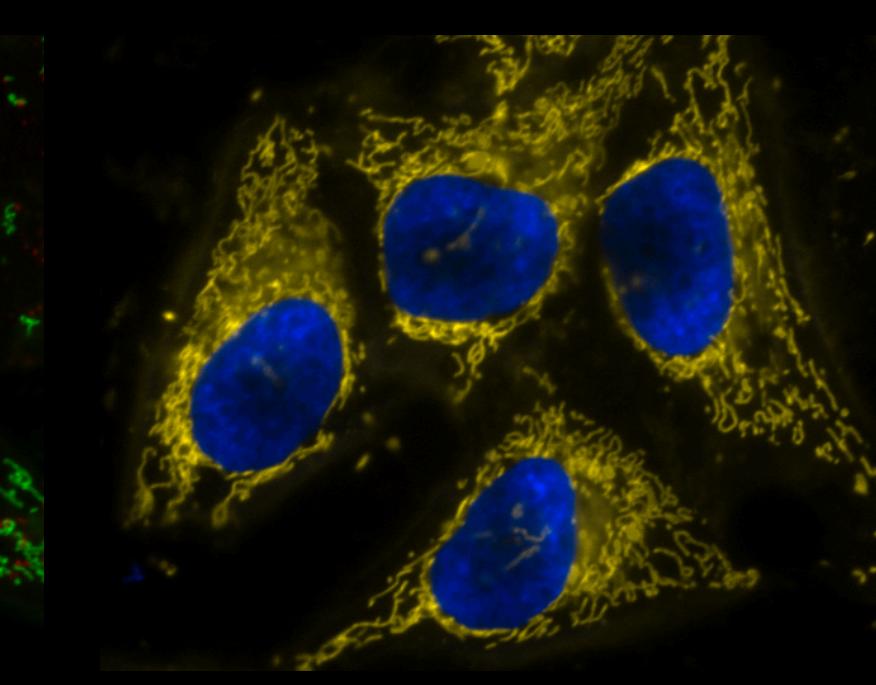
# Images and Colors - Choose the right LUT

Color blind people don't distinguish some colors

Protanope (no red)

