Introduction to Image Analysis

Micron Advanced Microscopy Course, 2017

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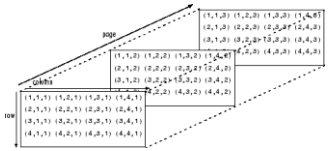
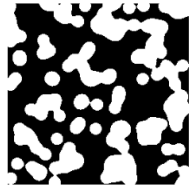
(the basement)

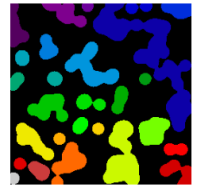
Microscope Image Analysis in 3 Parts

1. What is in a microscope image?
   - What is in an image?
   - Pixels and display
   - ImageJ
   - Considerations for acquisition

2. Arrays, Images, Dimensions
   - N Dimensional images
   - Filtering
   - Morphology
   - Connected Components

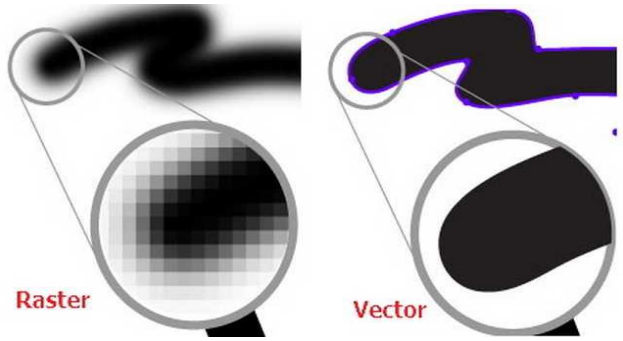






3. Don’t botch your data
   - File formats
   - Data storage
   - OMERO

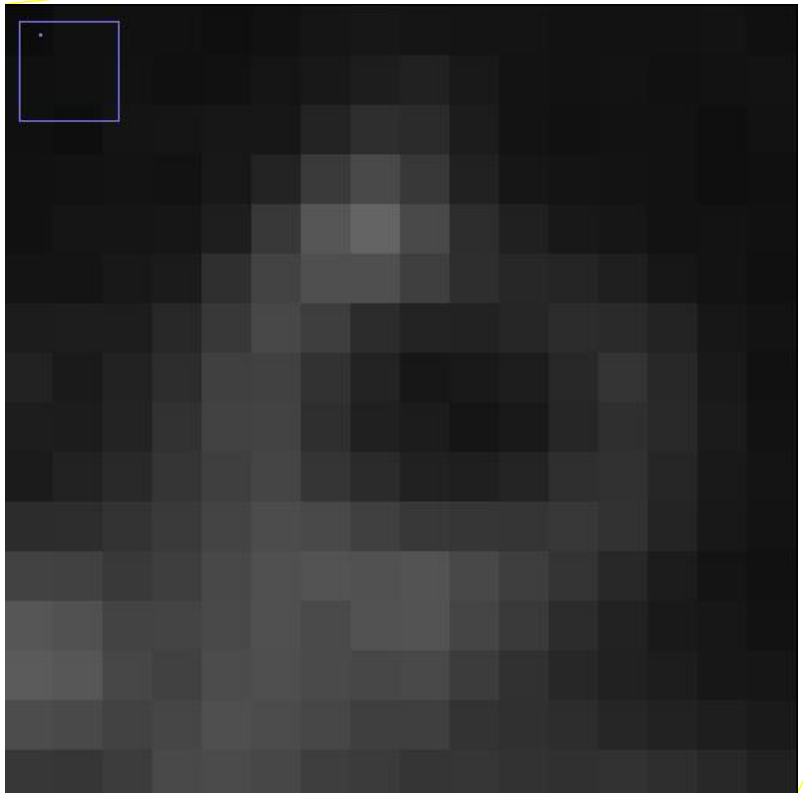
What is in an image?



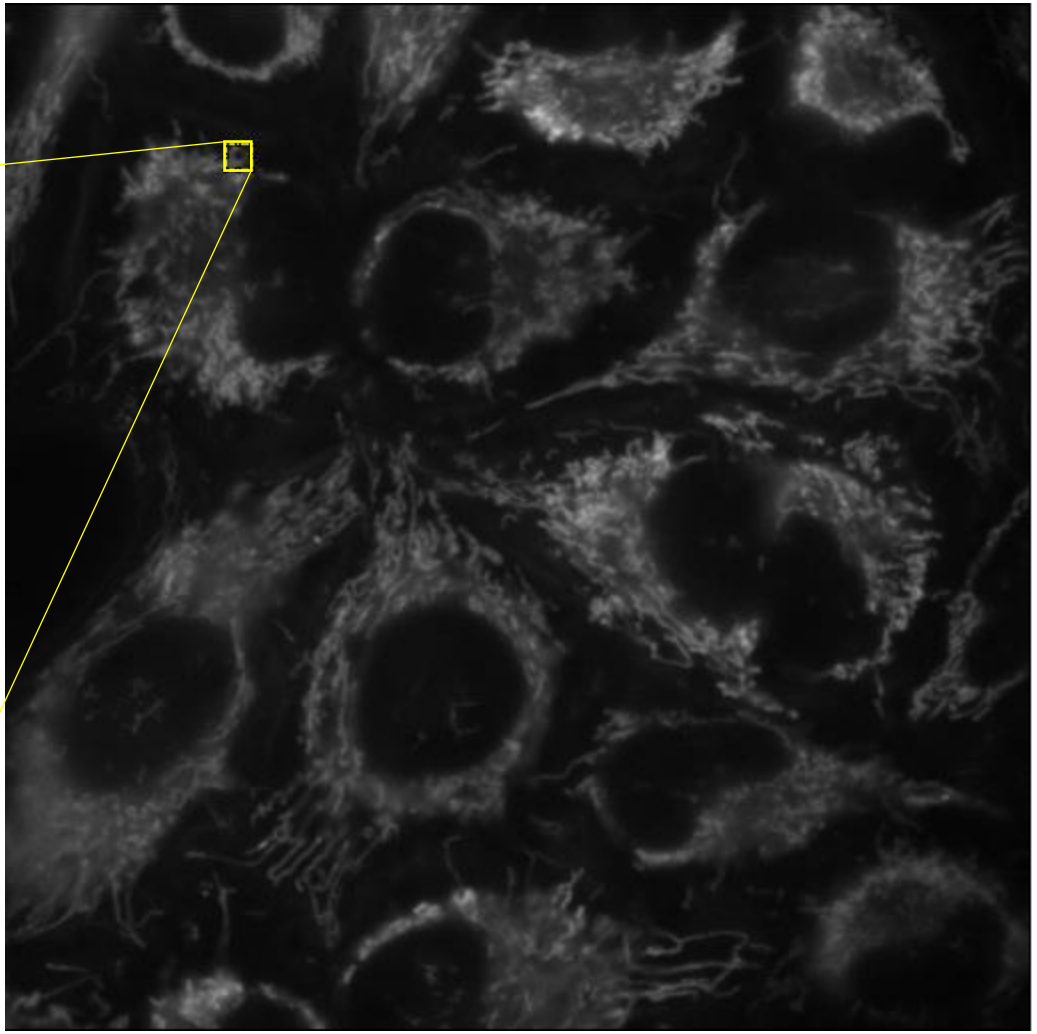
“pixel”:
<u>picture</u> (pix)
<u>element</u>

