Image Analysis Basics

-

Introduction to the Digital Image

PoL BioImage Analysis Symposium

- Training School -

Jan Brocher

Acknowledgement

Robert Haase

Marcelo Leomil Zoccoler

PoL for hosting

You for participating, your interest and questions !

Let’s get started…

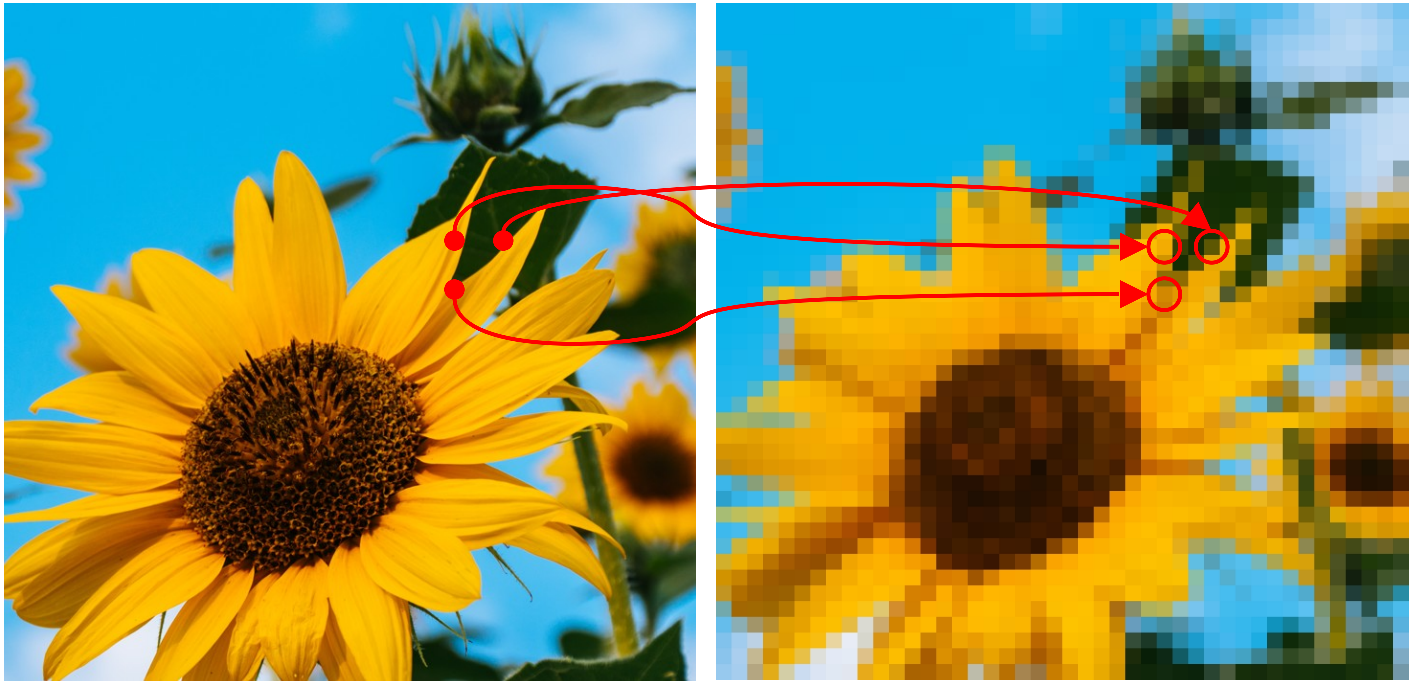
…at the beginning

What is a Pixel


Is a Pixel a Square ?

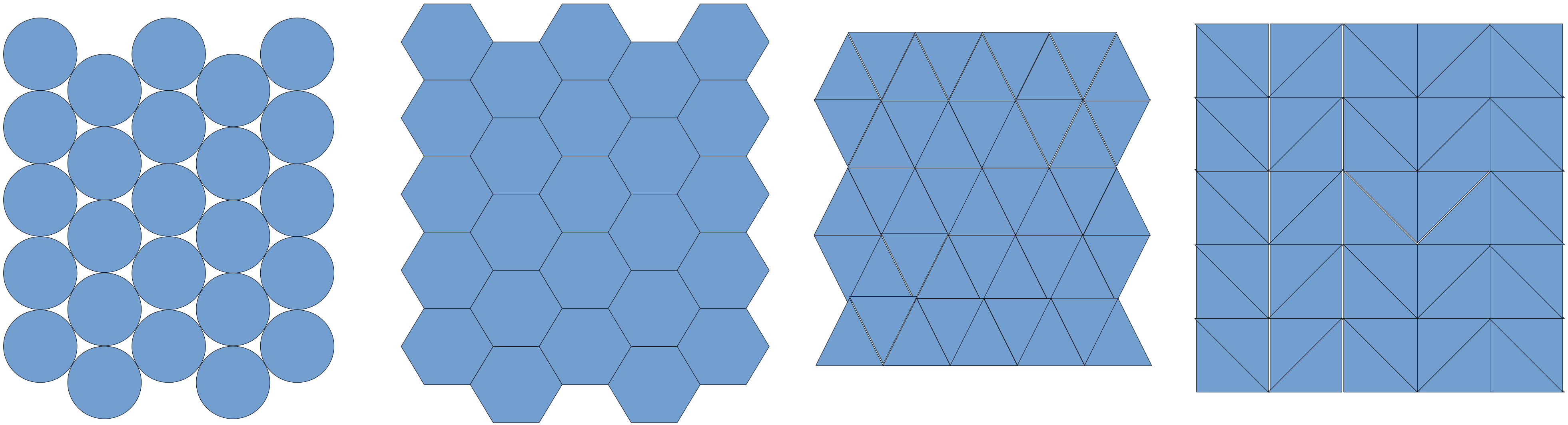
A Pixel IS NOT a SQUARE!

Pixel = picture element = point-sample of a real world signal



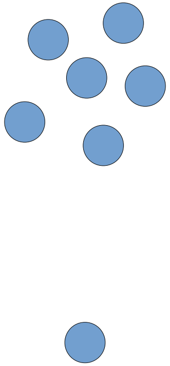
sunflower image: Brett Sayles (via Pexels)

Alternative Pixel Representations – There is no Optimum

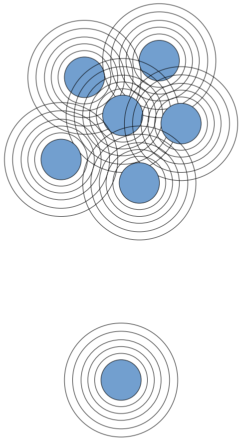


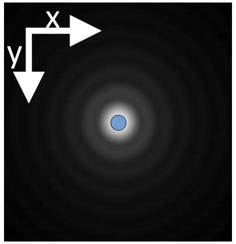
From Points to Pixels

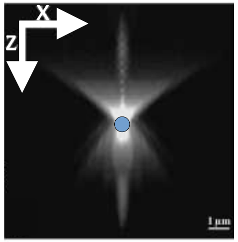
Fluorescent Molecules (e.g. beads)



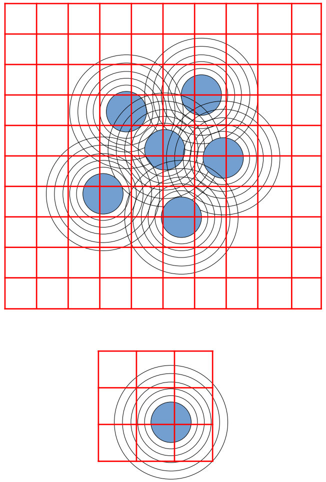
Occurrence in imaging system as PSF

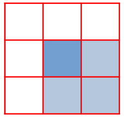






Areas covered by digital pixel representations





digital pixels

Pixels = Data !