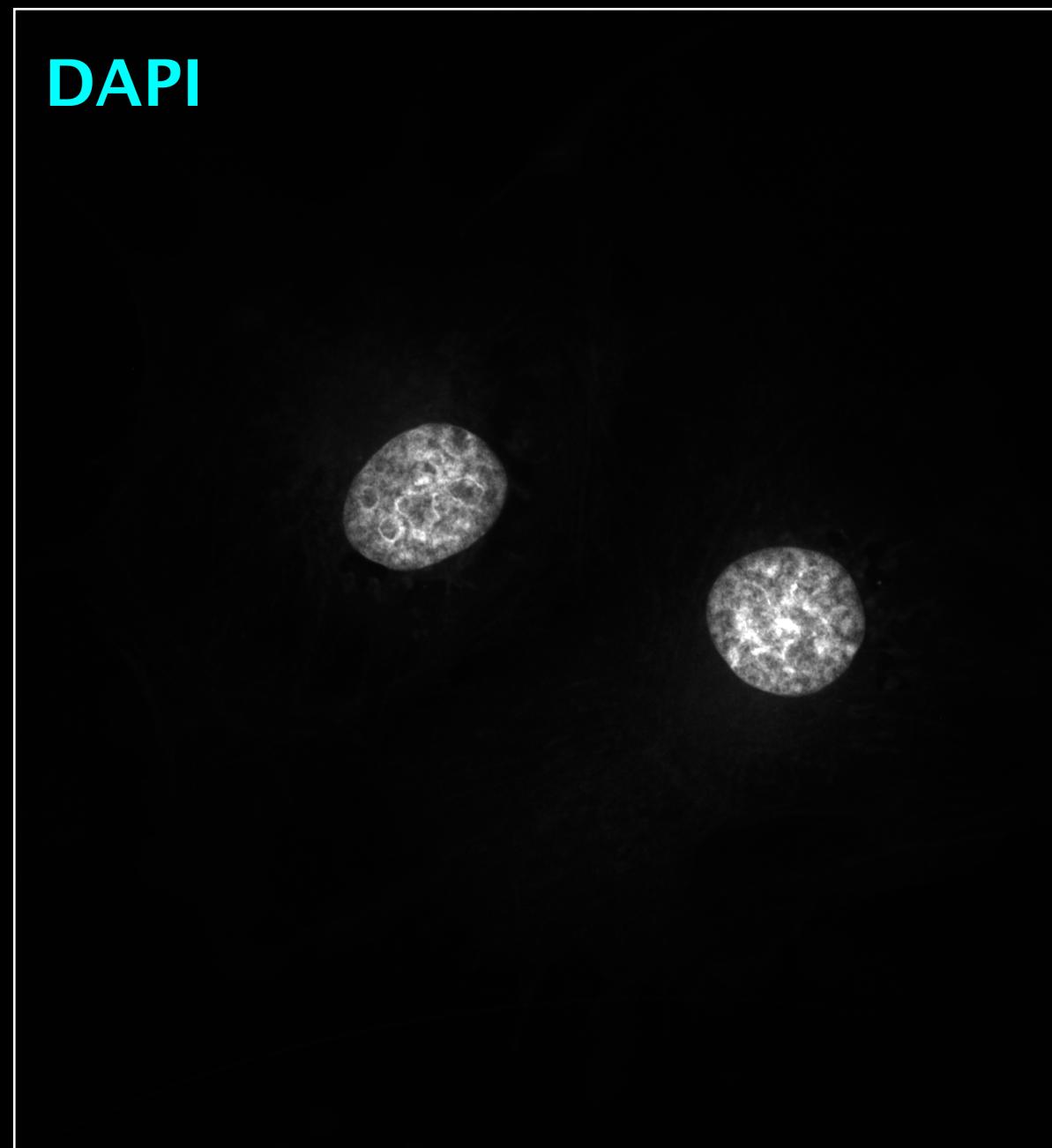


Tritanope (no blue)

