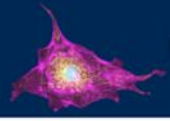
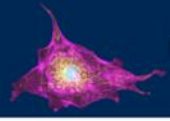


Introduction to image processing and analysis with ImageJ / Fiji. Part 1

Digital Images

Course by Dale Moulding

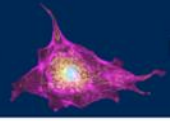


Session 1

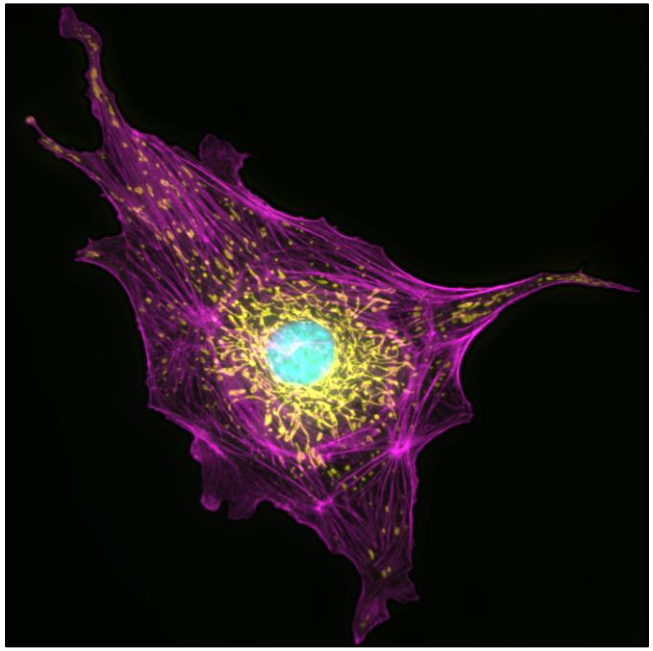
45 minutes
20 minute lecture
20 minutes exercises & answers
5 minutes break

Learning objectives:

- Explain the composition of a digital image
- Understand pixel values and image brightness
- Know the difference between an RGB colour image and a digital image with discrete channels and look up tables
- Show how different bit depths are used for image capture and processing



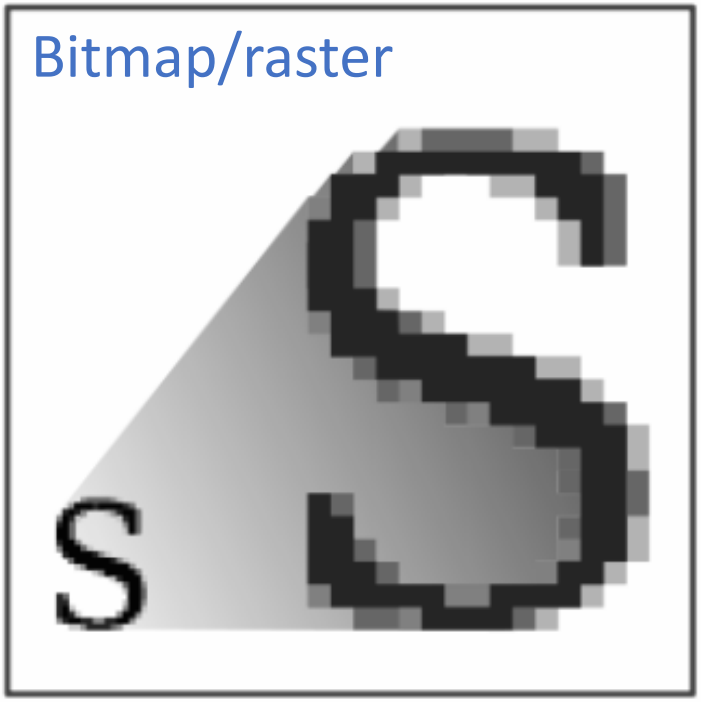
What is a digital image?



Quick answer: Just numbers.
Therefore, image analysis is just maths.

Digital images are either pixel or vector based

Bitmap/raster



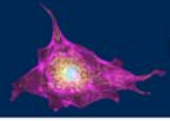
- Array of points (pixels)
- Not scalable (aliasing)

Microscopy Images
Photoshop/GIMP
ImageJ

Vector

- Mathematical/geometric equations
- Scalable

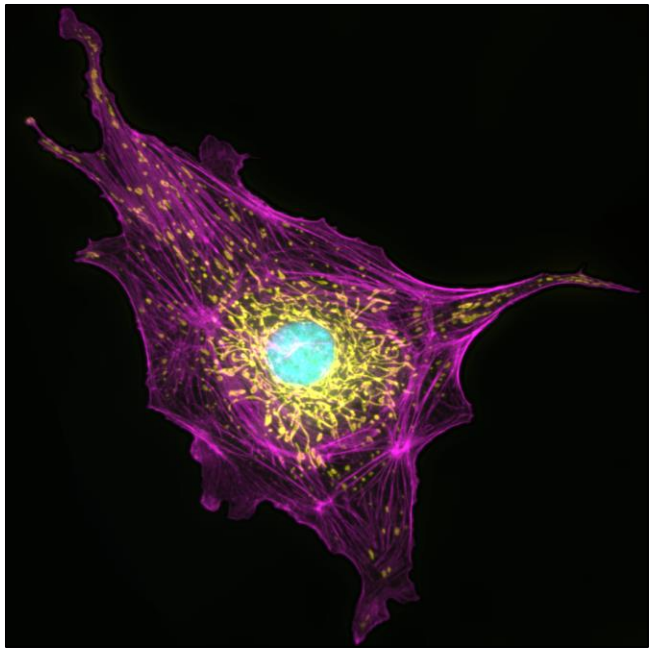
Illustrator/Inkscape
Acrobat



All microscope images are a digital representation of your sample.

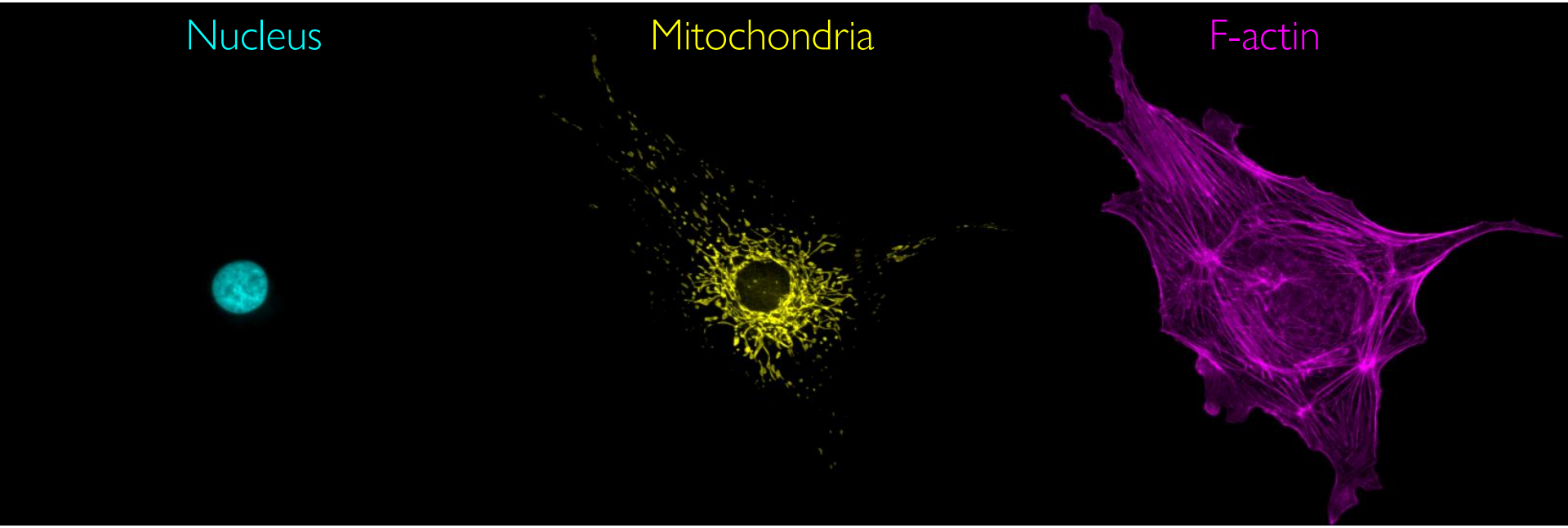
Array of pixels with defined spacing (size) & location

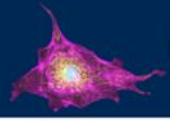
Each pixel is a single numerical value



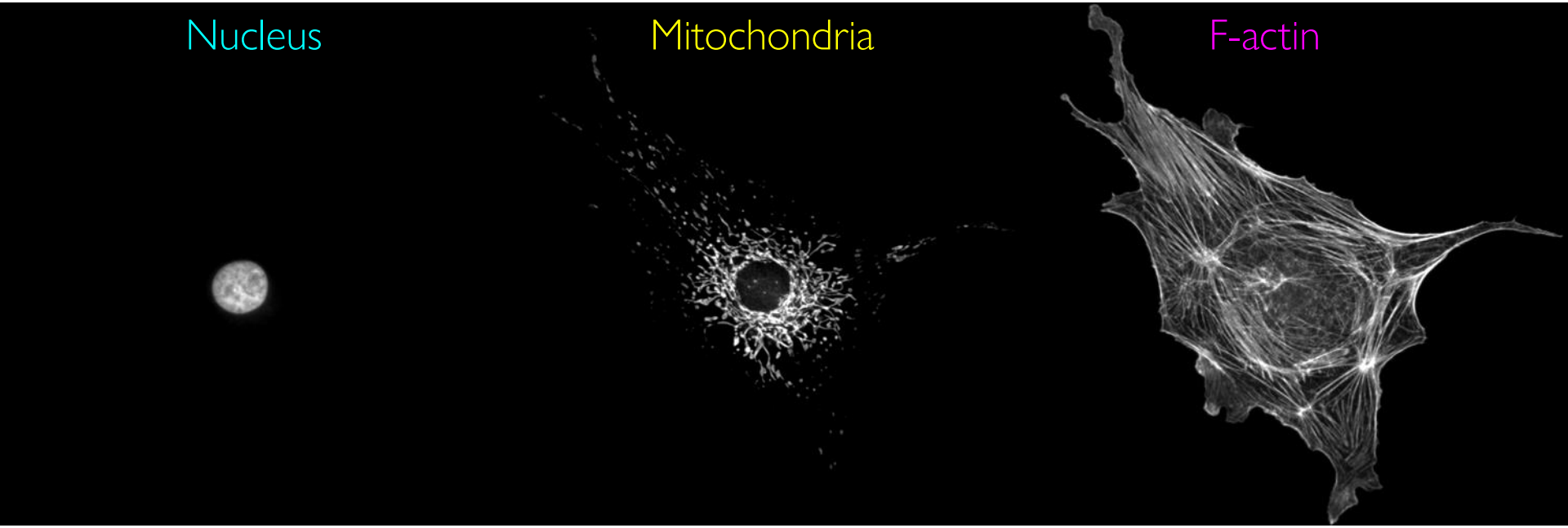
The pixel value represents the amount of light from the specimen that falls onto that pixel

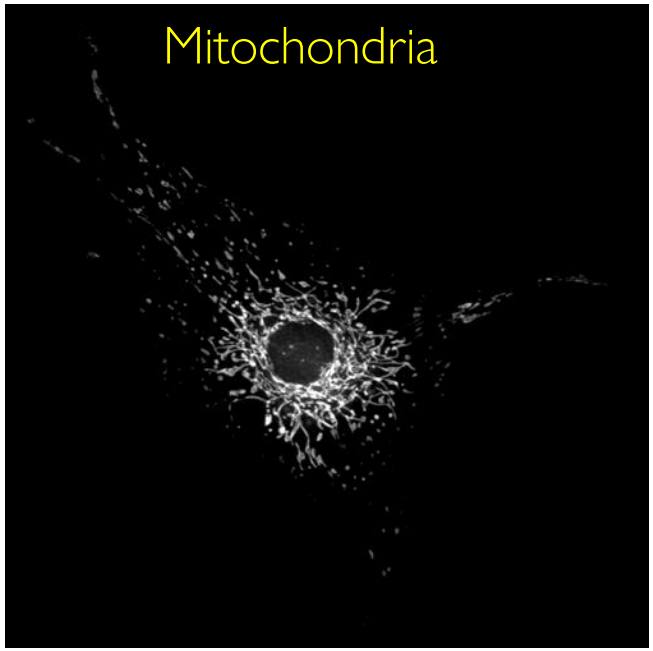
Digital microscope images are often composed of more than one channel (colour), and are rarely RGB images



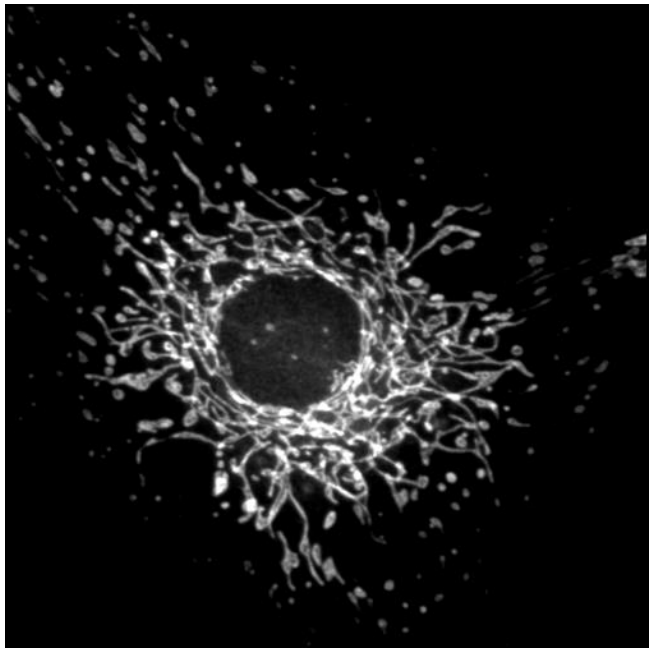


Colours are applied separately as Look Up Tables, each channel is a monochrome grayscale image.