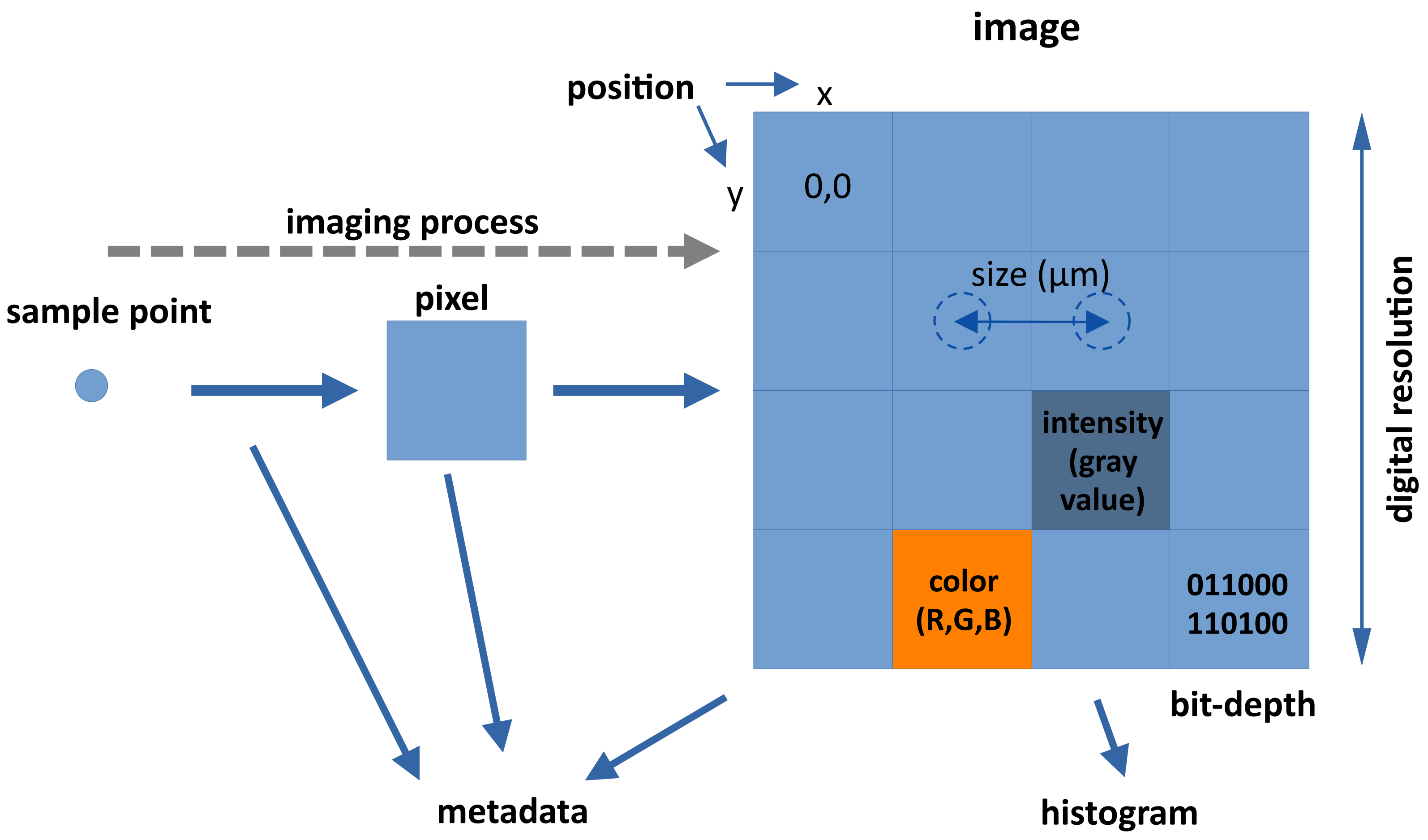
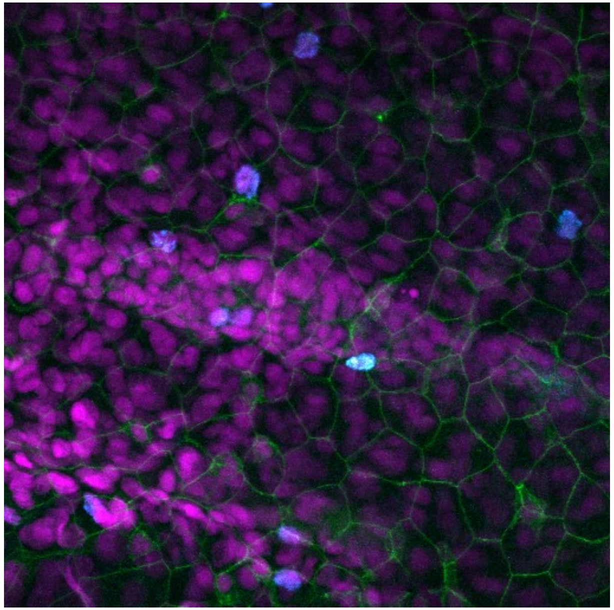


image
position
x
y
0,0
imaging process
sample point
pixel
size (µm)
intensity
(gray
value)
color
(R,G,B)
011000
110100
digital resolution
bit-depth
metadata
histogram

Always save your Raw Data in the Original File Format



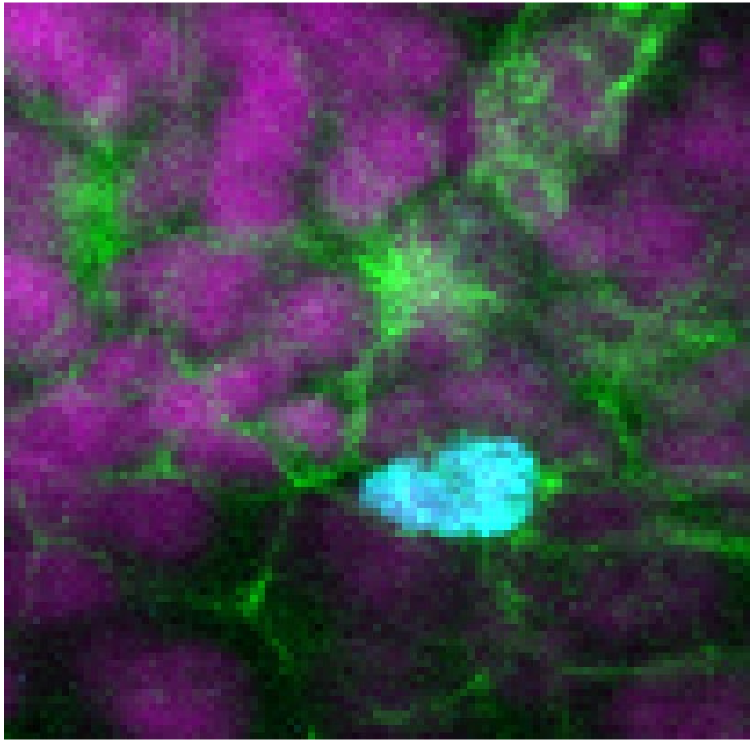
Original (commercial) File type — Metadata = **368 lines**

Exported Tiff — Metadata = **28 lines**

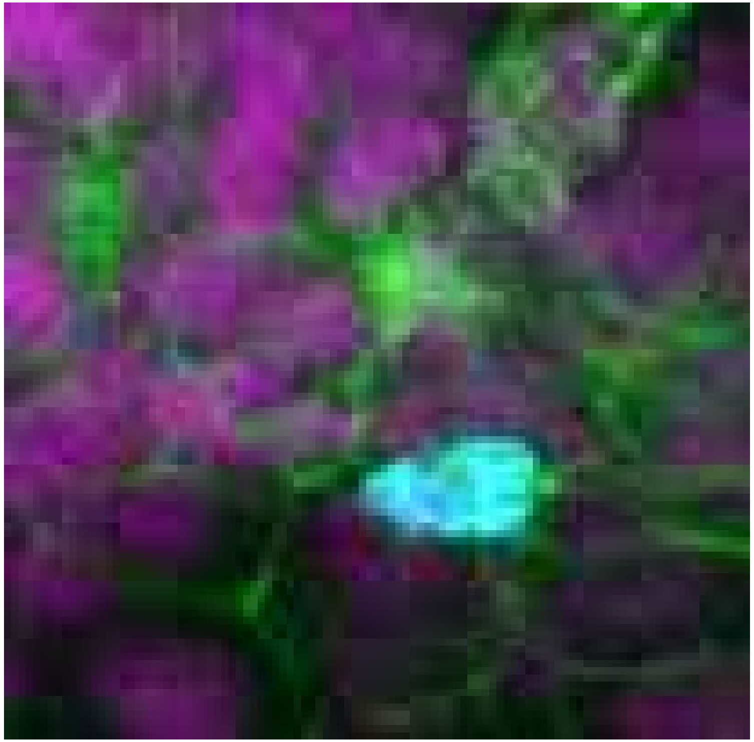
92% metadata loss

DON’T USE JPEG

Original

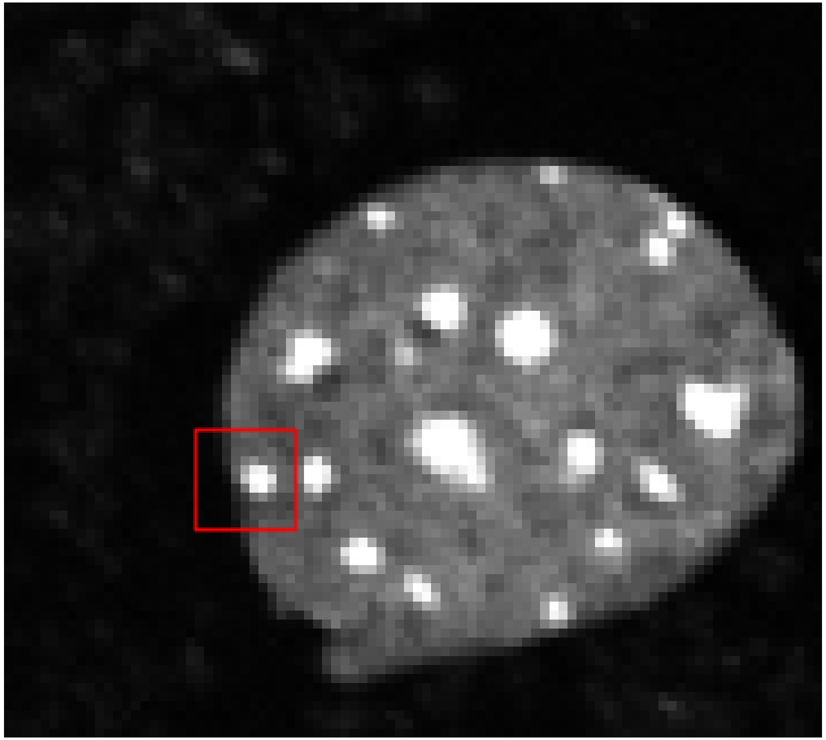


JPEG (66% compression)