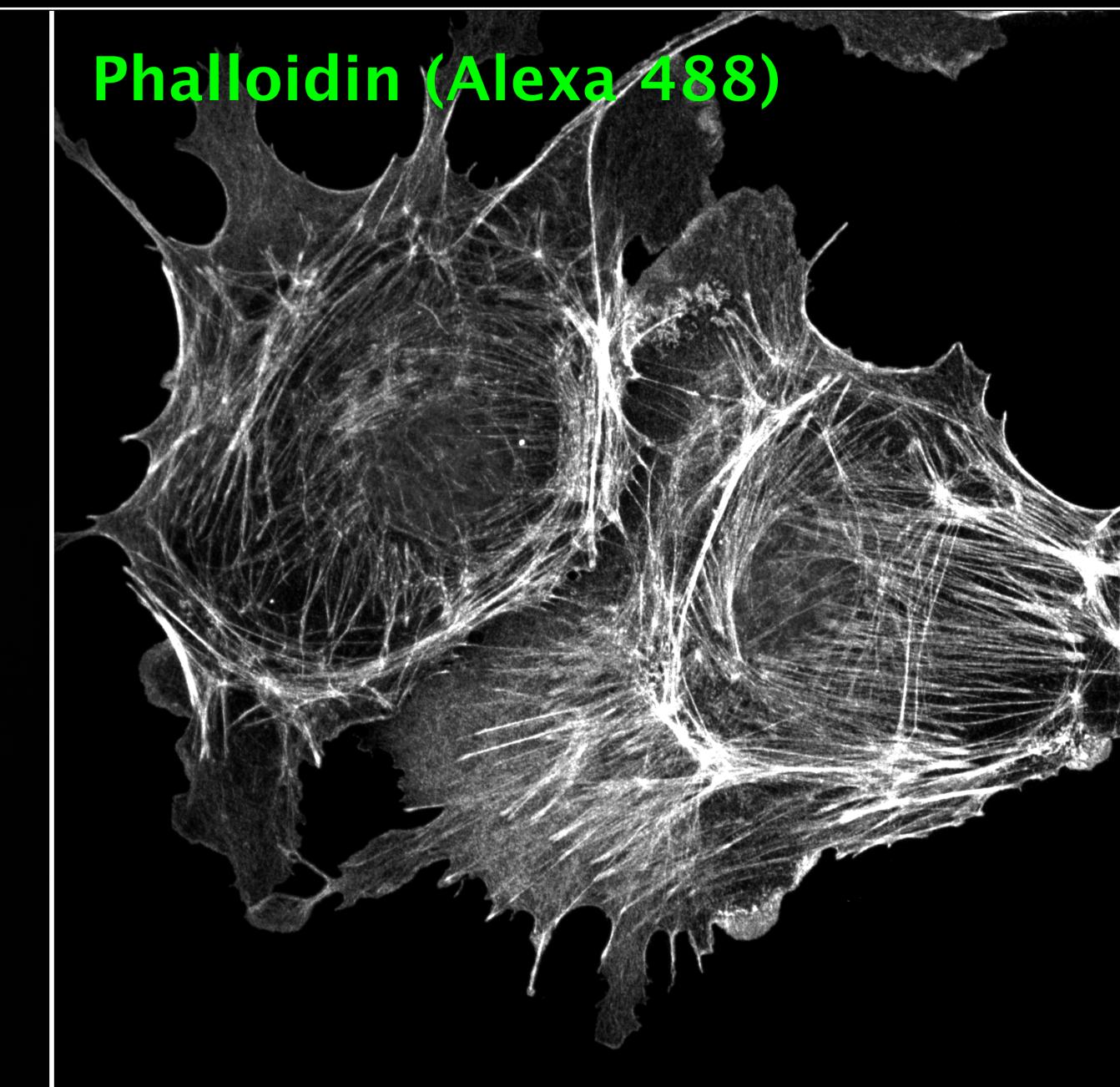




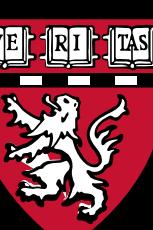
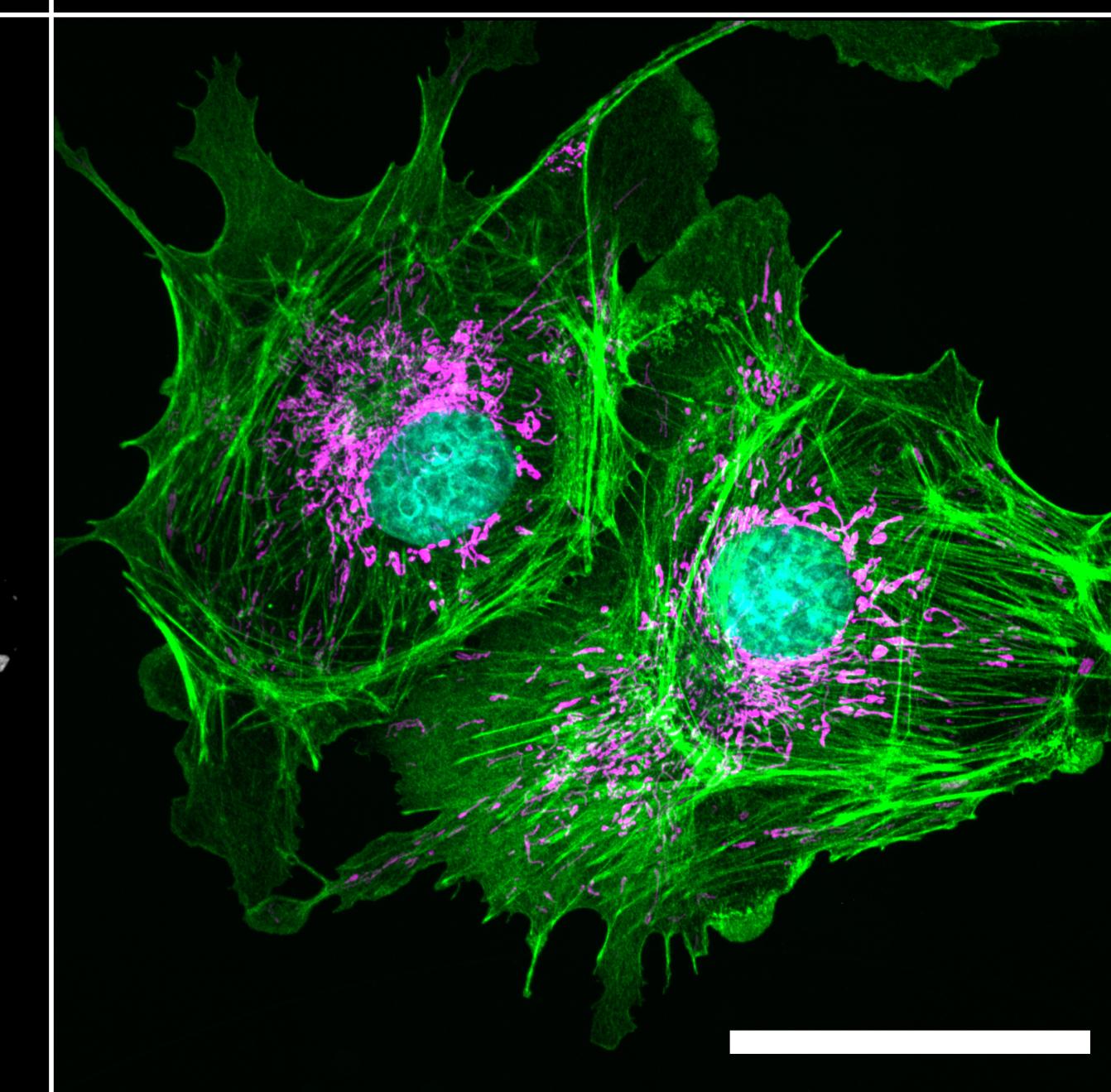
DAPI