The smallest addressable element of a raster image

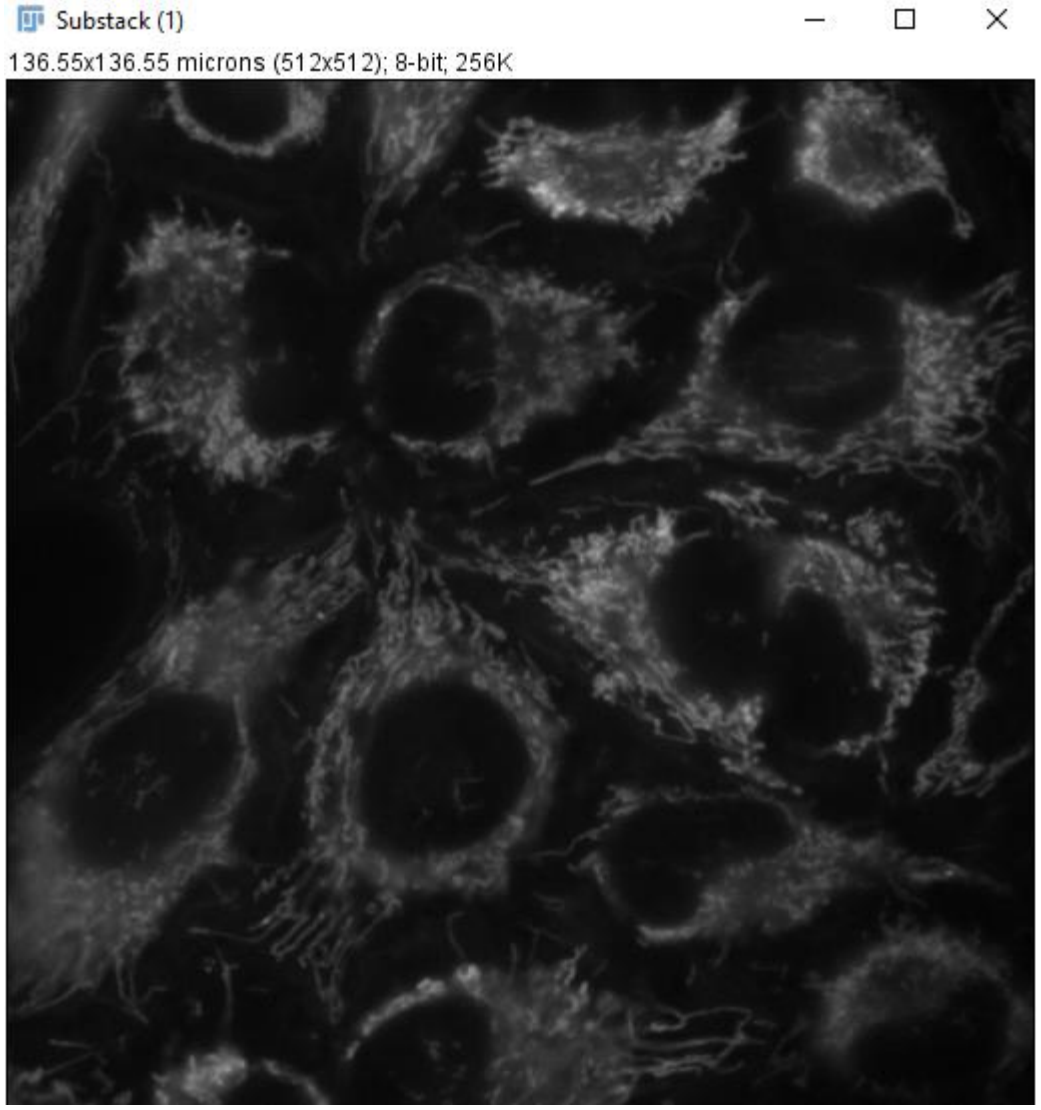
Zoom: 3200%



DeltaVision Confocal Microscope Image



Metadata



- Emission wavelength : 525 nm
- Excitation wavelength : 475 nm
- Exposure time : 0.1 s
- Objective :
- pixel size : 0.2667 x 0.2667 μm^2

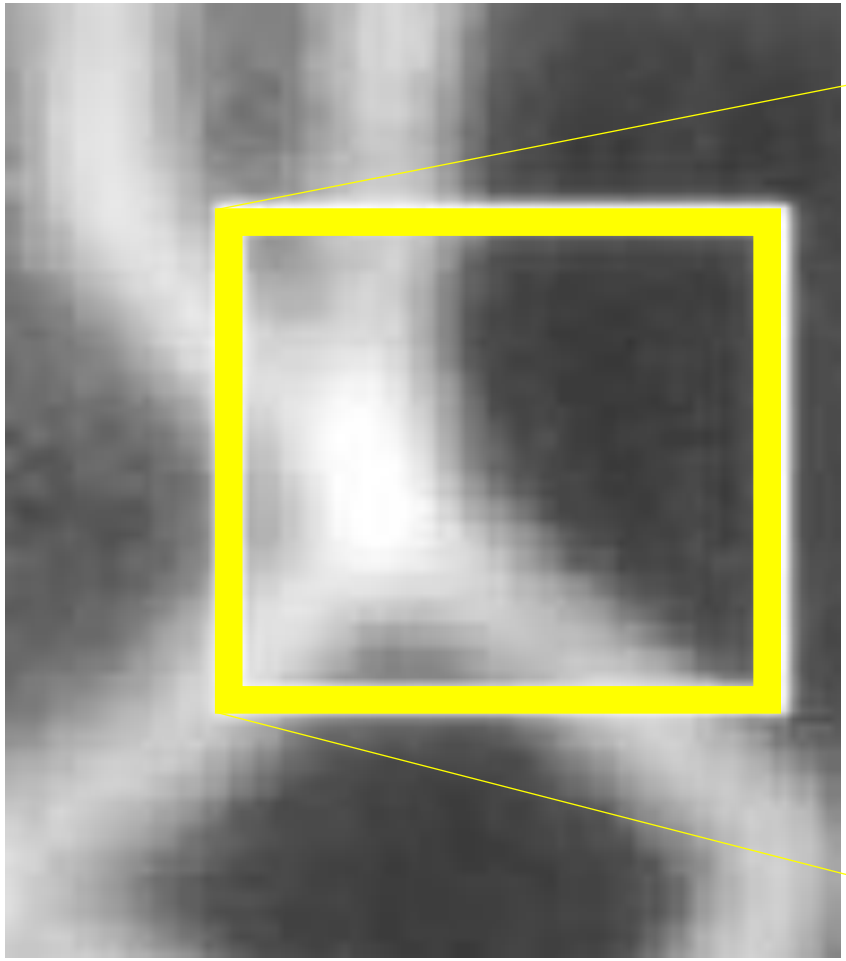
- Deconvolved
- 8bit conversion after contrast adjustment
- Full range displayed

- Plus treatment
- Liver cells
- Knockout

Digitization: Sampling and Quantization

An image *function* is digitized both spatially and in amplitude

(x, y, A*)* = (4, 15, 423)



