Introduction to Image Analysis

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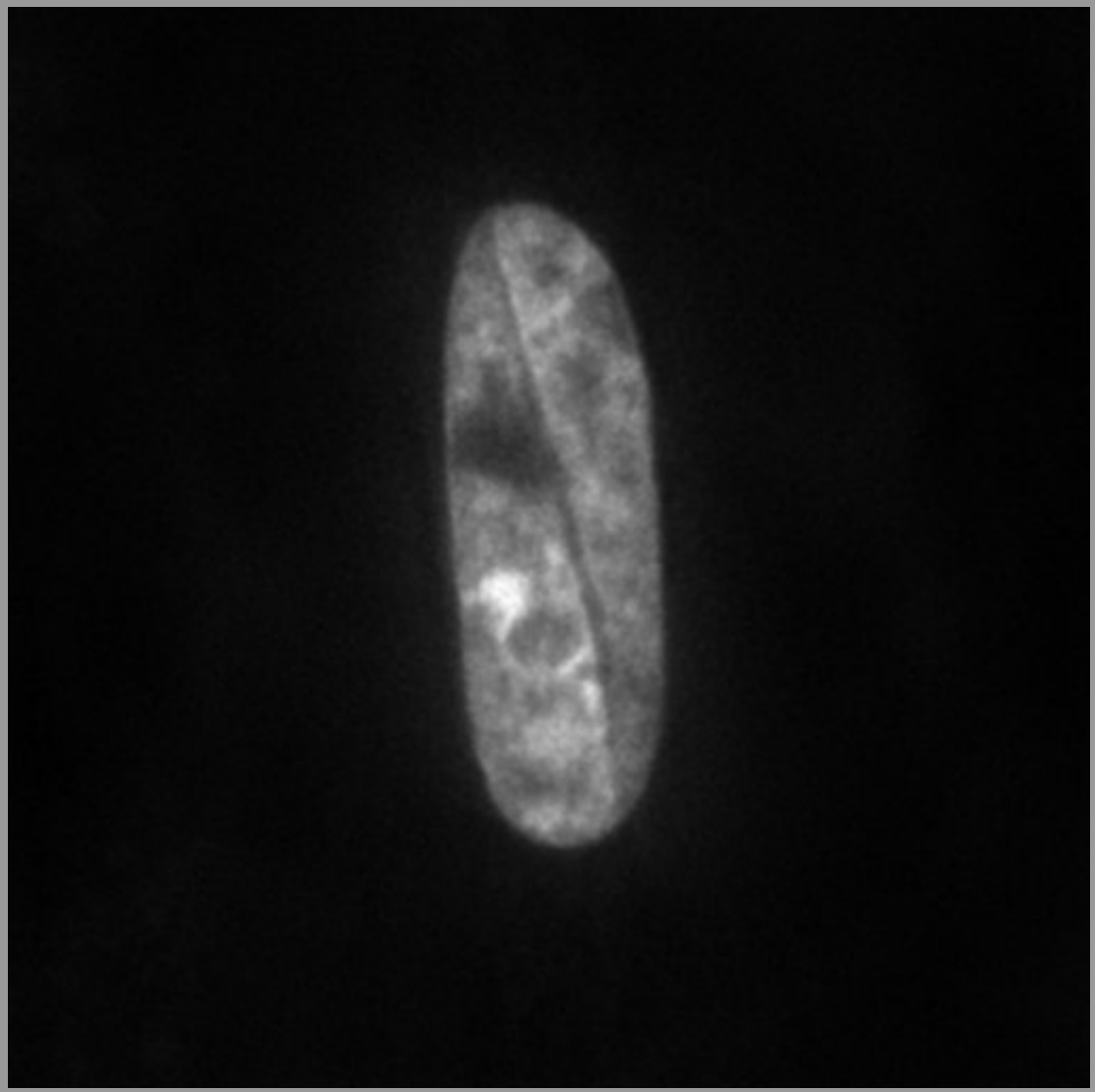
Micron Advanced Microscopy Course, 2016

Microscope Image Analysis in 3 parts

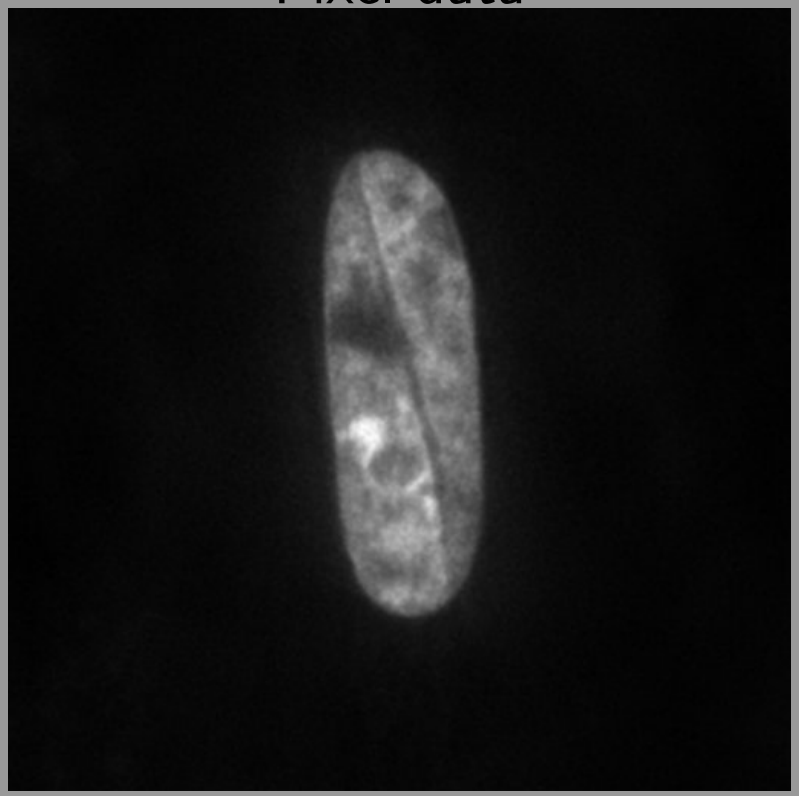
1. What is in a microscope image
   - What is in a image?
   - Image display
   - ImageJ
   - Image acquisition
2. Images as N dimensional numeric arrays
   - N dimensional images
   - Spatial filters
   - Morphology
   - Connected components
3. Don't botch your data
   - File formats
   - Data storage
   - OMERO