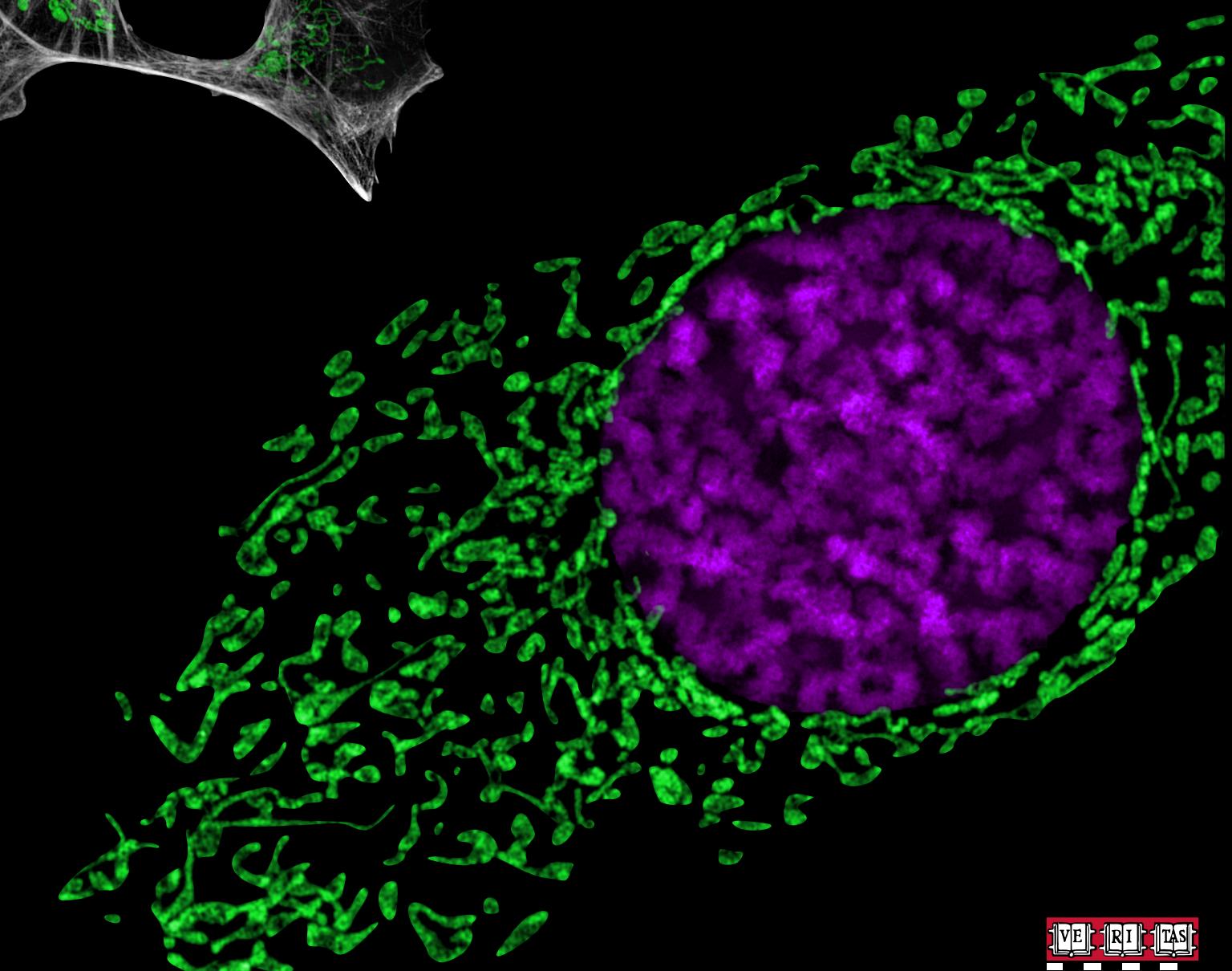
