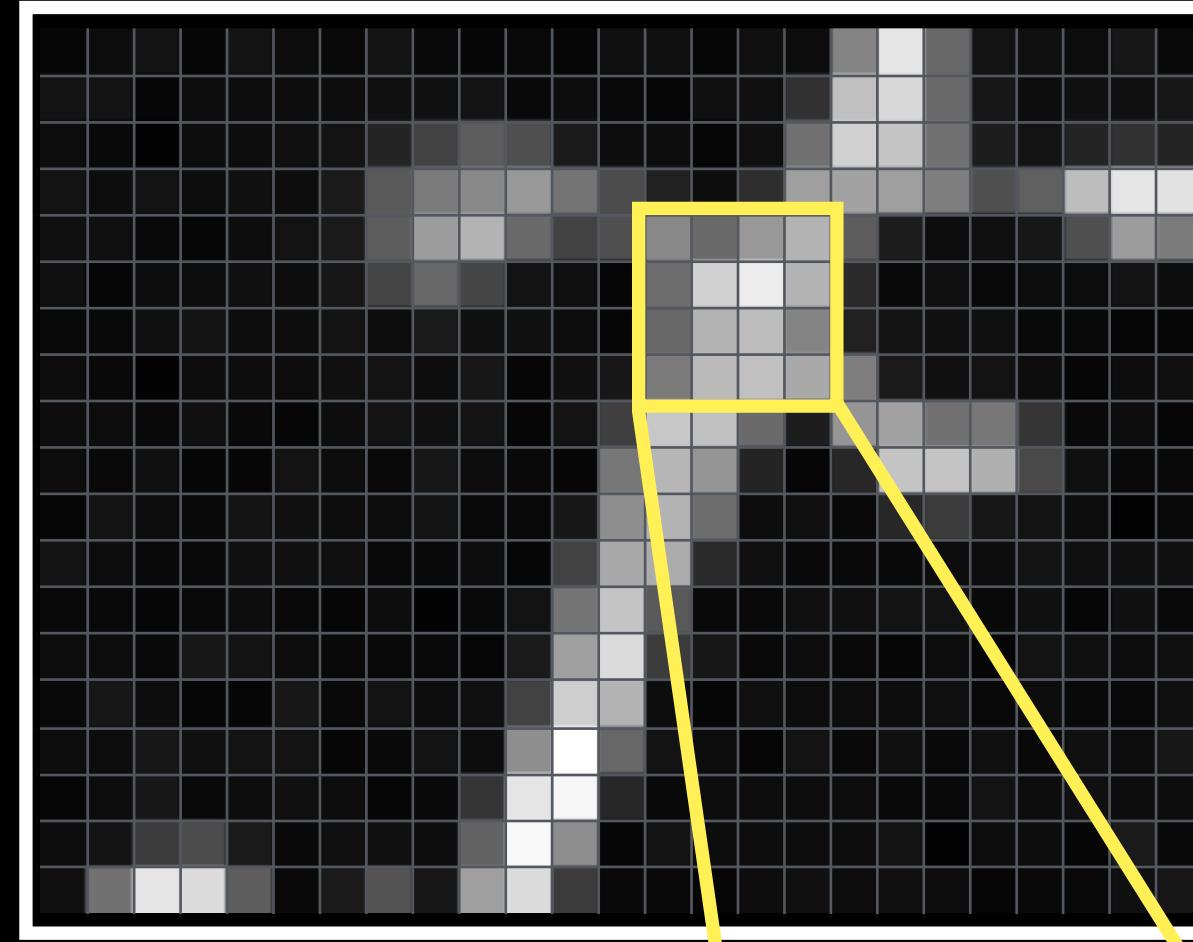




Introduction to Digital Images

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

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

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

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

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

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

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|-----|-----|-----|-----|
| 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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| | | | |
|-----|-----|-----|-----|
| 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 |

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

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

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

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

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|-----|-----|-----|-----|
| 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 |





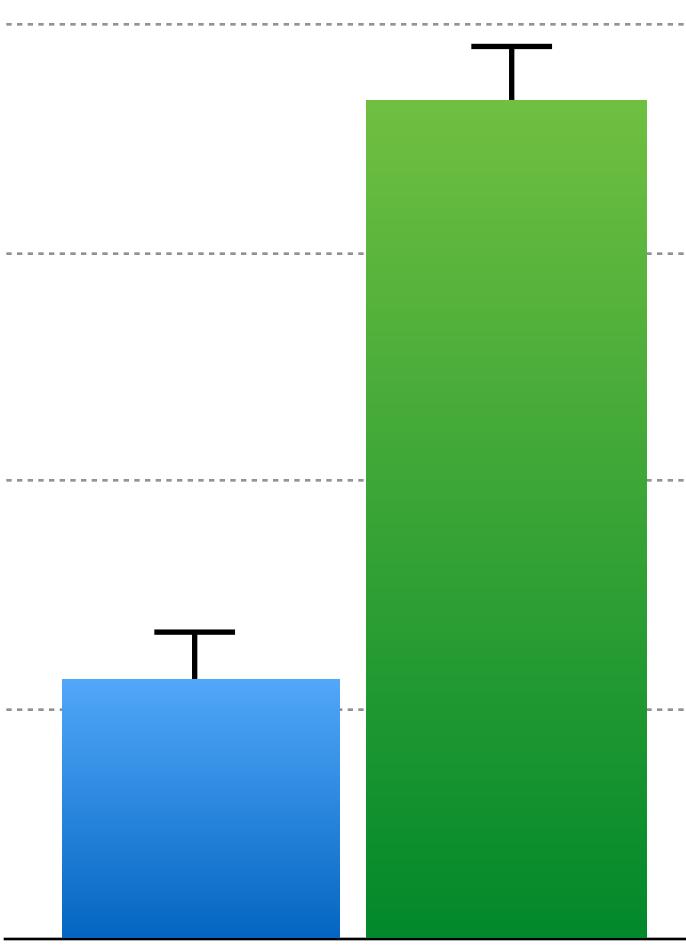
Introduction to Digital Images

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

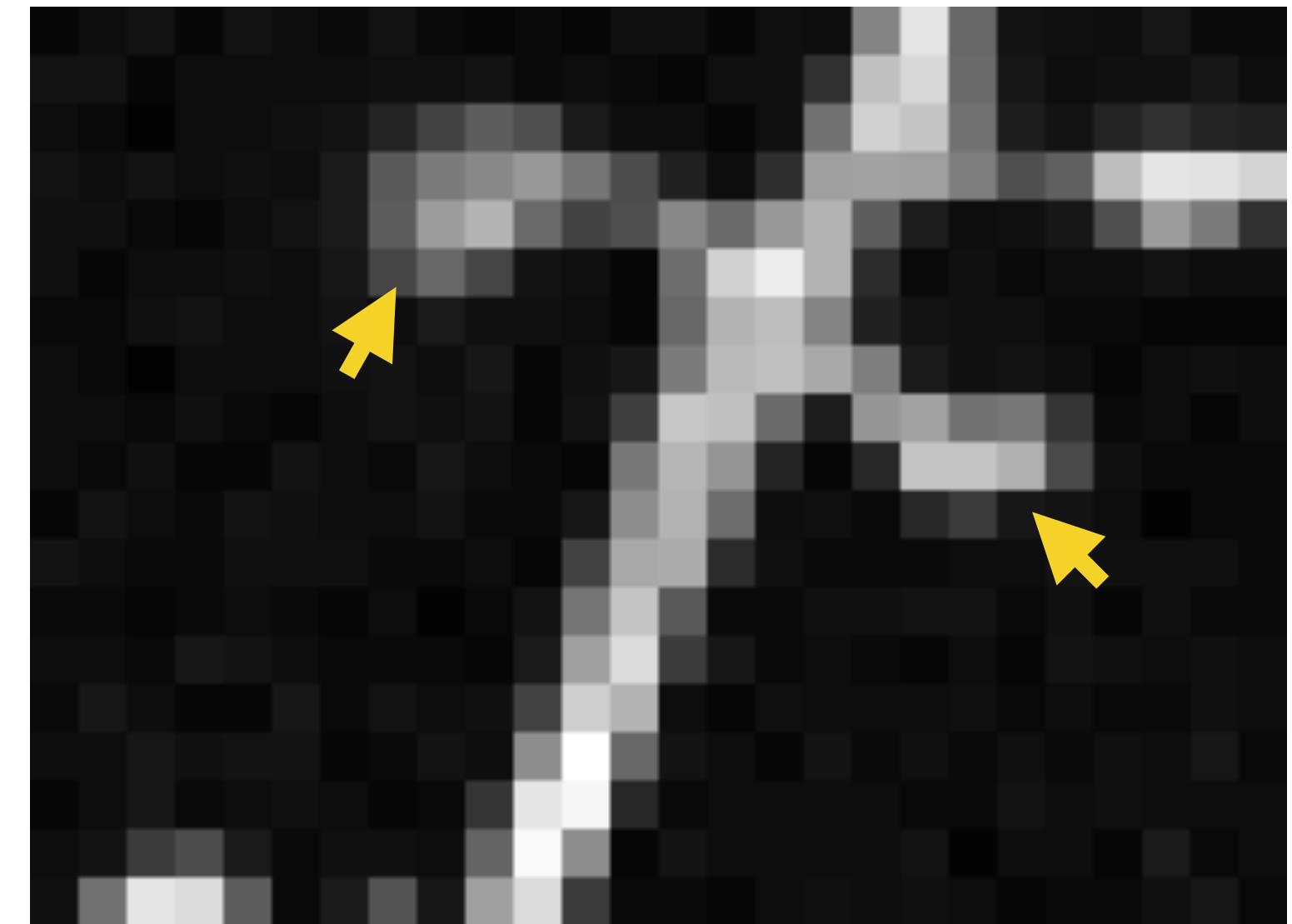
this is your **data**

| | | | | | | | | | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|-----|-----|-----|-----|
| 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 | 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 | 13 |
| 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 | 13 |