Digitization of spatial coordinates *(x, y)* is *image sampling*

Digitization of amplitude is *gray-level quantization*

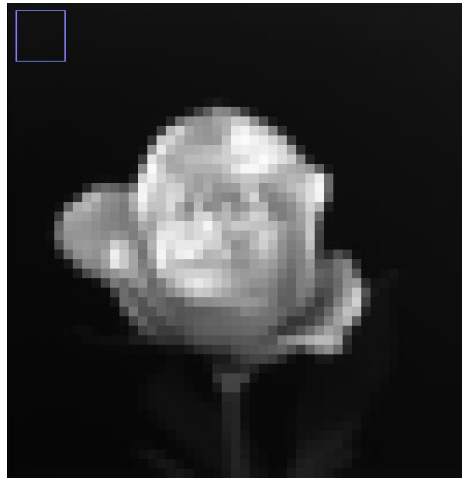
63	84	119	172	219	225	182	135	79	51	36	24	23	19	15	-1	14	14	8	0	-4	7	18
78	84	104	170	223	224	196	118	84	49	36	28	16	11	25	4	15	13	8	-4	9	11	7
61	80	115	153	209	204	170	113	73	46	41	29	9	17	11	11	0	12	-2	2	2	3	23
72	98	121	160	190	207	178	116	68	50	29	22	17	19	7	27	15	9	-3	-4	14	5	8
64	90	132	167	210	214	180	115	71	37	36	31	13	15	9	8	15	6	0	5	-14	4	12
75	93	124	169	216	229	196	107	71	56	19	18	22	24	7	5	15	11	8	-1	12	6	7
97	87	128	193	210	225	193	111	85	47	27	27	21	12	5	2	-1	4	1	-3	7	2	-10
103	108	134	180	201	233	185	115	55	38	26	25	15	20	18	6	2	2	1	4	-3	-13	0
142	132	161	216	238	223	160	90	59	45	17	10	9	13	10	11	4	-9	5	2	7	0	5
172	162	175	231	239	238	155	88	48	28	24	17	15	13	0	14	0	11	-3	4	9	0	-10
226	219	230	260	265	236	161	92	43	31	31	11	5	11	7	13	19	9	18	-11	-9	-2	6
234	247	256	302	311	253	174	97	48	27	12	15	7	7	0	16	8	5	3	-4	0	-6	4
260	263	297	346	349	303	196	126	55	27	30	24	3	6	7	1	12	3	9	0	-2	-13	2
244	293	340	388	399	321	223	130	74	29	24	30	17	4	3	11	0	8	7	-3	-2	-2	-2
209	273	359	423	436	365	264	141	80	57	32	45	13	3	18	8	-7	0	-6	4	-1	-2	-3
176	253	342	430	443	394	291	161	86	59	37	23	18	5	0	7	8	11	1	-3	13	-5	-2
152	218	311	425	470	420	325	208	111	66	52	29	28	9	4	7	8	4	-7	11	-18	-13	-2
129	199	294	413	469	441	384	257	148	111	69	34	20	20	6	3	15	4	-2	-6	-3	-10	9
140	206	294	385	439	442	365	310	223	157	114	78	45	28	9	21	5	15	-4	-13	0	-5	-1
173	233	309	354	392	375	333	303	261	214	135	92	51	47	18	12	13	12	20	-9	4	1	15
221	278	300	321	306	293	286	279	250	231	184	142	108	67	41	18	13	5	8	-8	0	7	5
267	302	291	244	228	211	201	215	241	227	205	184	136	110	68	51	26	11	8	3	0	8	-3
284	279	257	202	133	129	137	151	183	213	209	188	187	155	109	69	49	26	25	8	8	18	-4
275	248	191	143	95	85	87	98	122	166	184	192	206	194	176	135	98	50	44	19	21	0	1

1024, 1024

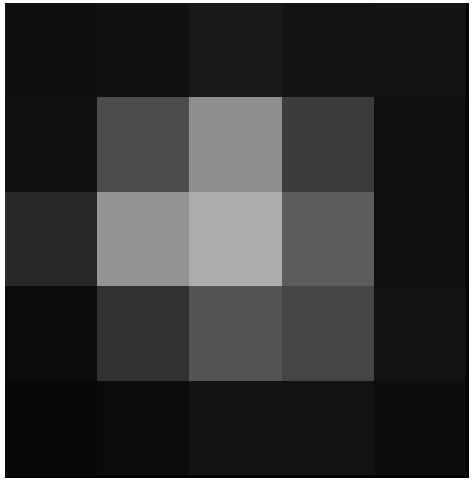
512, 512

100, 100

50, 50



5, 5



32-bit

16-bit

8-bit

4-bit

2-bit

Bit-depth and Dynamic Range

Storage of this data is limited by bits. Discrete quantization of gray values is expressed as integer powers of 2

0 black
1 white

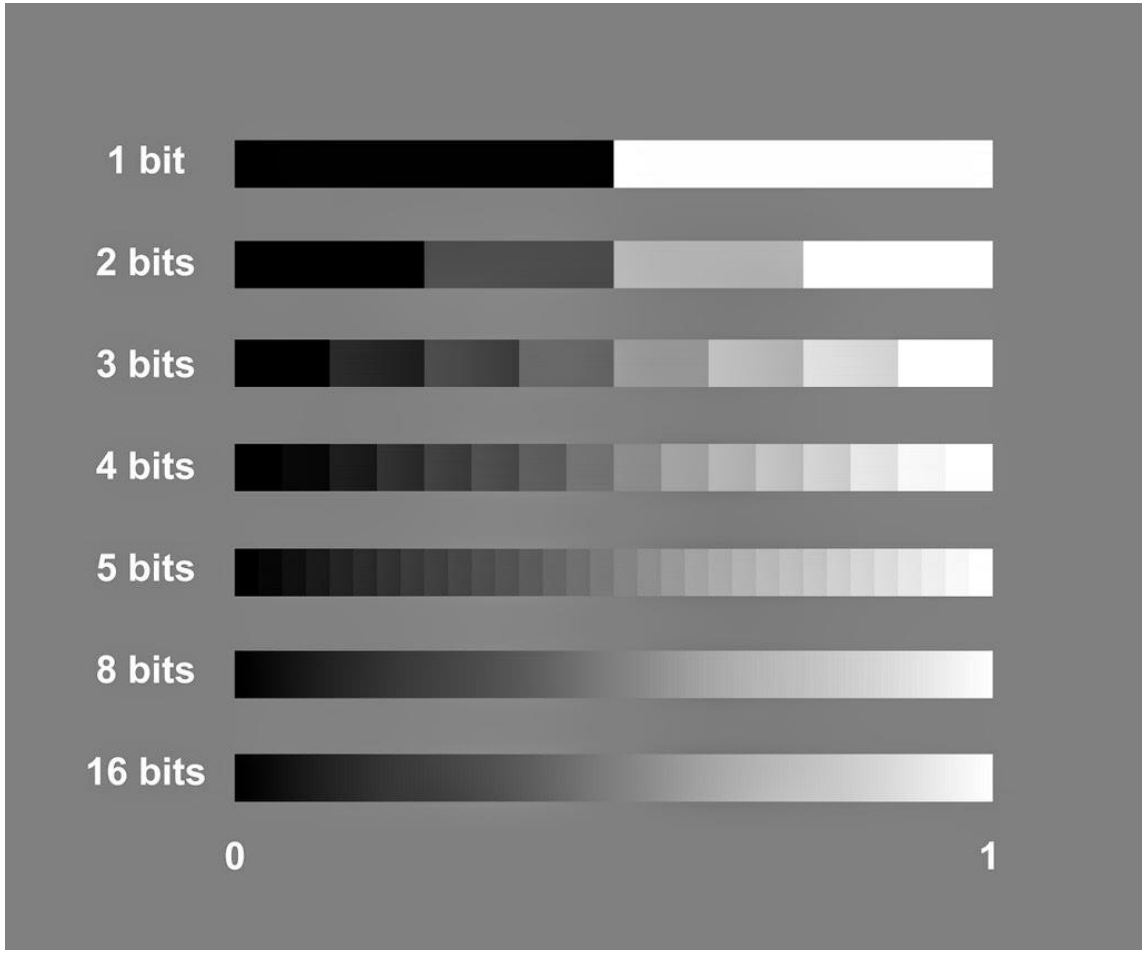
$2^1 = 2 \times 1 = 2$

0 0 black
1 0 dark gray
0 1 light gray
1 1 white

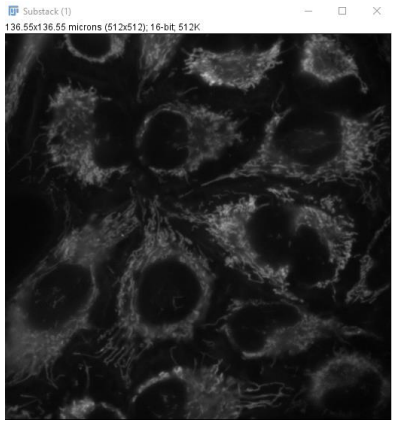
$2^2 = 2 \times 2 = 4$

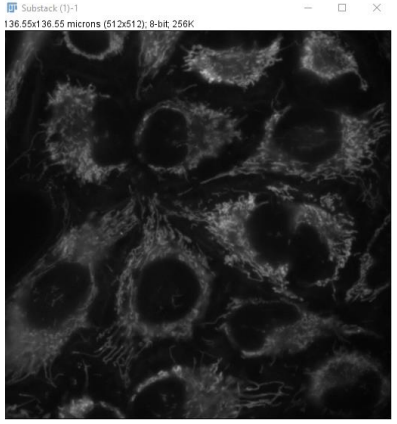
0 0 0
1 0 0
0 1 0
0 0 1
1 1 0
1 0 1
0 1 1
1 1 1