What is an image?



What is an image?

Pixel data



Metadata

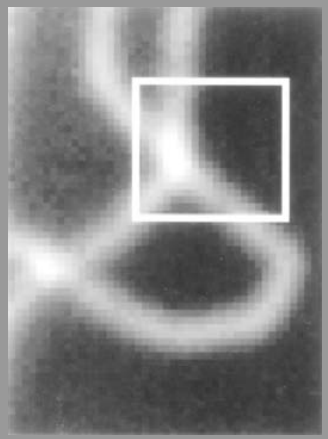
- emission wavelength 535nm
- excitation wavelength 500nm
- exposure time 2s
- objective Olympus 100X/1.40
- pixel size 64.4×64.4 nm

- deconvolved
- 8bit conversion after contrast adjustment
- full range displayed

- horse fibroblast cell line
- transient transfection of H2B-GFP
- treated with sugar lumps

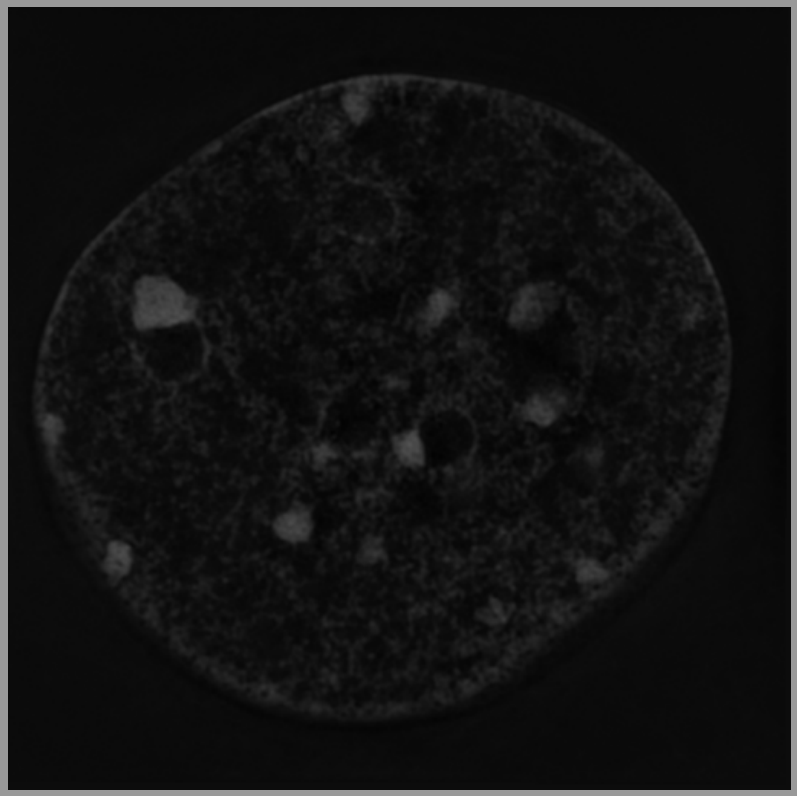
File $\Rightarrow$ `my-lovely-horse-H2B-GFP_01_R3D_D3D.dv`

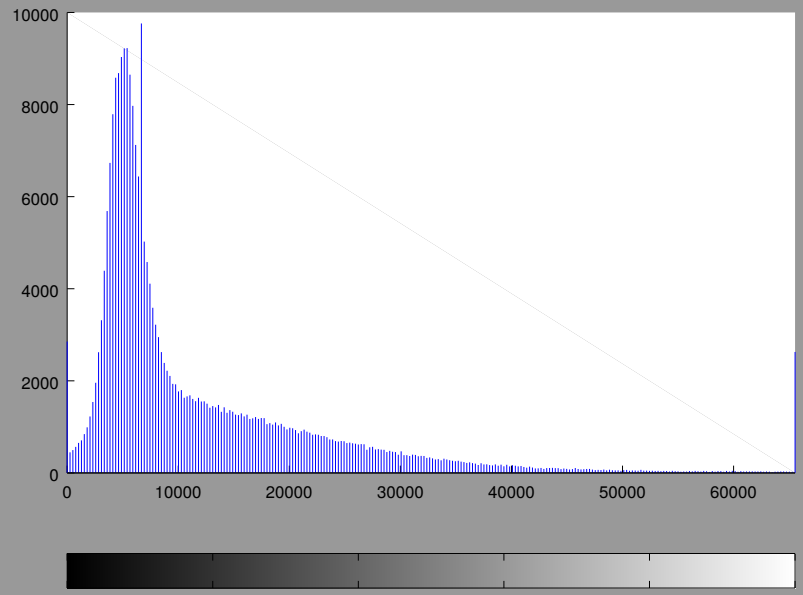
Pixel data



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	8
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	65	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	76	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