this is your **result**



this just helps to
communicate the result

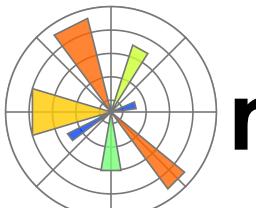




Visualize Images in Python



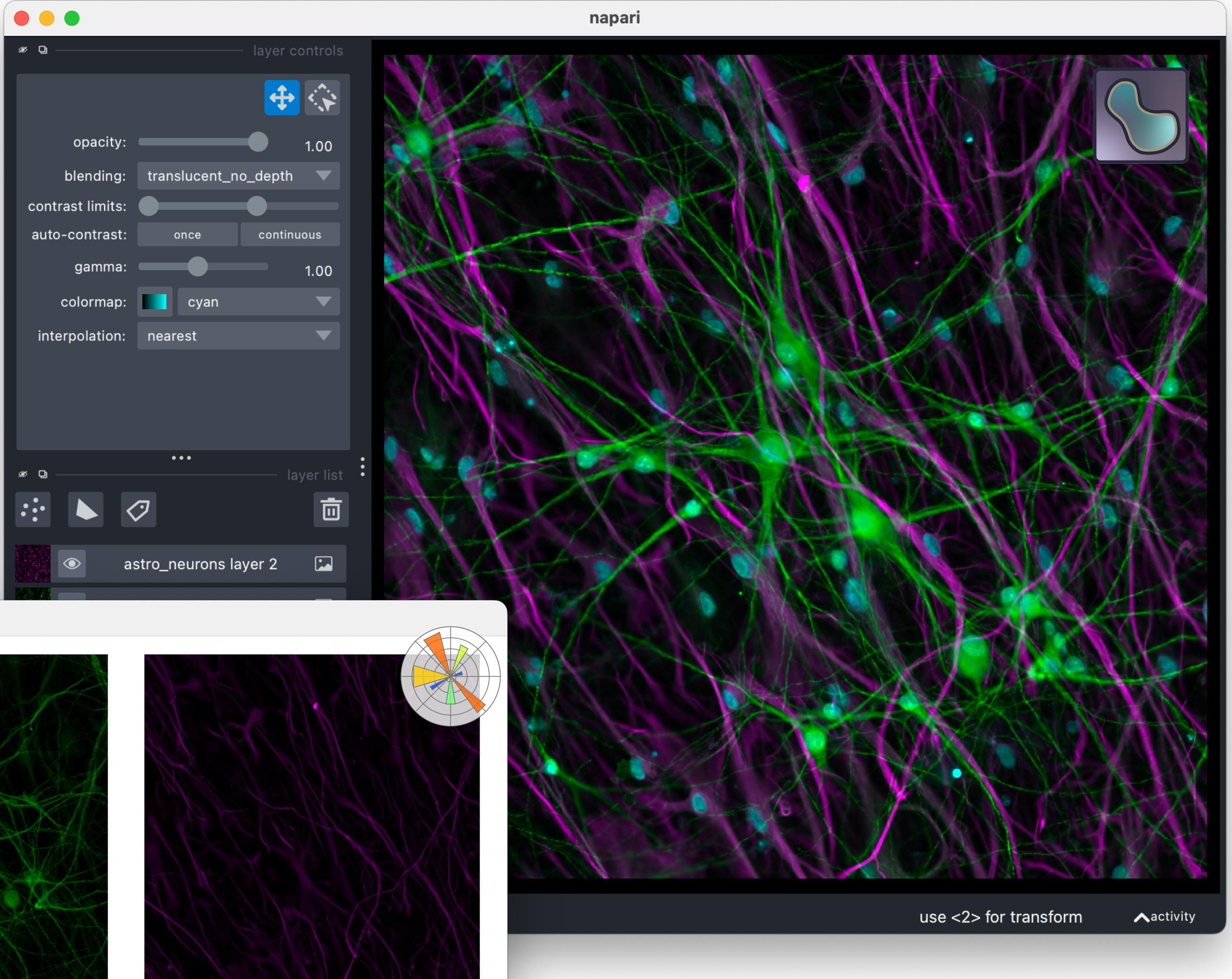
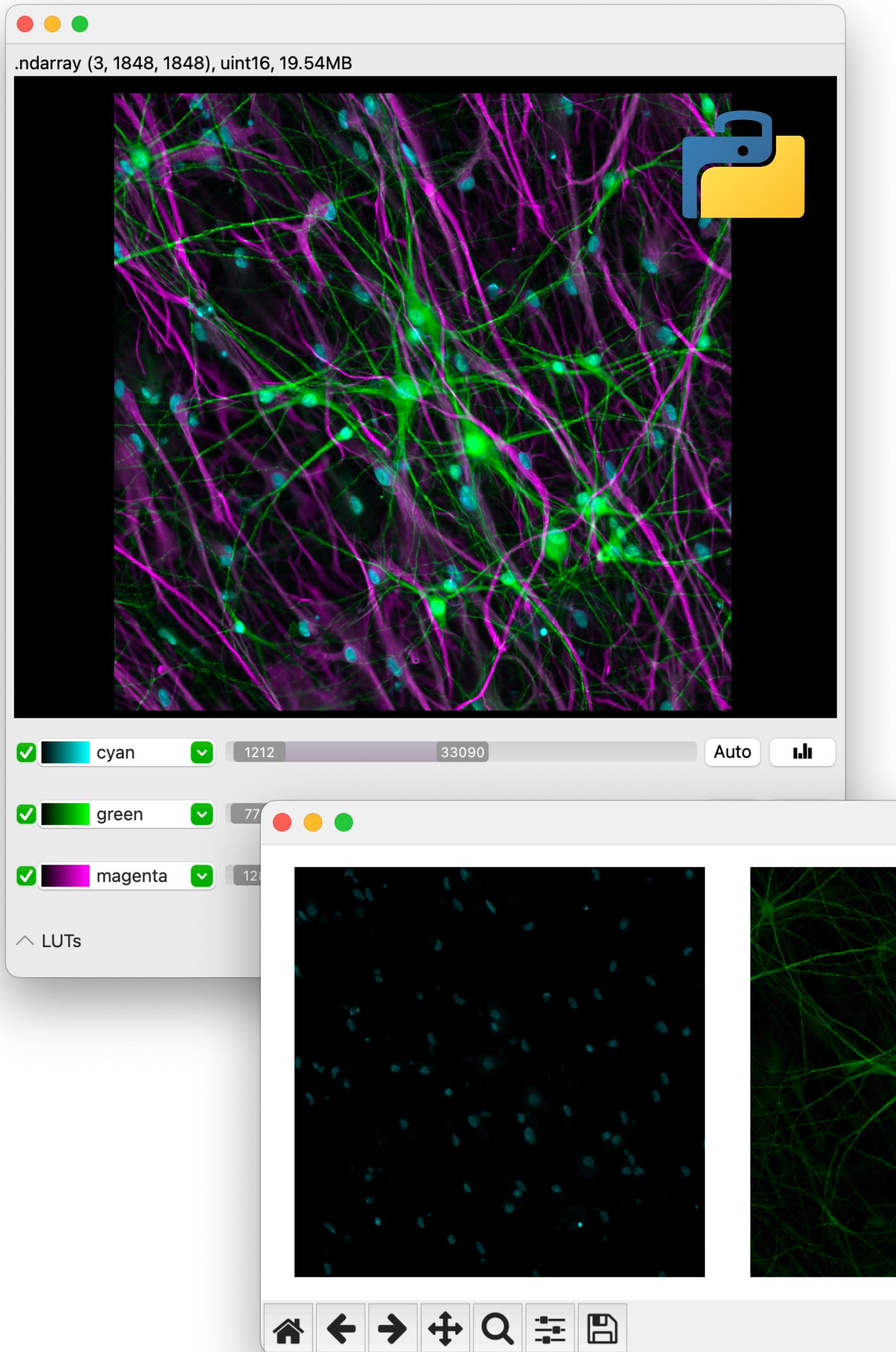
napari



matplotlib

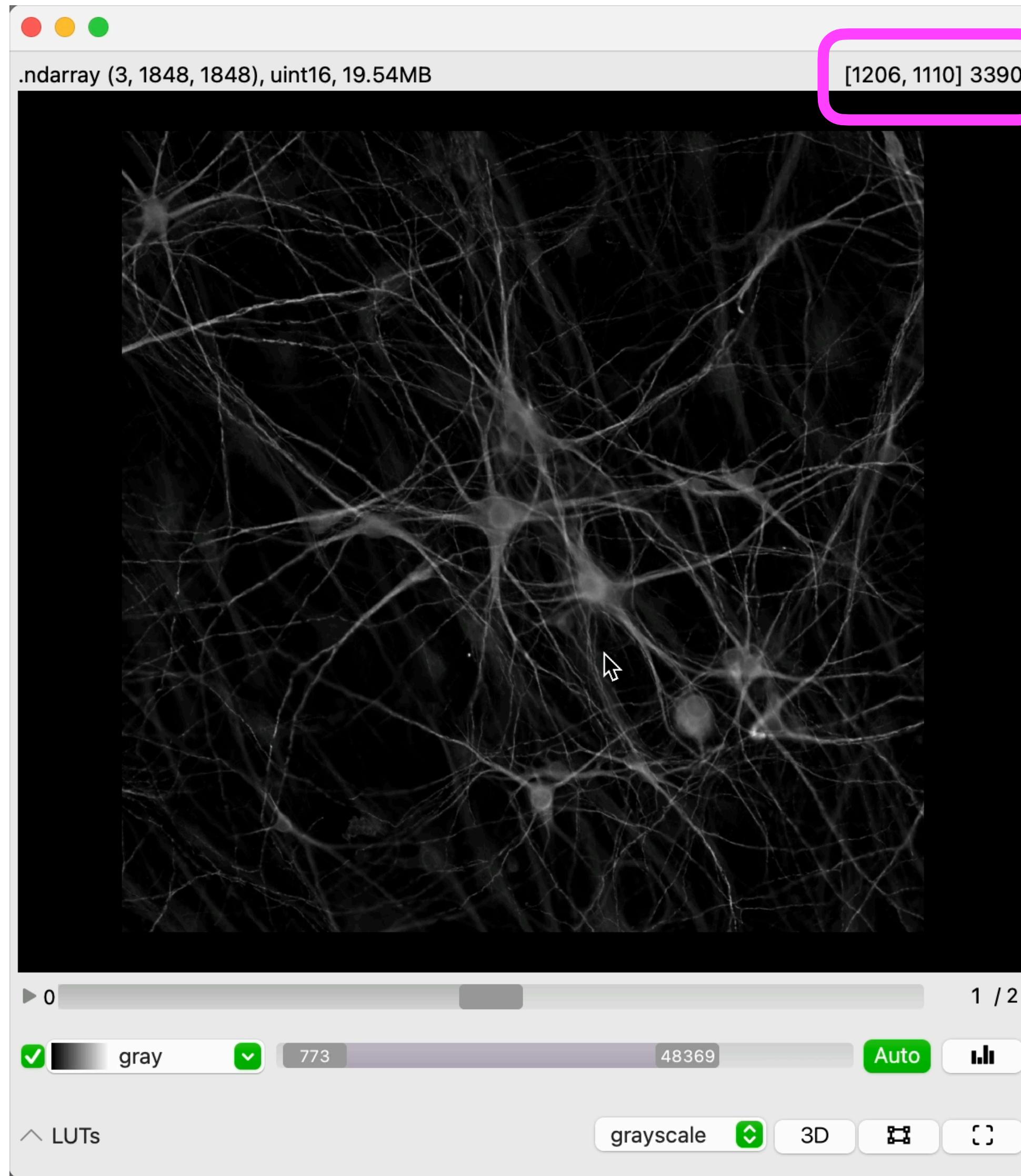


ndv





Inspect Individual Pixel Values



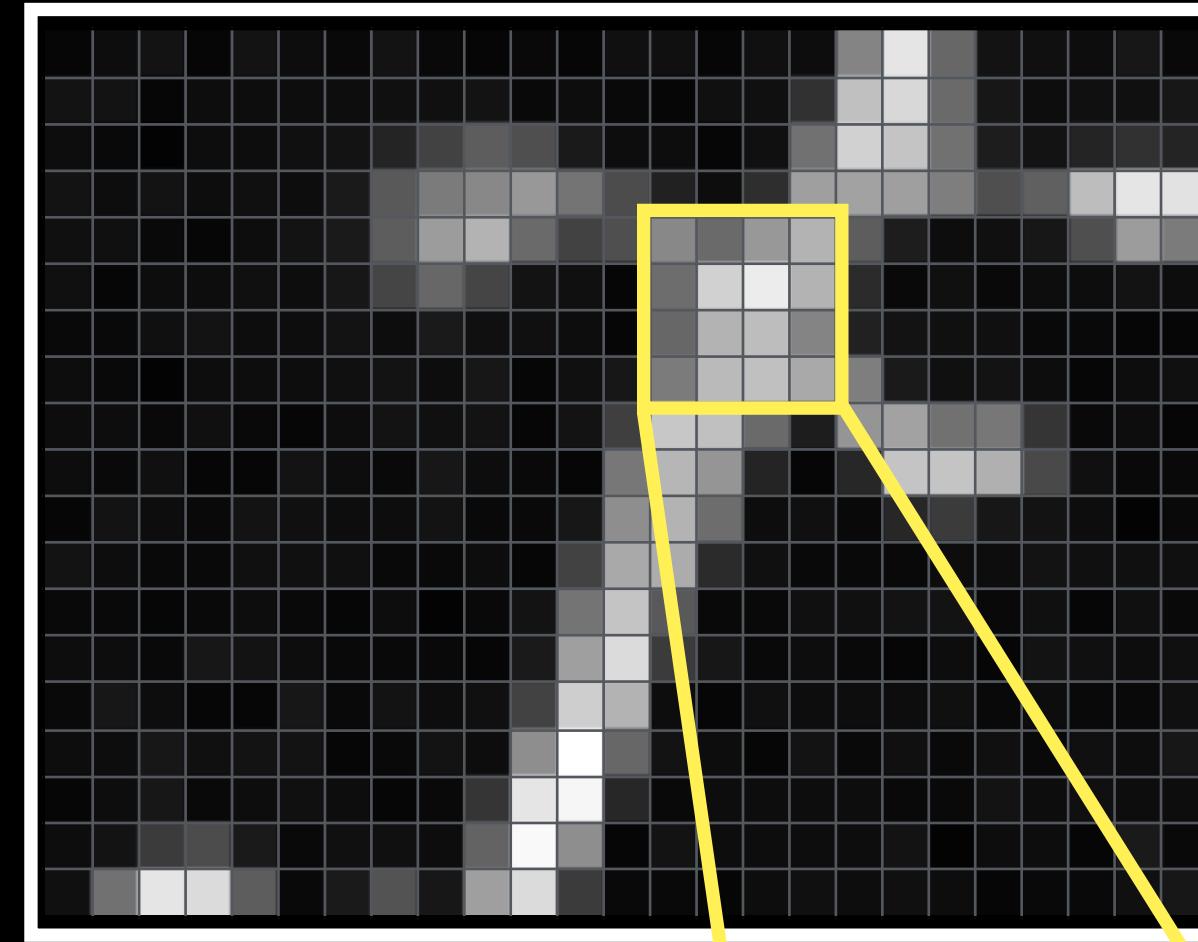
<https://pyapp-kit.github.io/ndv>





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 | 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?



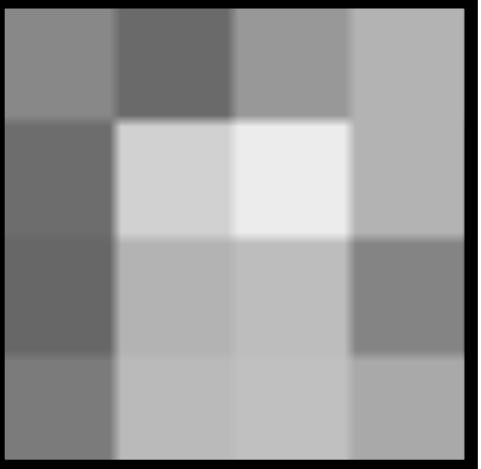


Detectors in Fluorescence Microscopy

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**.