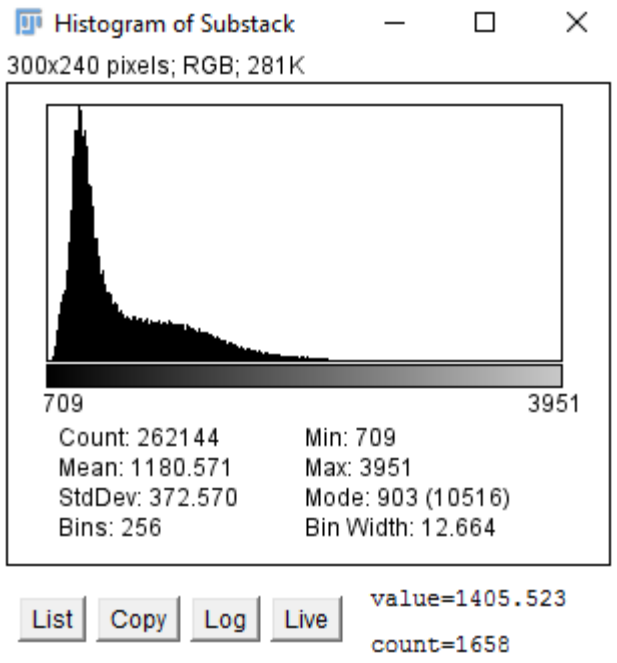
$2^3 = 2 \times 2 \times 2 = 8$

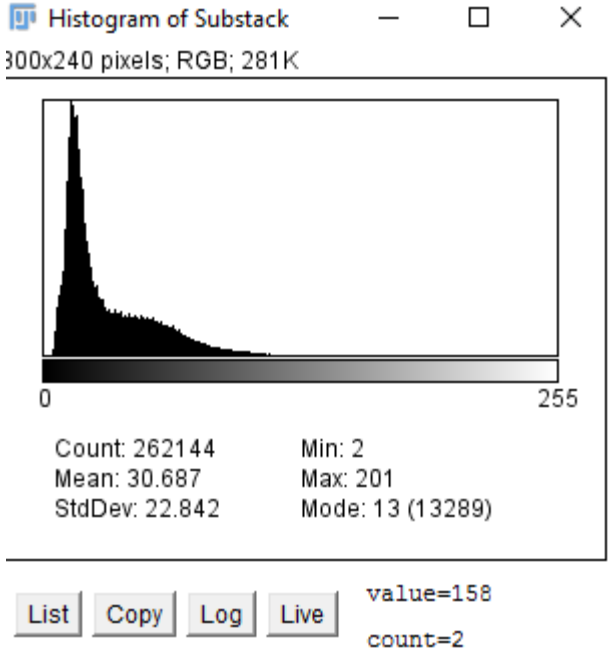


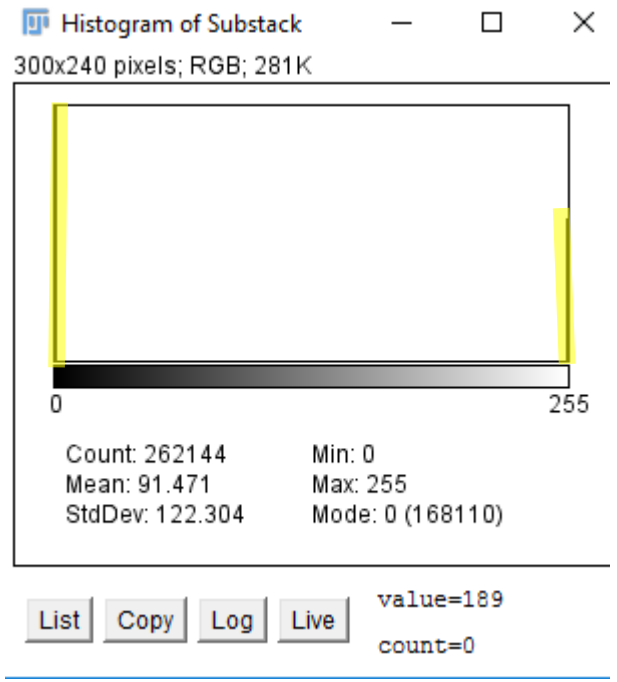
Histograms of pixel values and image display