Metadata = 0 lines

Pixel Gray Value reflect the Recorded Intensity



7	6	19	45	85	99	95	85	84	75	78
4	5	14	45	79	83	87	94	76	66	80
4	6	13	40	86	91	84	94	104	77	64
4	6	9	42	88	119	139	110	105	93	75
4	4	6	26	99	166	236	243	148	101	96
4	4	3	15	87	194	255	255	230	100	93
3	3	4	7	44	117	238	253	178	102	97
4	3	4	5	36	60	111	132	100	74	75
3	5	5	6	25	46	65	73	74	71	62
6	3	4	5	25	45	74	74	82	71	79
6	5	4	5	17	54	65	94	88	87	88

Counting all Pixels of each Intensity = Histogram

7	6	19	45	85	99	95	85	84	75	78
4	5	14	45	79	83	87	94	76	66	80
4	6	13	40	86	91	84	94	104	77	64
4	6	9	42	88	119	139	110	105	93	75
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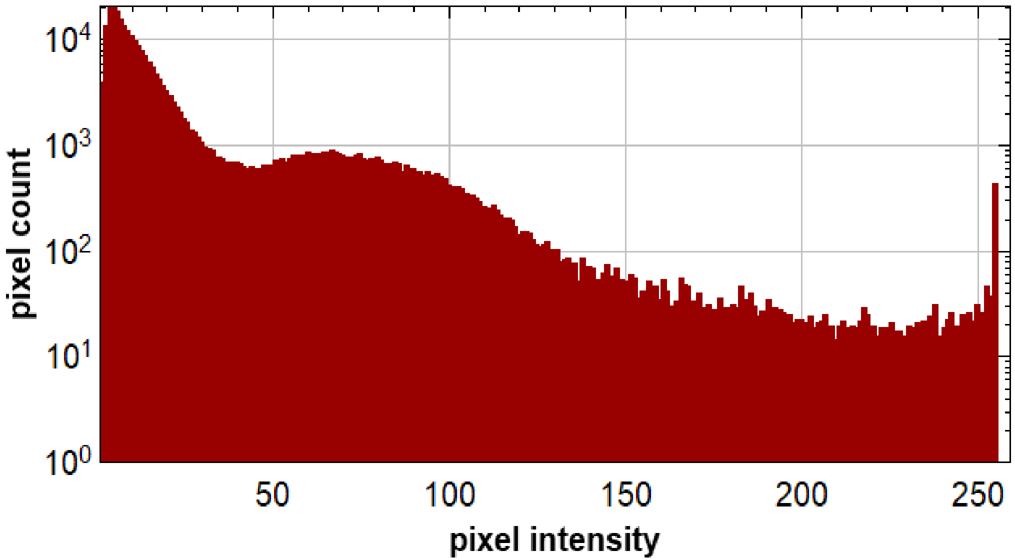

Dynamic / Intensity range

Counting all Pixels of each Intensity = Histogram

7	6	19	45	85	99	95	85	84	75	78
4	5	14	45	79	83	87	94	76	66	80
4	6	13	40	86	91	84	94	104	77	64
4	6	9	42	88	119	139	110	105	93	75
4	4	6	26	99	166	236	243	148	101	96
4	4	3	15	87	194	255	255	230	100	93
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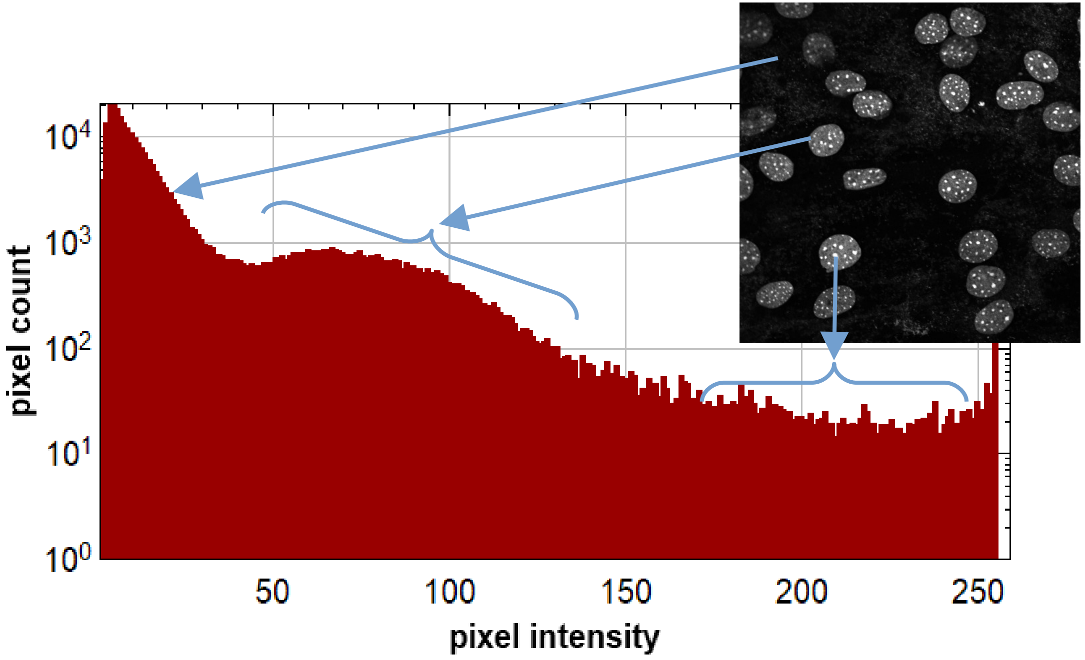
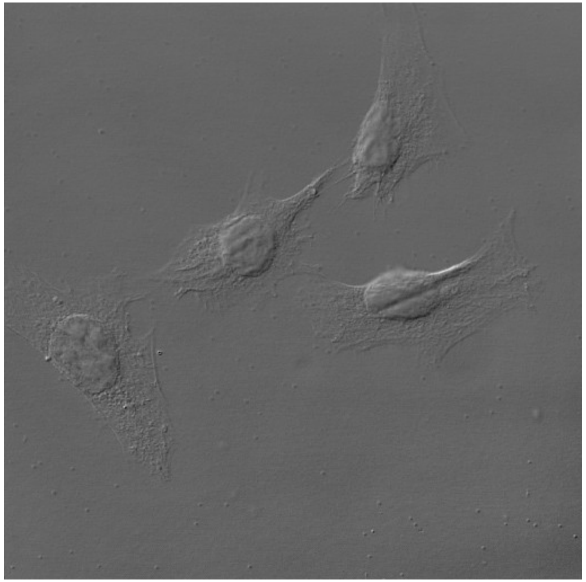
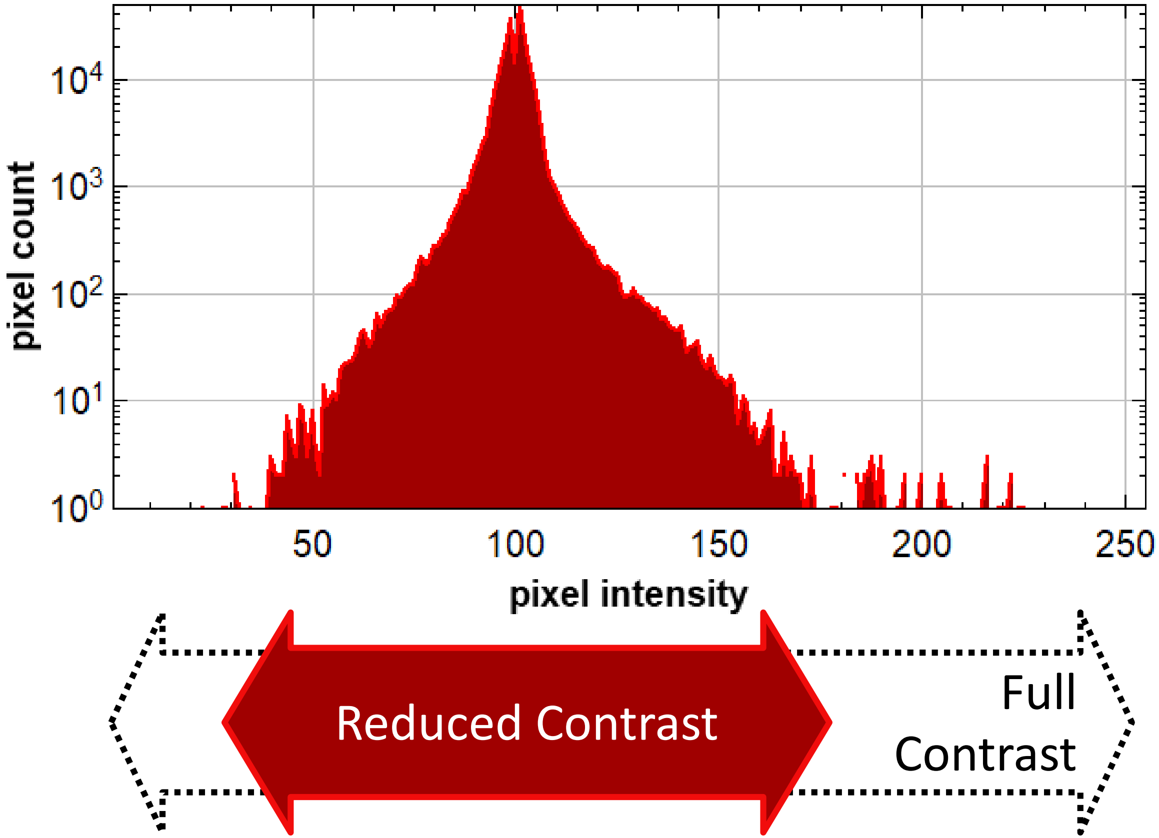
3	5	5	6	25	46	65	73	74	71	62
6	3	4	5	25	45	74	74	82	71	79
6	5	4	5	17	54	65	94	88	87	88