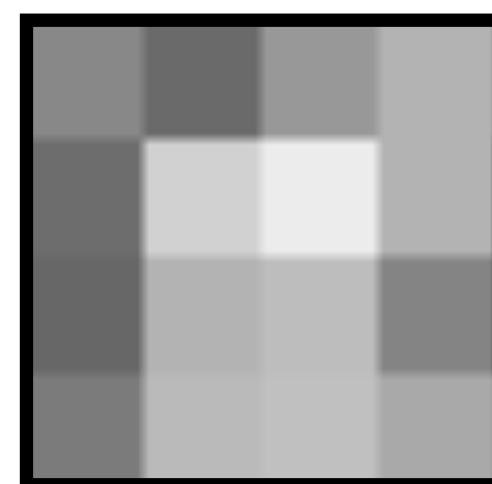
bit depth of the image = bit depth of the detector
(Unless you change that during acquisition)

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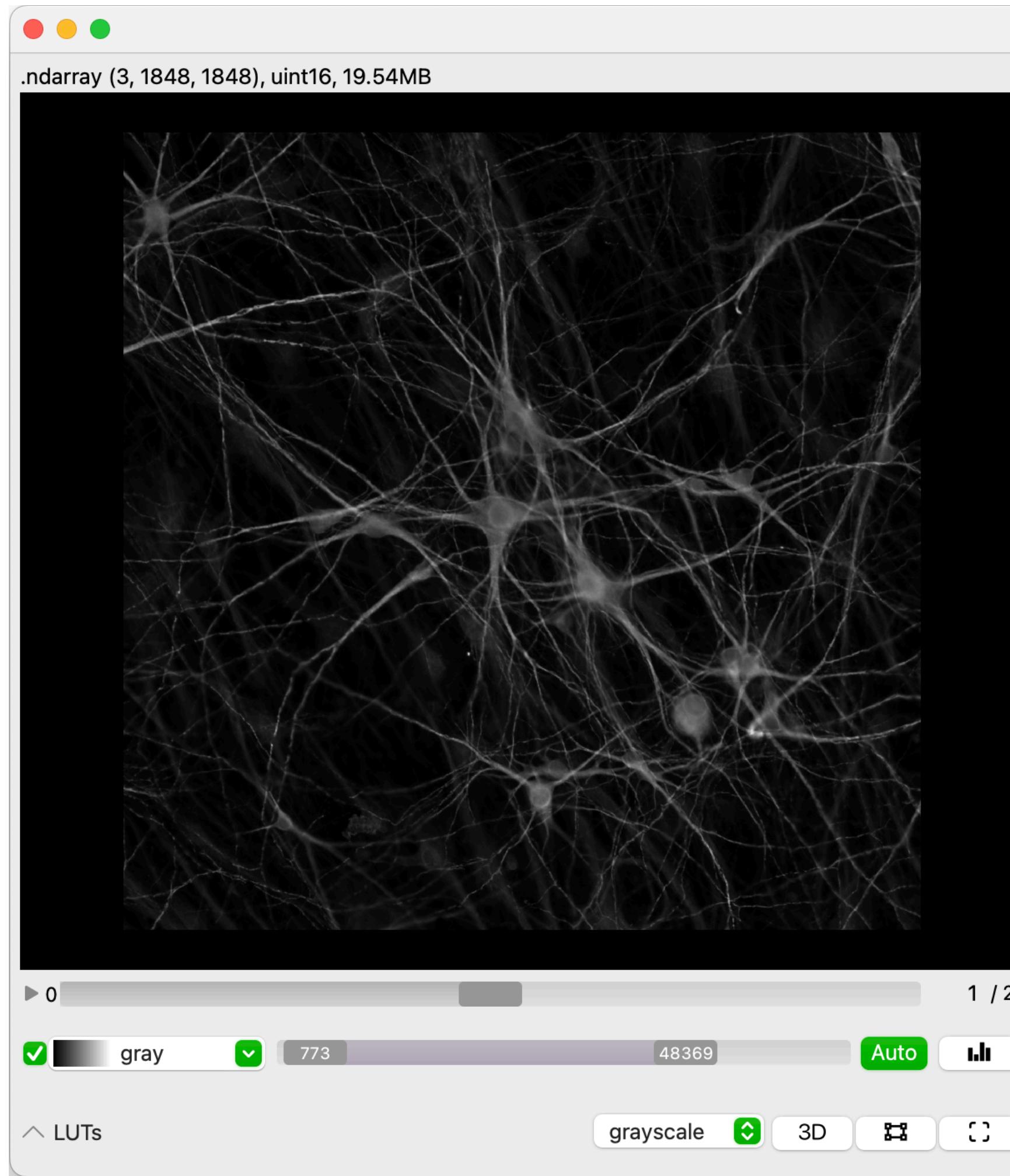
| | | | |
|-----|-----|-----|-----|
| 136 | 106 | 152 | 179 |
| 109 | 209 | 236 | 179 |
| 103 | 179 | 189 | 132 |
| 123 | 186 | 192 | 169 |