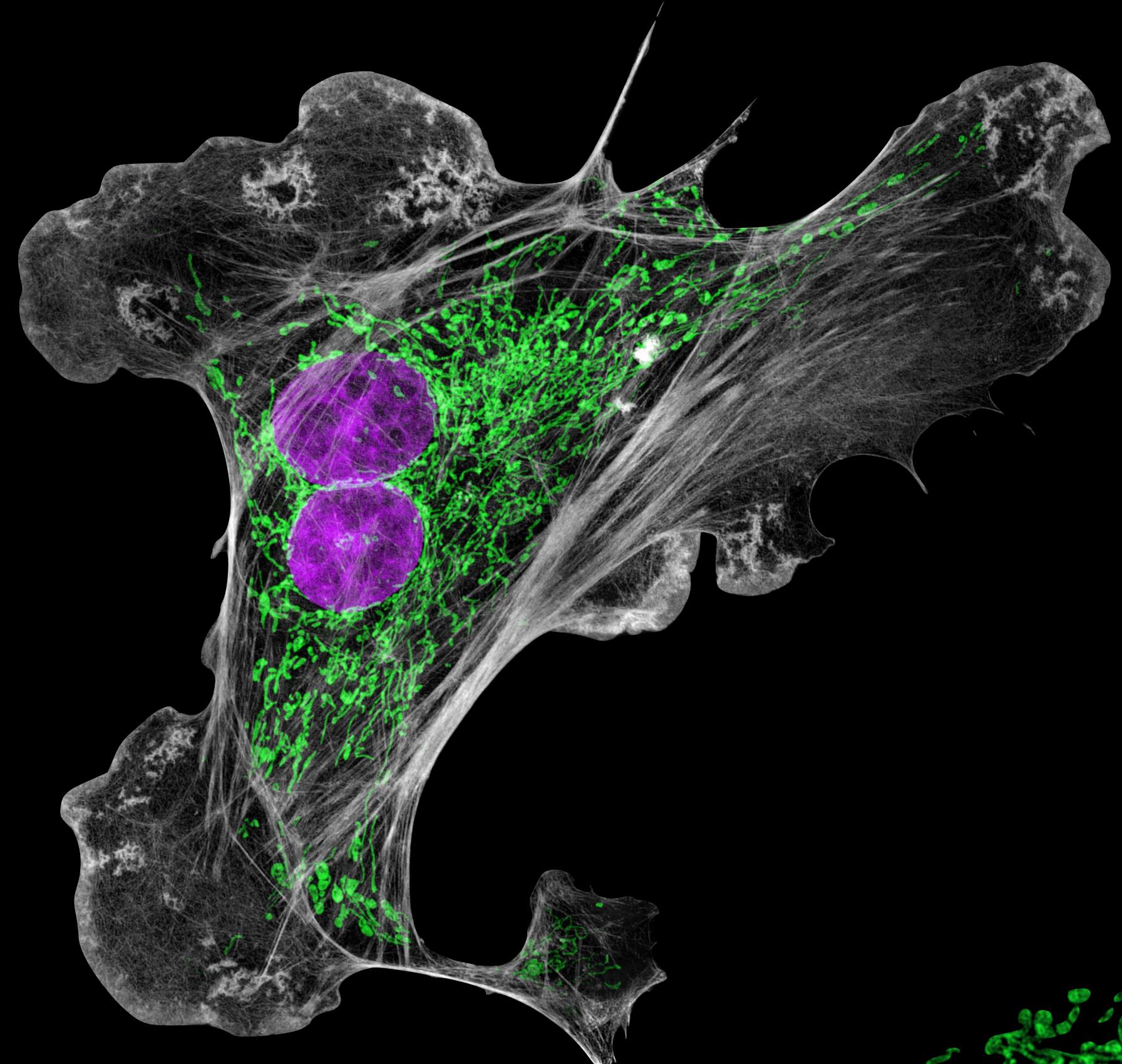
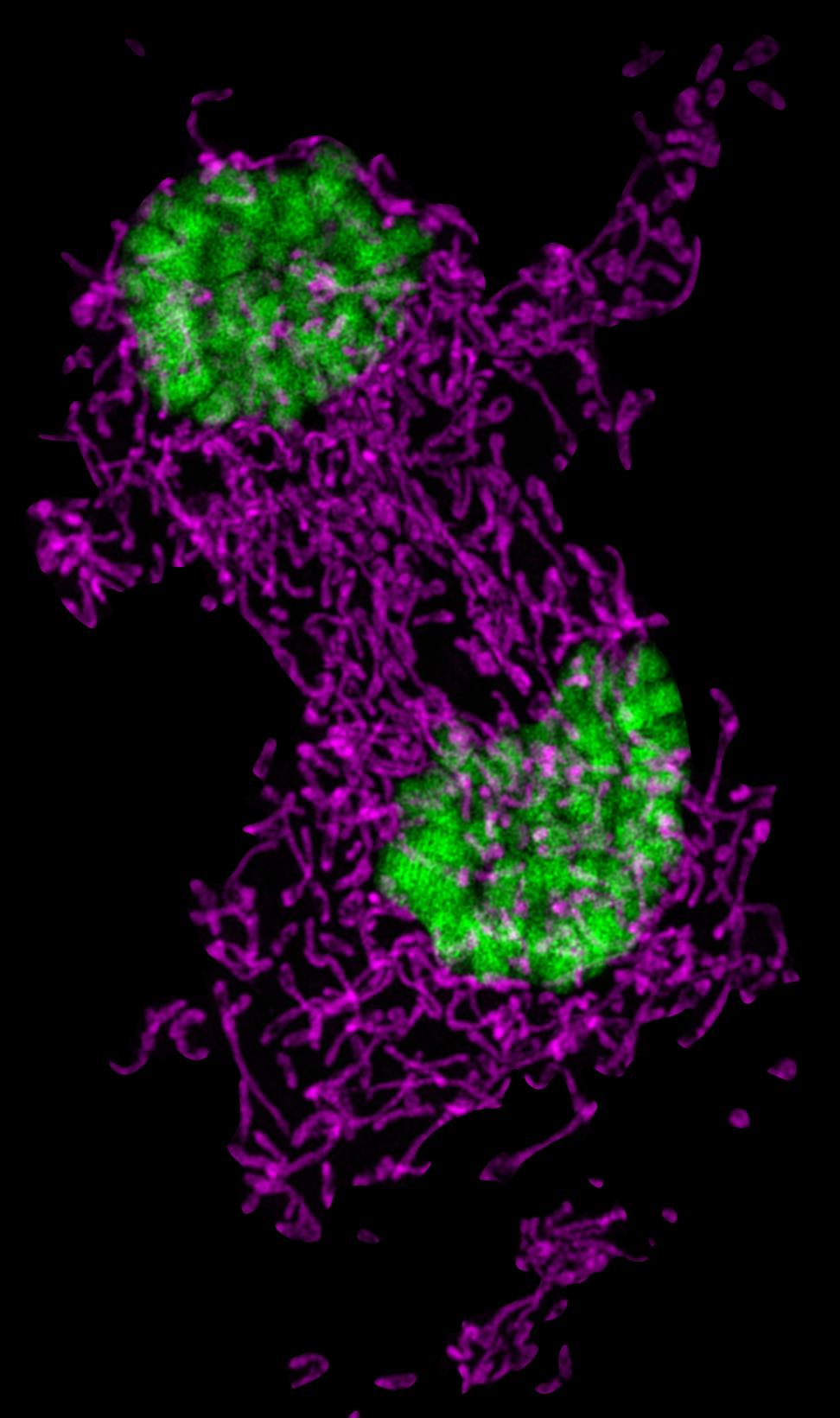