Histogram of pixel values

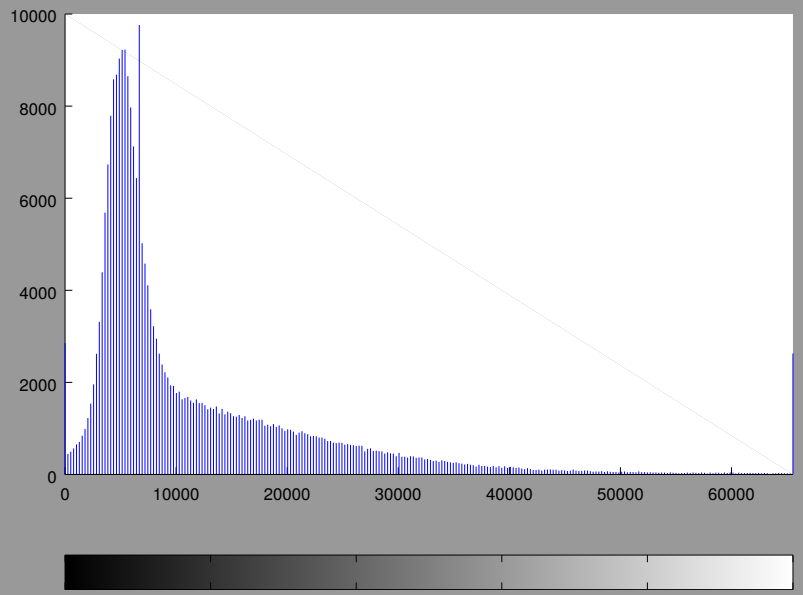




Adjust contrast/levels, see effect on display and pixel values.

Bit depth / dynamic range

2	2
2×2	4
$2 \times 2 \times 2$	8
...	
2^7	128
2^8	256
2^9	512
2^{10}	1024
2^{11}	2048
...	
2^{15}	32768
2^{16}	65536



More pixels in the same bin, less dynamic range.

ImageJ

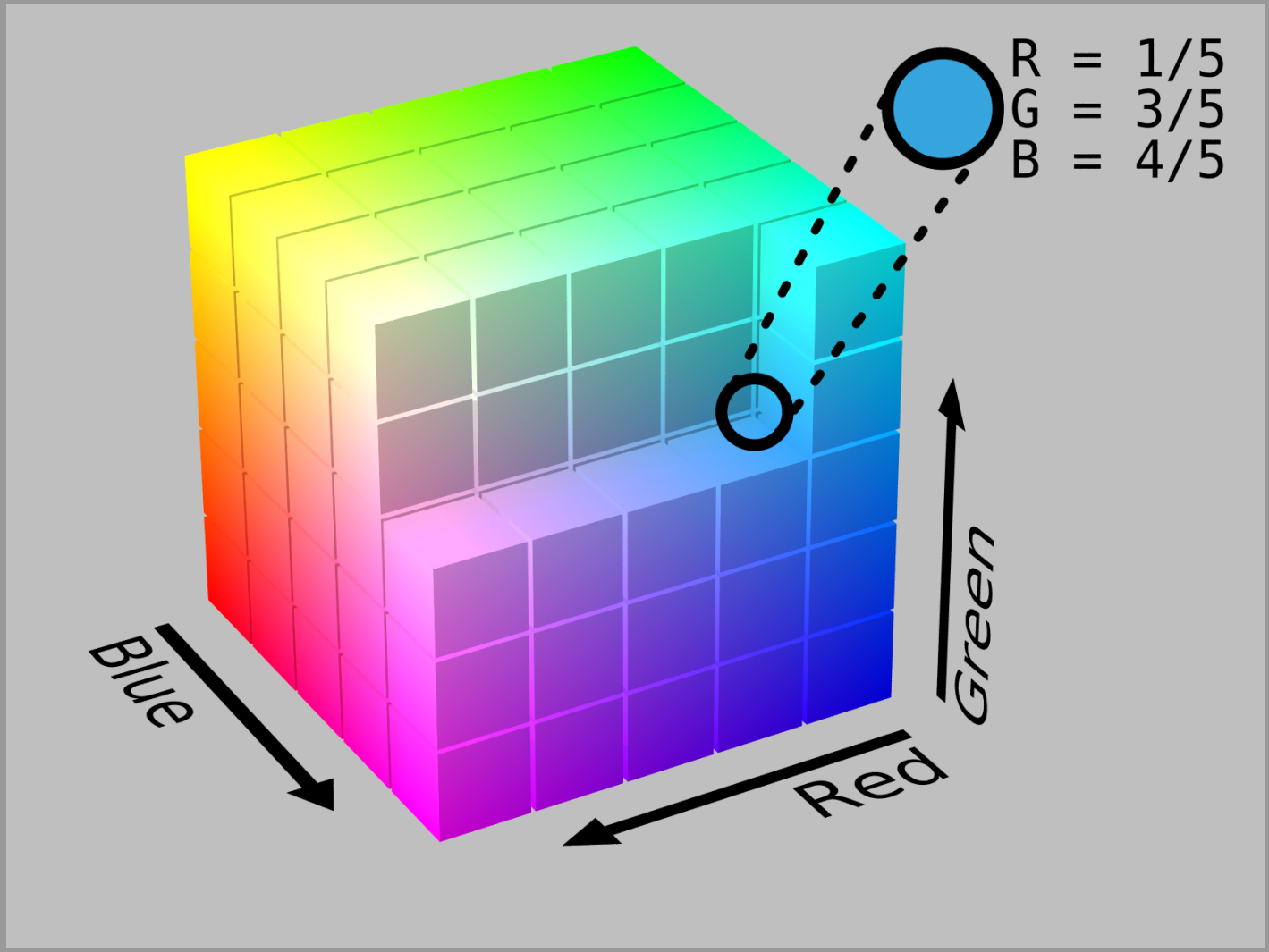
- *de facto* standard in medical sciences
- free and open source
- massive helpfully massive community