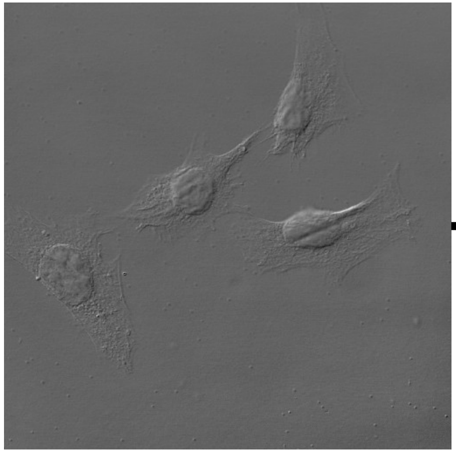
Intensity range

Histogram Inspection reveals Usage of the Dynamic Range



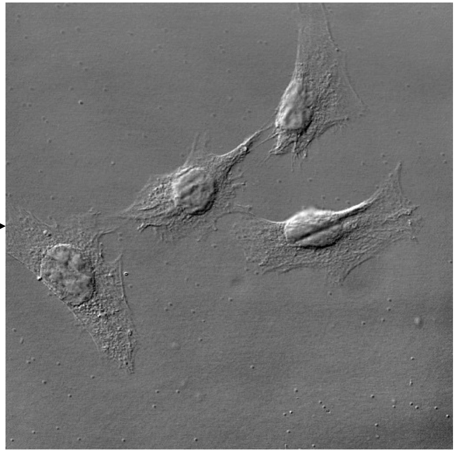


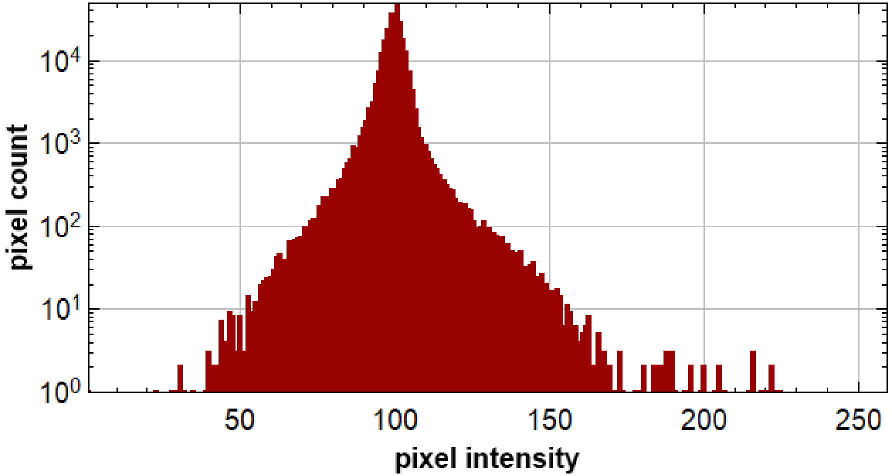
Histogram Inspection reveals Changes in Images

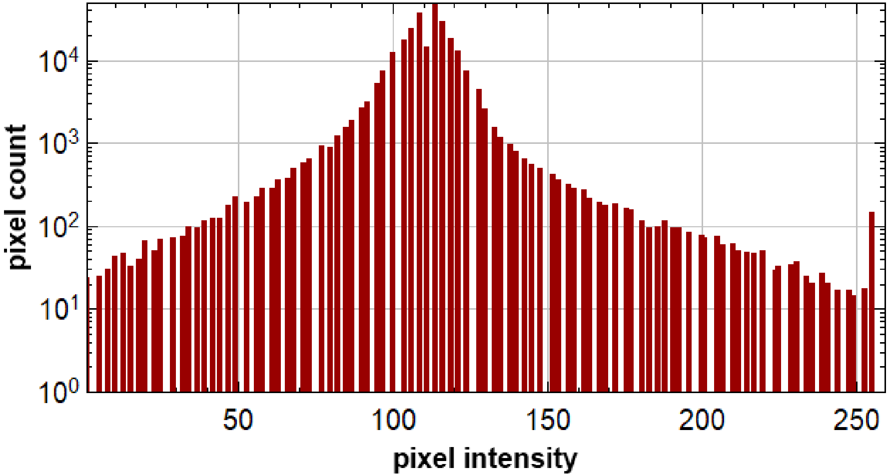


applied
contrast
enhancement

(point operation)