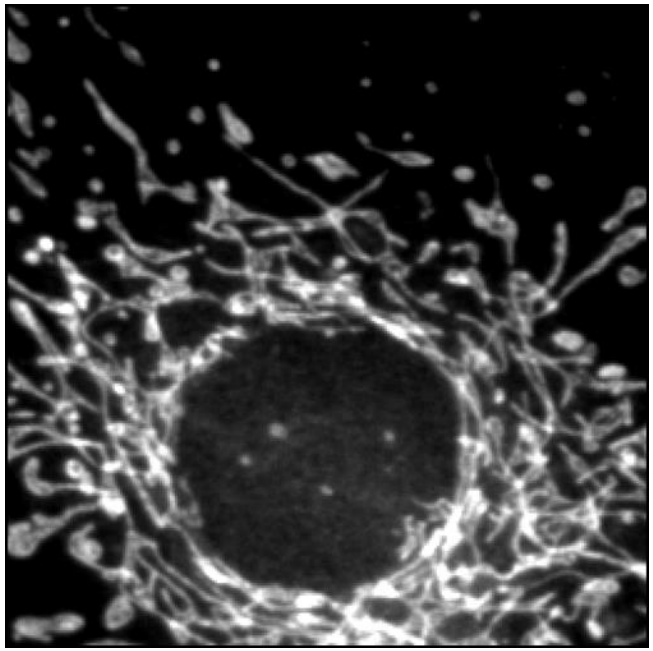



Mitochondria

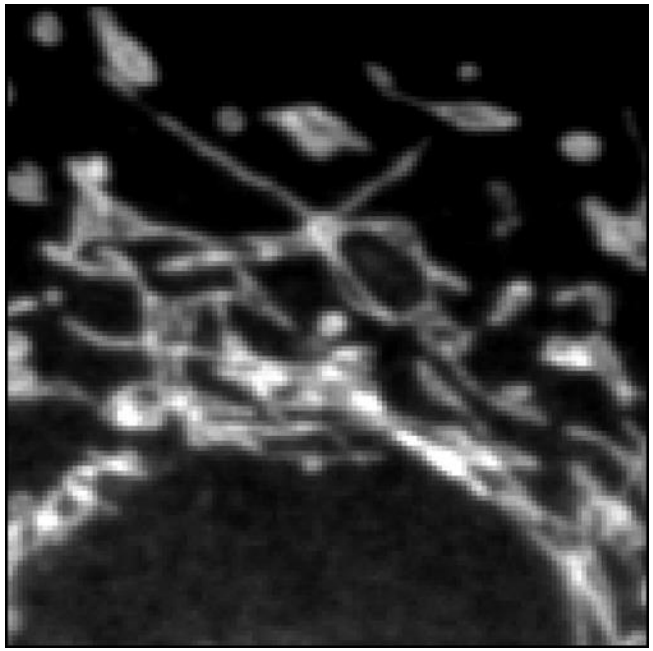
2 x



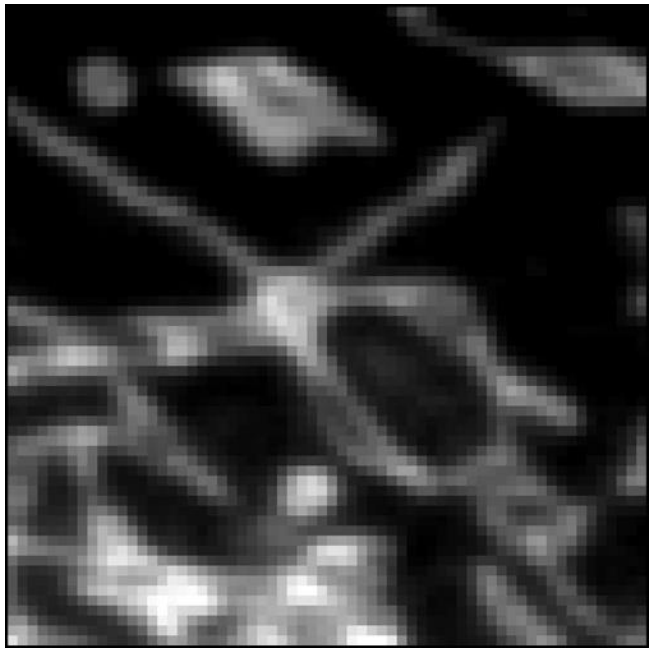
4 x



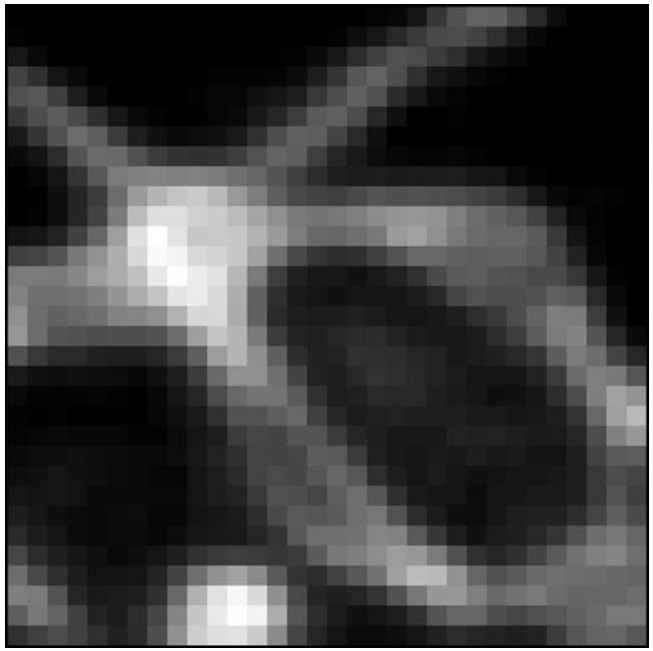
8 x



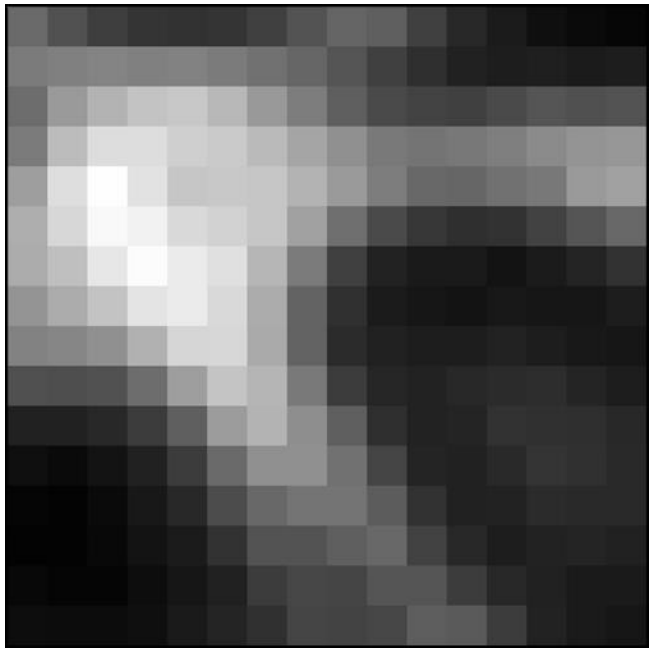
16 x



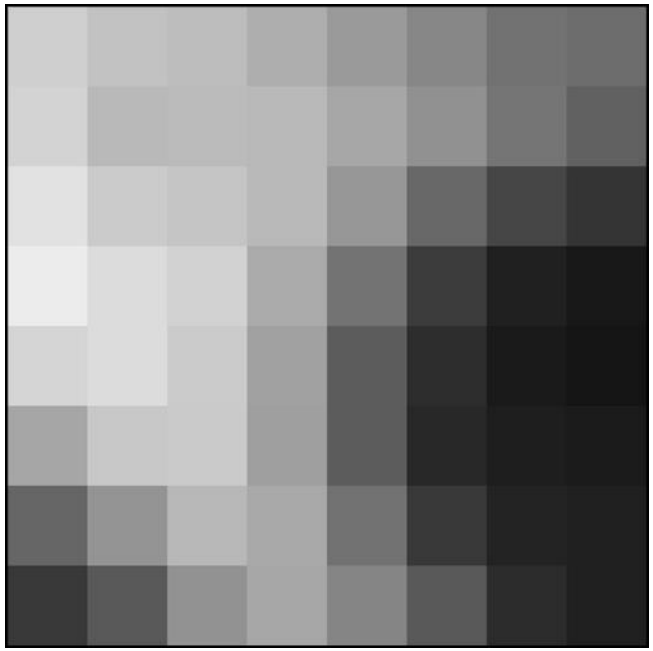
32 x



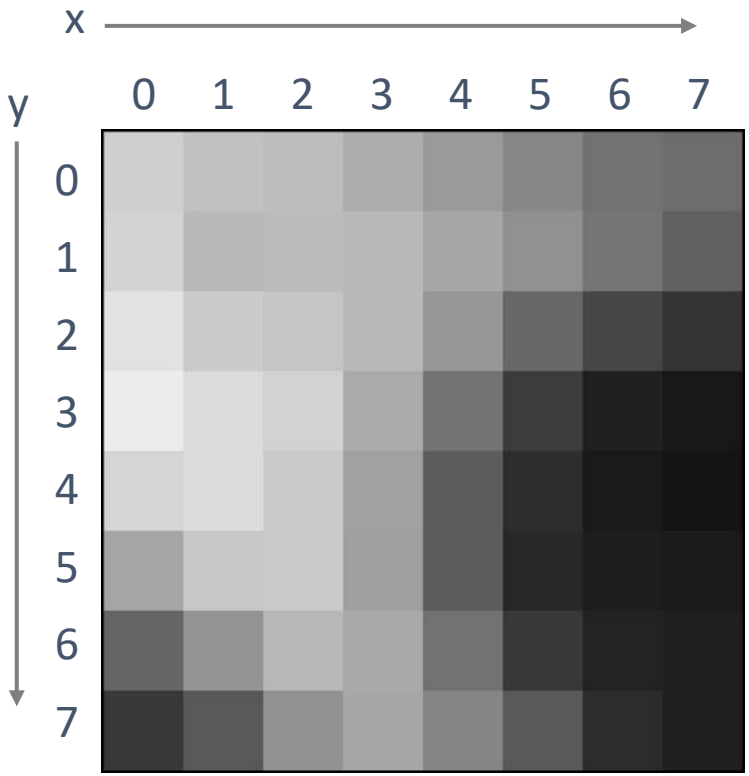
64 x



128 x



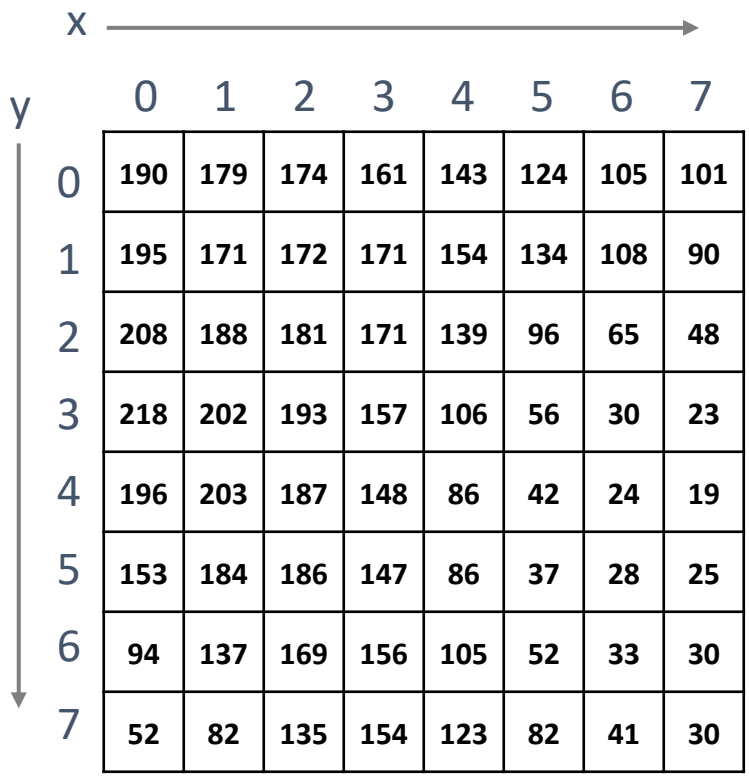
Array of pixels with defined spacing (size) & location (x & y)



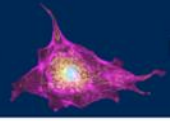
Each pixel is a single numerical value

y \ x	0	1	2	3	4	5	6	7
0	190	179	174	161	143	124	105	101
1	195	171	172	171	154	134	108	90
2	208	188	181	171	139	96	65	48
3	218	202	193	157	106	56	30	23
4	196	203	187	148	86	42	24	19
5	153	184	186	147	86	37	28	25
6	94	137	169	156	105	52	33	30
7	52	82	135	154	123	82	41	30

The pixel value represents the amount of light from the specimen that falls onto that pixel



Simply an 2D array (table of values).