ImageJ1

ImageJ2

FIJI

RGB

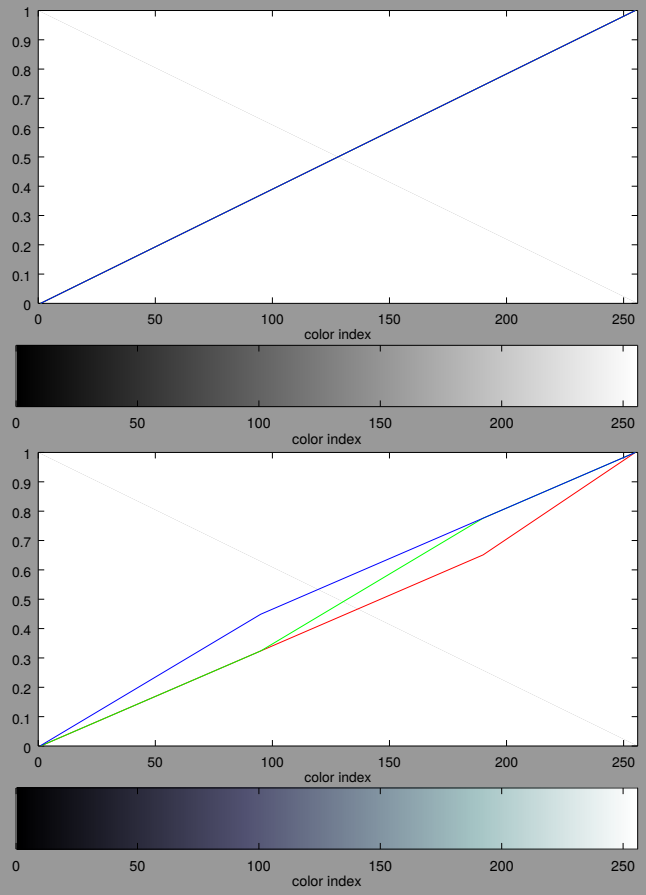


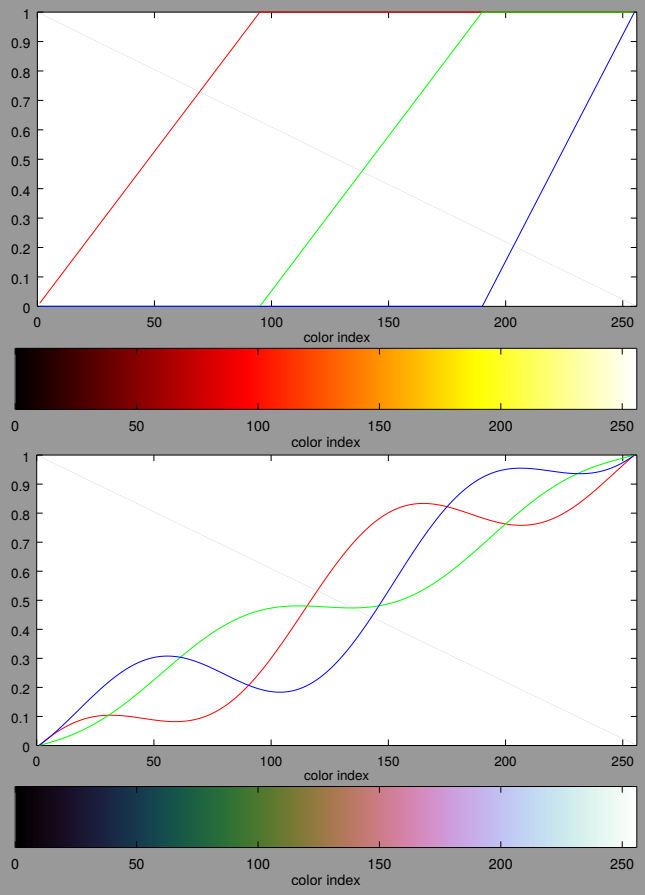
If you ever get an RGB image, you did something wrong.

multi channel

Multichannel image is a series of grayscale images.