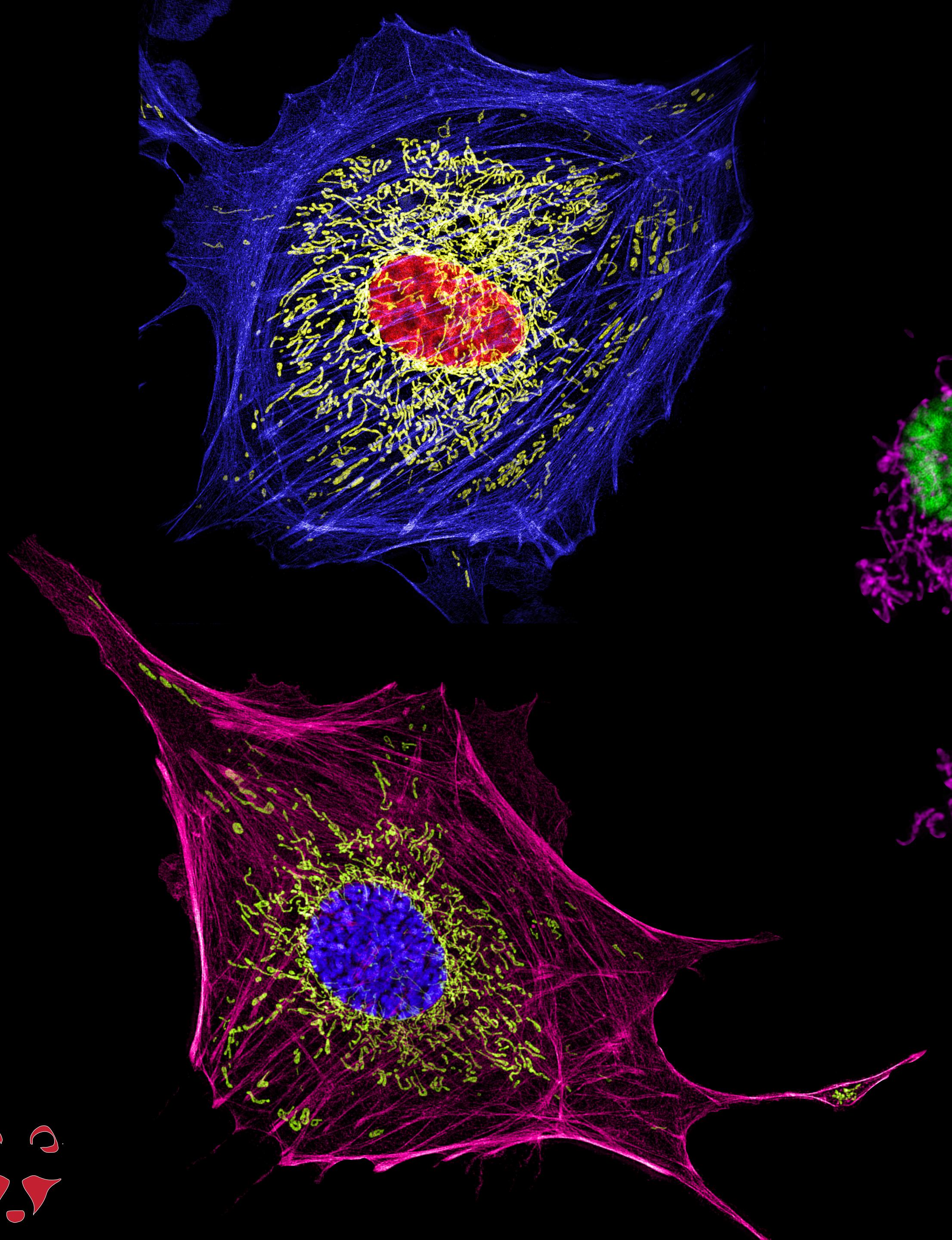