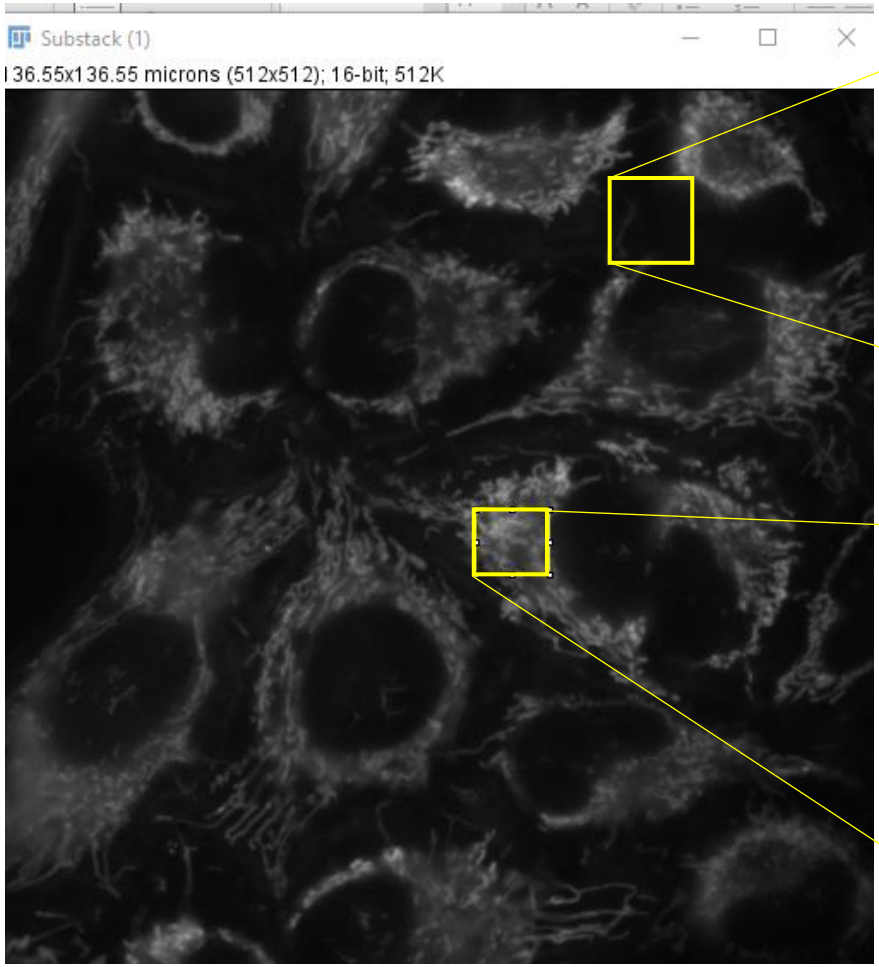


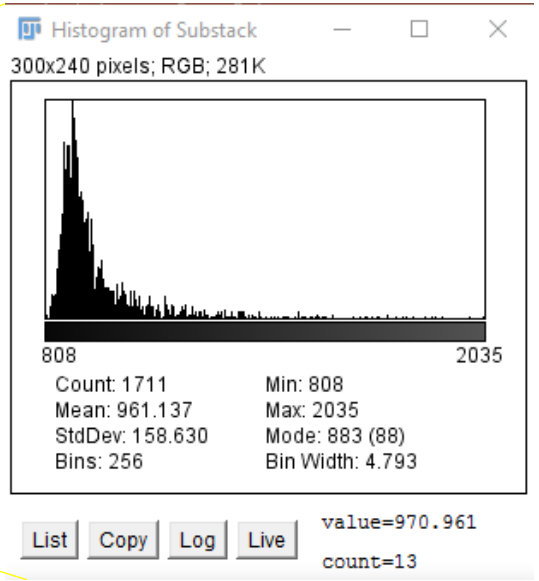


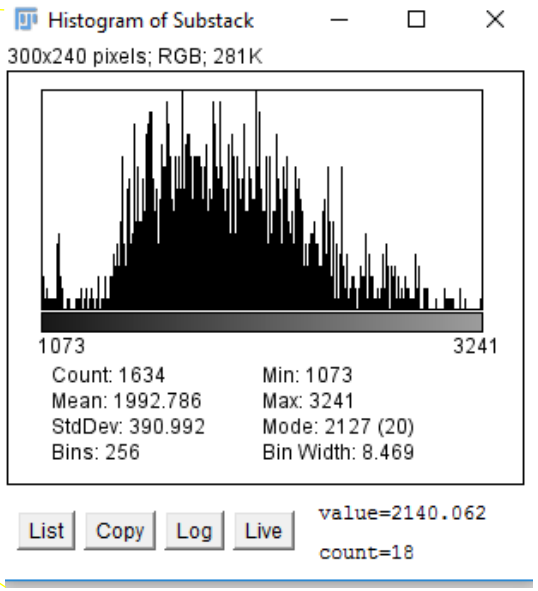






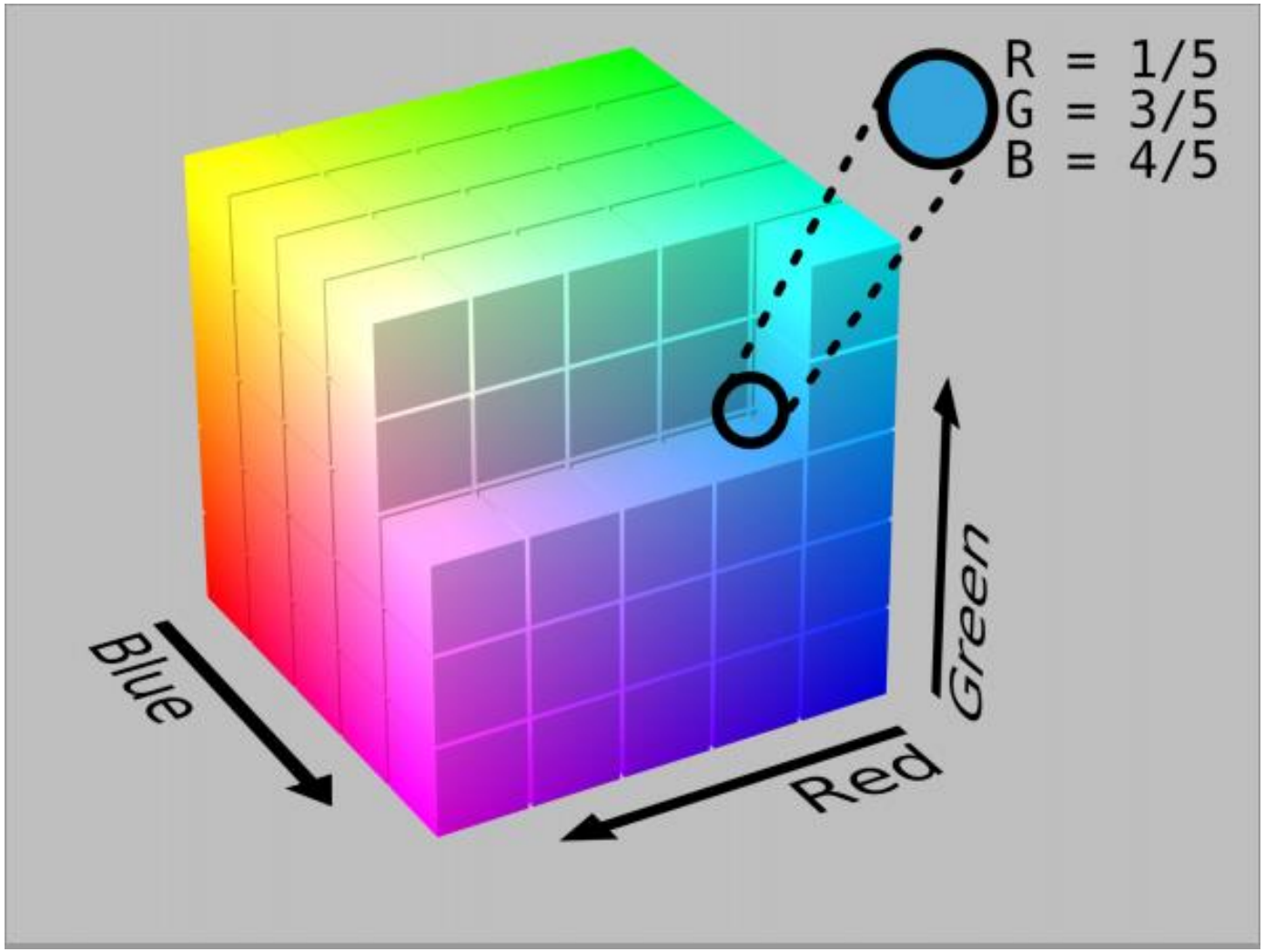

Substack (1)
136.55x136.55 microns (512x512); 16-bit; 512K


Histogram of Substack
300x240 pixels; RGB; 281K
808
2035
Count: 1711
Mean: 961.137
StdDev: 158.630
Bins: 256
Min: 808
Max: 2035
Mode: 883 (88)
Bin Width: 4.793
List
Copy
Log
Live
value=970.961
count=13


Histogram of Substack
300x240 pixels; RGB; 281K
1073
3241
Count: 1634
Mean: 1992.786
StdDev: 390.992
Bins: 256
Min: 1073
Max: 3241
Mode: 2127 (20)
Bin Width: 8.469
List
Copy
Log
Live
value=2140.062
count=18