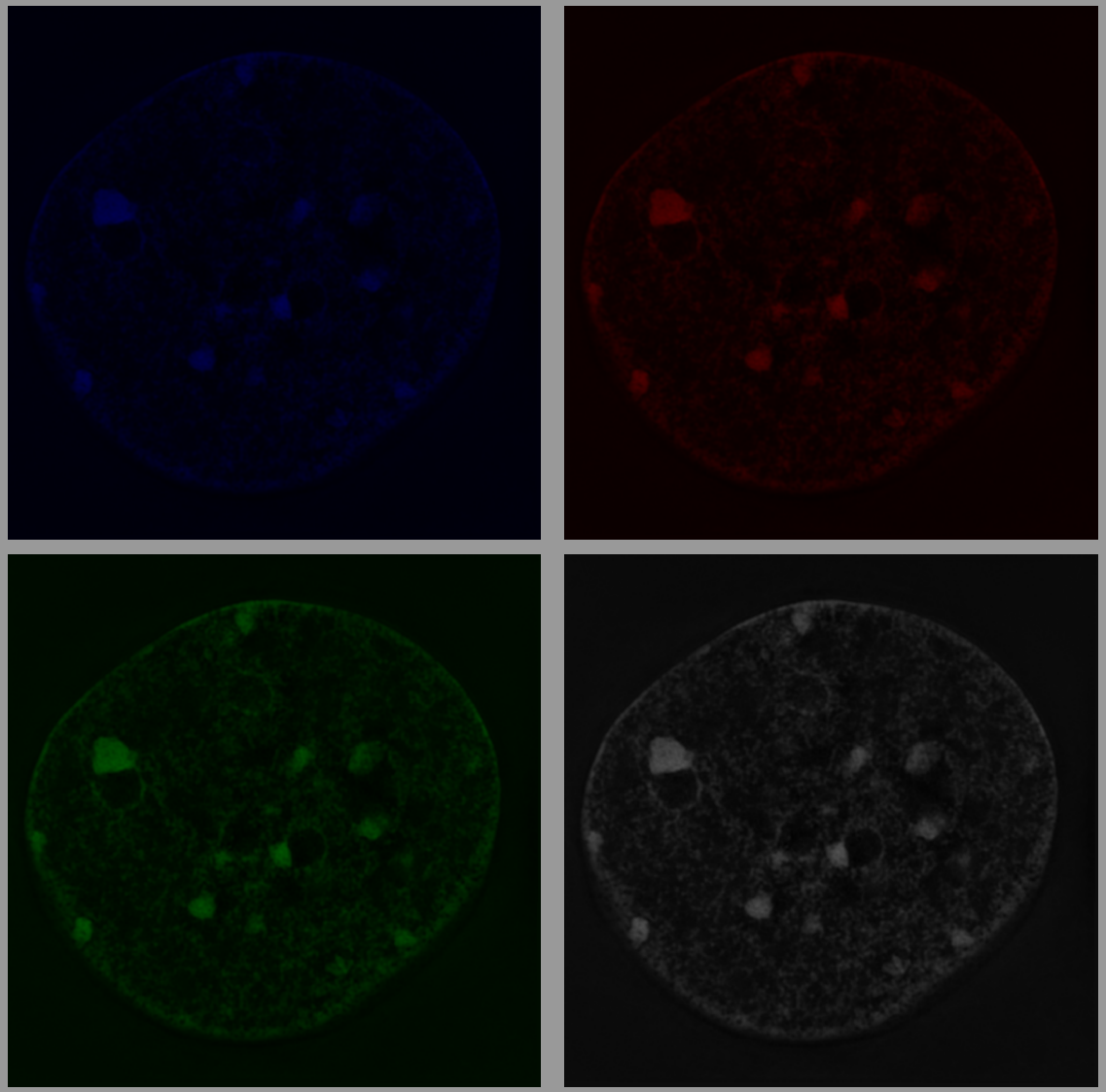
Lookup tables or colormaps



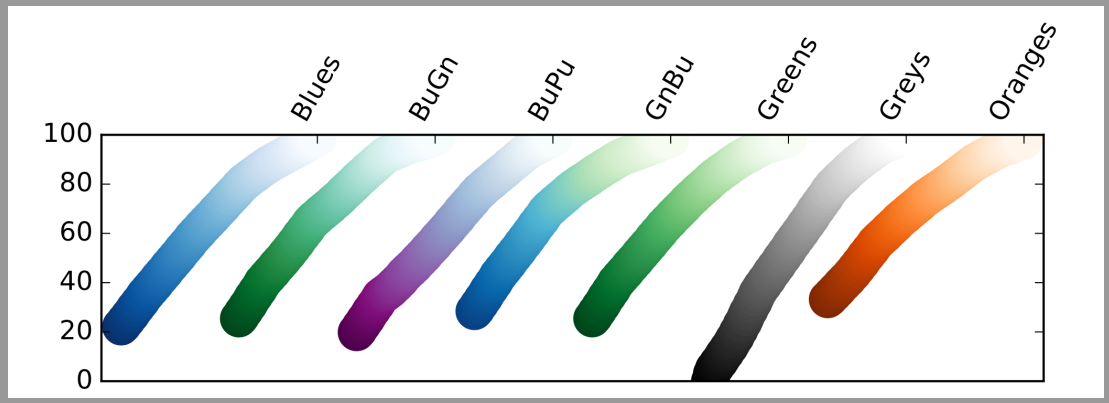


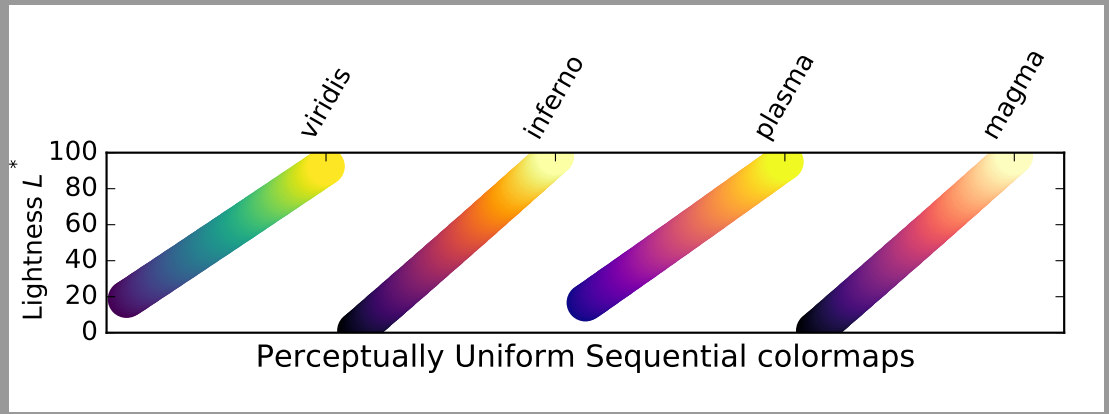
See ImageJ “Display LUTs”

Lightness perception



Lightness perception





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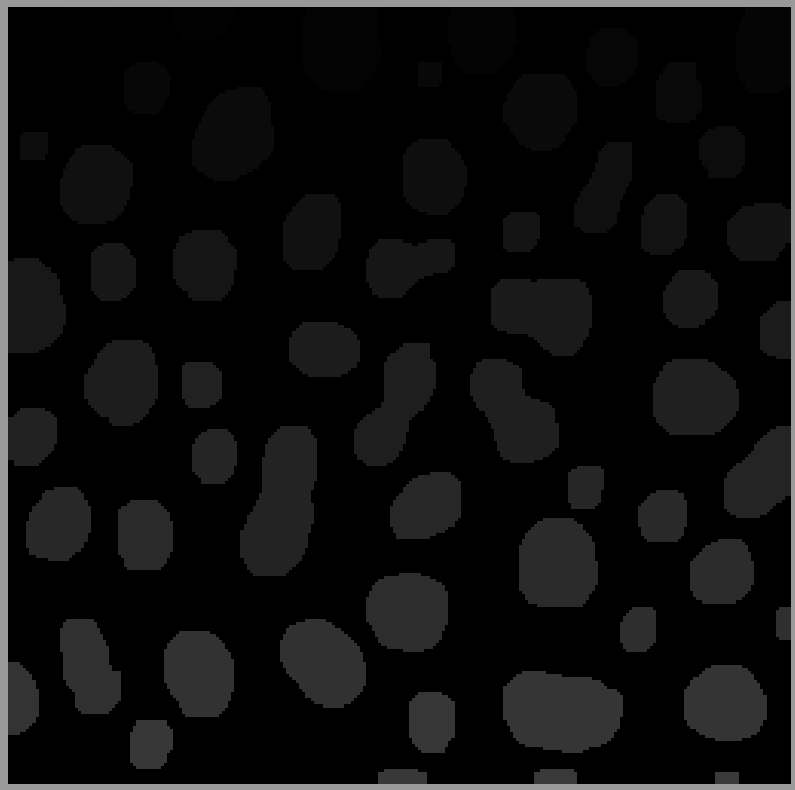
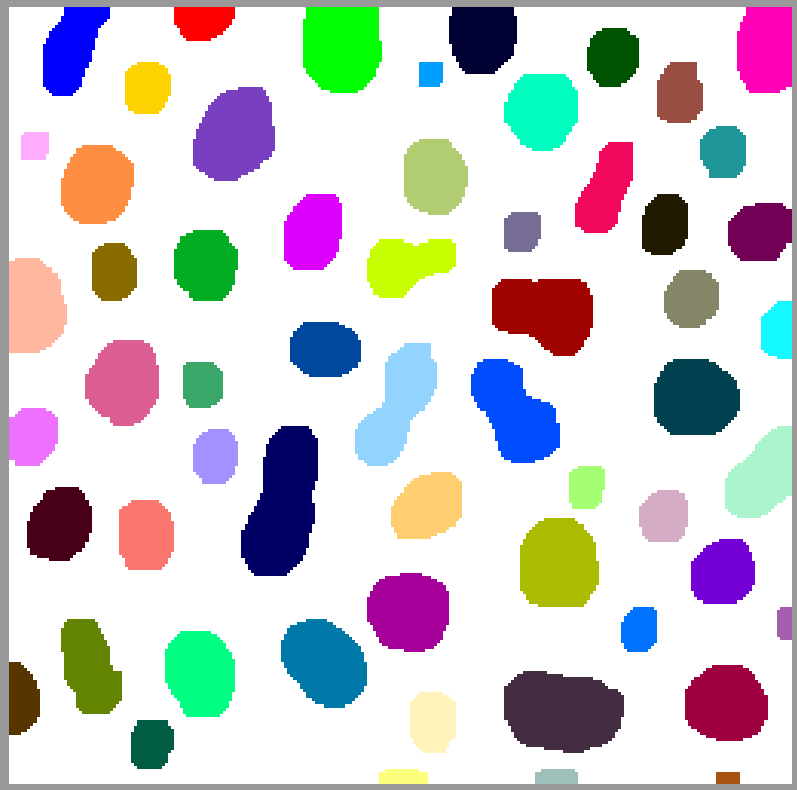




correct image acquisition

- The system must be correctly set up and aligned. PSF verification.
- The specimen should not cause undue optical aberration.
- Avoid underflow and overflow but fill dynamic range while keeping same settings across all images. Use a HiLo LUT. Beware of automatic intensity caling.
- Check dark signal or background image.
- Be aware of XYZ optical resolution of the system and sample appropriately.
- Take care with signal to noise limitations. Binning, gain, exposure time, fluorophore. Make sure you collect enough light.