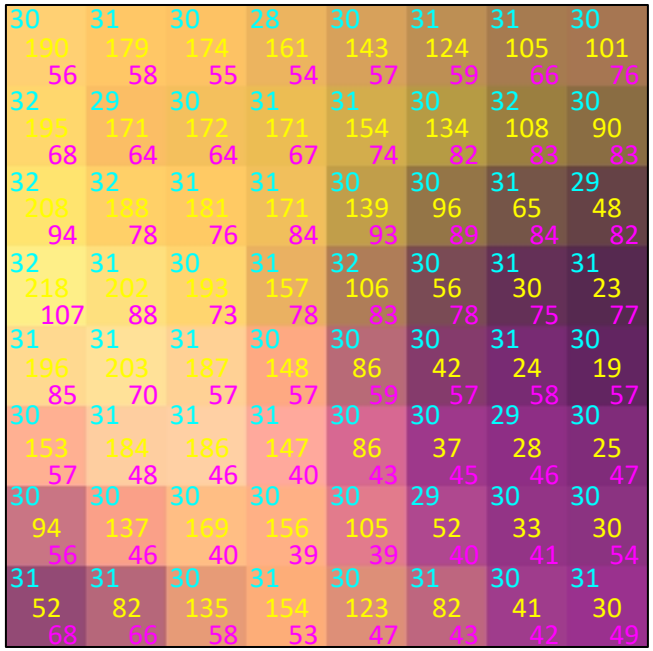


3 arrays (channels /colours) each with its own Look Up Table (LUT)

30	31	30	28	30	31	31	30	190	179	174	161	143	124	105	101	56	58	55	54	57	59	66	76
32	29	30	31	31	30	32	30	195	171	172	171	154	134	108	90	68	64	64	67	74	82	83	83
32	32	31	31	30	30	31	29	208	188	181	171	139	96	65	48	94	78	76	84	93	89	84	82
32	31	30	31	32	30	31	31	218	202	193	157	106	56	30	23	107	88	73	78	83	78	75	77
31	31	31	30	30	30	31	30	196	203	187	148	86	42	24	19	85	70	57	57	59	57	58	57
30	31	31	31	30	30	29	30	153	184	186	147	86	37	28	25	57	48	46	40	43	45	46	47
30	30	30	30	30	29	30	30	94	137	169	156	105	52	33	30	56	46	40	39	39	40	41	54
31	31	30	31	30	31	30	31	52	82	135	154	123	82	41	30	68	66	58	53	47	43	42	49

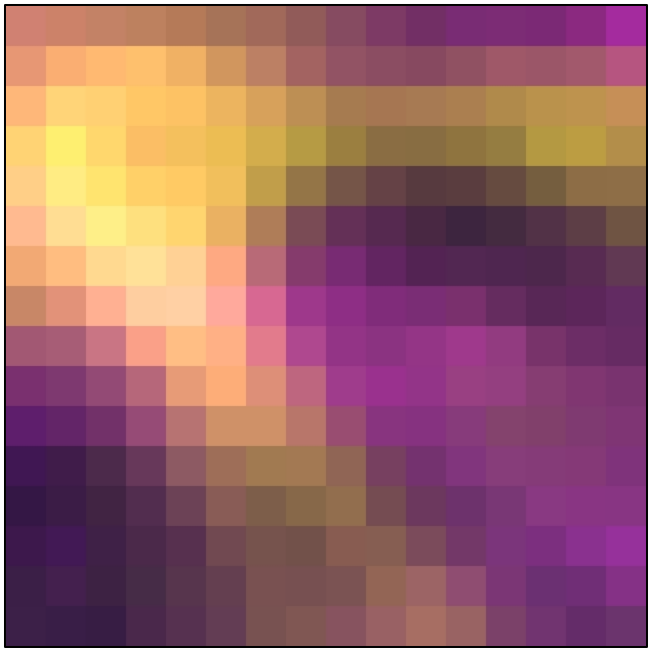
Composite image of the 3 channels

128 x

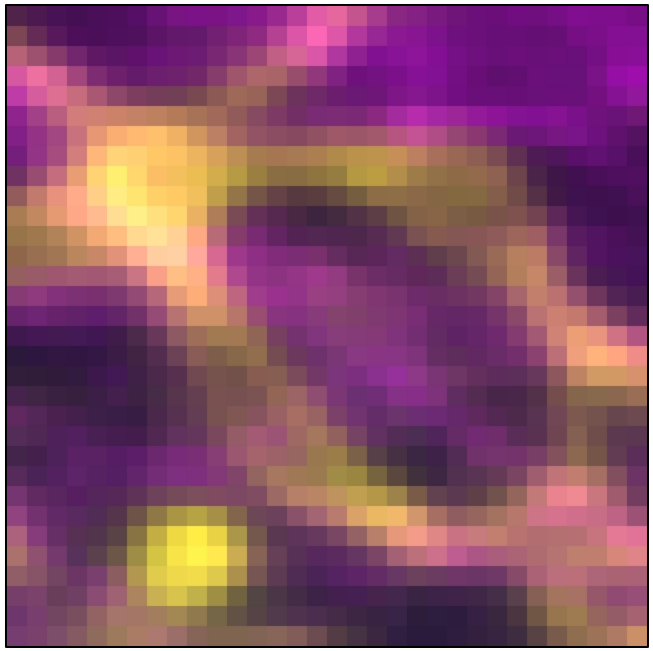


3 arrays / channels (each with its own LUT) are overlayed

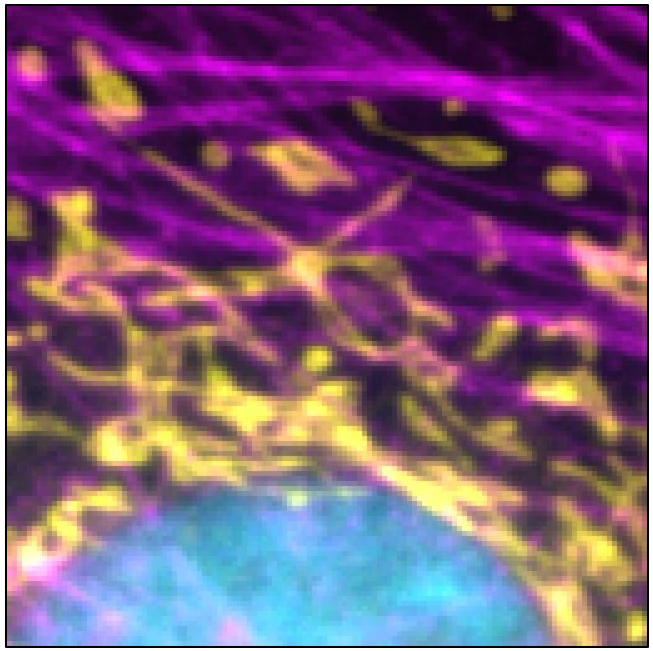
64 x



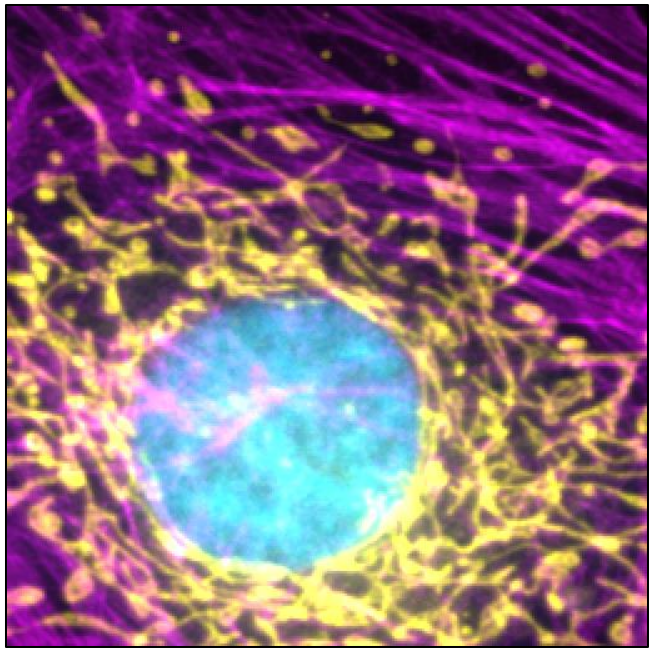
32 x



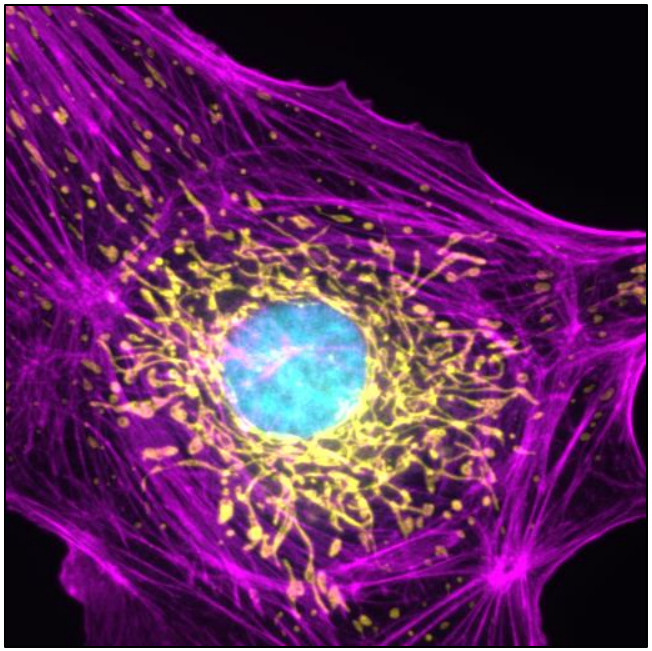
8 x



4 x



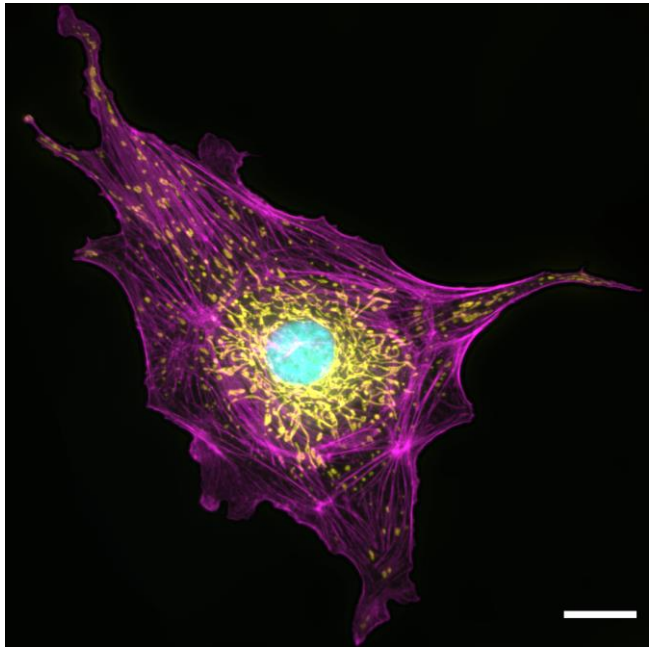
2 x



What is a digital image?

0-1199 (1200) pixels

0
-
1199
(1200)
pixels



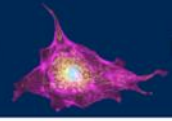
Array(s) of pixels

Fixed size / spacing

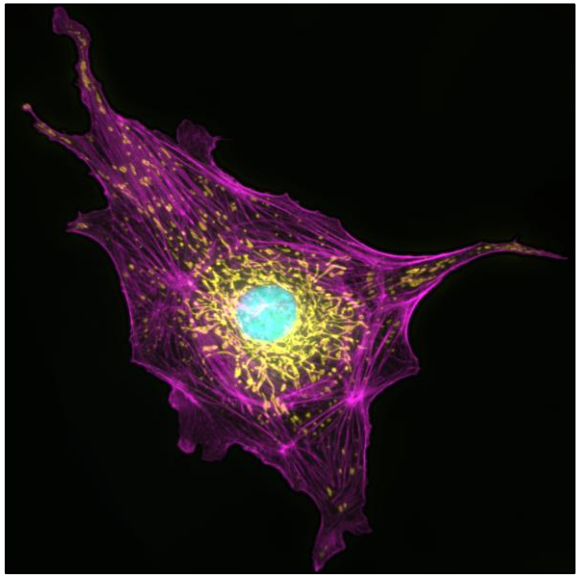
Defined Location (x,y)

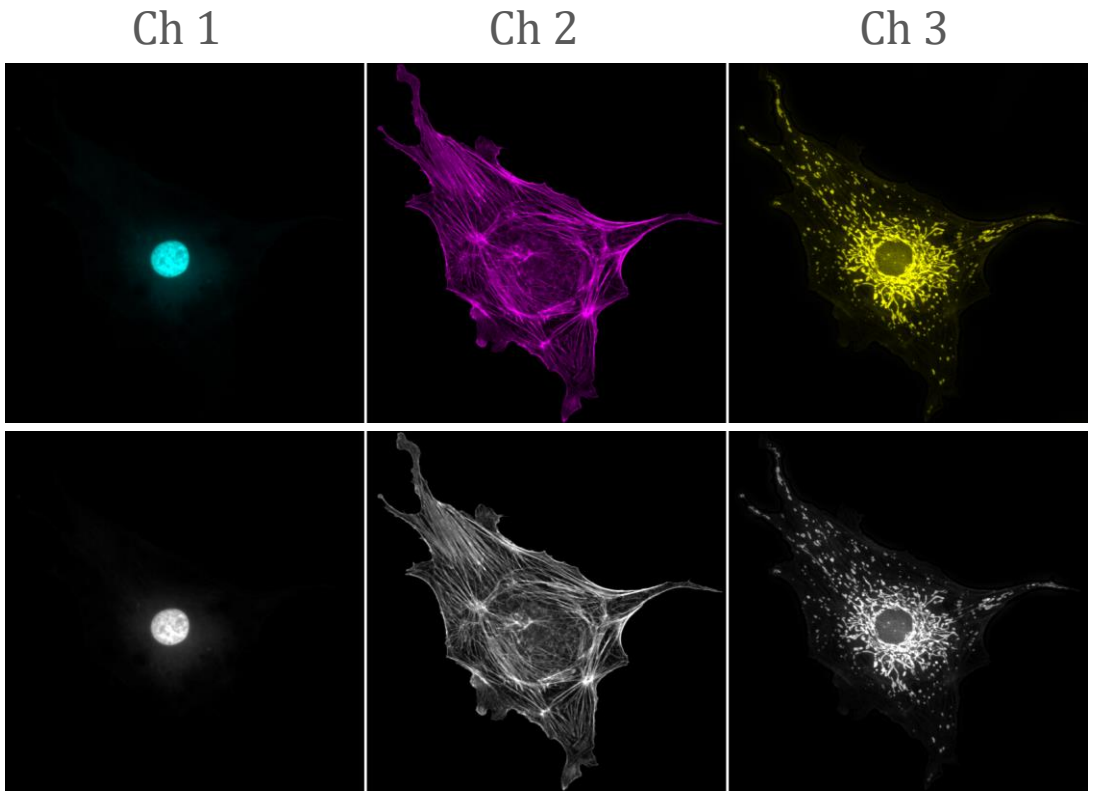
Pixel value represents the total intensity of light from the sample that falls on that pixel

162.5 nm / Pixel
Image size: 178 µm x 178 µm
Scalebar 20 µm

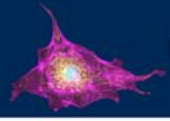


What colour are digital images?