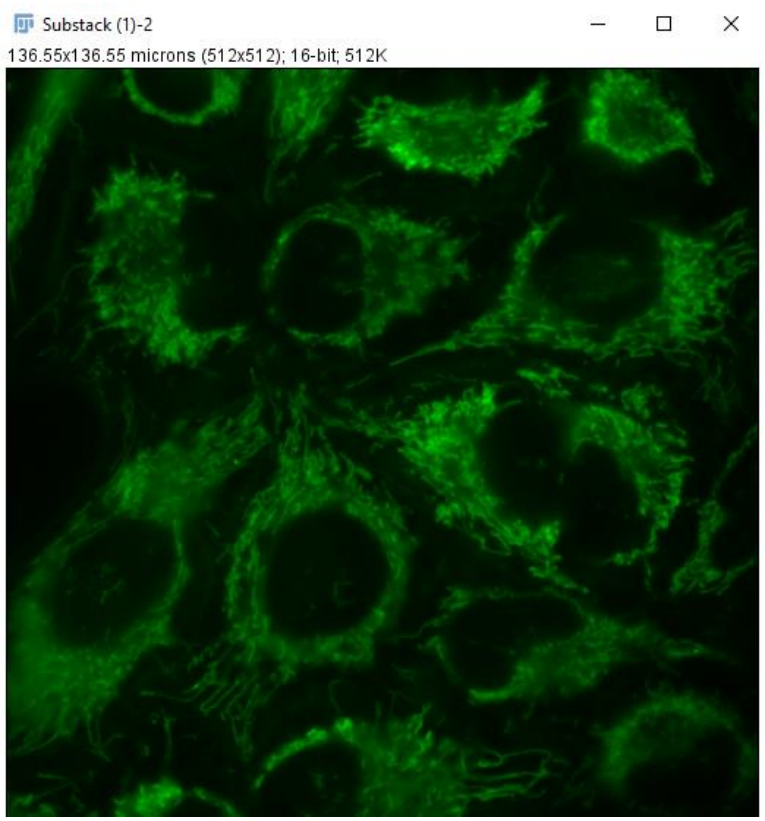
RGB

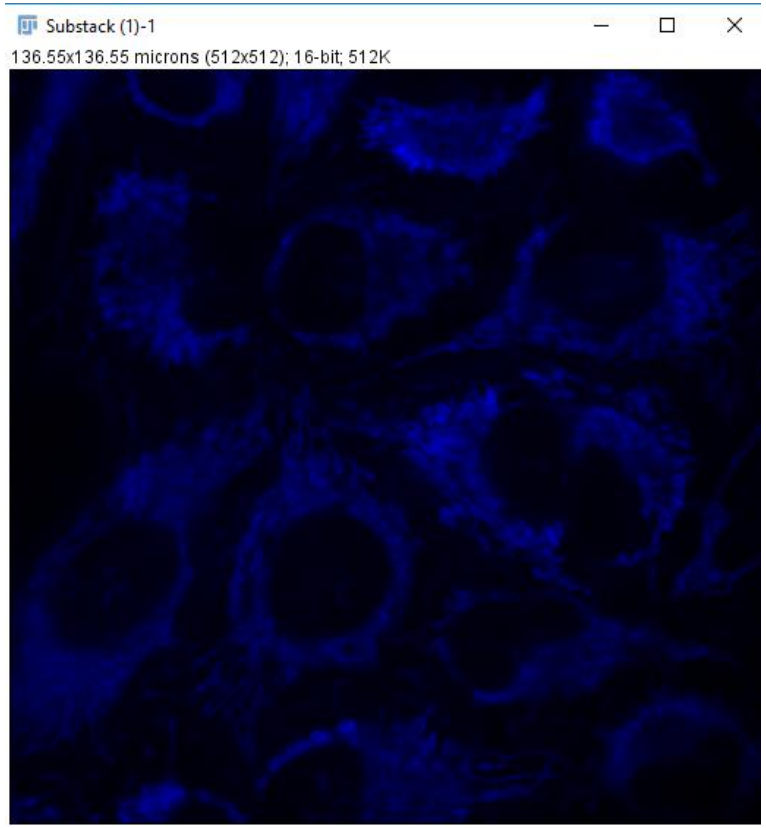
RGB Images are not accurate for fluorescence microscopy.