Digital Value = Pixel Intensity Value = Grey Value





Bit Depth



numpy 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)
```



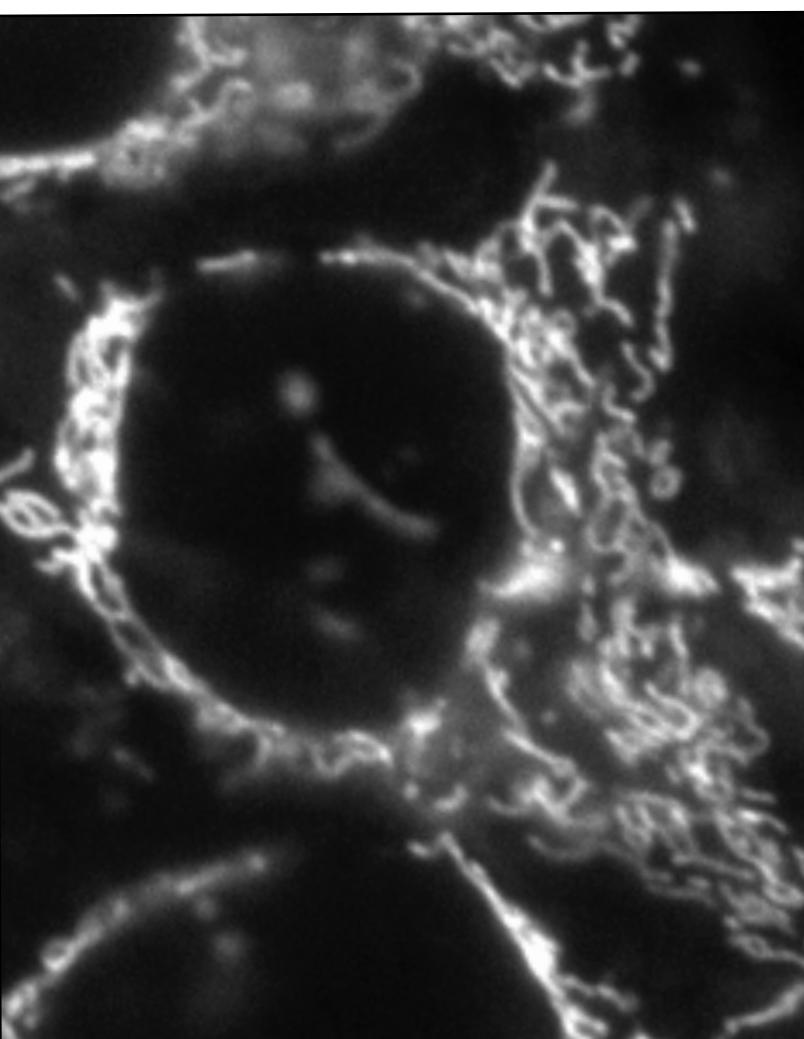
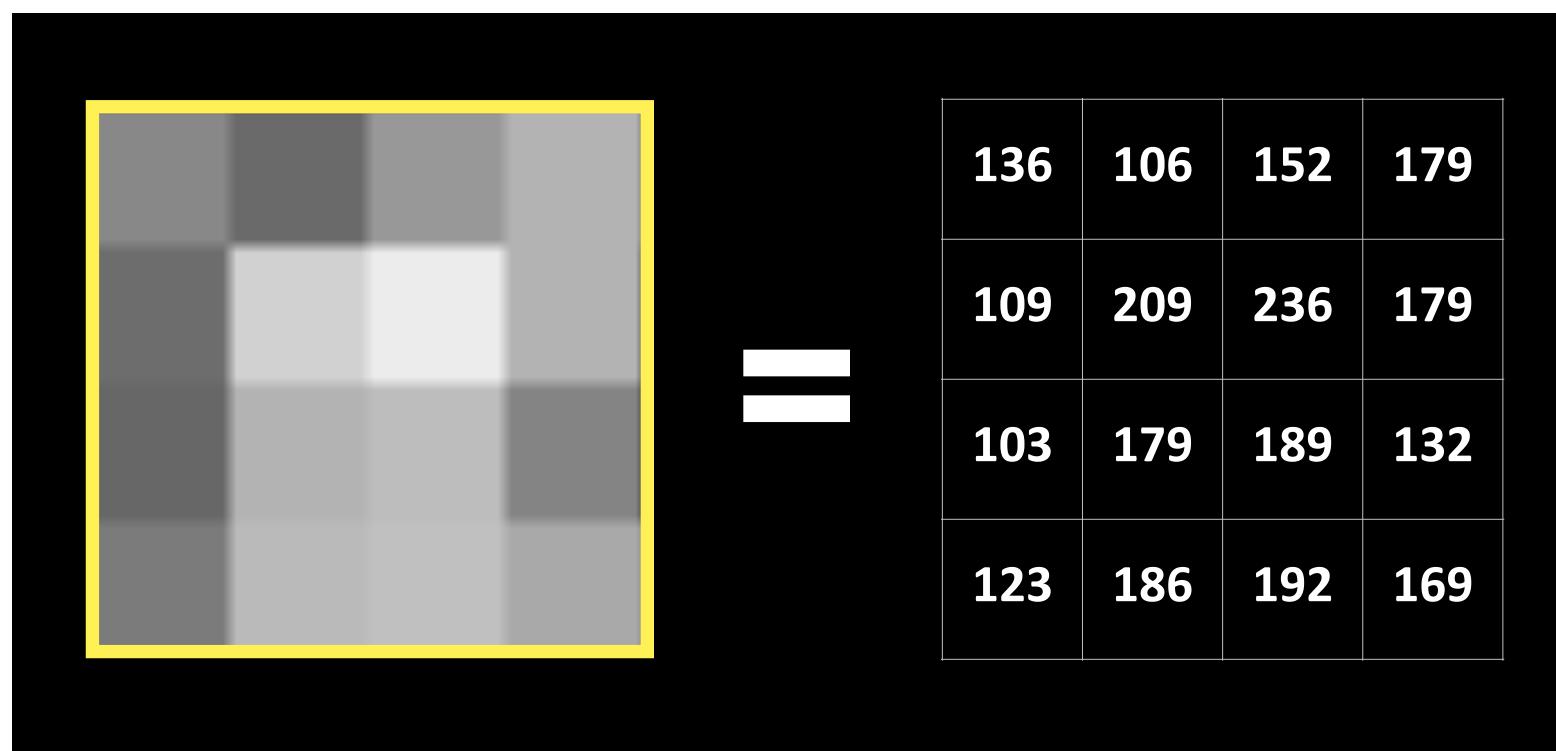


Display your Images

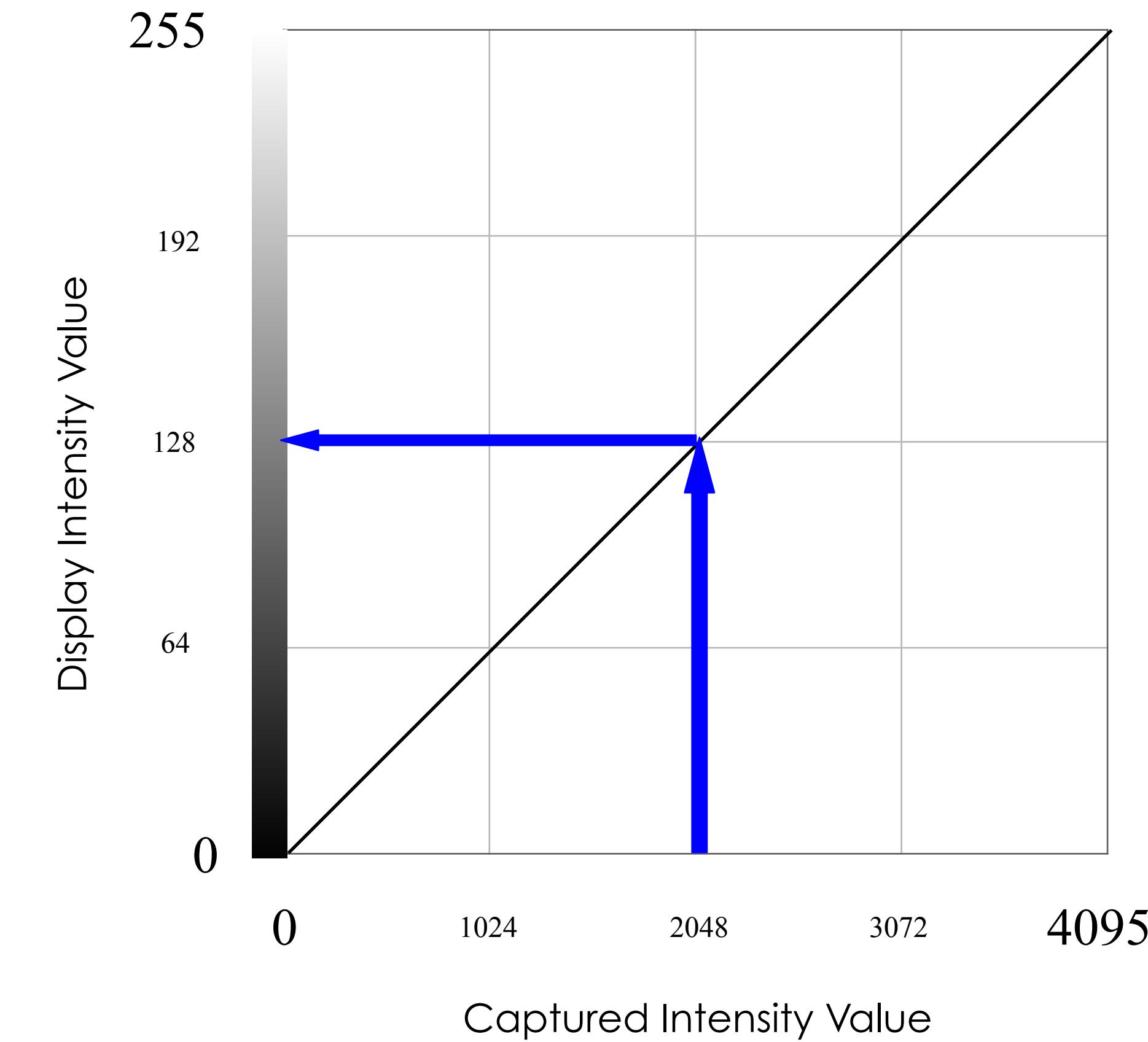
Mapping Image Intensity to Monitor Intensity (**LookUp Tables**)

LUT = *how the grey values are displayed*

LUTs do not change the pixel values

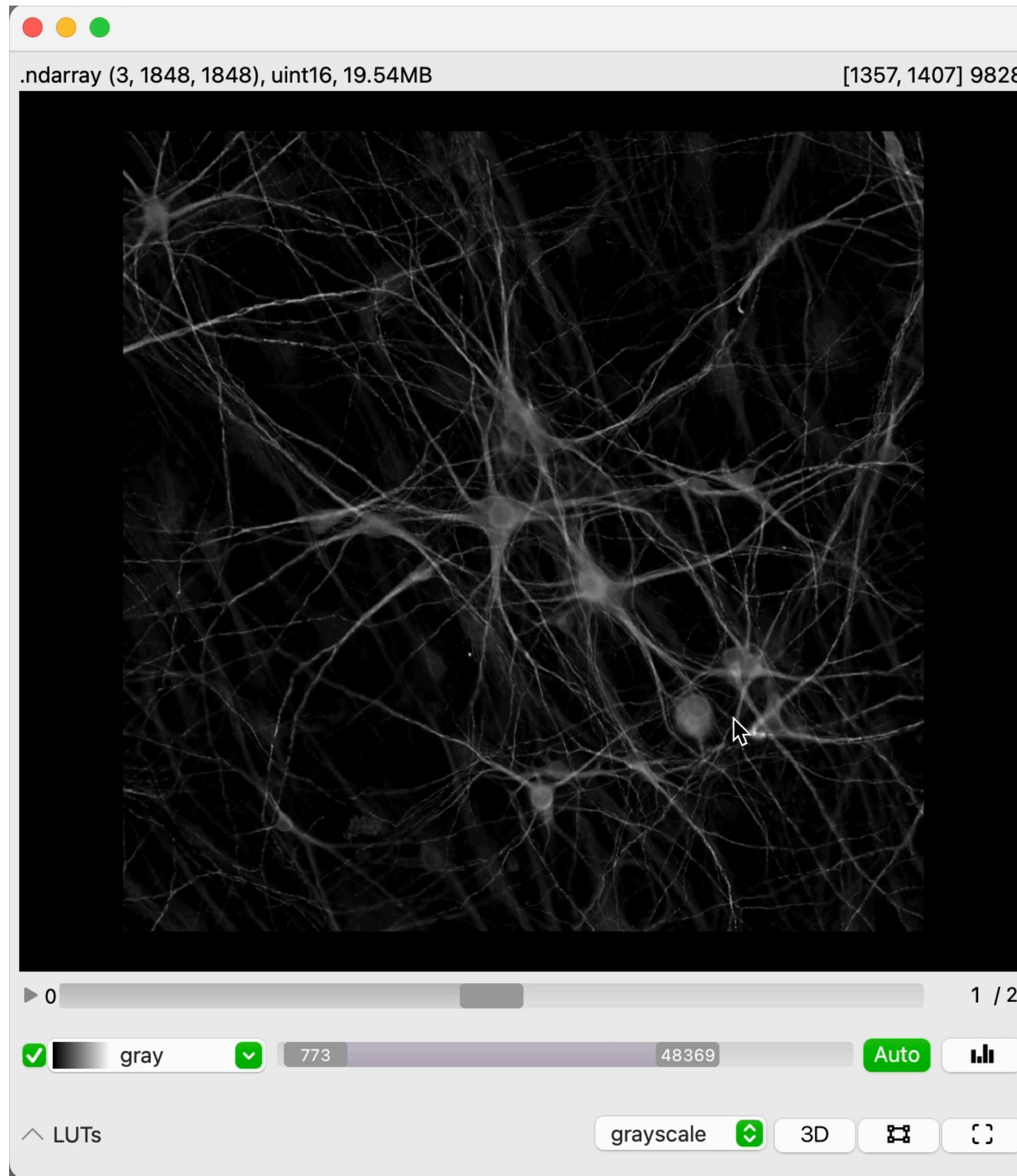


| Image (12 bit) | Displayed color |
|----------------|-----------------|
| 0 | |
| 1 | |
| ... | |
| 2000 | |
| ... | |
| 4095 | grey LUT |





Display your Images - Brightness & Contrast



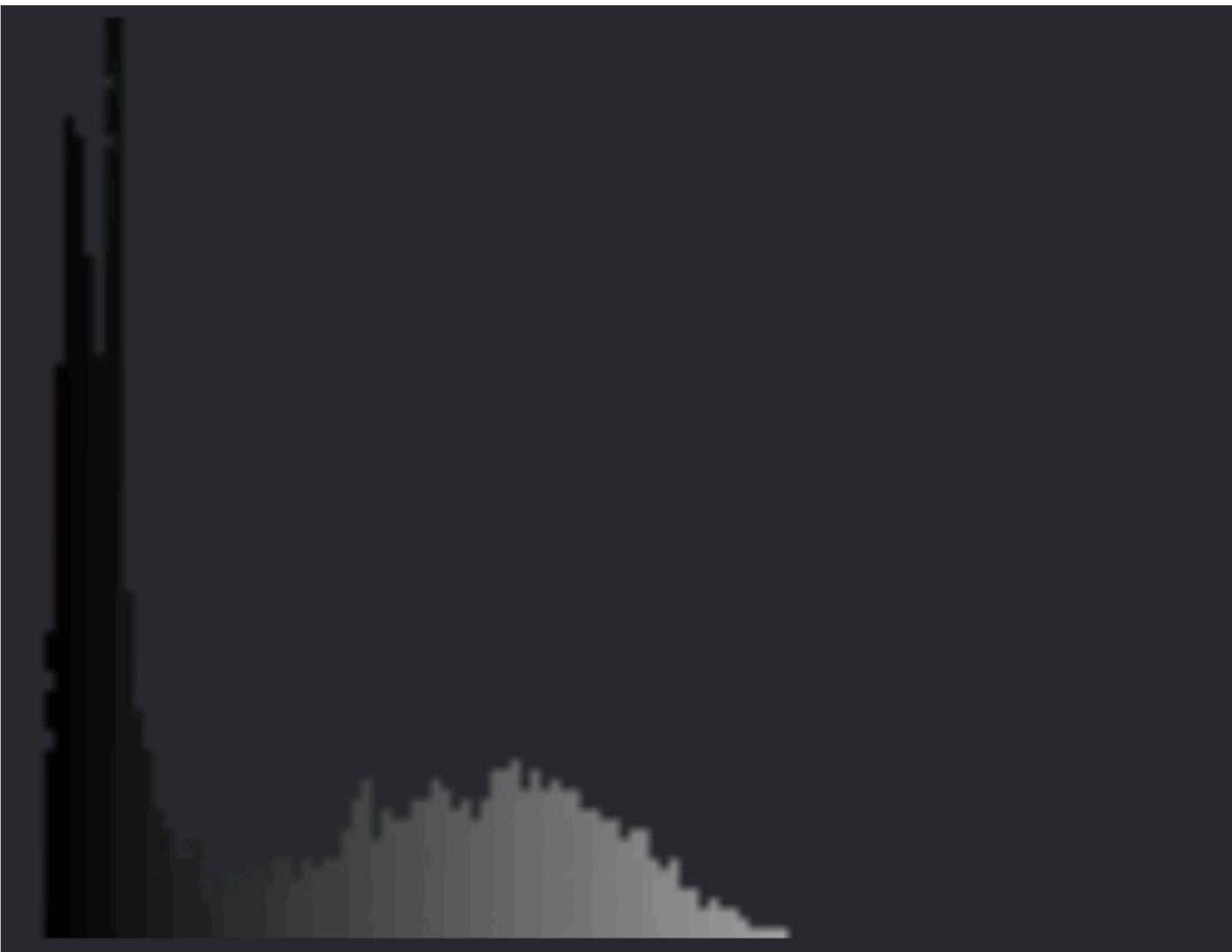


Histogram

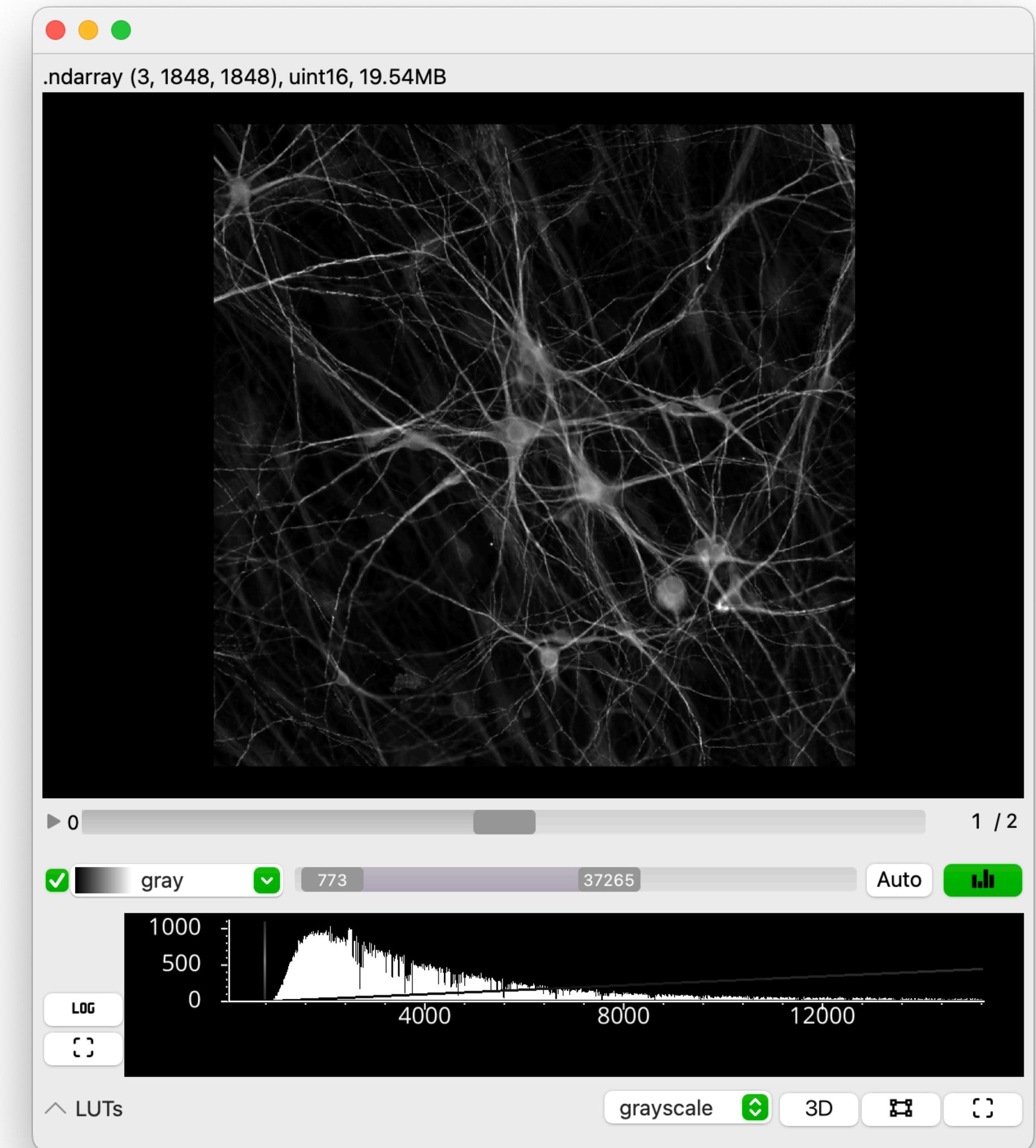