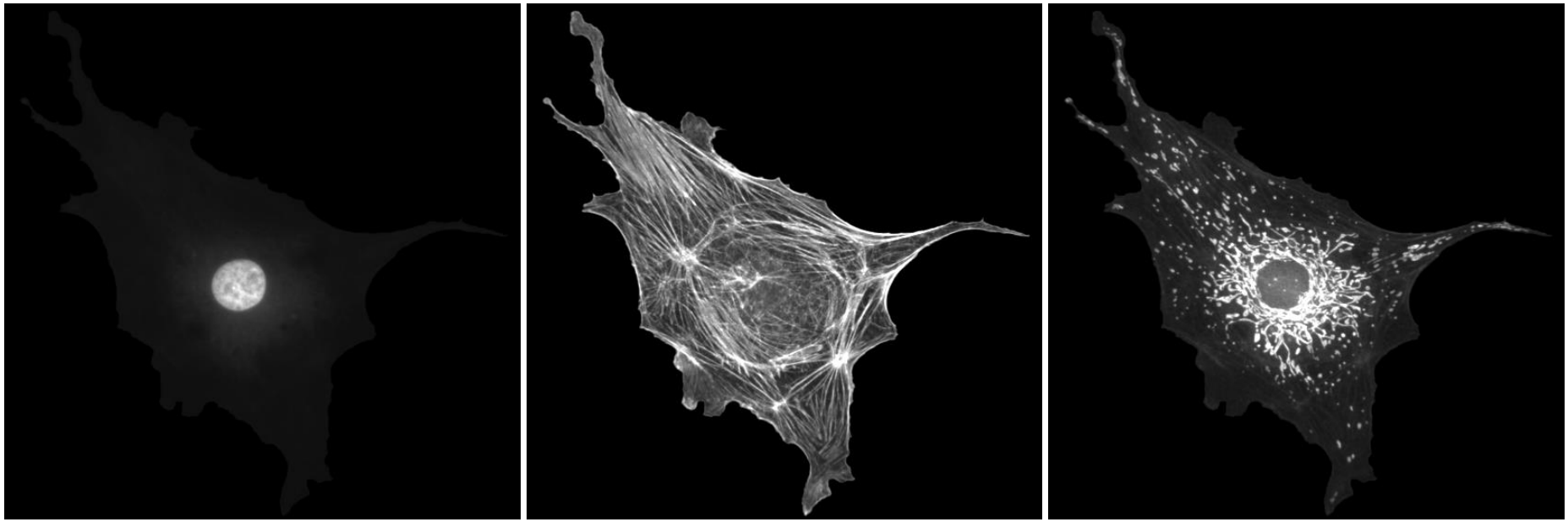


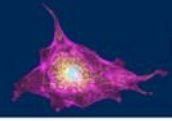
Quick answer: They don't have a colour, they are just numbers.
The numbers represent brightness, so they are just shades of grey.



Each channel can be assigned any colour (look-up table - LUT)

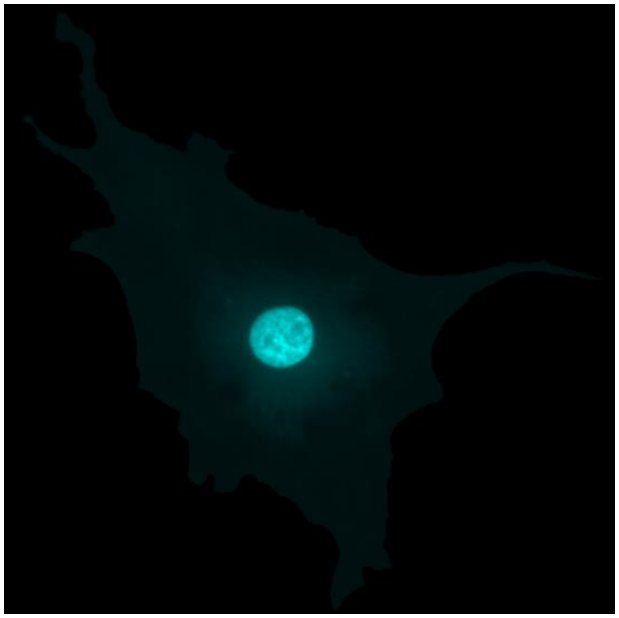
Ch 1 Ch 2 Ch 3





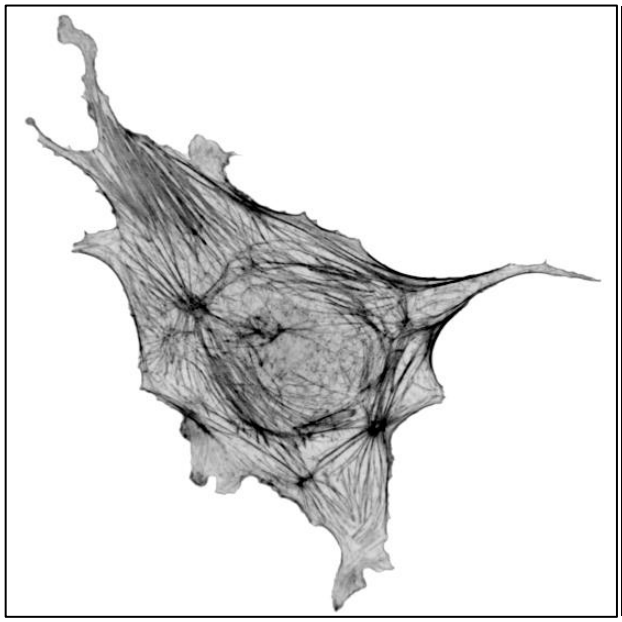
Each channel can be assigned any colour (look-up table - LUT)

Ch 1



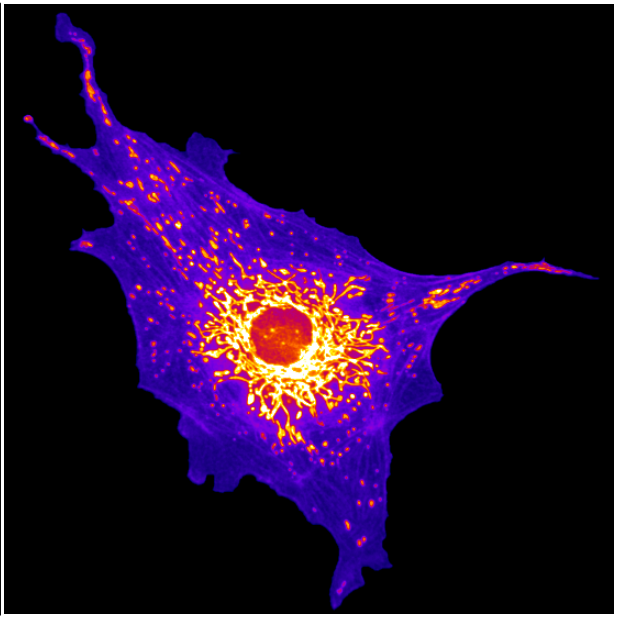
Cyan

Ch 2



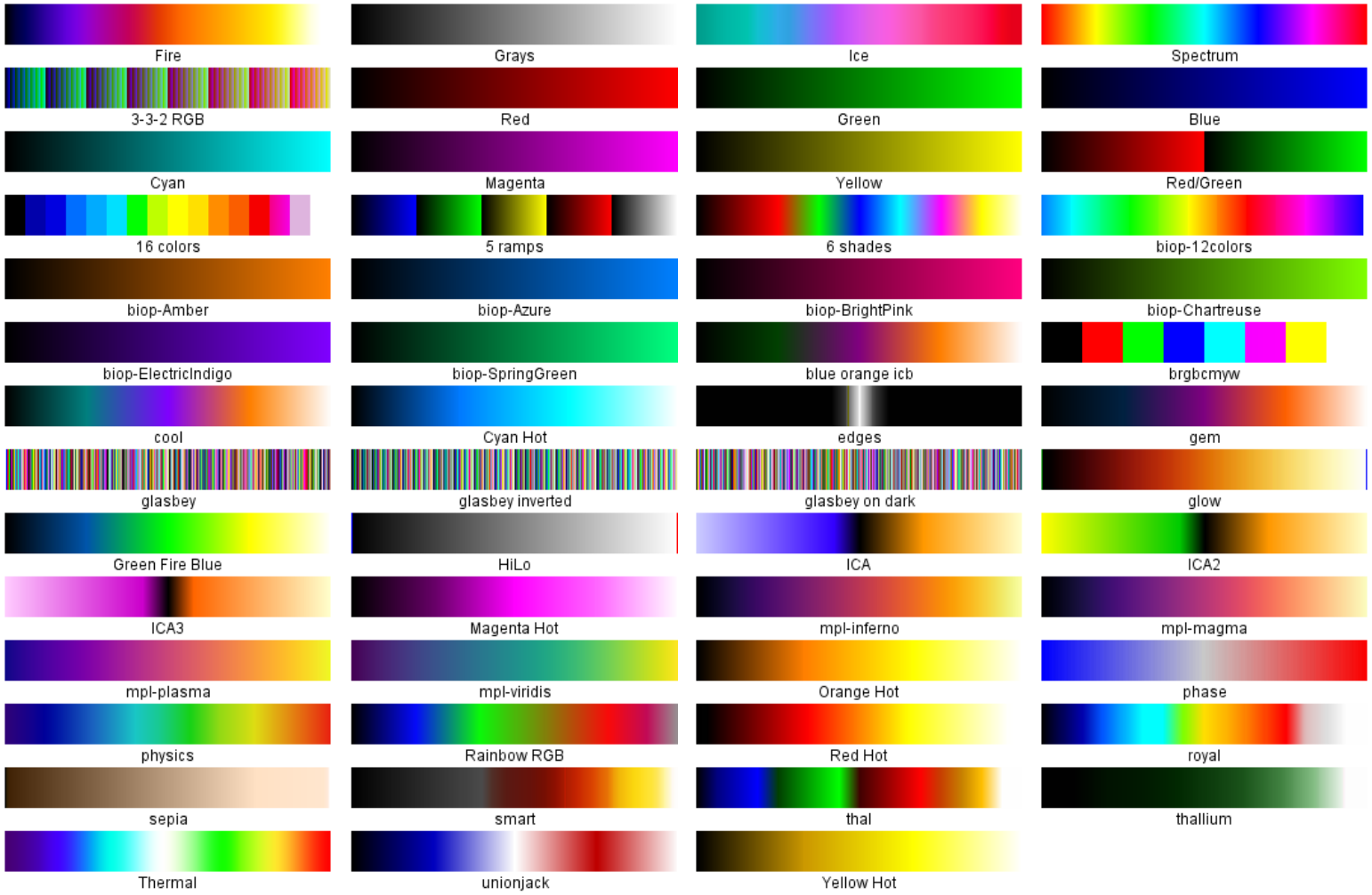
Invert LUT

Ch 3



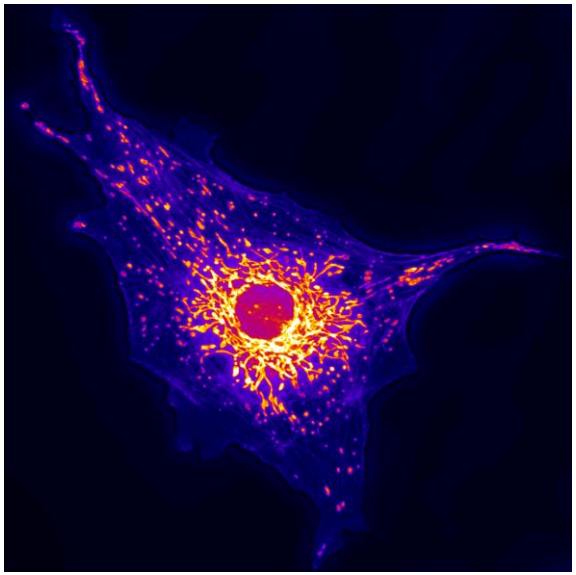
Fire

Each channel can be assigned any colour (look-up table - LUT)

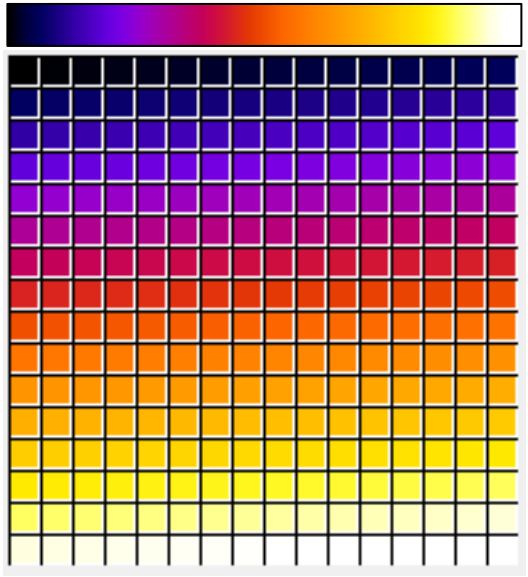


What range of pixel values are used in scientific digital images?

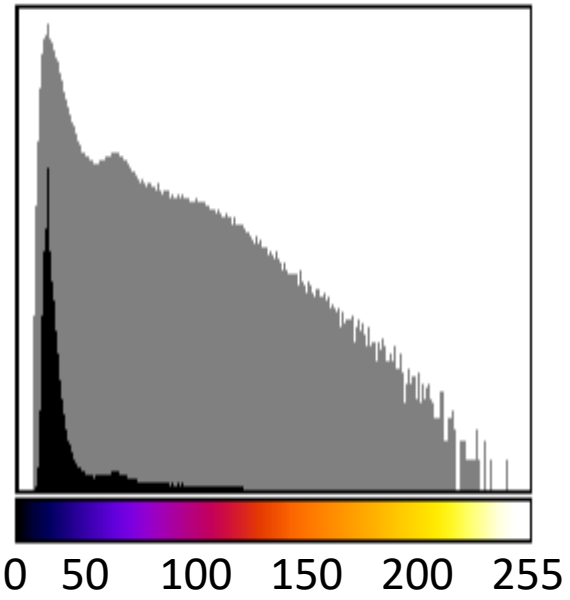
Image



LUT



Histogram



8 bit image

2^8

$2x2x2x2x2x2x2x2$

= 256 (0-255)

0	=	Black / No signal
1-254	=	Darker to lighter pixels
255	=	White / Brightest signal

What range of pixel values are used in scientific digital images?