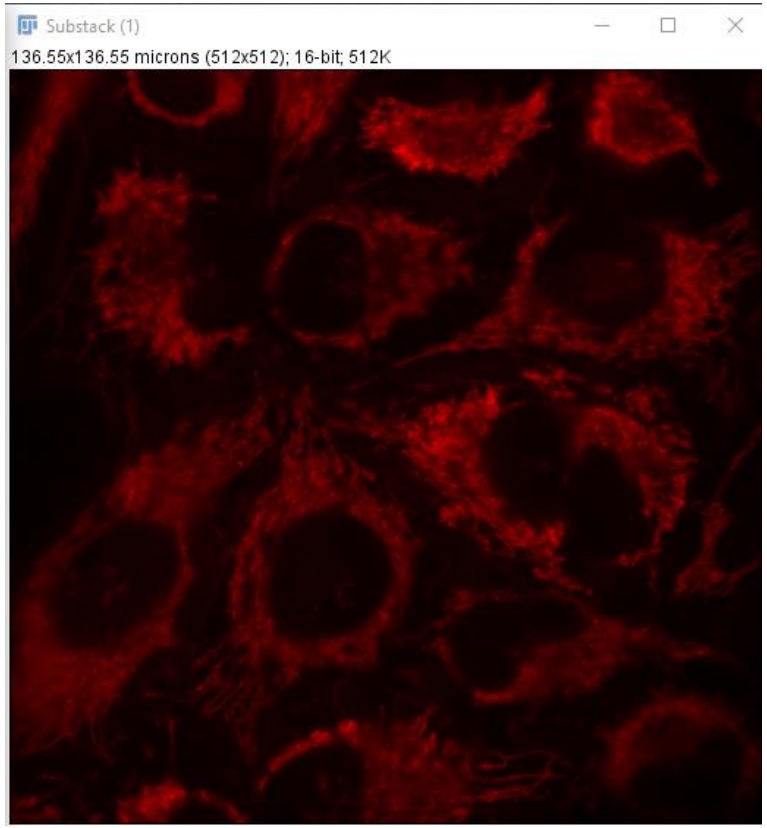


If you ever get an RGB image, something has gone wrong

Look up tables or colour maps

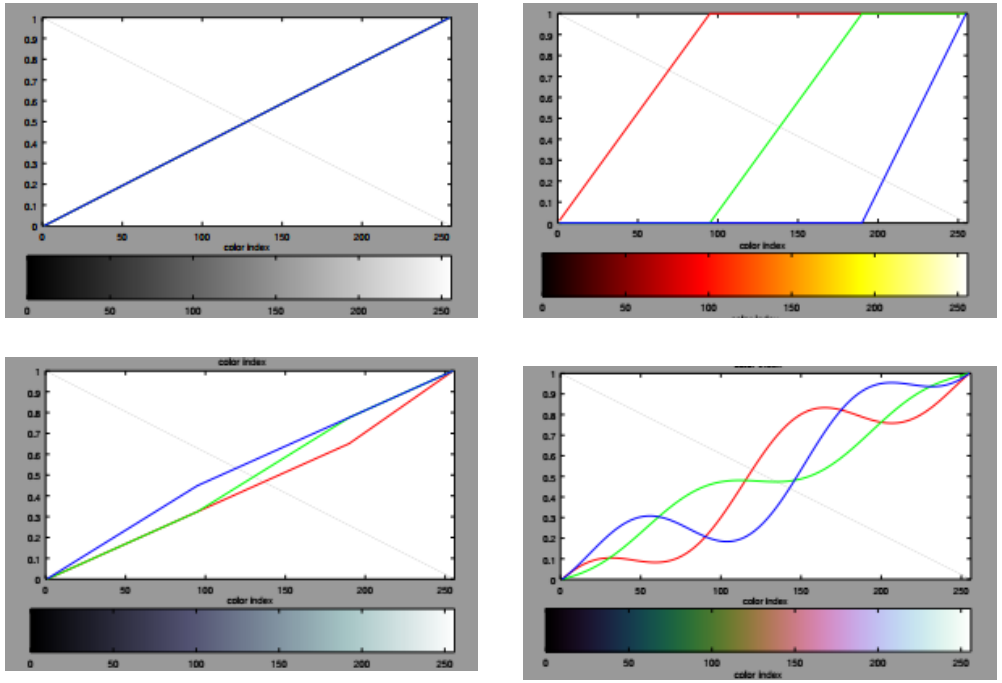


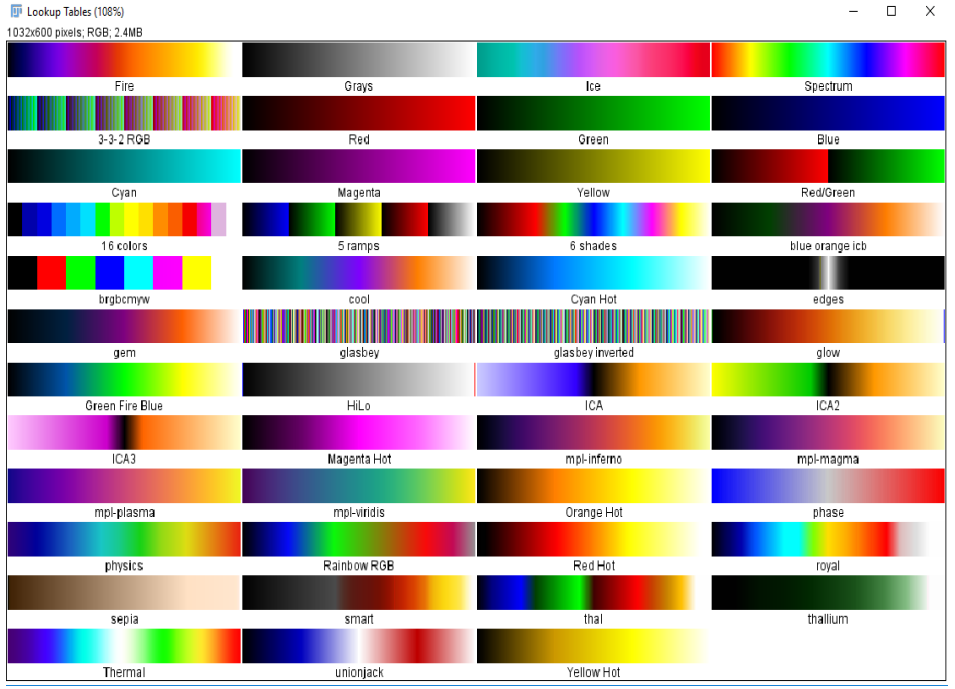


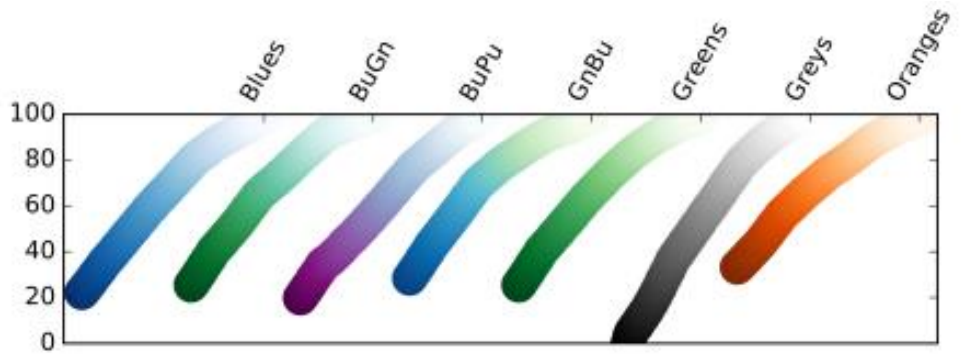


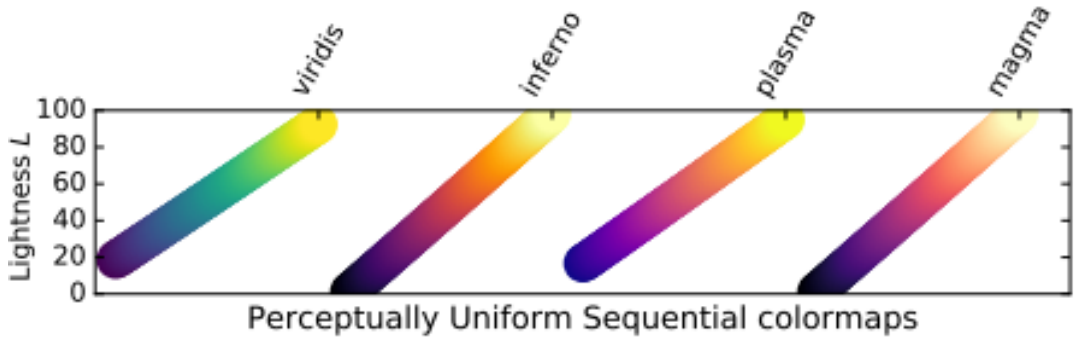
A multichannel image is just multiple grayscale images