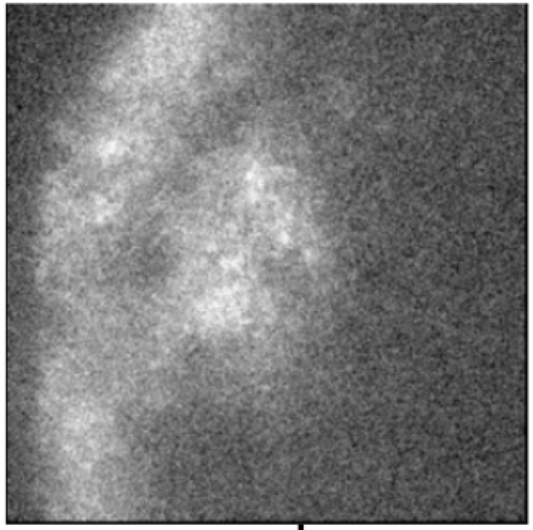
Micron

Come speak with us first.

Noise

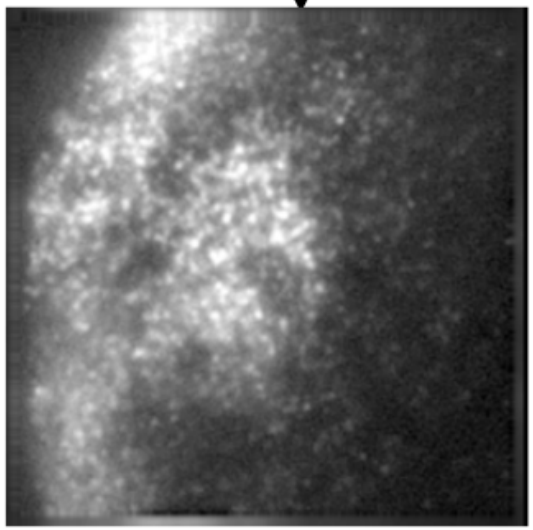
increased signal increases S/N = improved contrast

noisy image (scaled)