jaehyuk-lee: <https://jaehyuk-lee.com/animated-image-histogram/>

Pixel Count



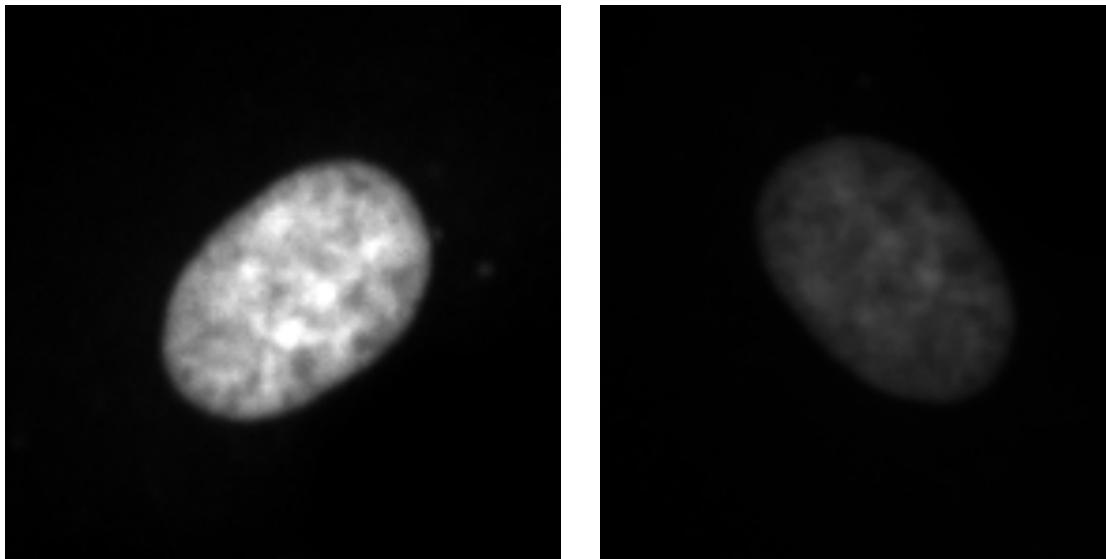
Pixel Values



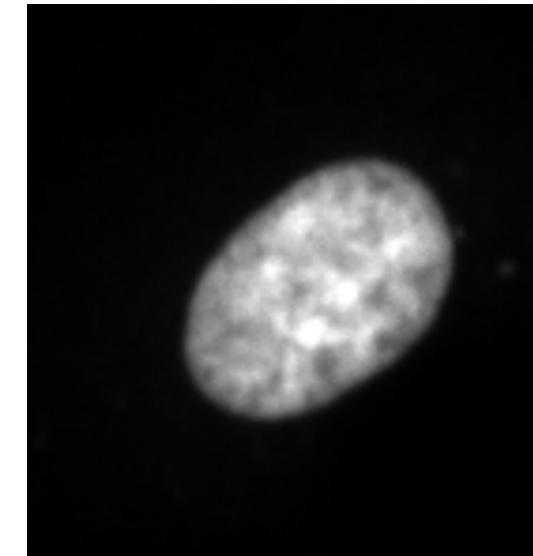


Display your Images - Brightness & Contrast

Which image has more fluorescence?



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



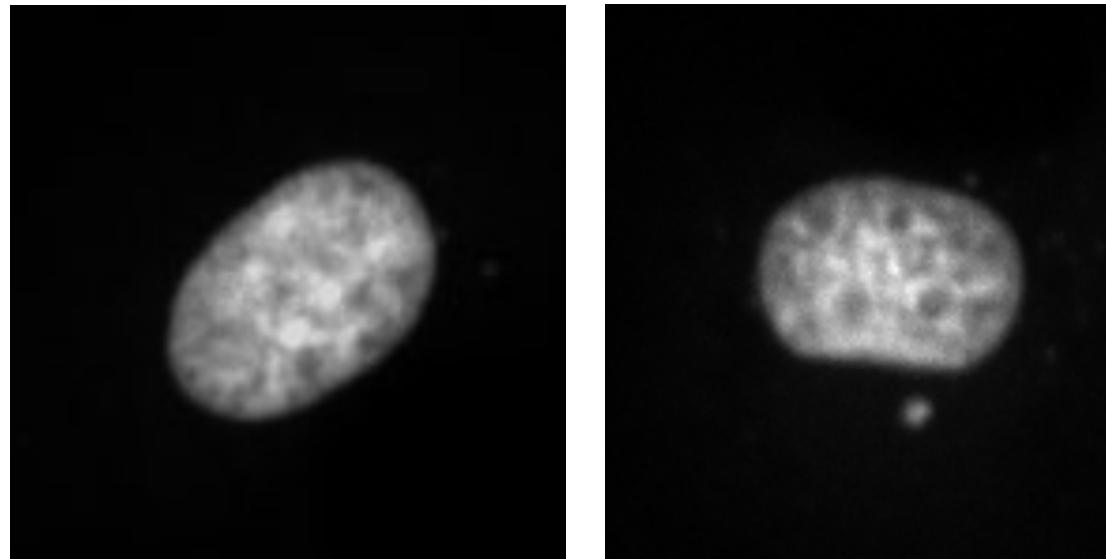
| | | |
|----------------|-------------------|-------------------|
| Mean: | 4803 | 4803 |
| Display range: | 188- 16828 | 188- 16828 |





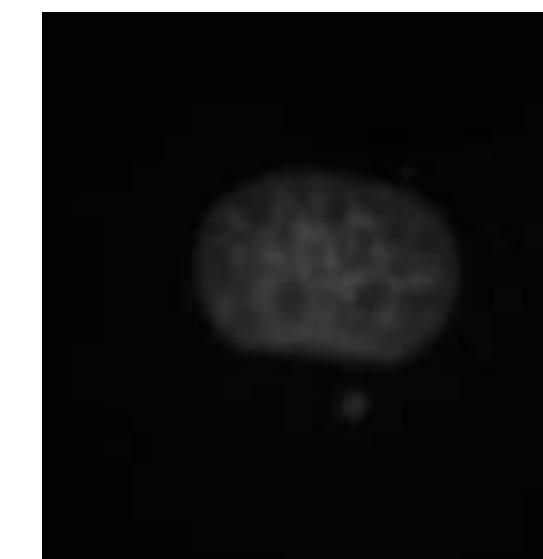
Display your Images - Brightness & Contrast

Which image has more fluorescence?



| | | |
|----------------|-------------------|------------------|
| Mean: | 4803 | 2074 |
| Display range: | 188- 19540 | 112- 7768 |

Do NOT trust your eyes,
rely on numbers!



| | | |