Scales in colour









Tools for processing and analysis

ImageJ1

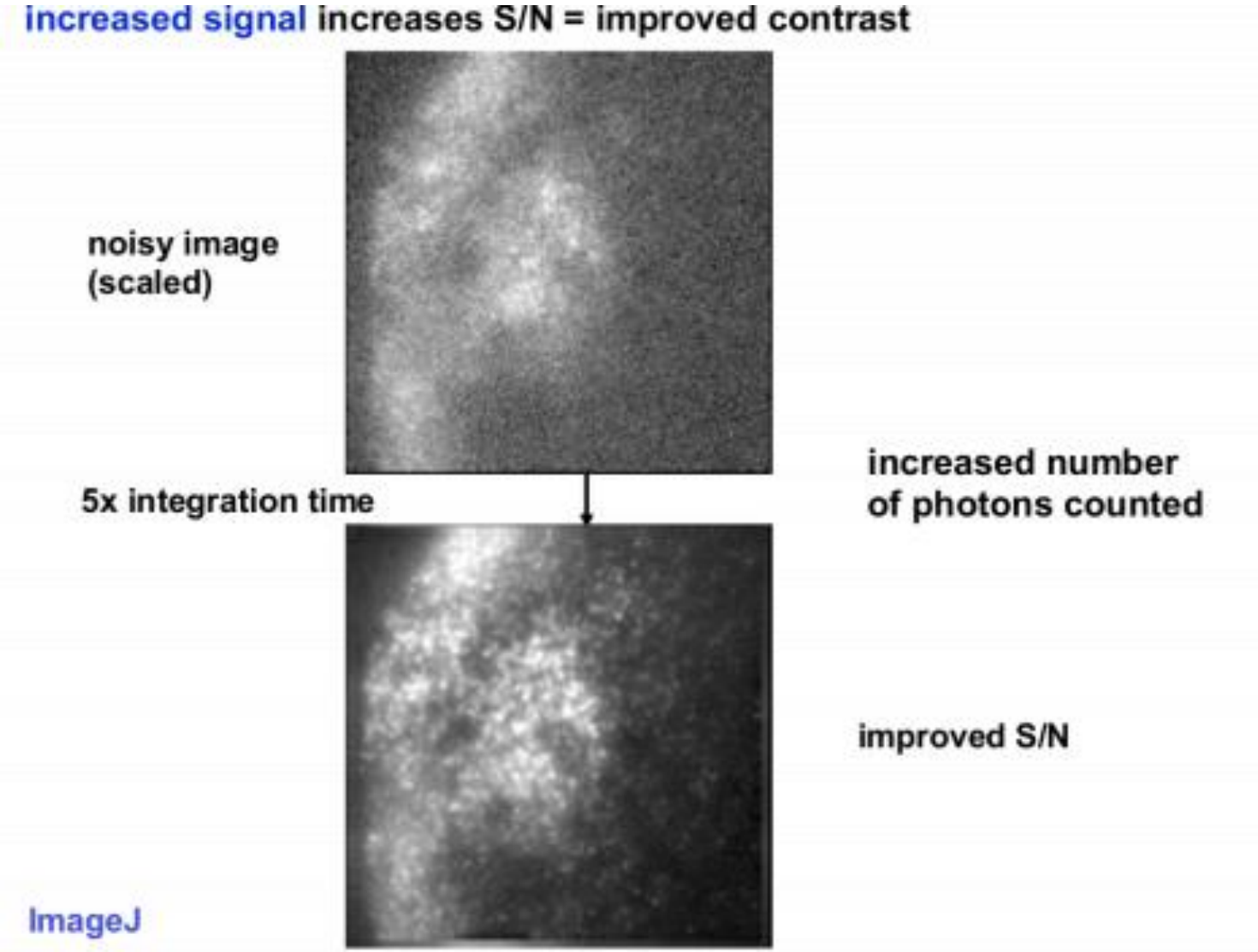
ImageJ2

FIJI – Is Just ImageJ

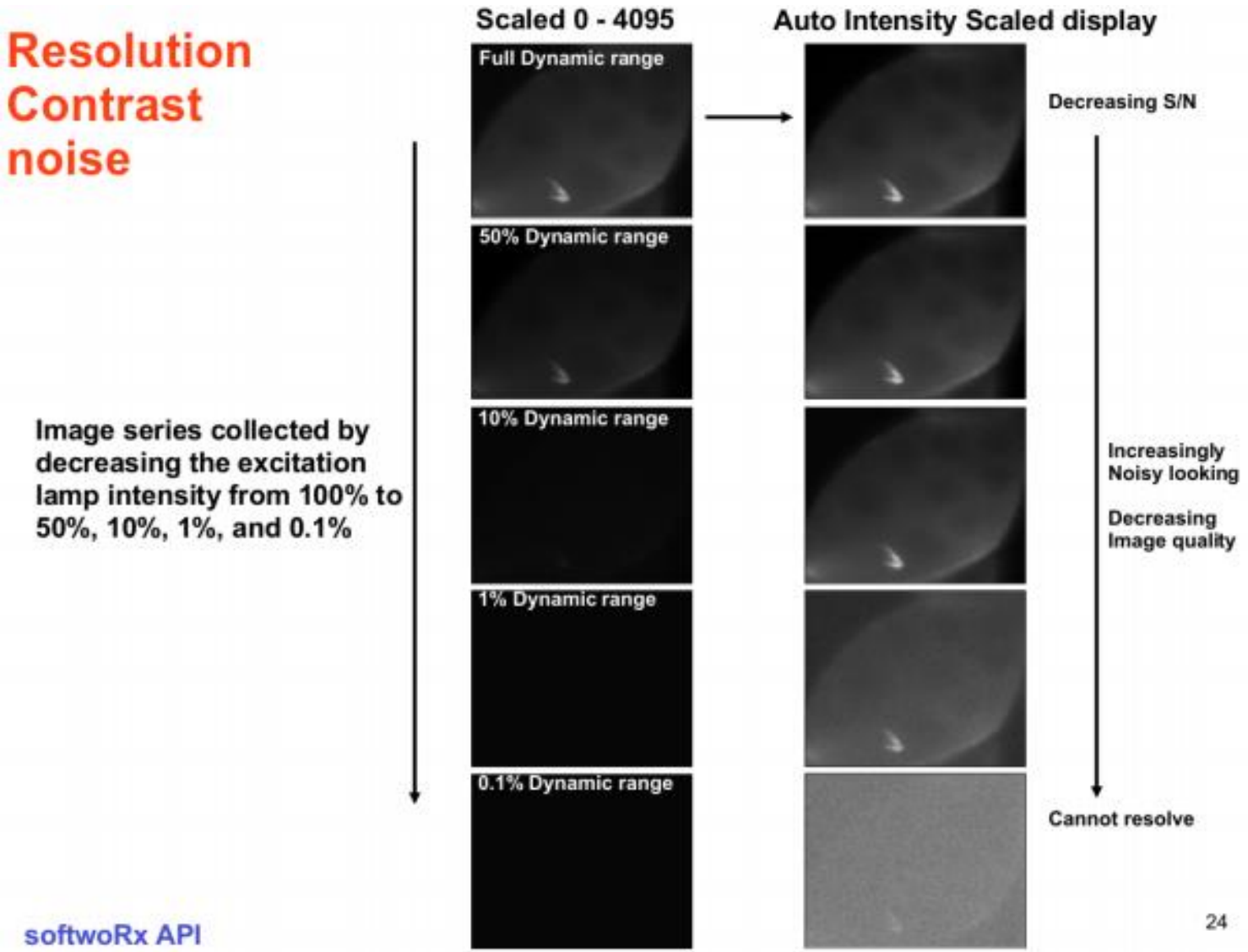
Considerations for Aquisition

- The system must be correctly set up and aligned. Check this with a PSF if necessary
- The specimen should not cause undue optical aberration
- Avoid saturation and underflow but fill the dynamic range while keeping settings the same across all images
- Use a HiLo LUT, beware of intensity scaling
- Check dark signal with a background image
- Be aware of x,y,z optical resolution of the system and sample appropriately
- Take care with signal to noise limitations

Improving Signal to Noise during Acquisition

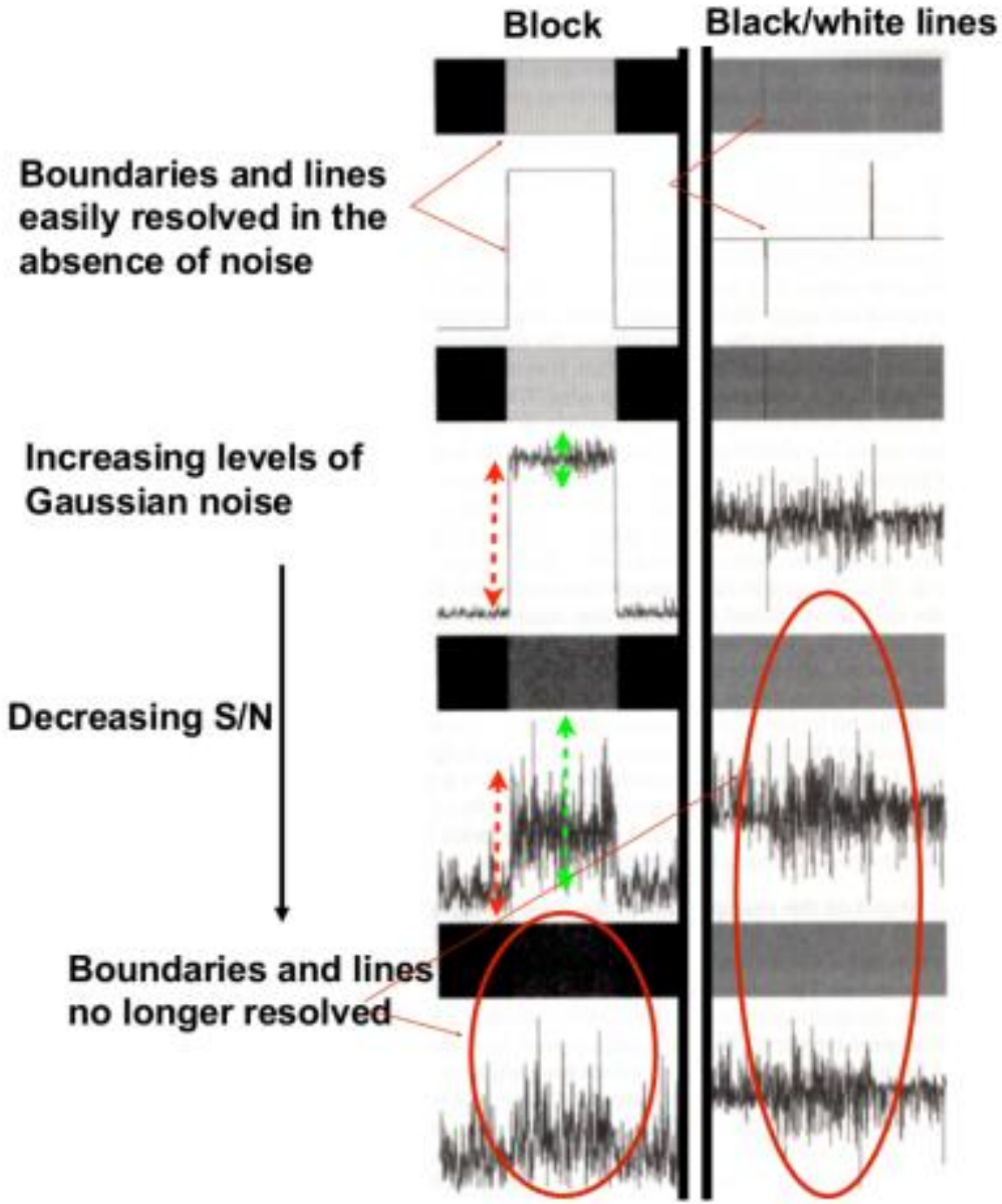


How does noise affect my resolution?



Resolution, contrast, noise

The difference between signal and background must be at least 3X the noise to be detectable



Micron

Come speak with us!

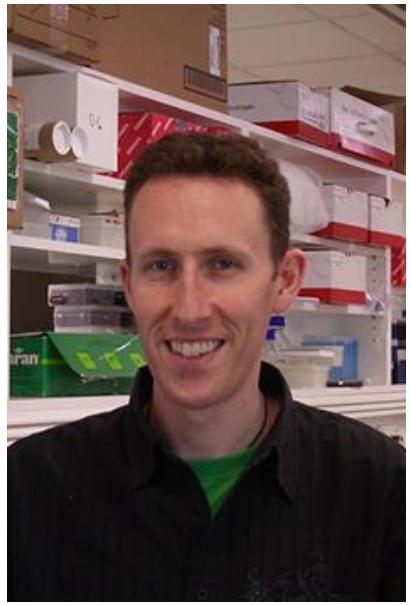




Figure Preparation Guidelines

- Carry out all processing and analysis before making figures by using software for raster images (i.e. ImageJ)
- Use vector graphics for lettering, arrows, diagrams, arranging panels (Inkscape)
- Both can rotate, size and crop
- Do not use Office suite of applications: Word, Powerpoint, Keynote, Impress, Writer
- Be consistent with processing steps, especially contrasting