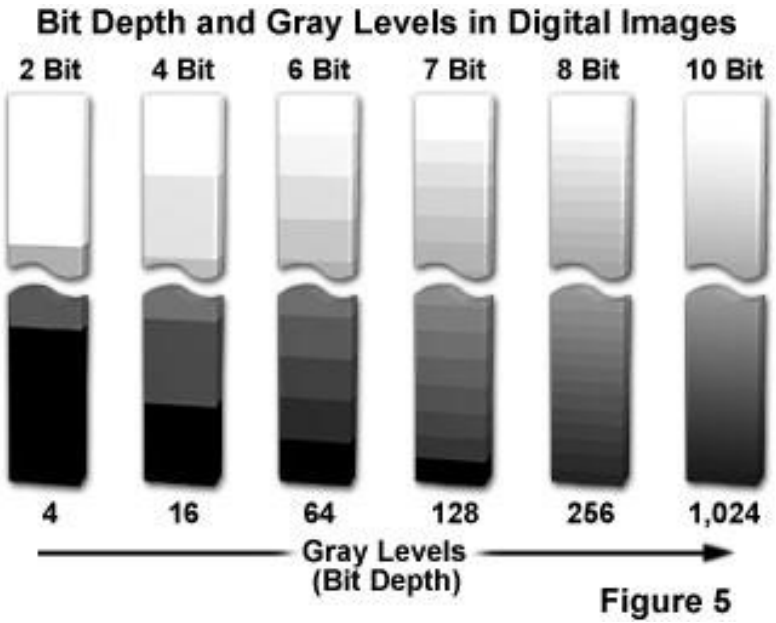


Figure 5

8-bit : from 0 to 255 (2^8=256 steps - integer)
TV, websites, mobiles, printed images

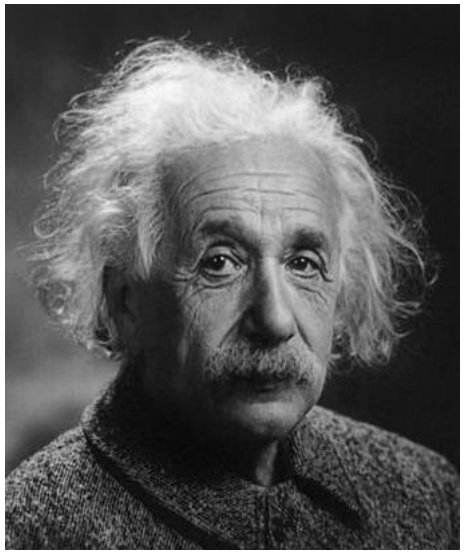
12-bit : from 0 to 4095 (2^{12}=4096 steps)
Scientific cameras, PMTs

16-bit : from 0 to 65535 (2^{16}=65536 steps)
Scientific cameras, PMTs

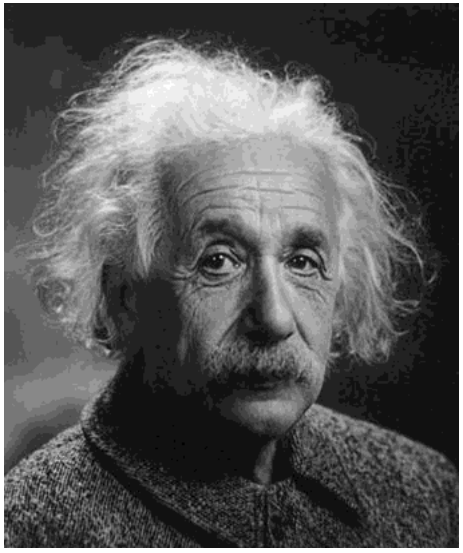
32-bit : 4,294,967,296 (2^{32} floating point values)
Computer processing

RGB : three 8-bit grayscale channels for red, green and blue

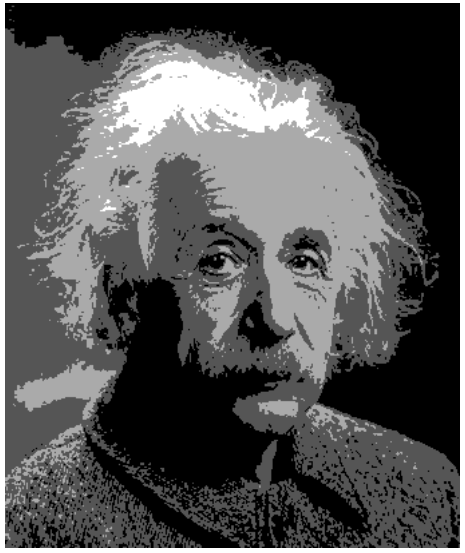
8 bit - 256 levels



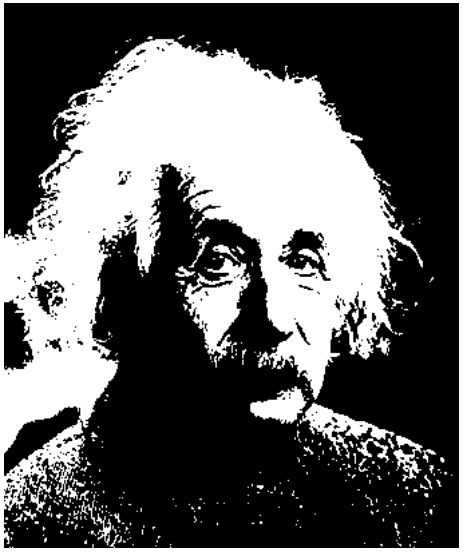
4 bit - 16 levels

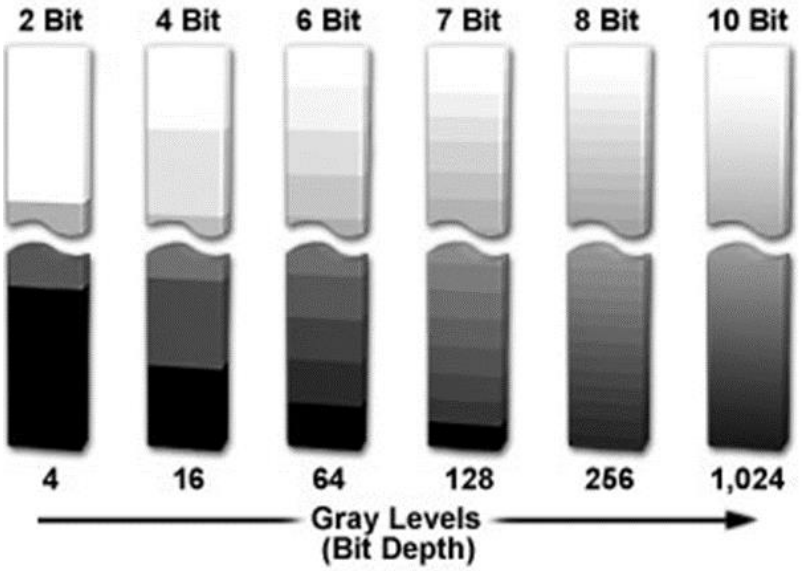


2 bit - 4 levels

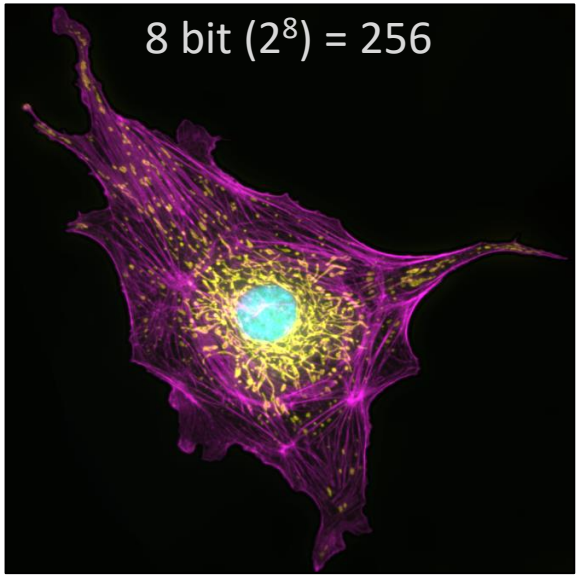


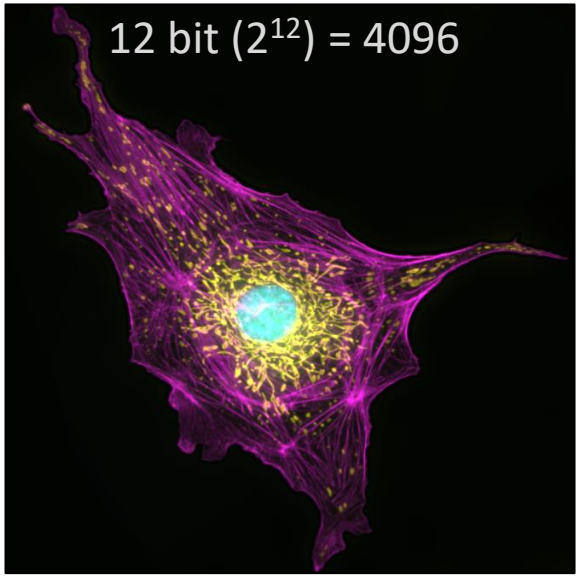
1 bit - binary

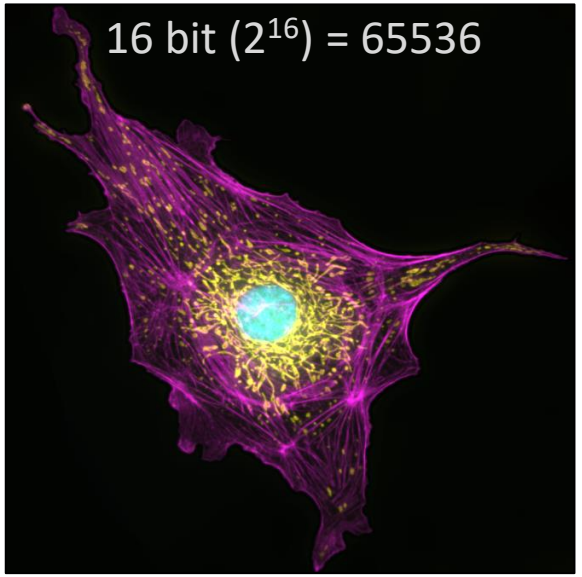


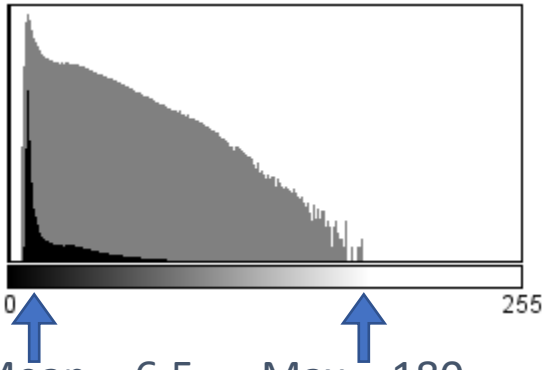


Why use more than 8 bits?



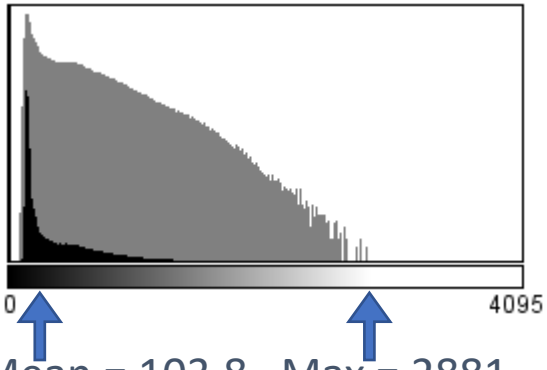






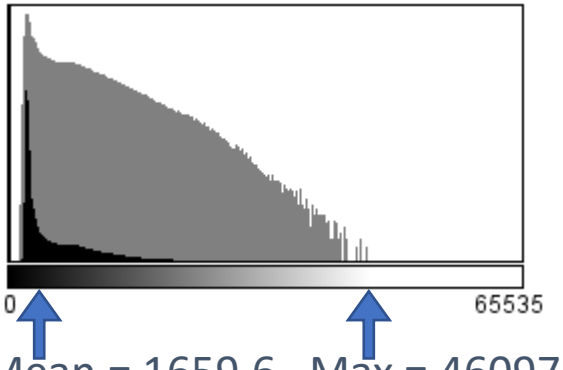
Mean = 6.5 Max = 180

180/6.5 = 27.7



Mean = 103.8 Max = 2881

2881/103.8 = 27.7



Mean = 1659.6 Max = 46097

46097/1659.6 = 27.7

Why use more than 8 bits? Image analysis is Maths

Image 1

	0	1	2	3	4
0	40	64	80	120	152
1	48	64	80	120	152
2	48	64	80	112	144
3	48	64	80	112	136
4	48	64	80	104	128

Multiply

	0	1	2	3	4
0	2560	4096	5120	6720	8512
1	2688	3584	4480	6720	8512
2	2688	3584	4480	5376	6912
3	2688	3584	3840	4480	6528
4	2688	3584	3840	4160	6144

8 bit result

	0	1	2	3	4
0	255	255	255	255	255
1	255	255	255	255	255
2	255	255	255	255	255
3	255	255	255	255	255
4	255	255	255	255	255

Image 2

	0	1	2	3	4
0	64	64	64	56	56
1	56	56	56	56	56
2	56	56	56	48	48
3	56	56	48	40	48
4	56	56	48	40	48

Divide

	0	1	2	3	4
0	0.63	1.00	1.25	2.14	2.71
1	0.86	1.14	1.43	2.14	2.71
2	0.86	1.14	1.43	2.33	3.00
3	0.86	1.14	1.67	2.80	2.83
4	0.86	1.14	1.67	2.60	2.67

8 bit result

	0	1	2	3	4
0	1	1	1	2	3
1	1	1	1	2	3
2	1	1	1	2	3
3	1	1	2	3	3
4	1	1	2	3	3

Why use more than 8 bits? Image analysis is Maths

Image 1

	0	1	2	3	4
0	40	64	80	120	152
1	48	64	80	120	152
2	48	64	80	112	144
3	48	64	80	112	136
4	48	64	80	104	128

Multiply

	0	1	2	3	4
0	2560	4096	5120	6720	8512
1	2688	3584	4480	6720	8512
2	2688	3584	4480	5376	6912
3	2688	3584	3840	4480	6528
4	2688	3584	3840	4160	6144

16 bit result

	0	1	2	3	4
0	2560	4096	5120	6720	8512
1	2688	3584	4480	6720	8512
2	2688	3584	4480	5376	6912
3	2688	3584	3840	4480	6528
4	2688	3584	3840	4160	6144

Image 2

	0	1	2	3	4
0	64	64	64	56	56
1	56	56	56	56	56
2	56	56	56	48	48
3	56	56	48	40	48
4	56	56	48	40	48

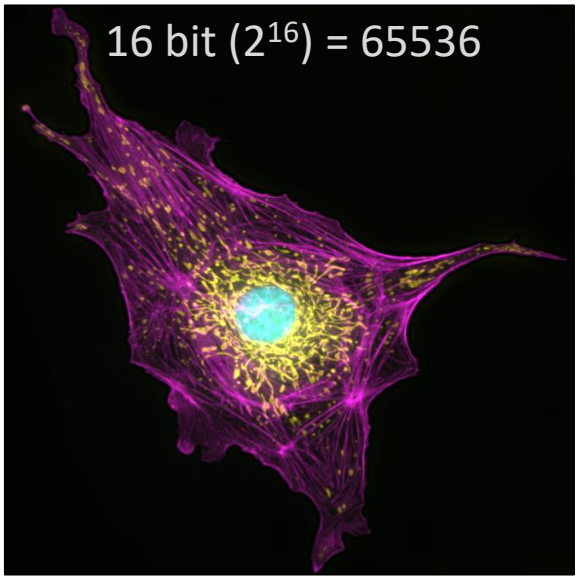
Divide

	0	1	2	3	4
0	0.63	1.00	1.25	2.14	2.71
1	0.86	1.14	1.43	2.14	2.71
2	0.86	1.14	1.43	2.33	3.00
3	0.86	1.14	1.67	2.80	2.83
4	0.86	1.14	1.67	2.60	2.67