|----------------|-------------------|-------------------|
| Mean: | 4803 | 2074 |
| Display range: | 188- 19540 | 188- 19540 |

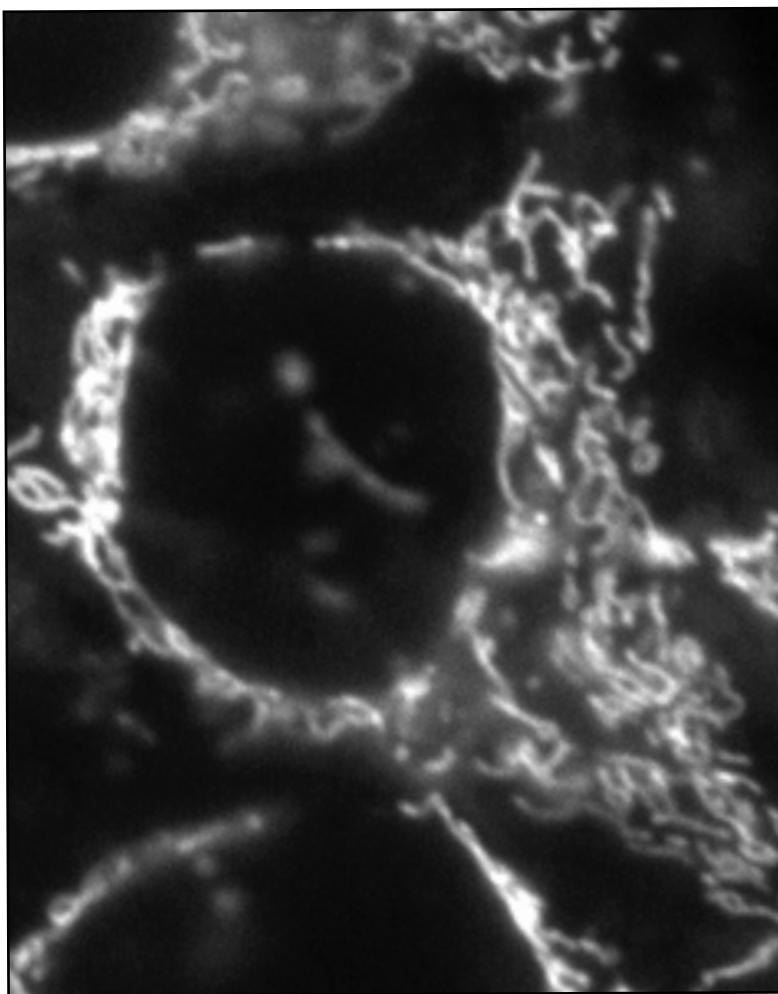




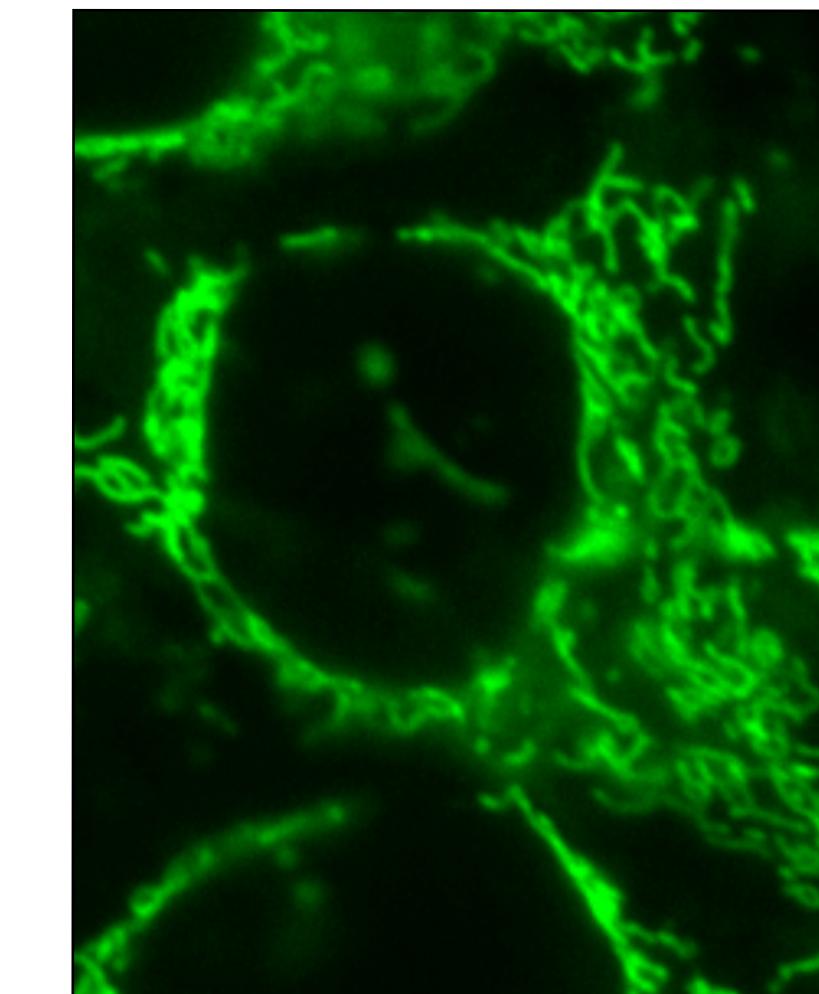
Images and Colors - Lookup Tables (LUTs)

LUT = how the grey values are displayed

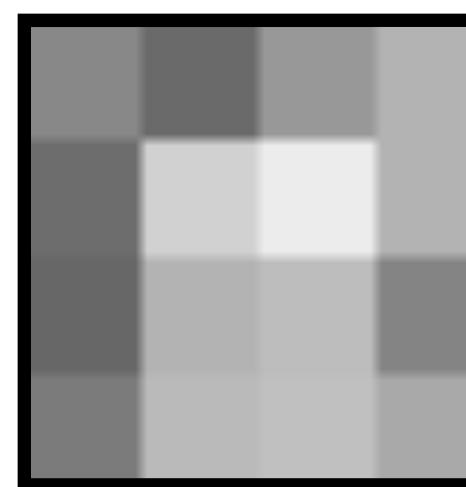
LUTs do not change the pixel values



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

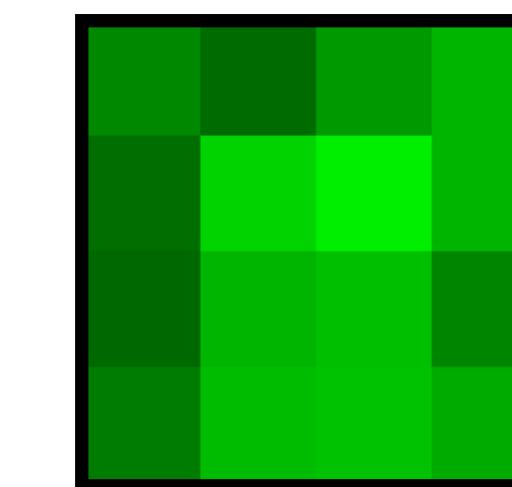


| Image (8 bit) | Displayed color |
|------------------|--------------------|
| 0 | |
| 1 | |
| ... | |
| 100 | |
| ... | |
| 255 | |



=

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



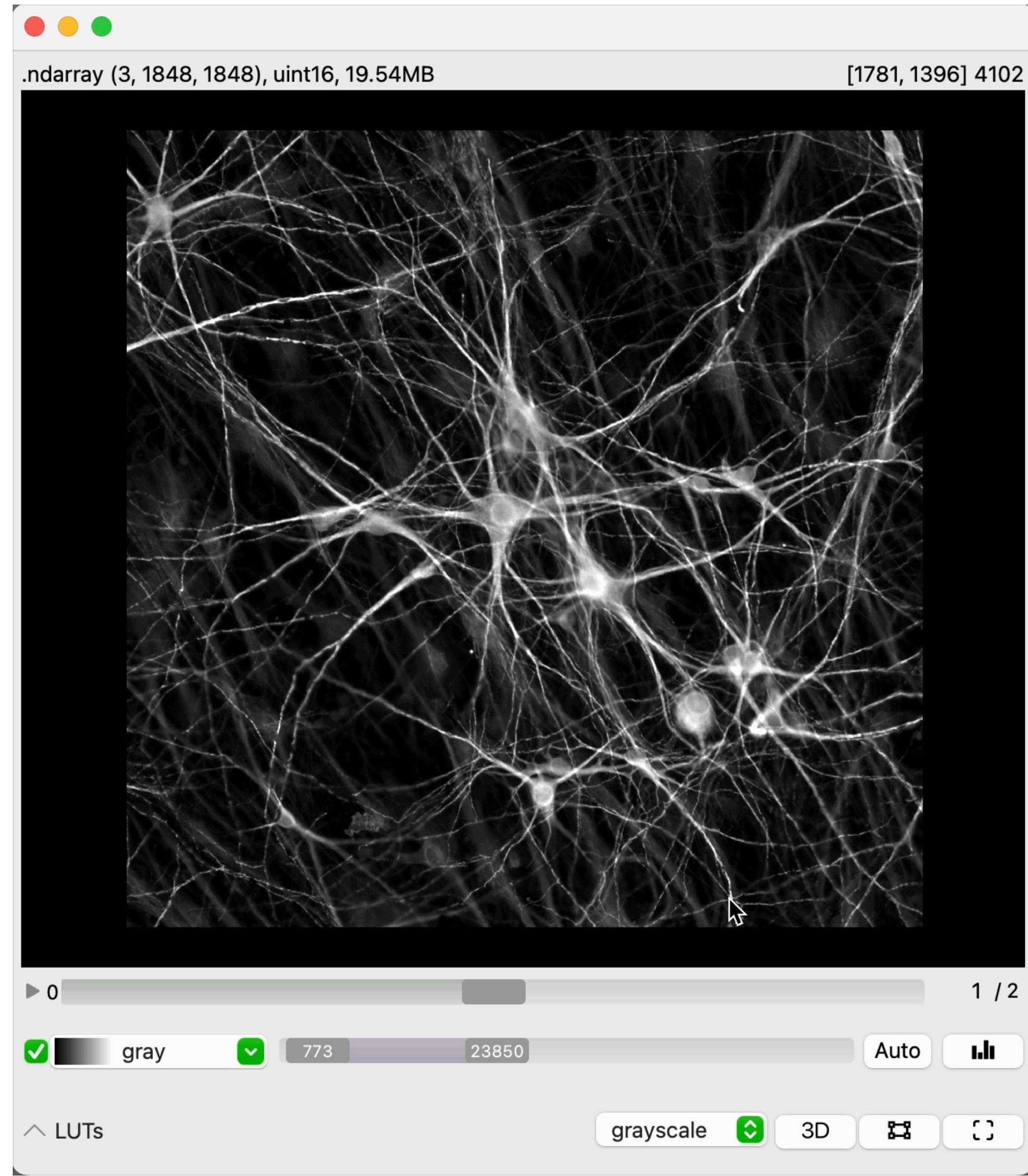
=

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