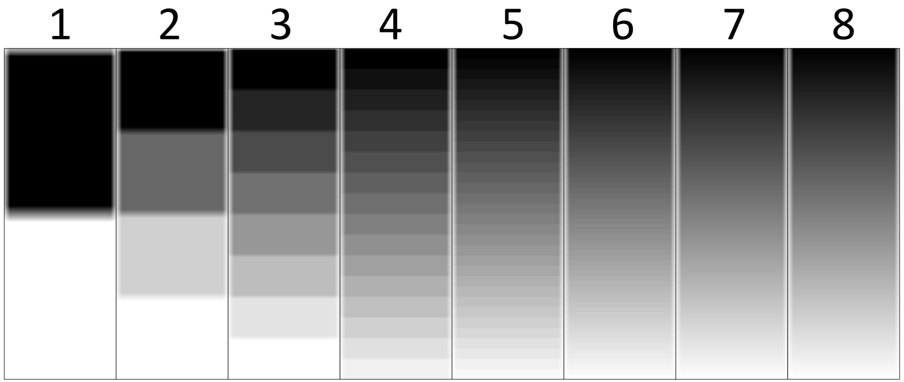




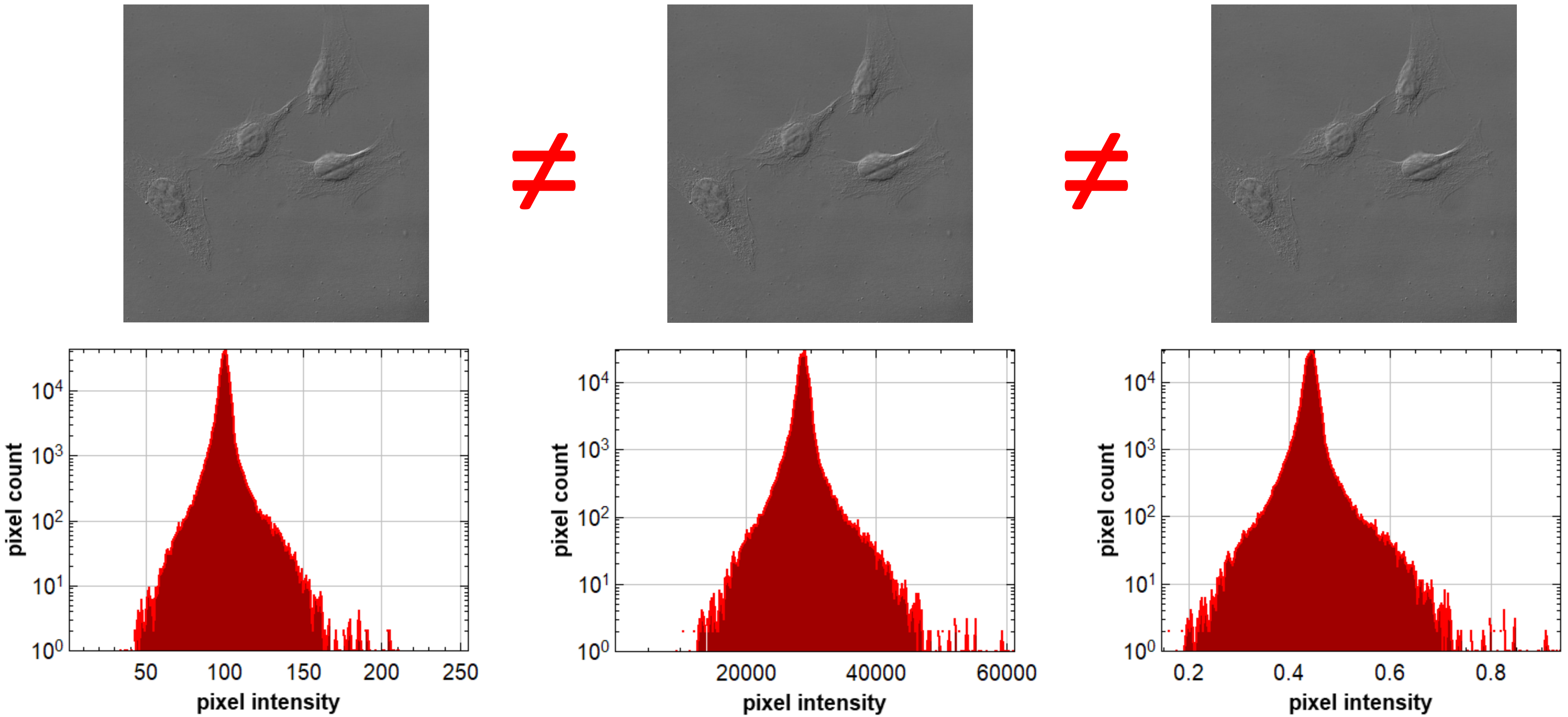


Bit-Depth defines Range of Image Intensity Values

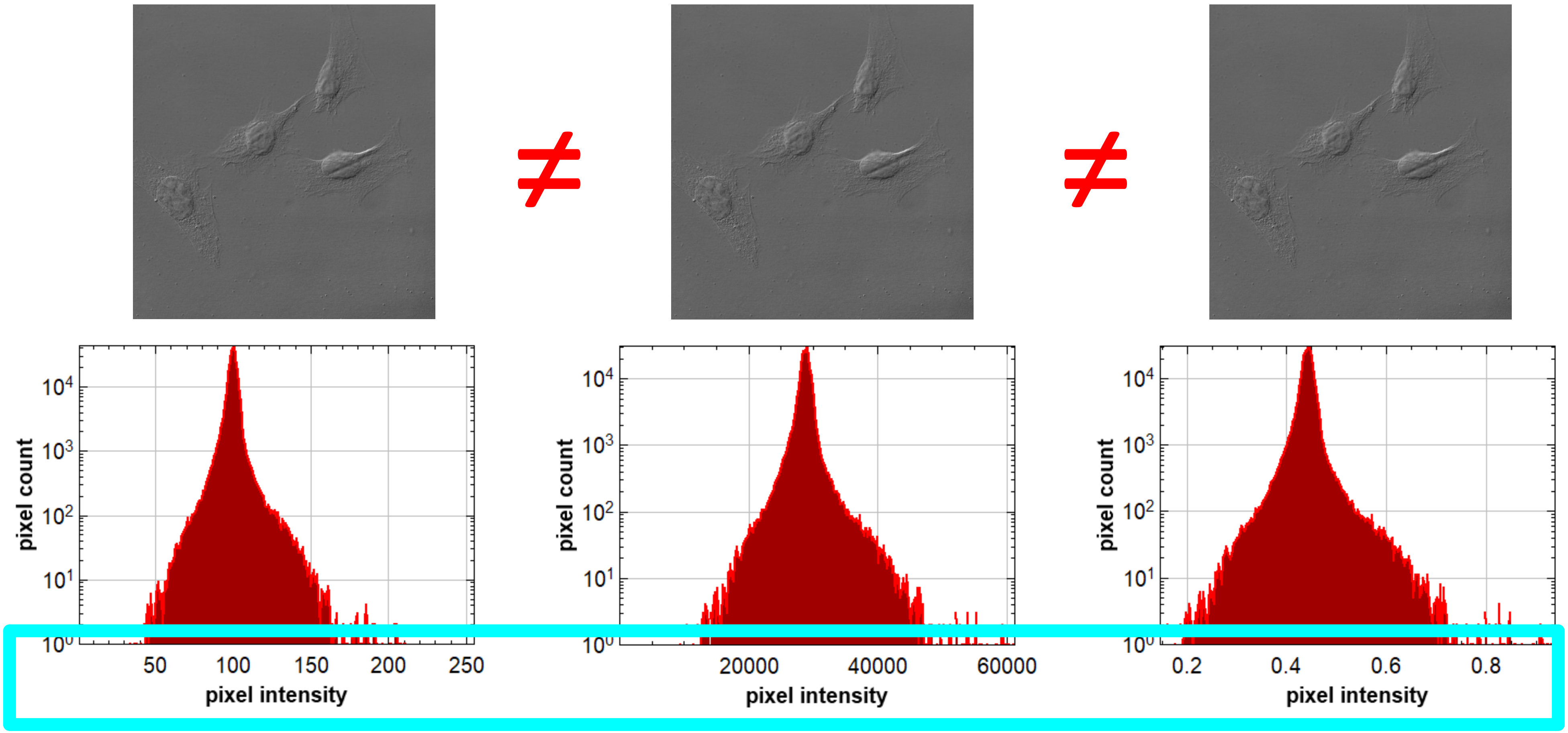
- A bits is the smallest memory unit in computers, *atomic data*.
- The bit-depth *n* enumerates how many different intensity values are present in an image:
    - 2^n grey values
- In microscopy, images are usually stored as 8, 12, or 16-bit images.



Changes in Bit-Depth change the Pixel Intensity Range

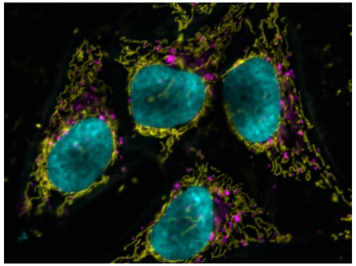


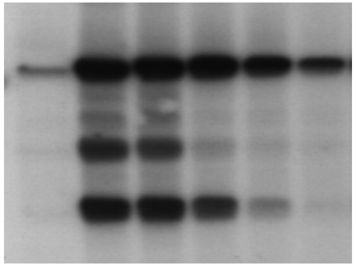
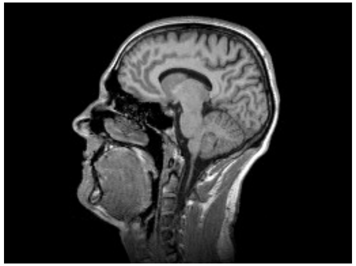
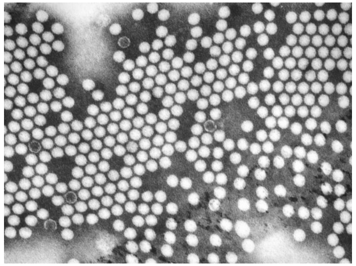
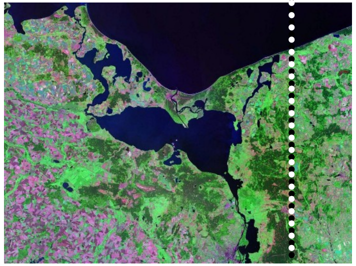
Changes in Bit-Depth change the Pixel Intensity Range



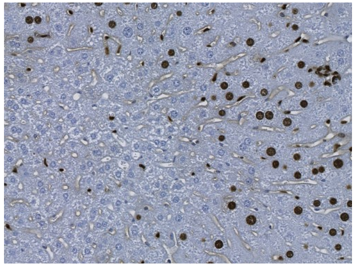
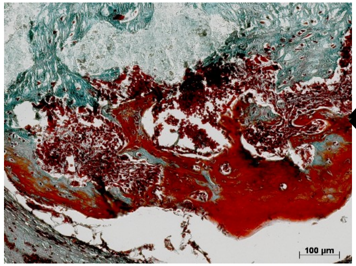
Examples of Intensity-based versus True Color Images