Phalloidin (Alexa 488)

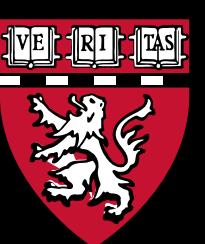


MitoTracker Red





Talley Lambert





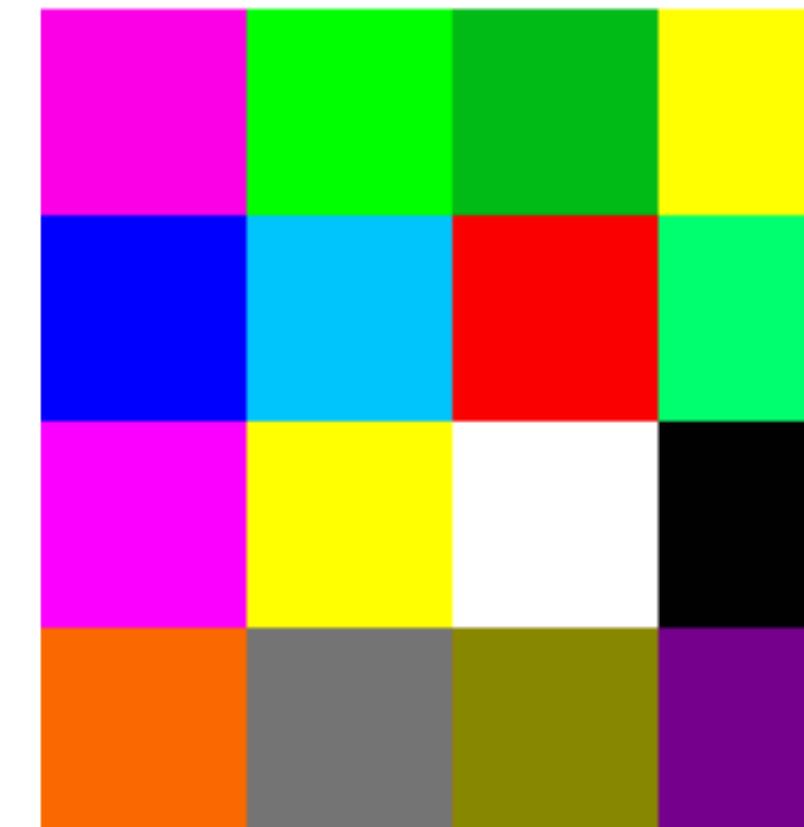
# Images and Colors



## RGB Images (still matrix of numbers)

LUTs **cannot** be applied to RGB Images

*RGB image*



=

255	0	0	255
0	255	186	255
234	0	28	1
0	1	255	0
0	199	0	255
255	255	0	111
255	255	255	0
0	255	255	0
255	1	255	0
254	117	141	118
105	117	137	0
0	117	0	143