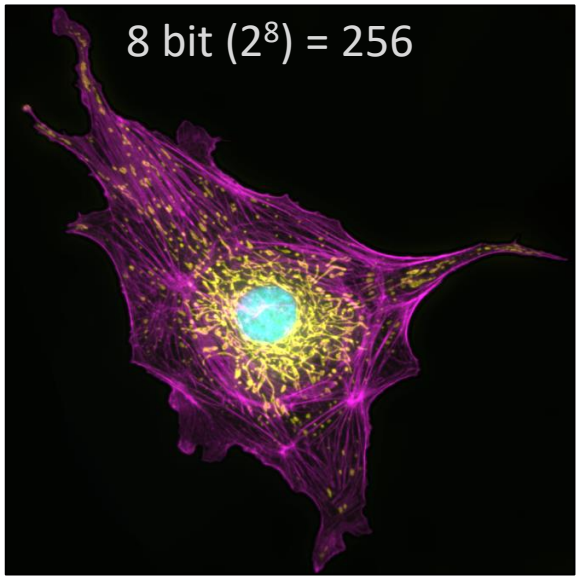
32 bit result

	0	1	2	3	4
0	0.63	1.00	1.25	2.14	2.71
1	0.86	1.14	1.43	2.14	2.71
2	0.86	1.14	1.43	2.33	3.00
3	0.86	1.14	1.67	2.80	2.83
4	0.86	1.14	1.67	2.60	2.67

Reducing bit depth loses data

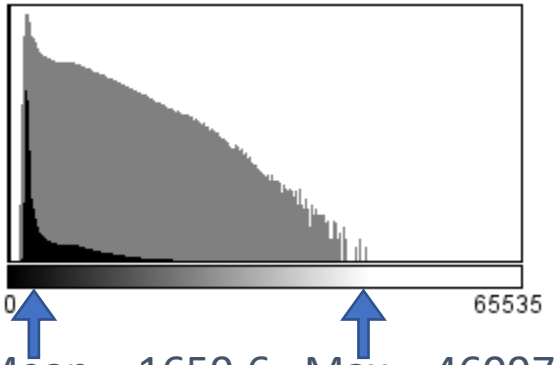


Save as a
Jpeg, Bmp, PNG, Gif



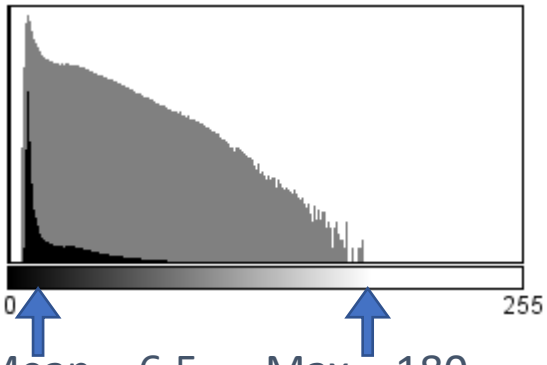
All photography formats use 8-bit RGB

Save scientific images as a .tif



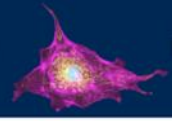
Mean = 1659.6 Max = 46097

46097/1659.6 = 27.7

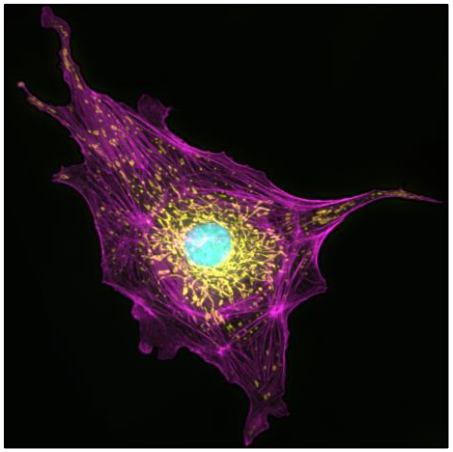


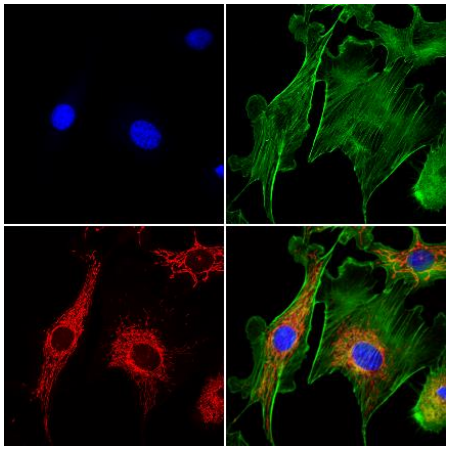
Mean = 6.5 Max = 180

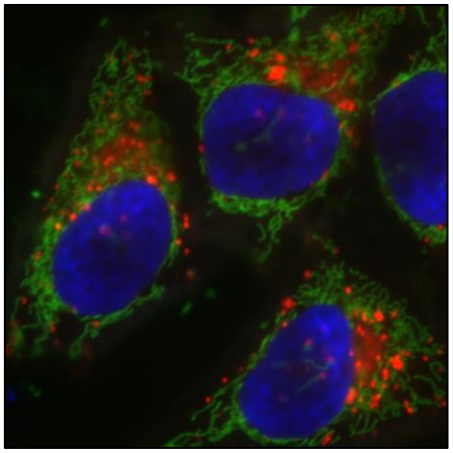
180/6.5 = 27.7

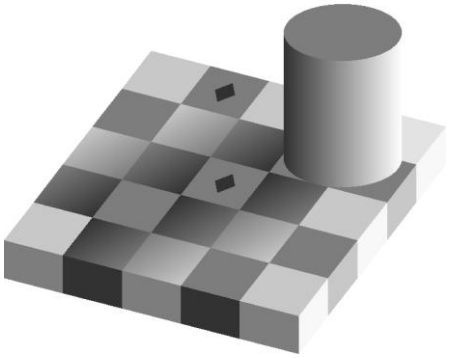


Publishing your images loses data!
All printed / website / TV / Powerpoint / Word images are RGB

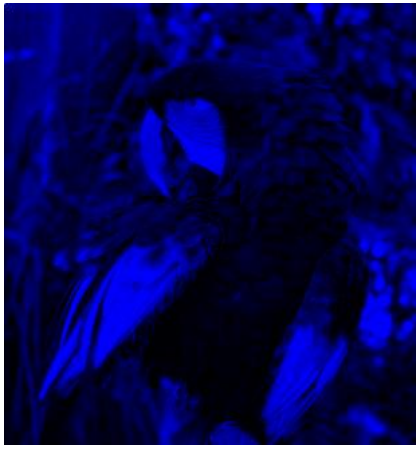




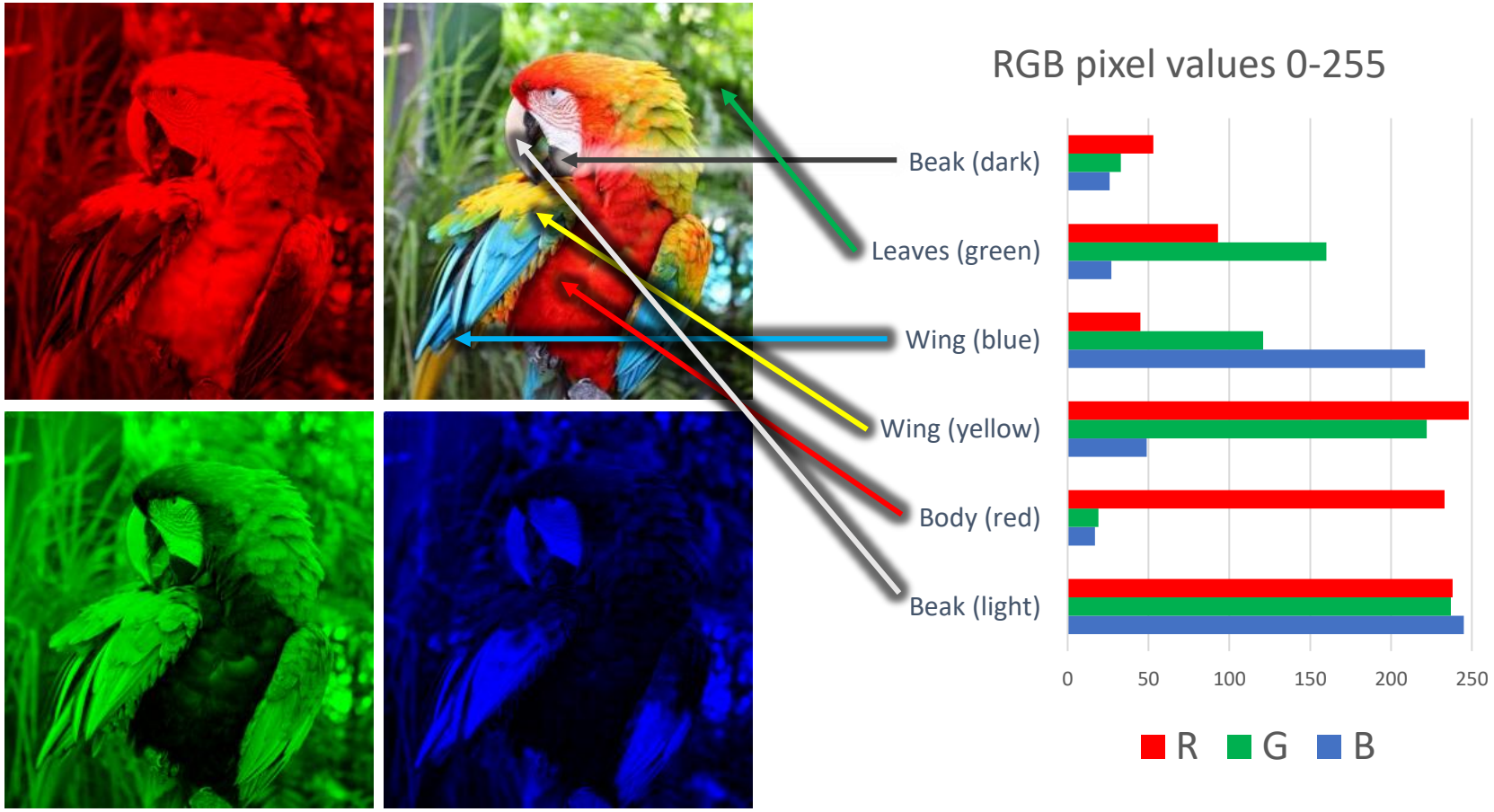


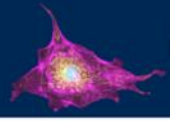


What is an RGB image?

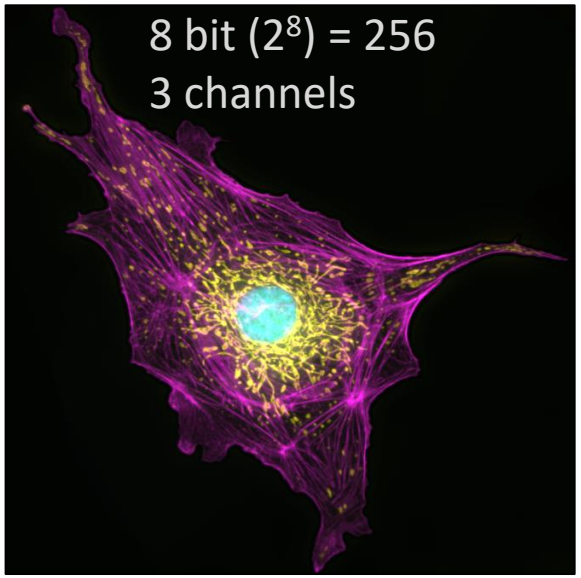


What is an RGB image?