Intensity-based images
8-, 10-, 12-, 14-, 16- and 32-bit

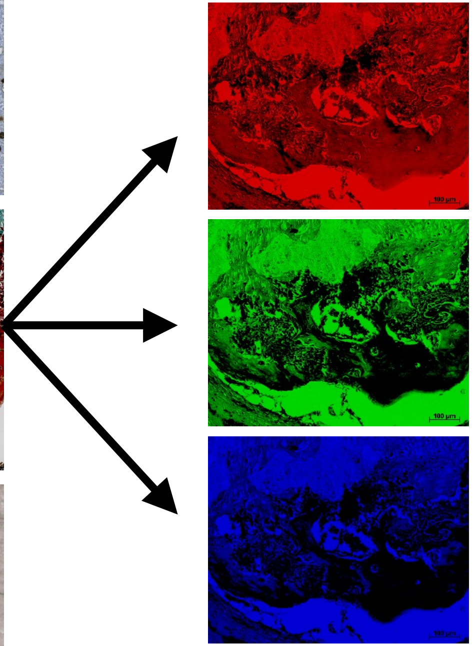


True-color images
24- / 48-bit

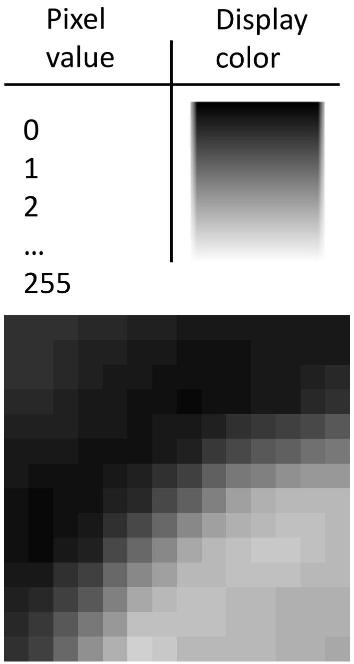



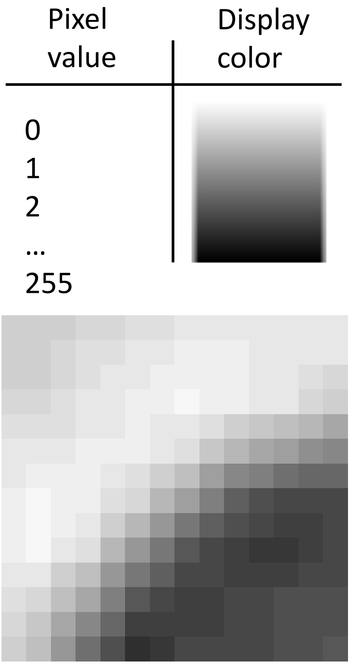
True-color channels
8-bit

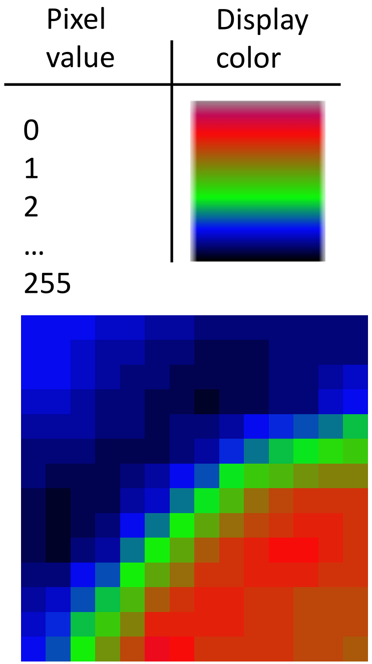


Colormaps / Pseudocolor / Lookup tables (LUTs)

- The lookup table decides how the image is displayed on screen.
- Applying a different lookup table does not change the pixel values, just there appearance





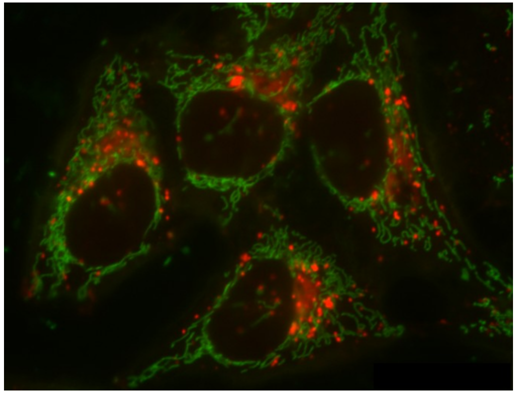


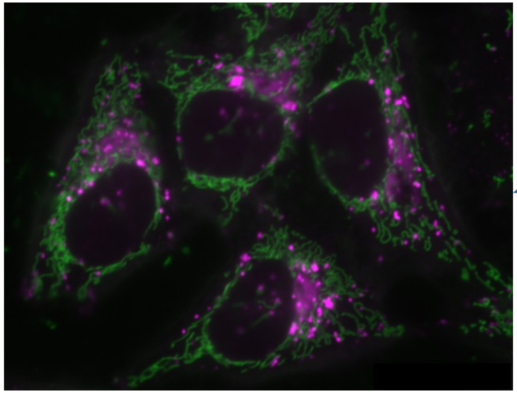
Robert Haase

Colormaps / lookup tables

- Choose visualization of your color tables wisely!
- Think of people with red/green blindness!

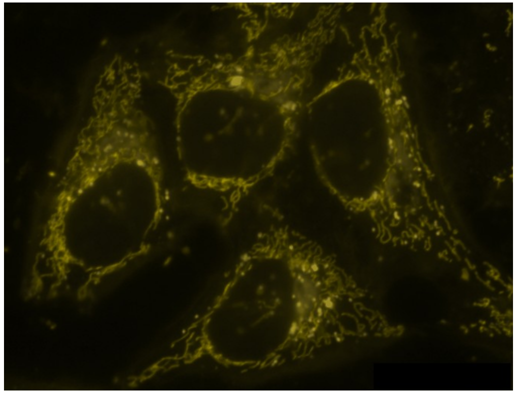
Common view

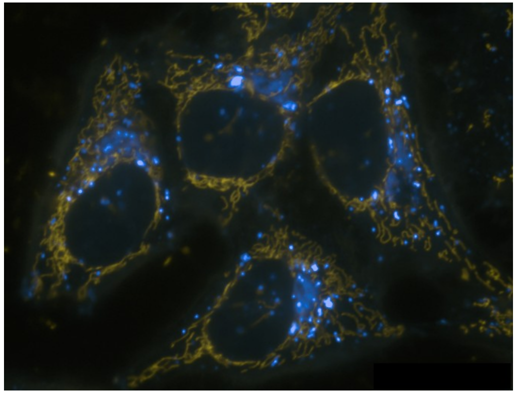




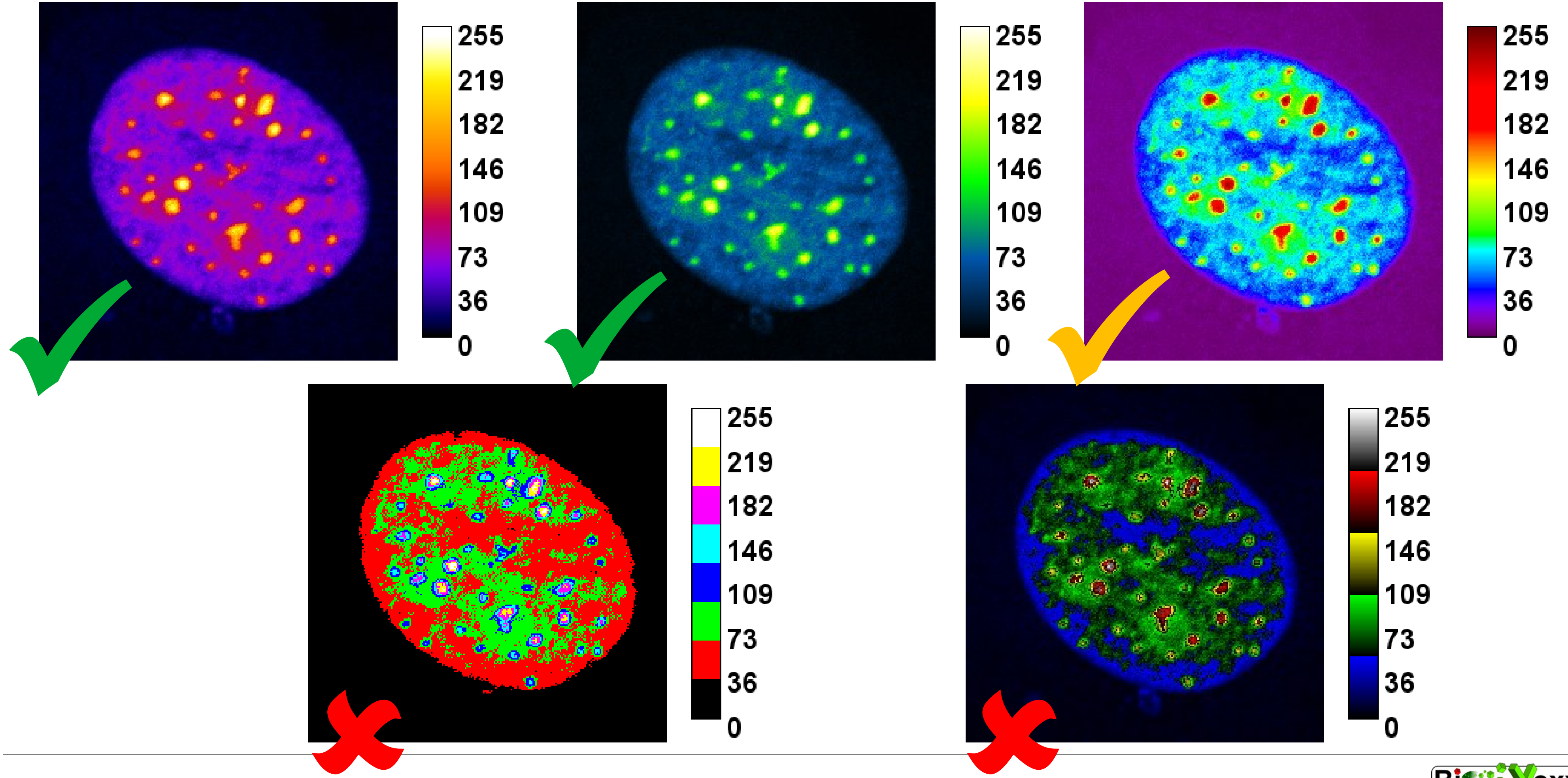
Replace red with magenta!