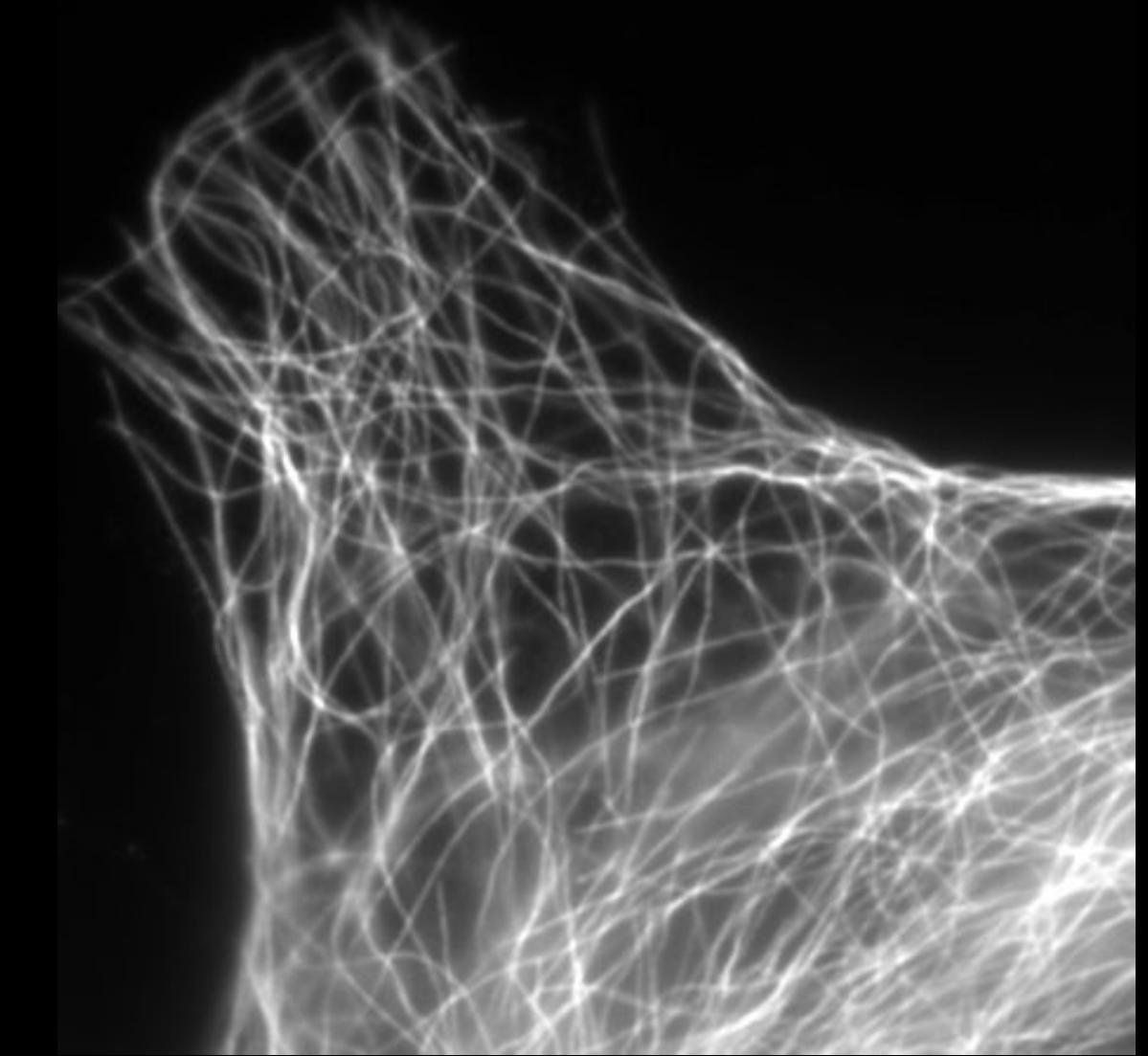
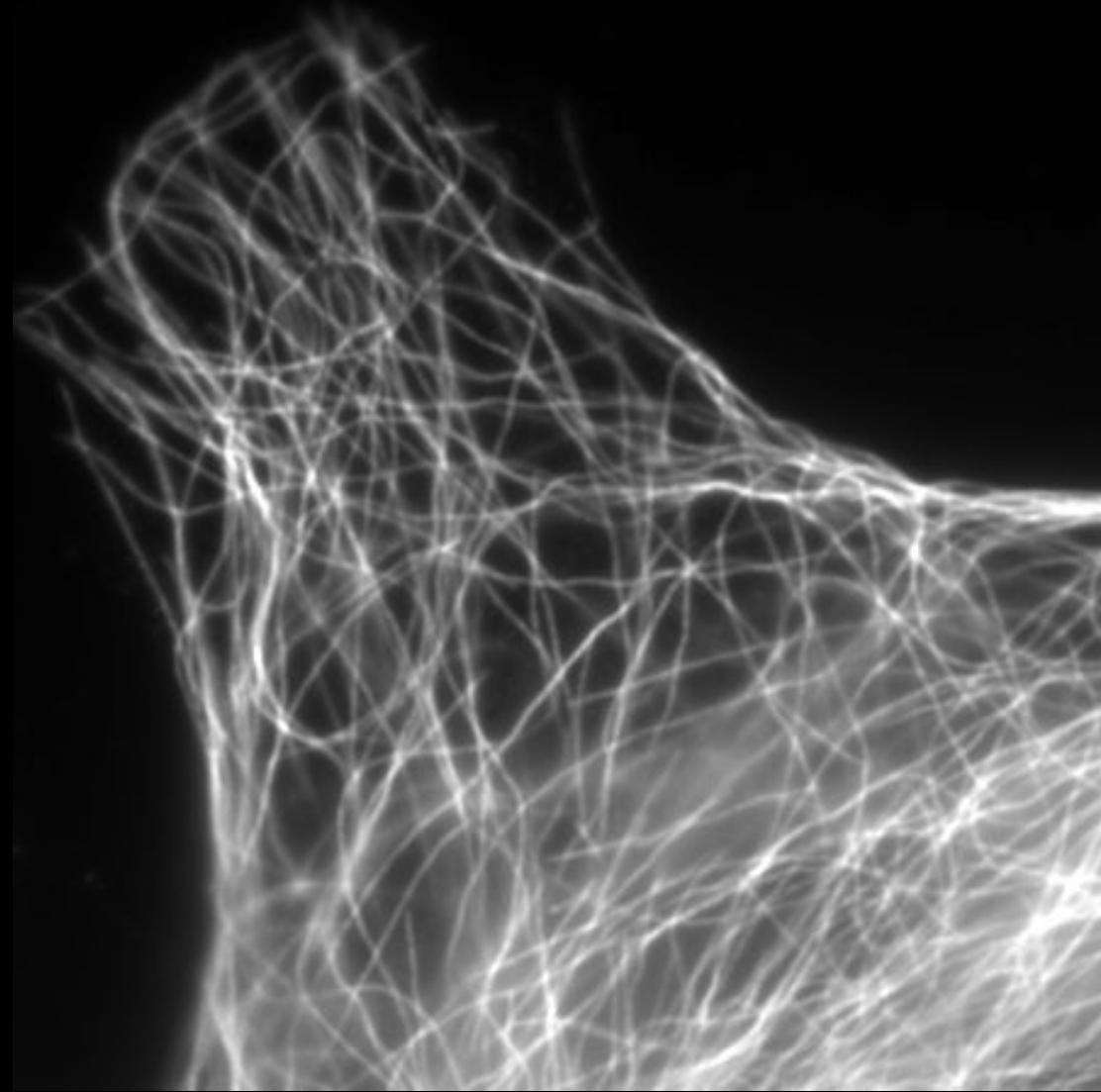
Images and Colors - Lookup Tables (LUTs)





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!

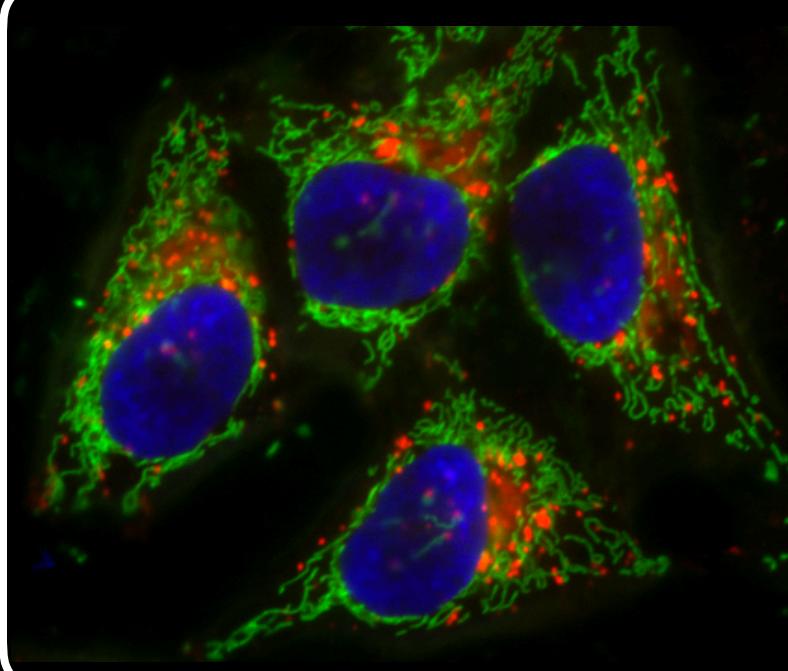




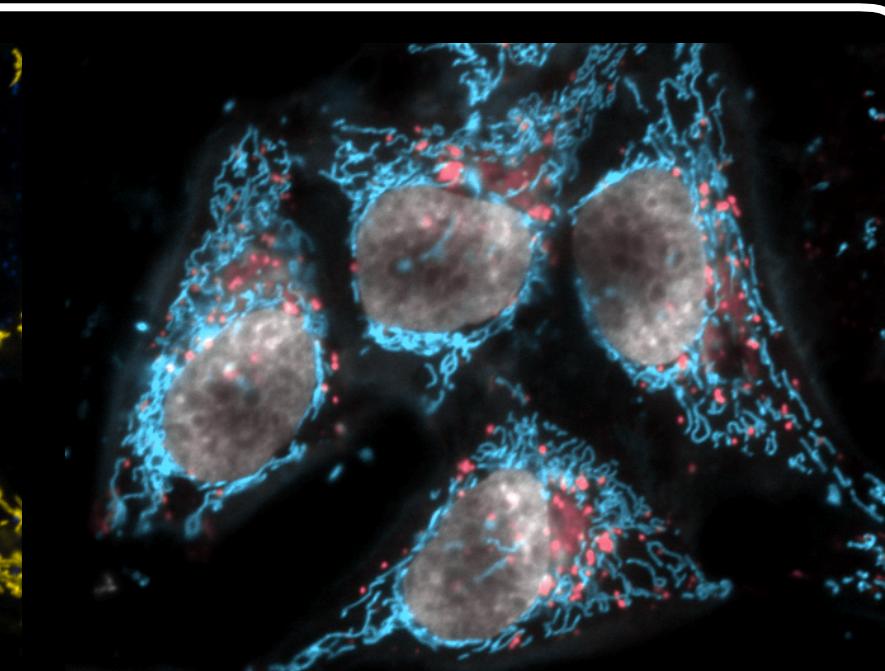
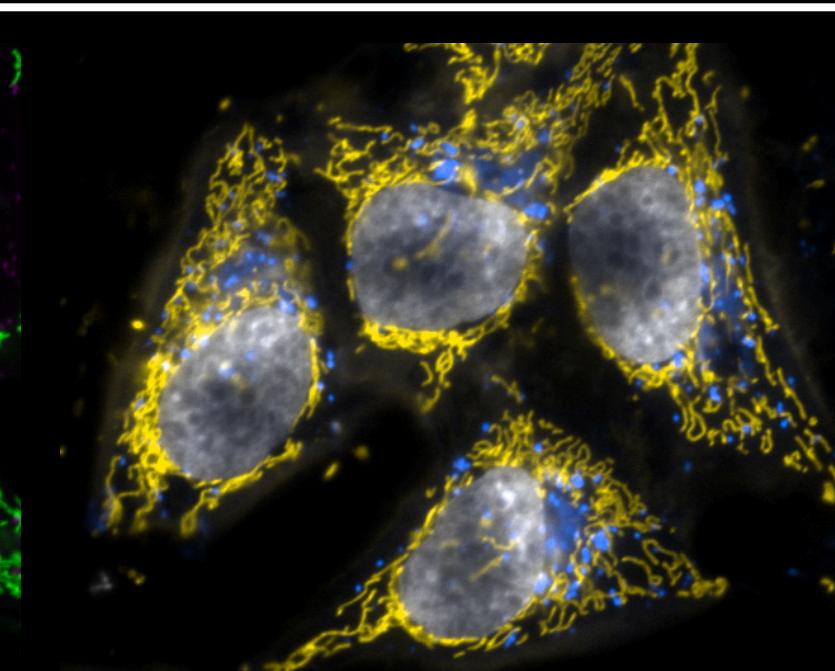
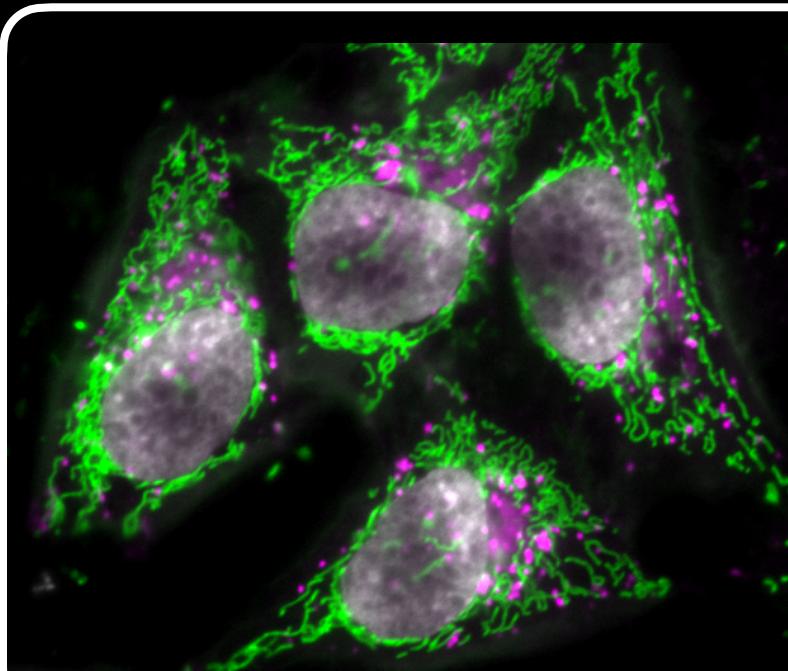
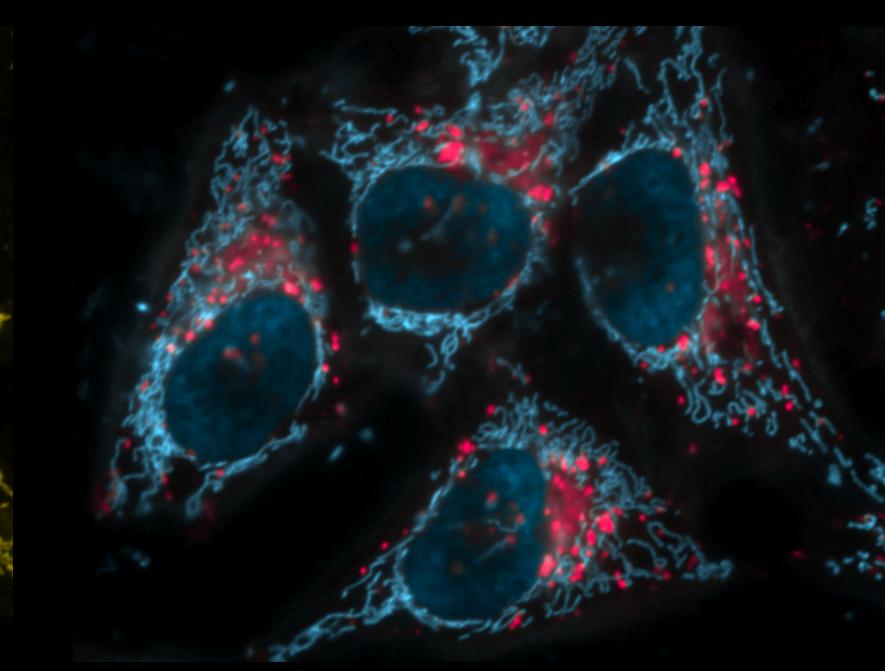
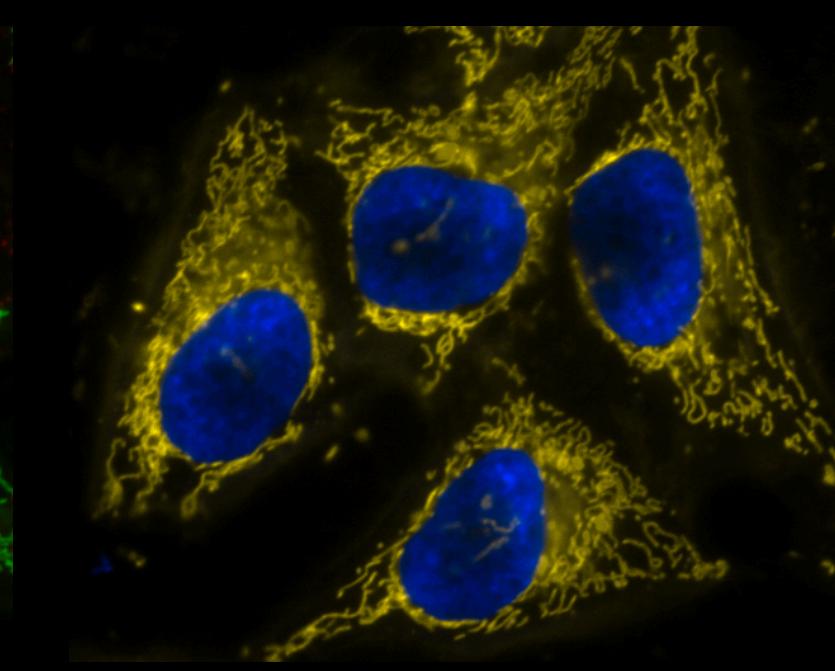
Images and Colors - Choose the right LUT

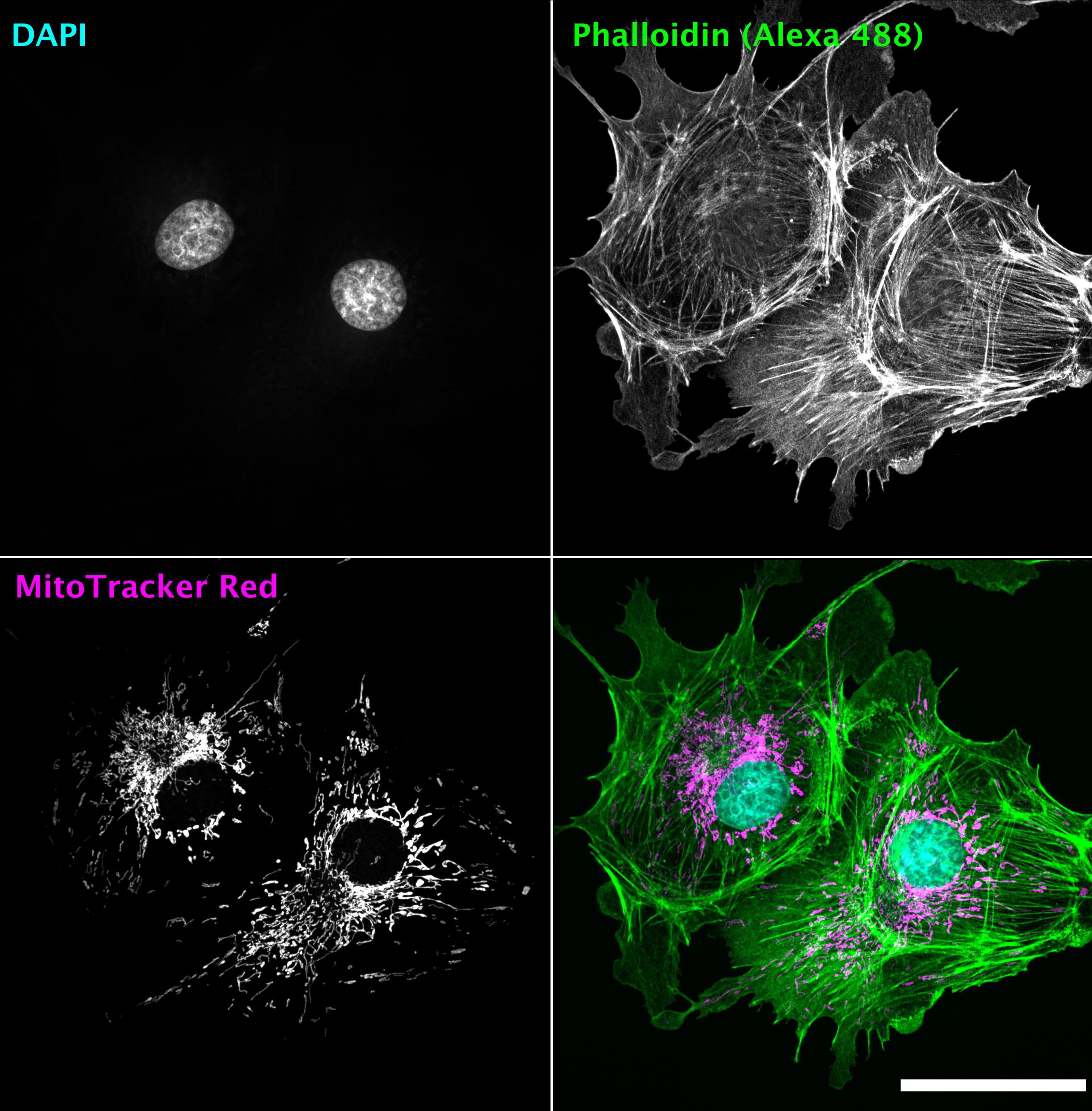
Color blind people don't distinguish some colors

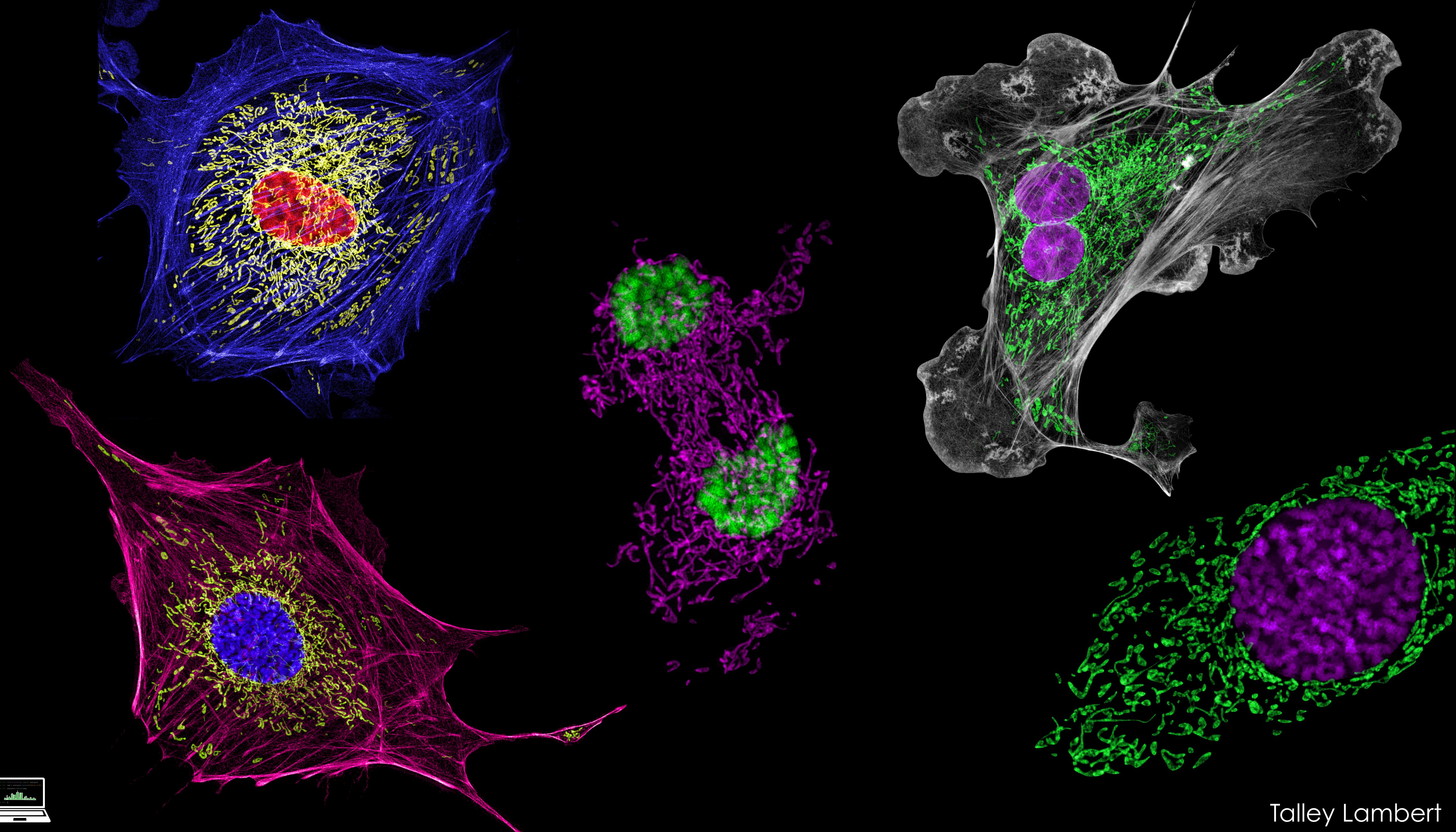
Protanope (no red)



Tritanope (no blue)







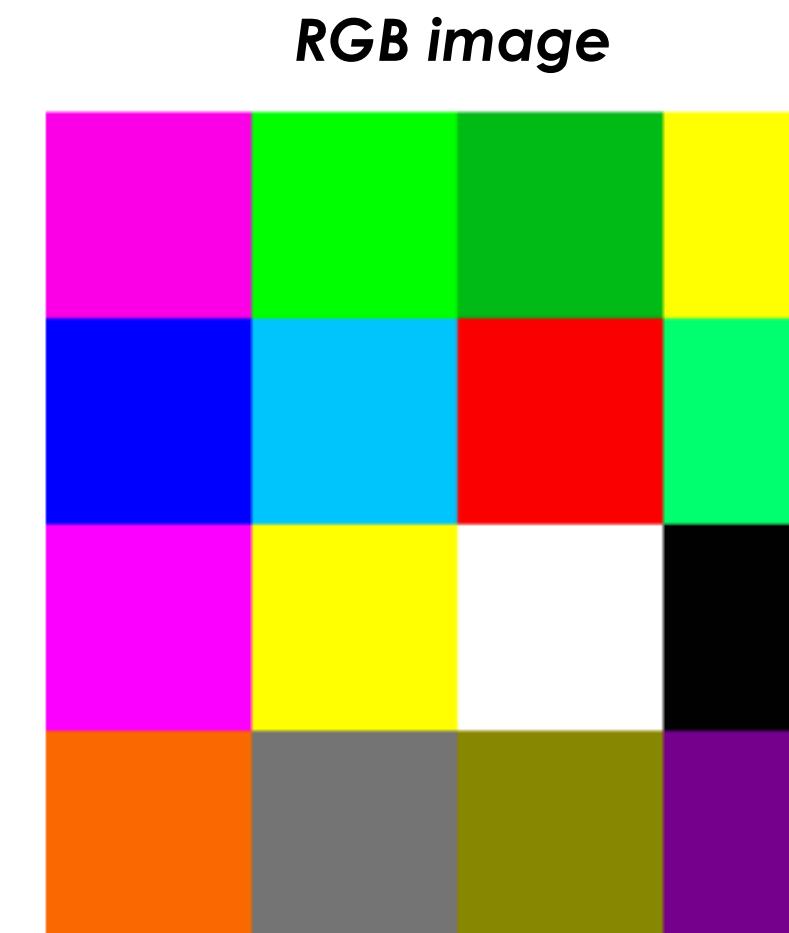
Talley Lambert



Images and Colors

RGB Images (still matrix of numbers)

LUTs **cannot** be applied to RGB Images