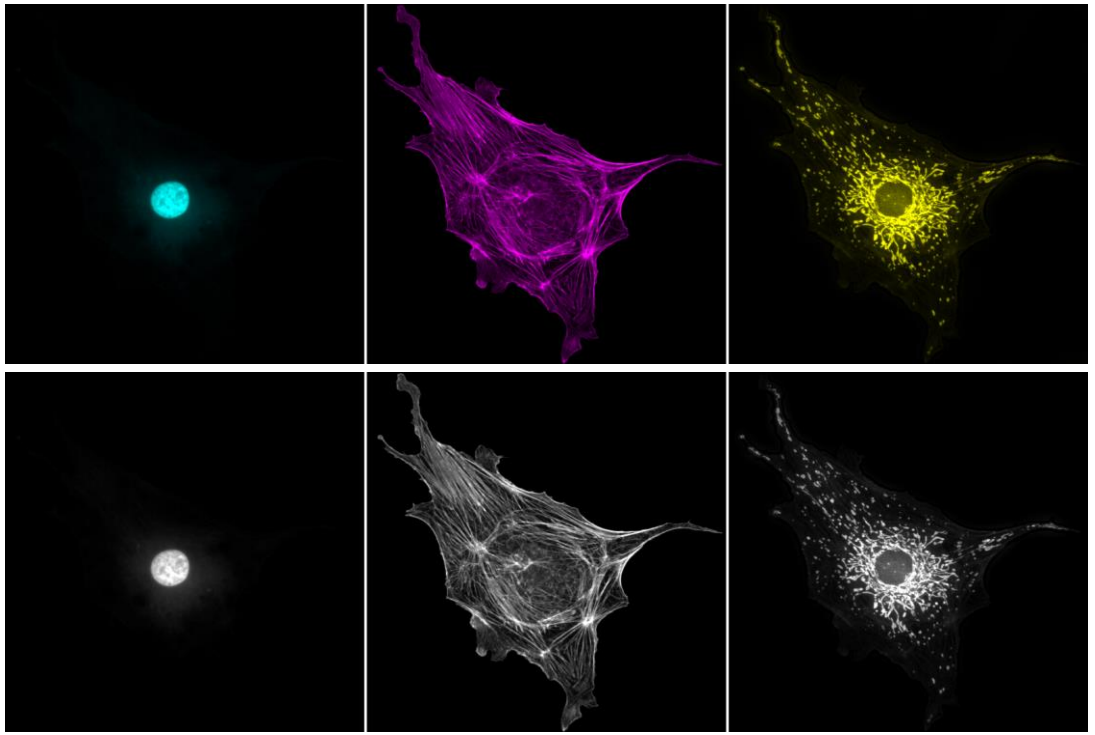




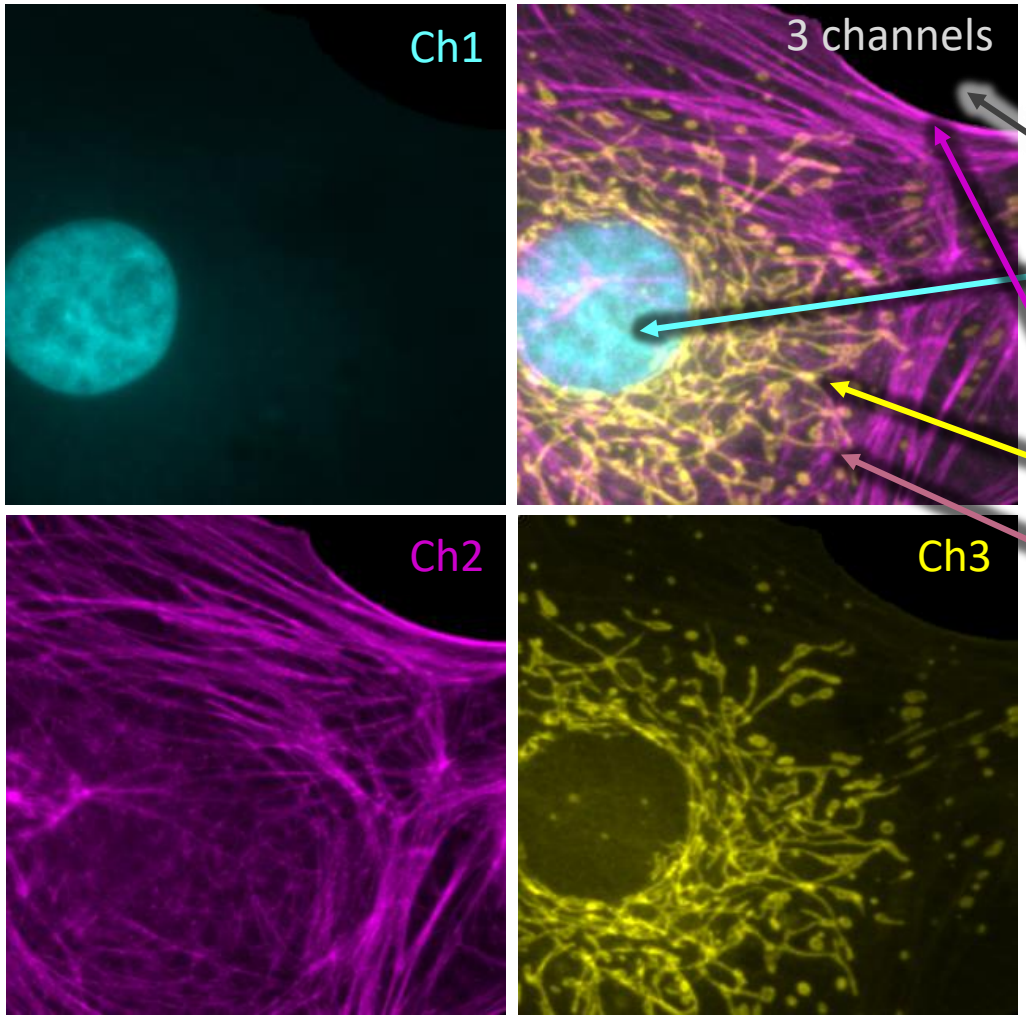
How are scientific images converted to RGB?

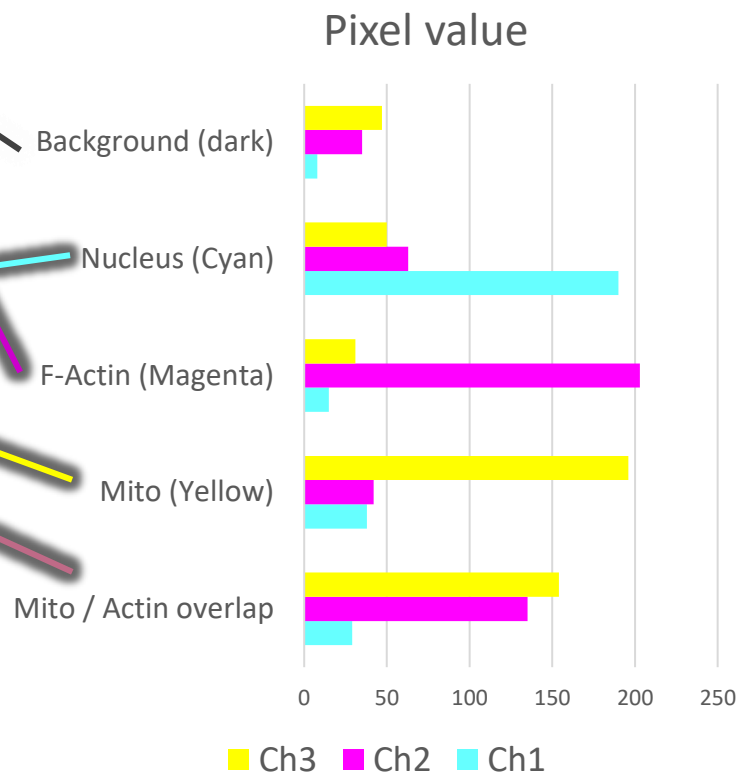


Ch 1 Ch 2 Ch 3

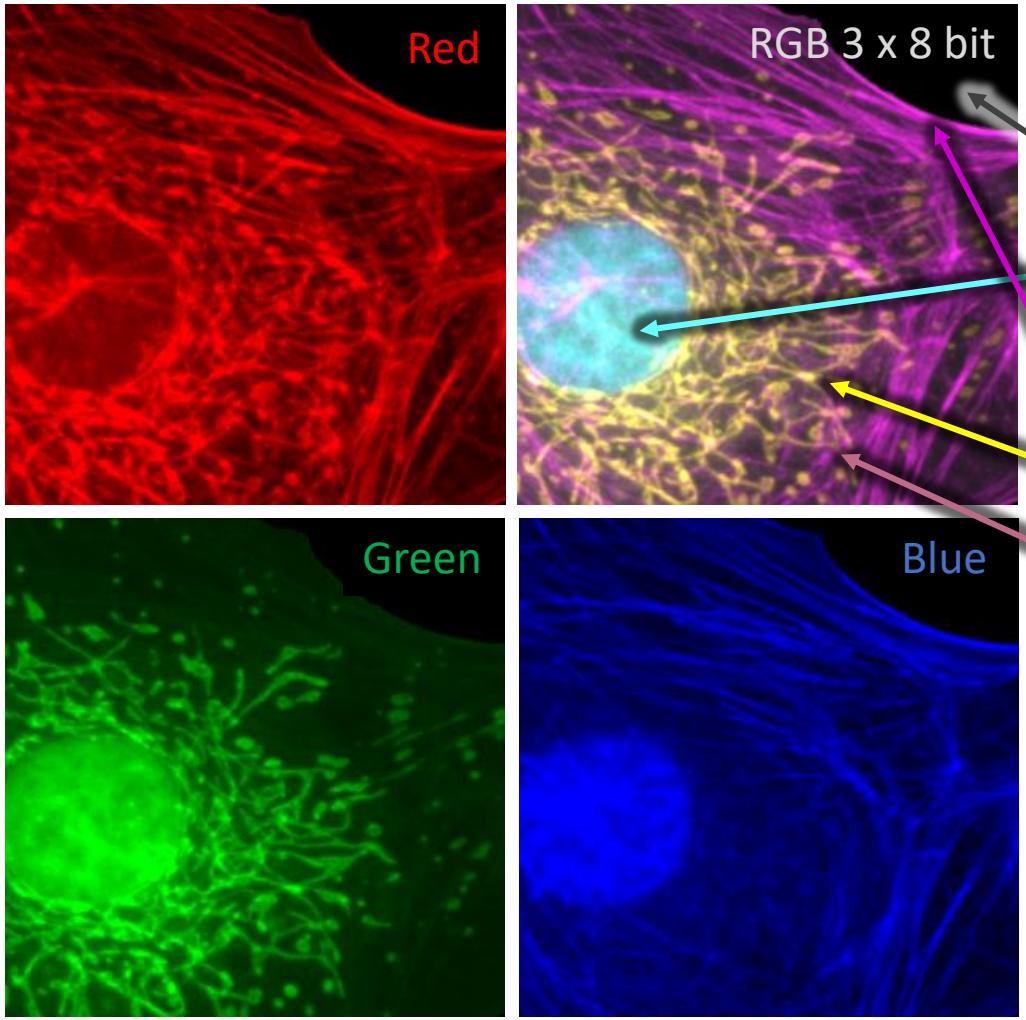


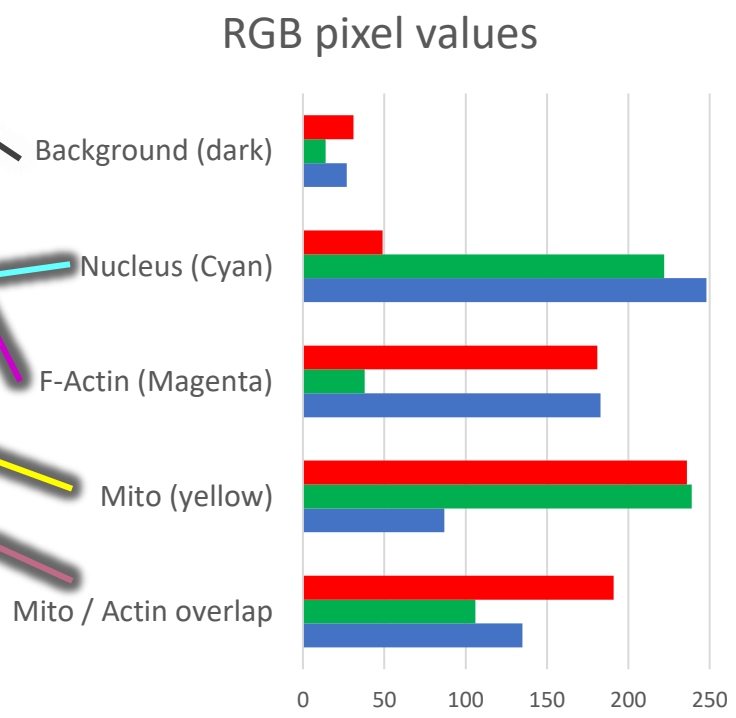
How are scientific images converted to RGB?



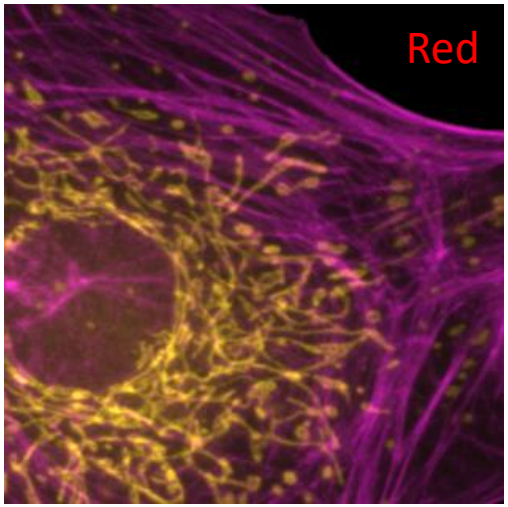


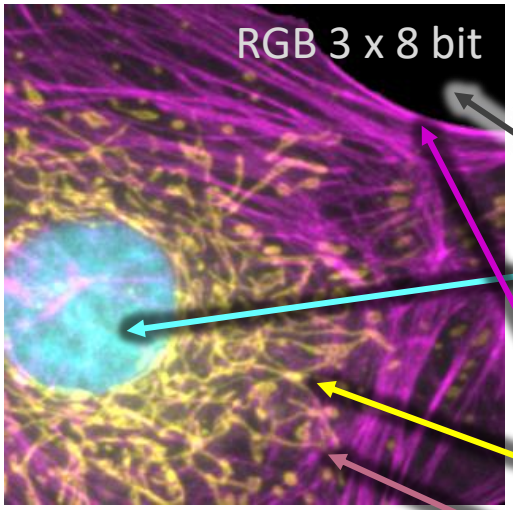
How are scientific images converted to RGB?