Red/green blind people may see it like this

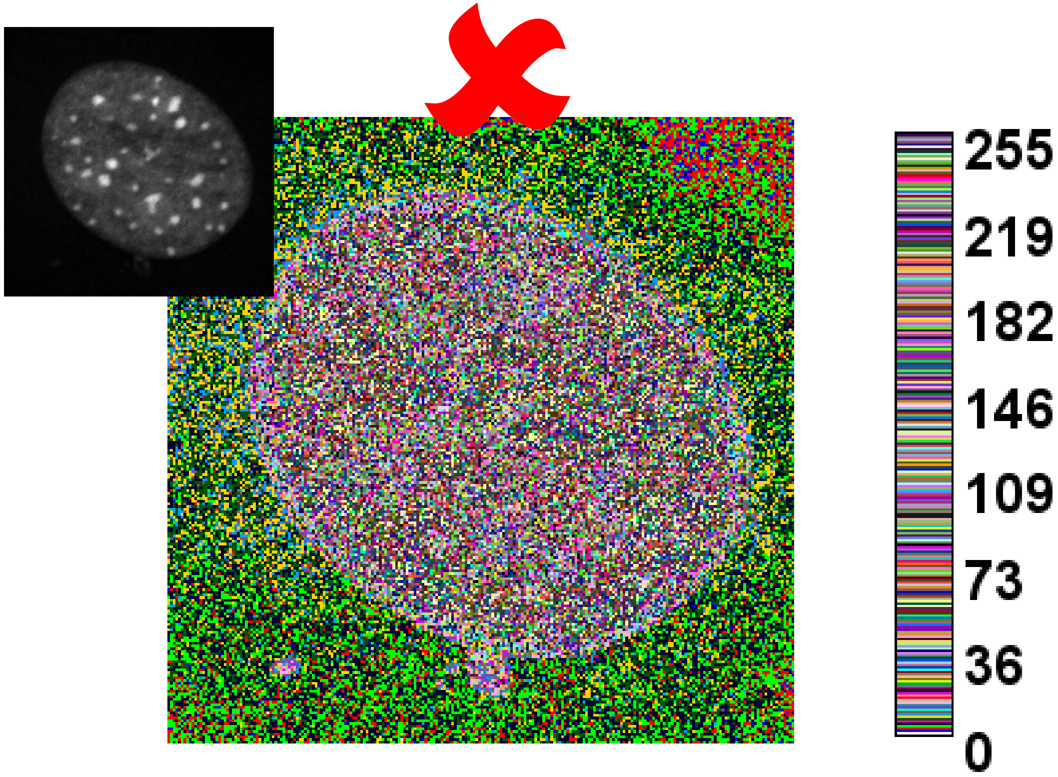


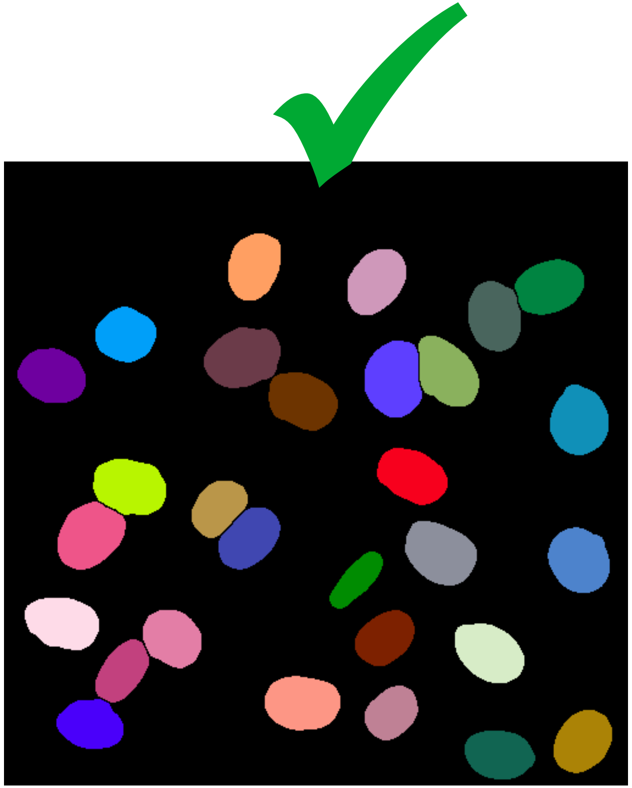


Choose LUTs / Colormaps wisely !

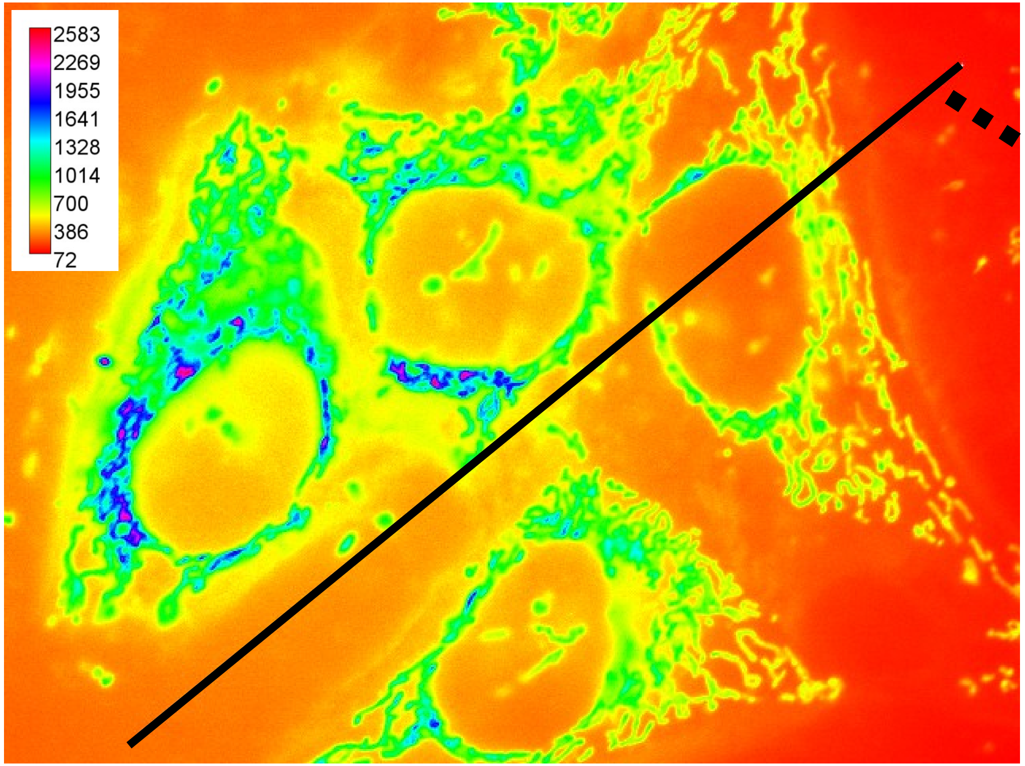


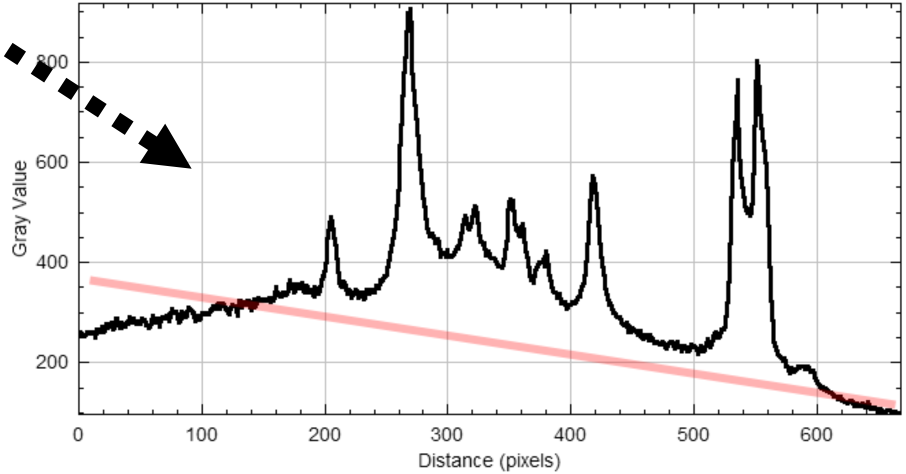
Usability of LUTs / Colormaps depends on Context