5x integration time

increased number of photons counted

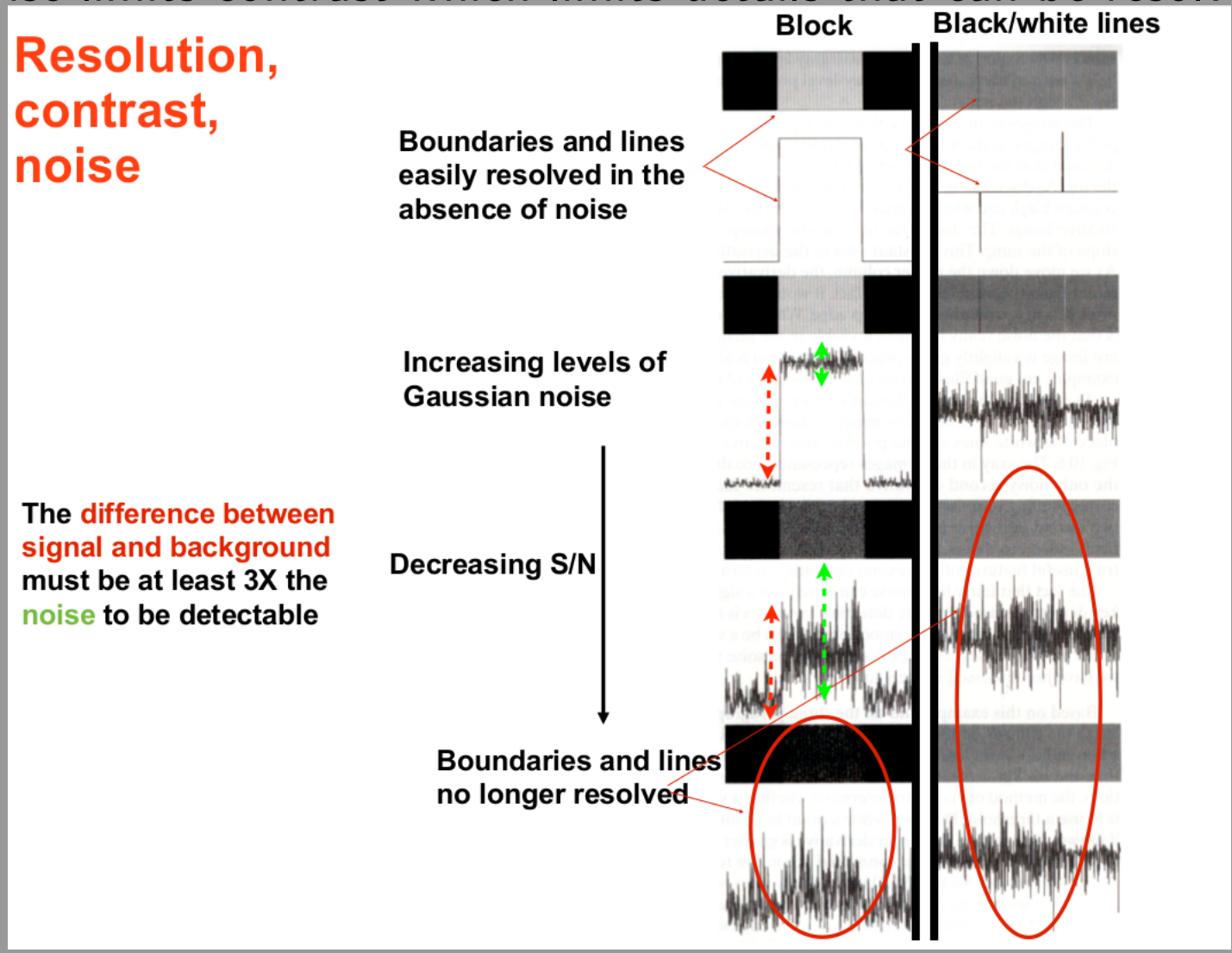


improved S/N

ImageJ

Noise

Noise limits contrast which limits details that can be resolved.



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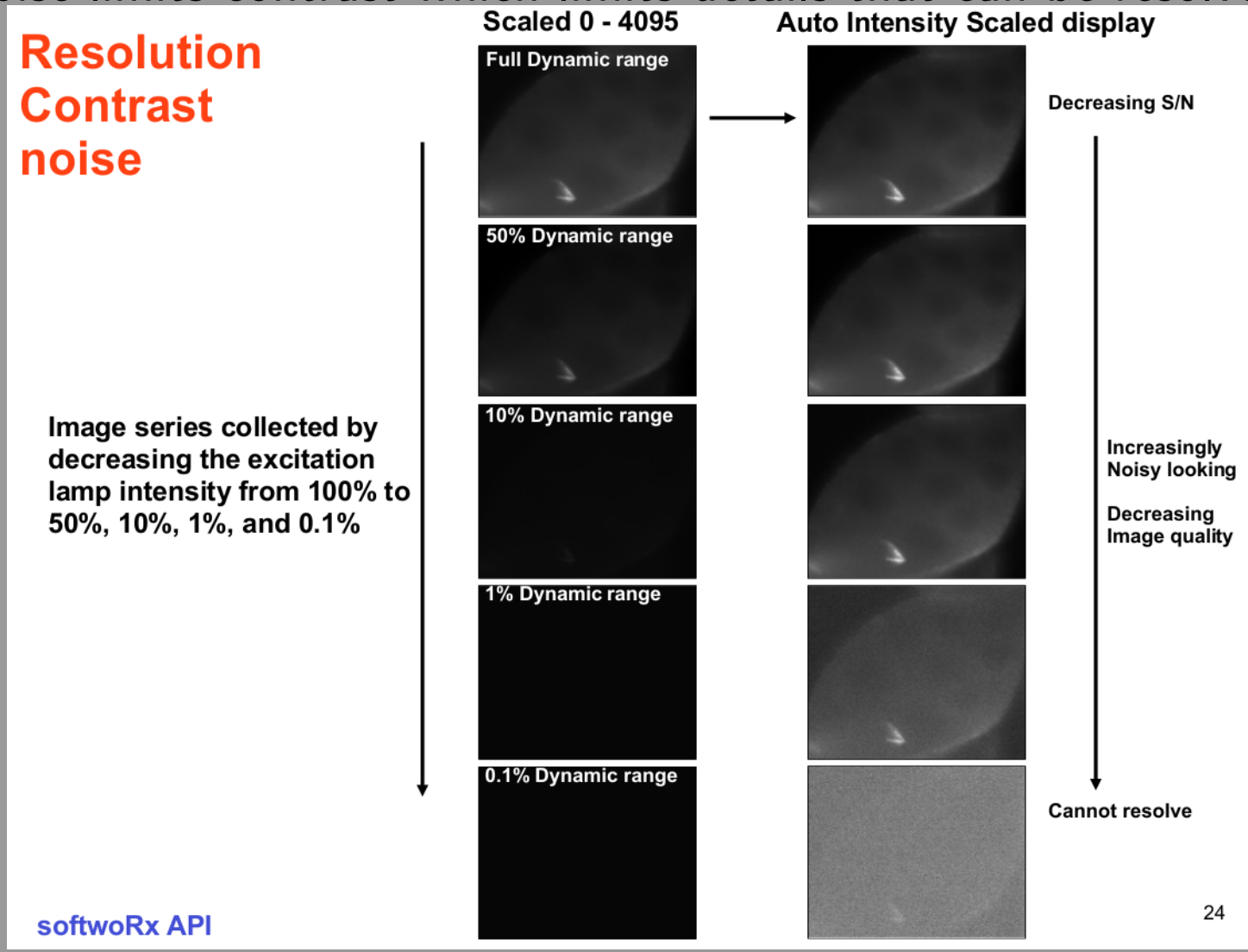

raster vs vector

raster individual pixel values. Use ImageJ.

vector polygons with attributes. Use inkscape.

ImageJ

Inkscape

GIMP

Figure preparation guidelines

- Carry out all processing and analysis of images before making figures by using pixel based (raster) programs.
- Handling of images for figures should use pixel based (raster) programs.
- Vector graphics for lettering, arrows, diagrams, arranging panels.
- Both can rotate, resize, and crop.
- Do not use office applications like powerpoint, keynote, writer, word, or impress.
- Do not use screenshoot.
- Be consistent with processing steps, especially contrasting.