**RGB Color** image (e.g. jpeg, png) = **R**ed + **G**reen + **B**lue

**RGB Color** image = **8 bit Red, 8 bit Green, 8 bit Blue** = **R (0-255), G (0-255), B (0-255)**



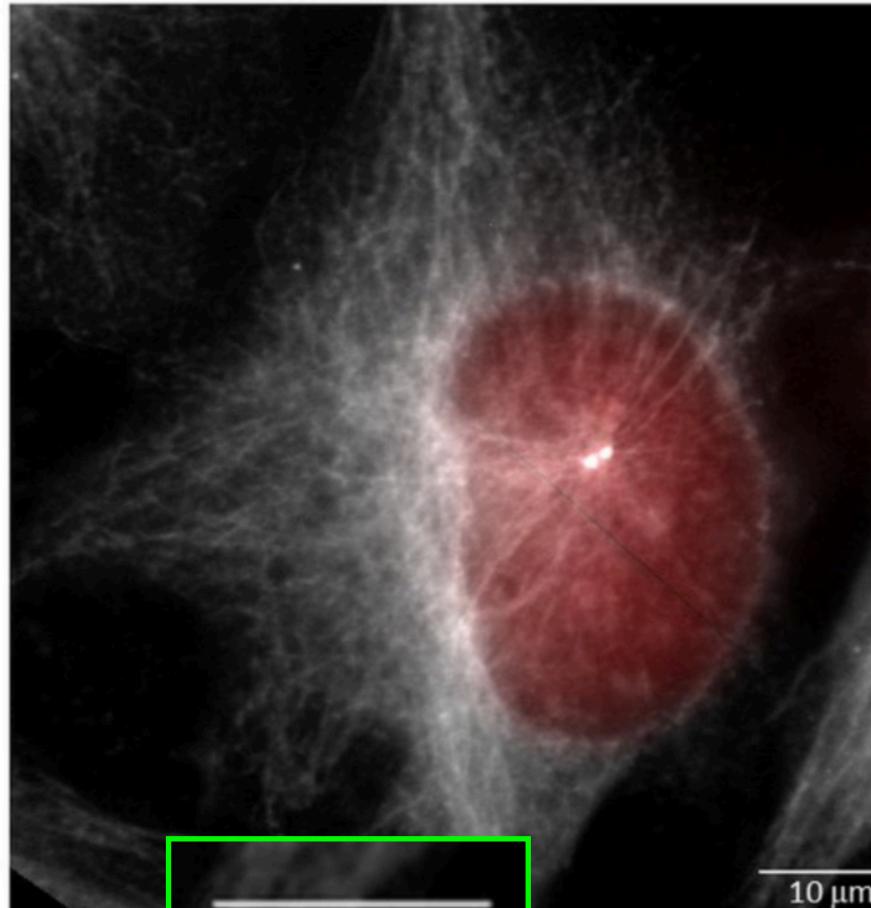


# Pixel Size & image Scale

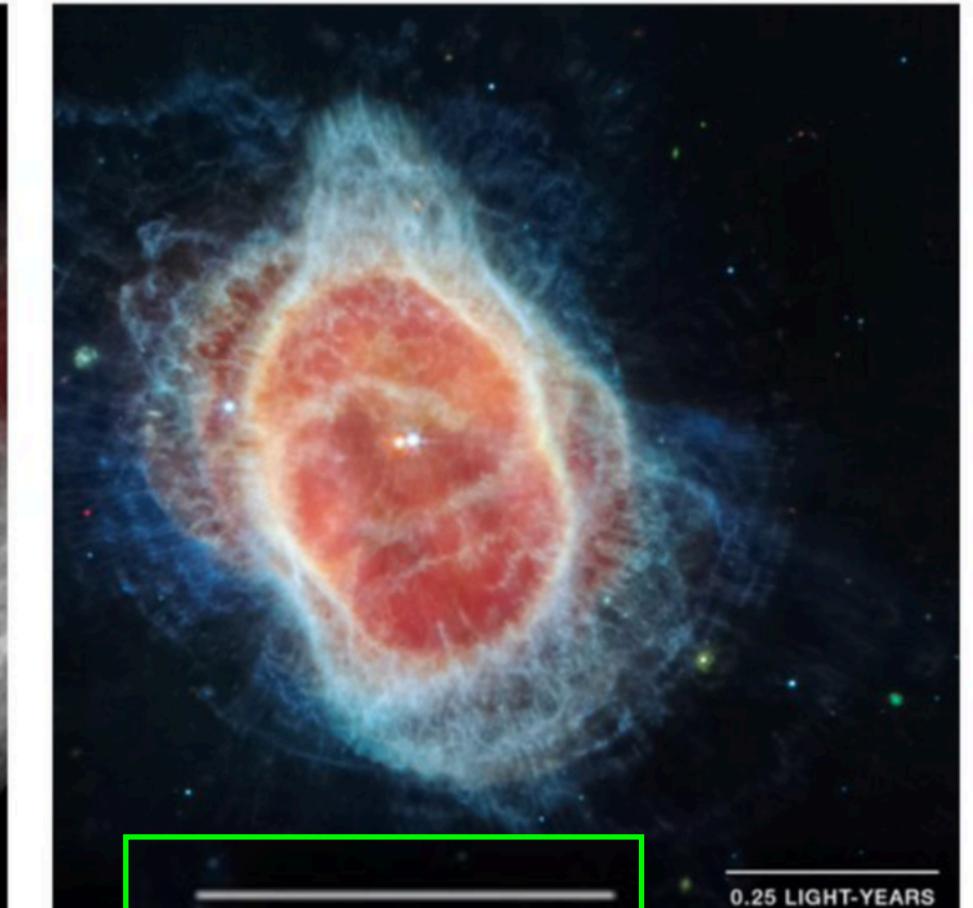


 Laurence Haren  
@HarenLaurence ...

never forget the scale bar! [@StearnsLab](#)  
when biology meets astronomy: cell vs nebula,  
centrosome vs dying star! [@EtienneKlein](#)



10  $\mu\text{m}$



0.25 LIGHT-YEARS





# Pixel Size & image Scale



What if the pixel size is not stored in the metadata?

If you know the **magnification** and the **camera** you used for the acquisition,  
you can estimate the image pixel size.

**image pixel size = camera pixel size / magnification**

**Example:**

Magnification = 100x Objective

Camera = Hamamatsu Orca Flash 4

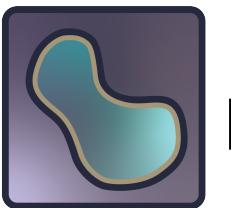
Product number	C13440-20CU
Imaging device	sCMOS
Cell (pixel) Size ( $\mu\text{m}^2$ )	6.5×6.5
Pixel Array (horizontal by vertical)	2048×2048
Effective Area (horizontal by vertical in mm)	13.312×13.312

**pixel width and height:  
 $6.5 \mu\text{m} / 100X = 0.065 \mu\text{m}$**

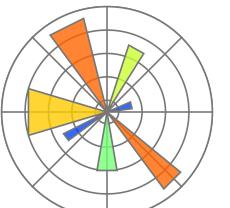




# Visualize Images in Python



napari



matplotlib



ndv