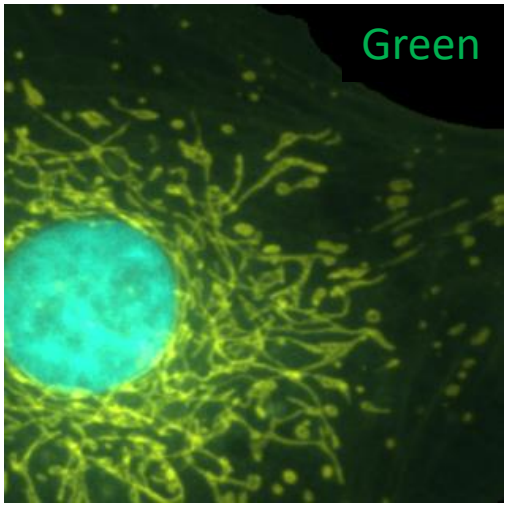


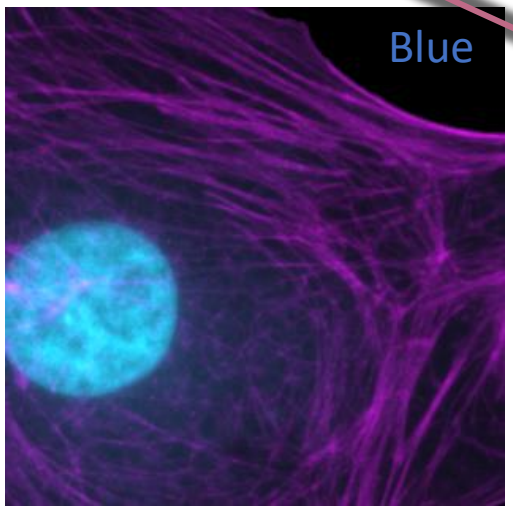


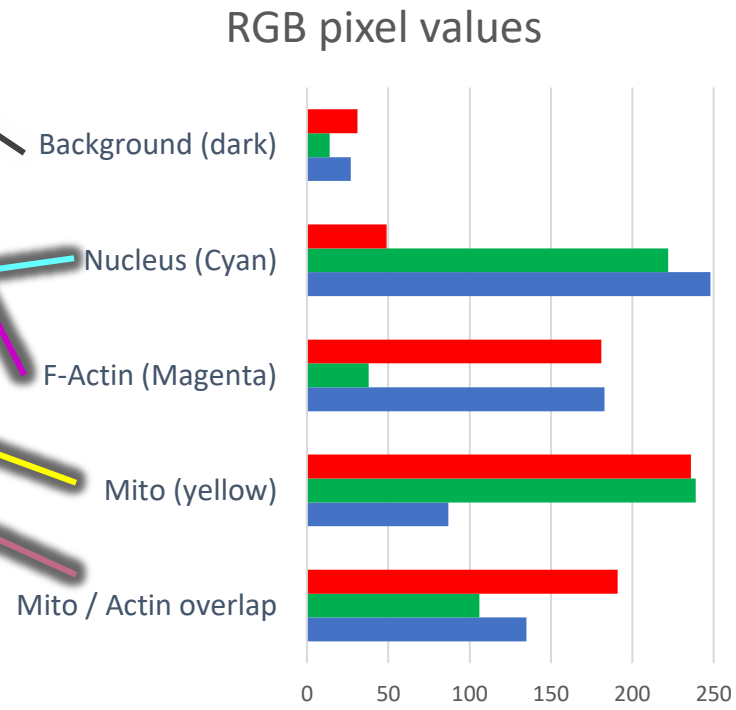
RGB images cannot be transformed back to multi channel images



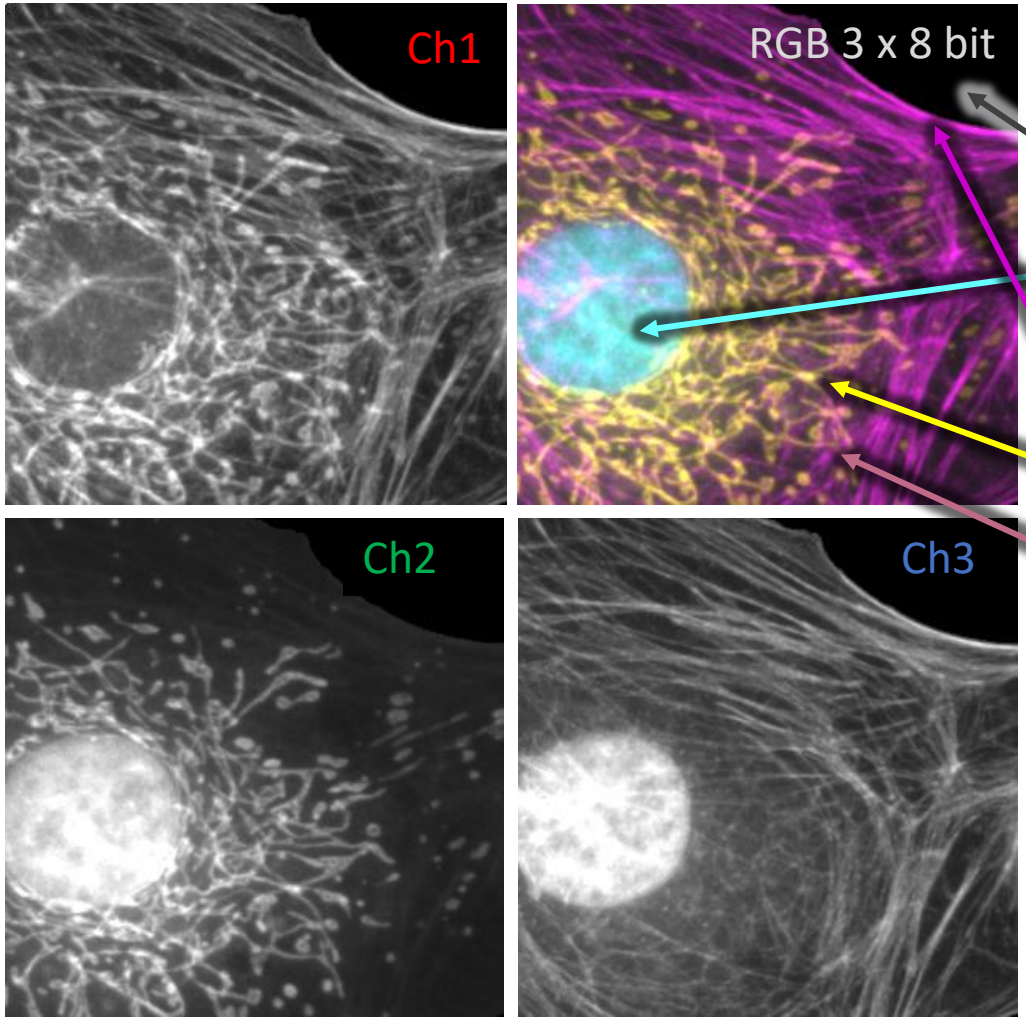


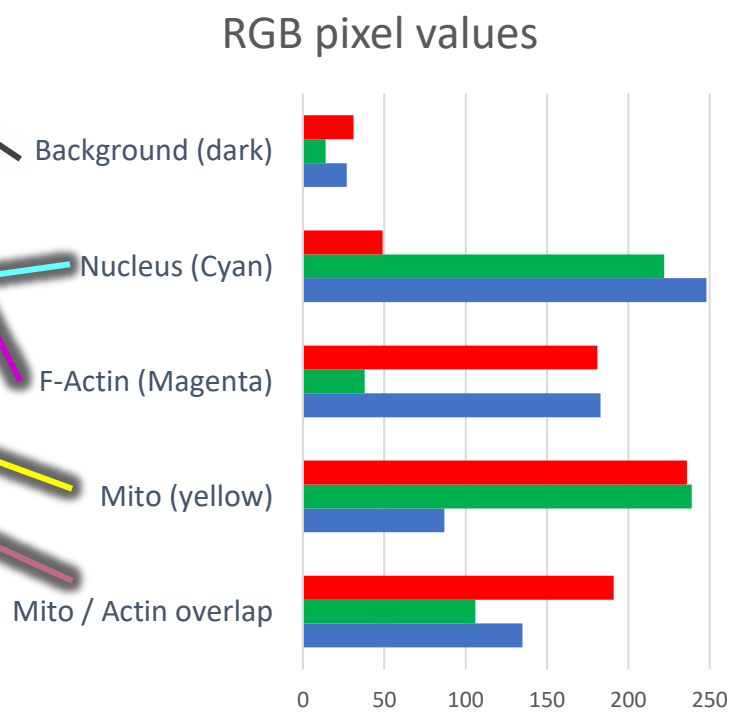




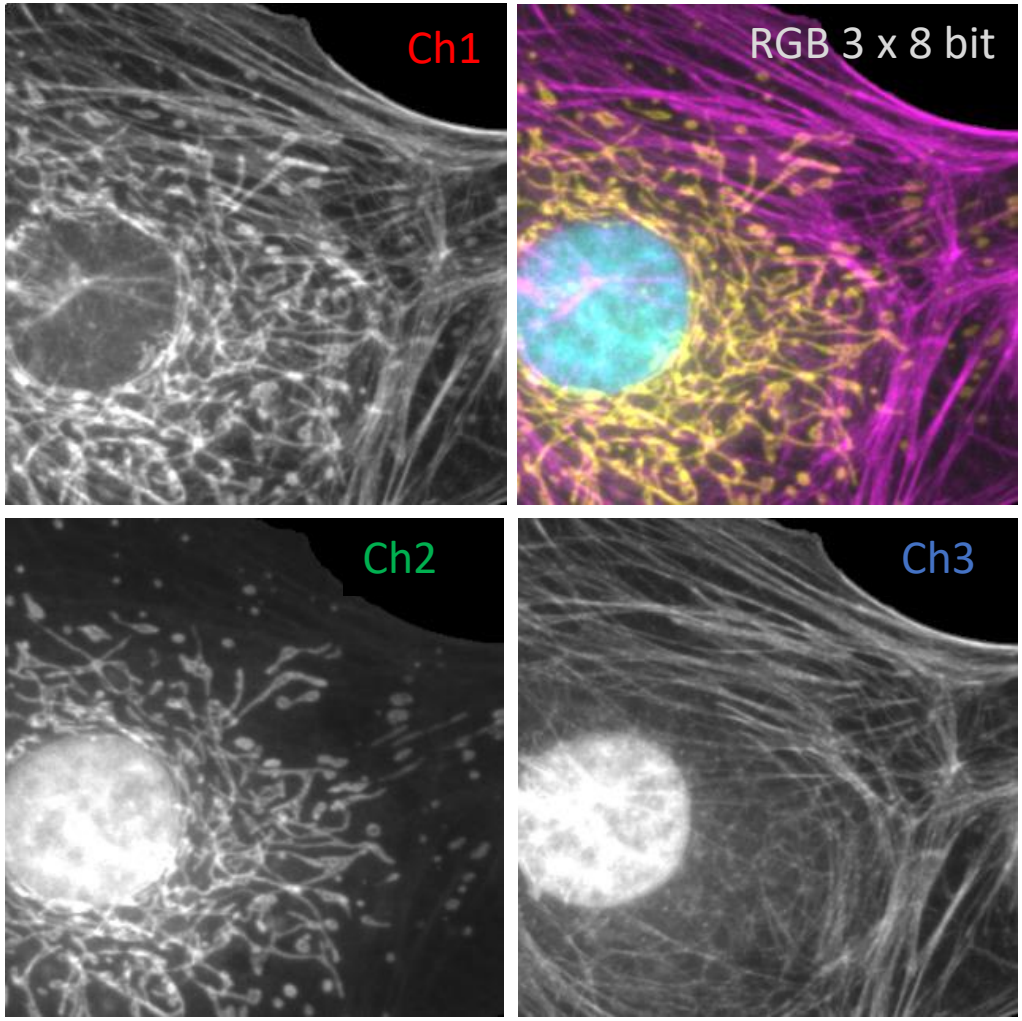


RGB images cannot be transformed back to multi channel images





RGB images cannot be transformed back to multi channel images

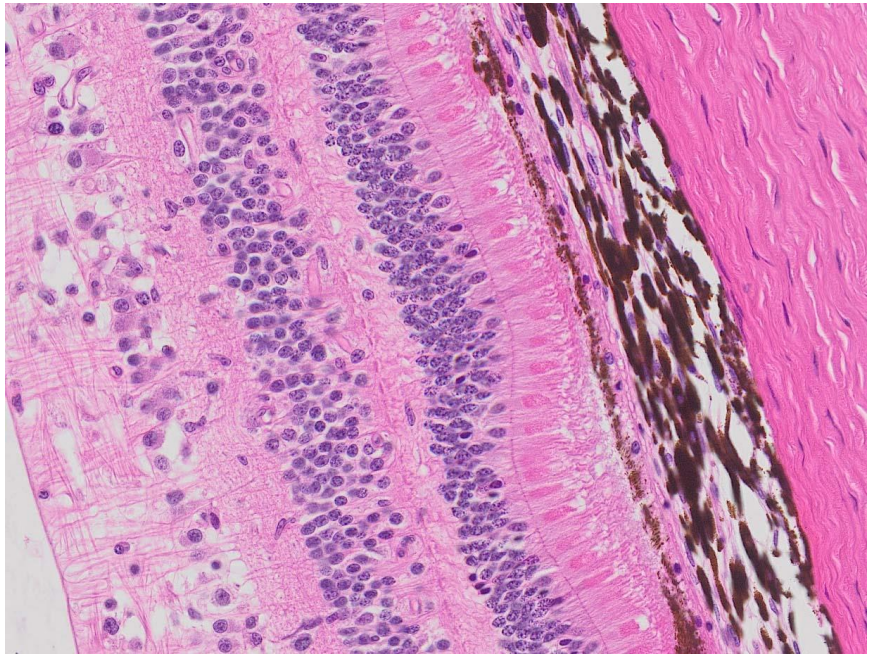


The only scientific image that can be converted back from an RGB image is a 8 bit 3 channel image that was originally Red, Green and Blue.

Any other colour combination isn't (easily) recovered.

Higher bit depths (i.e. 12, 16 bit), 4 or more colours, colours other than RGB cannot be recovered at original quality from a RGB image.

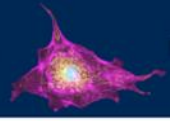
Histology images are captured with a colour camera = RGB



Fluorescence images are ideally captured with a monochrome camera or PMT.

Colour camera images are RGB.

They do not have discrete channels.
DAB = Brown (R 70, G 40, B 50)
Eosin = Pink (R 230, G 150, B 200)



What is a digital image?

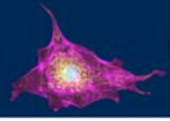
Image

- Array(s) of pixels
- Fixed size / spacing
- Defined Location (x,y)
- Also z, time, channel
- Pixel value represents image signal intensity

Metadata

Info about the image…

- # channels, slices, timepoints
- Filters, lasers, LEDS, excitation & emission wavelengths
- Microscope type, objective, detector
- Exposure, gain, offset
- Scale, zoom, voxel size



Digital image - File types recognised by ImageJ / Fiji

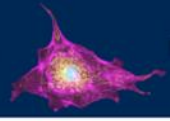
Image file types

tif
gif
bmp
png
ics
jpg
mpeg
quicktime
swf
pict
eps
pdf
photoshop

Microscope / IA software propriety files

Amira .am .amiramesh
Andor .sif
Bitplane Imaris .ims
Deltavision .dv .r3d etc
Hamamatsu .ndpi
Leica .lei .lif
Nikon .nd2 .nef
Olympus .oib .oif .sld .spl
Volocity .mvd2
Zeiss .lsm .czi . zvi

Save images as propriety microscope format.
Includes all metadata.
Use **.tif** for all image analysis.
Includes metadata, can be any bit depth

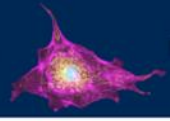


Digital image – What file type is best?

- Save images as propriety microscope format.
  - Includes all metadata.
  - Is identifiable as the original unprocessed image.

- Use **.tif** for image analysis.
  - Includes metadata
  - All bit depths supported
  - The processed image.

- Publish as an RGB image
  - Do not use this image for analysis
  - Lossy compression gives a small file – good for emailing, websites etc. Jpeg, Gif
  - Lossless compression (Tif, PNG) – larger files, finer detail.
  - Lossless RGB files are still not appropriate for image analysis.



Exercises using Fiji. Session 1 – Digital Images

Getting to grips with digital images…

1) Blobs to numbers and back again.

2) What bit depth is this image?

3) Advanced exercise (optional / homework):
   Changing bit depth when converting images.