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| | | | |
|-----|-----|-----|-----|
| 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)**

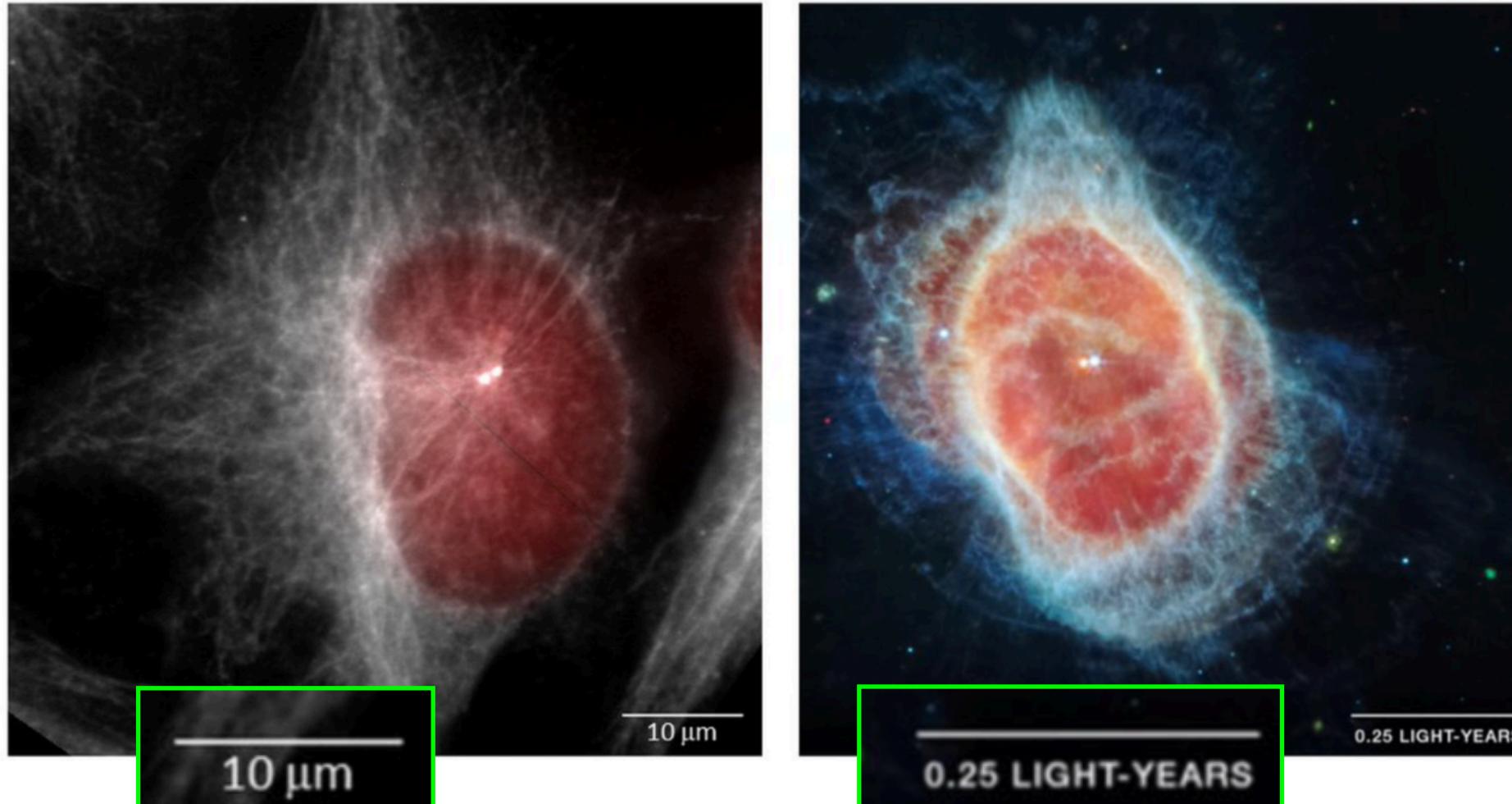




Pixel Size & image Scale

 Laurence Haren
@HarenLaurence ...

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







Pixel Size & image Scale

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

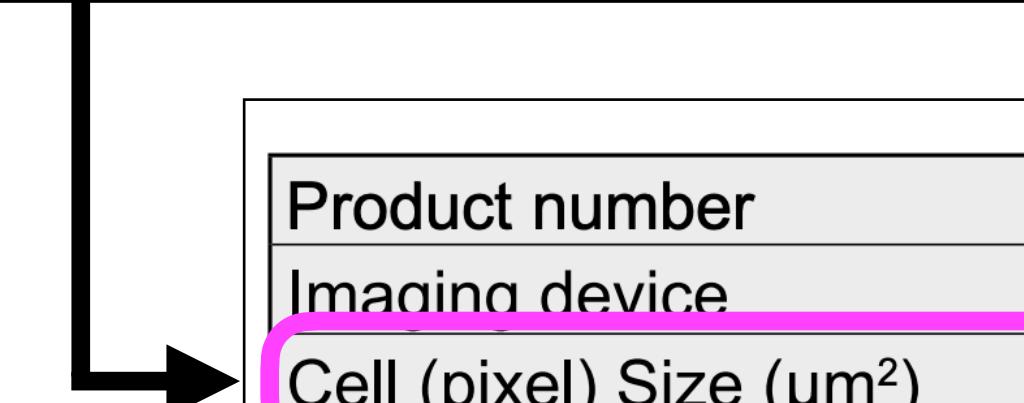
If you know the **magnification** and the **camera** you used for the acquisition,
you can estimate the image pixel size.

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 (μm^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:
 $6.5 \mu\text{m} / 100X = 0.065 \mu\text{m}$**



? Questions



iac.hms.harvard.edu/bobiac/2025