LUTs can be used to detect Uneven Lighting



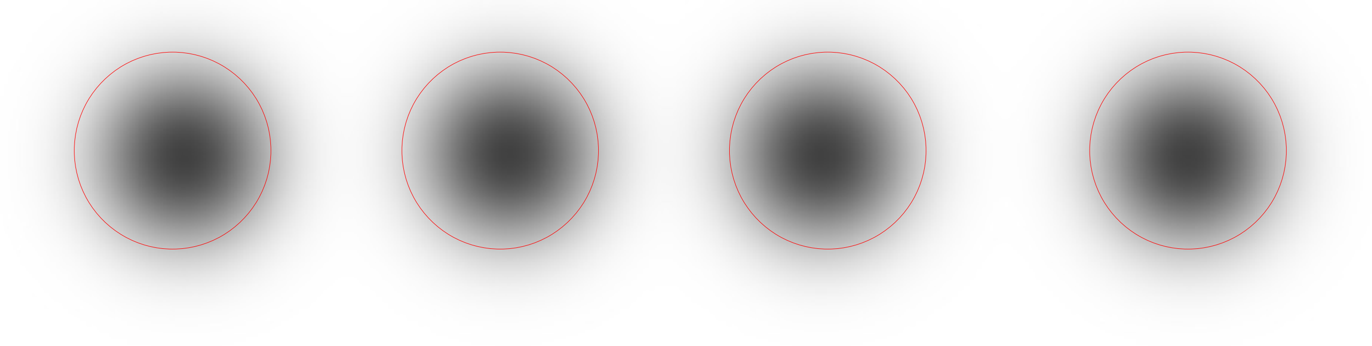


Uneven illumination

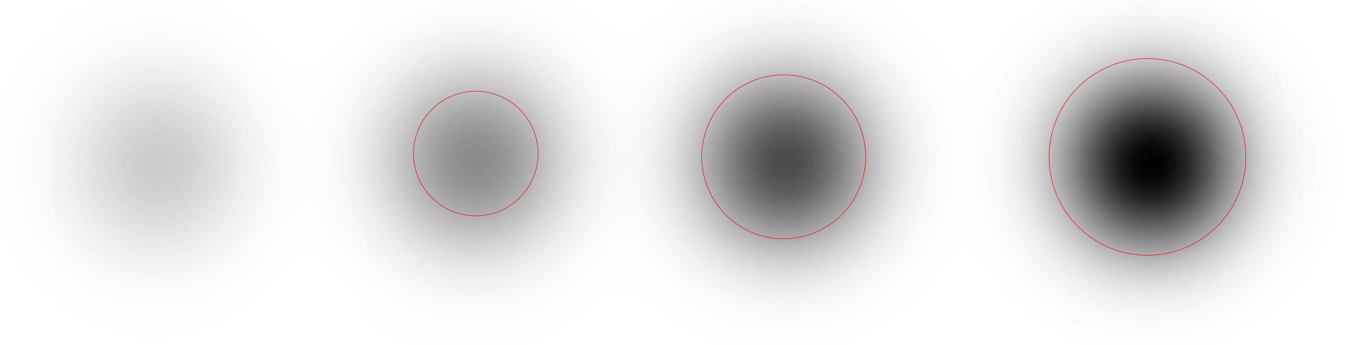
Uneven illumination needs to be corrected before analysis

Why do we need to address illumination problems?

4 objects of the same size, **NO** uneven illumination



4 objects of the same size, **WITH** uneven illumination



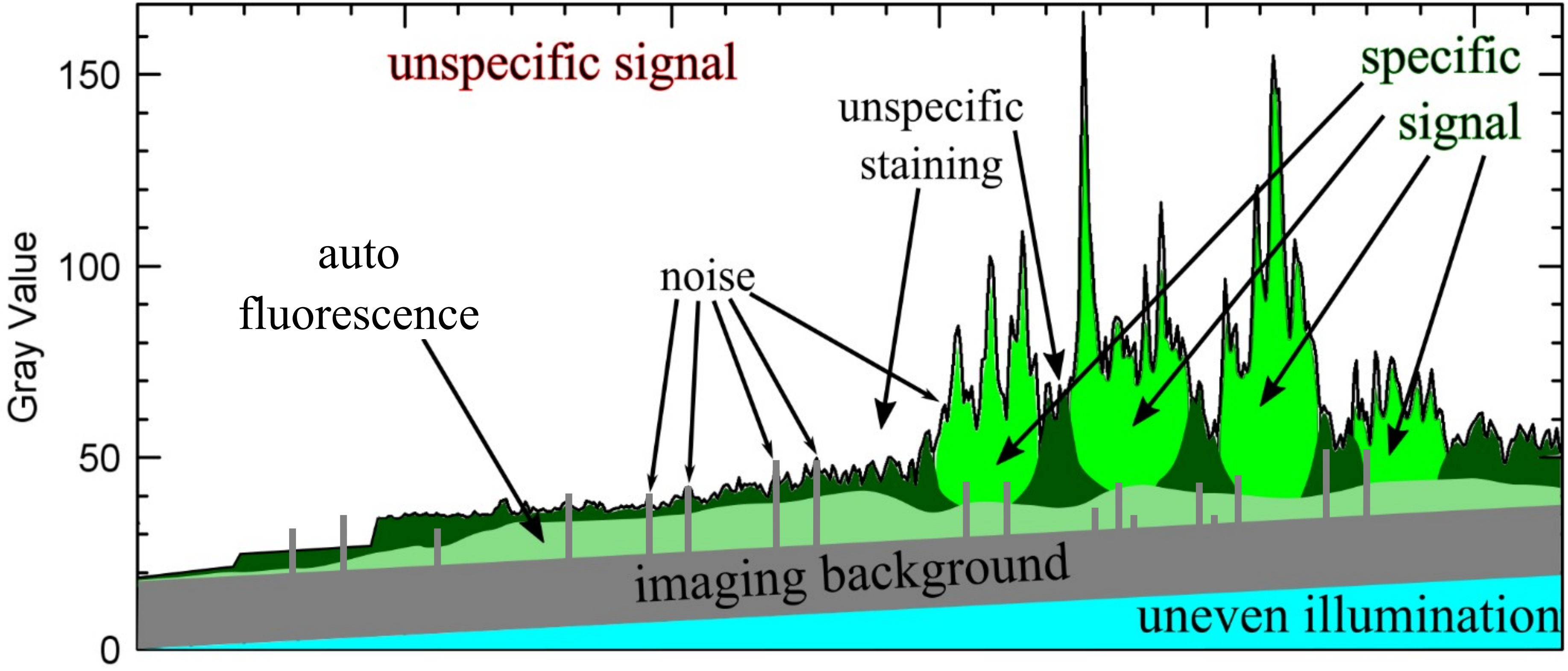
There is no such thing as "Background-Noise"

"Background-Noise" ≈

The Wolpertinger

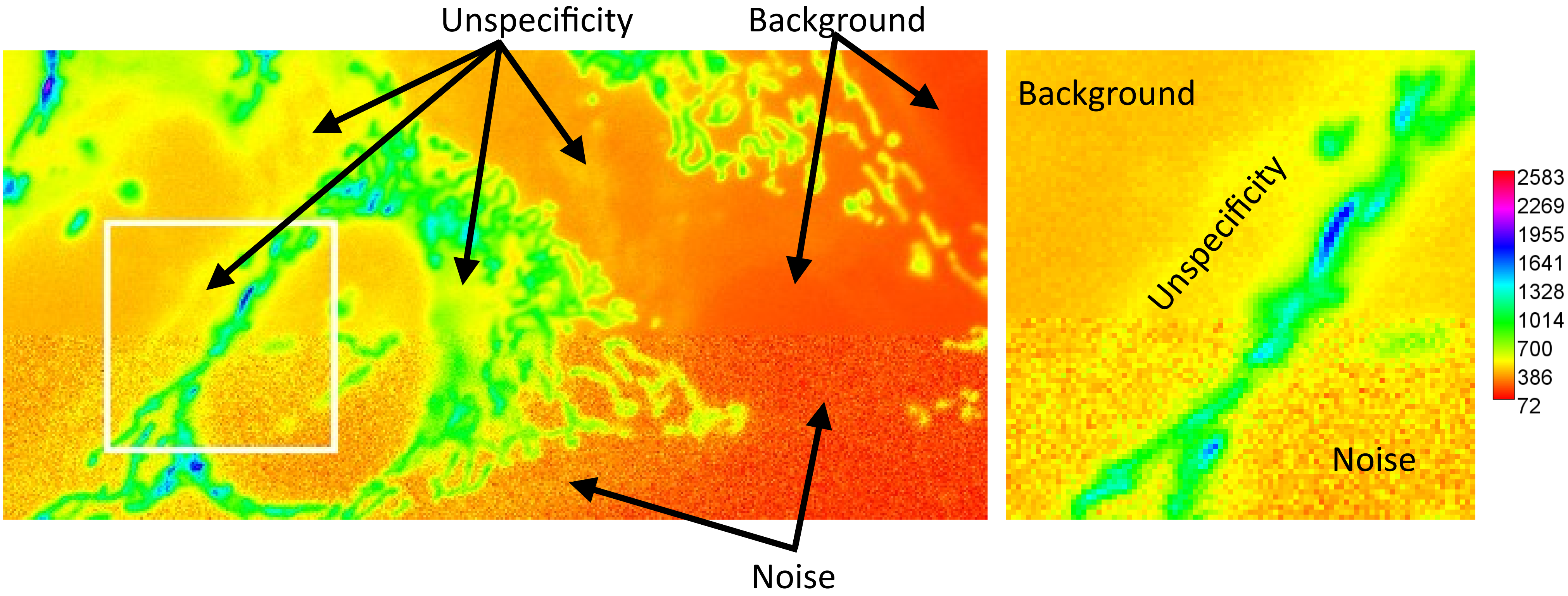
Bavarian mystical creature

Sometimes you don‘t see your signal in all the other signals



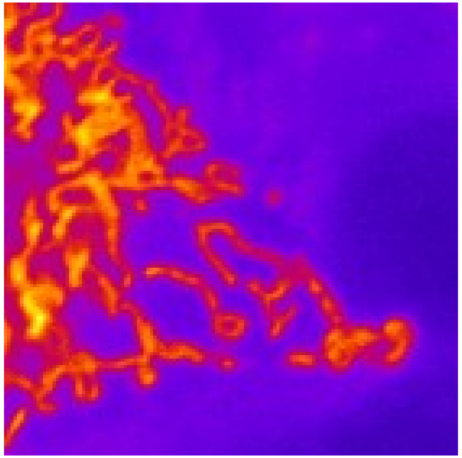
Unspecific signals need correction to not influence image analysis negatively

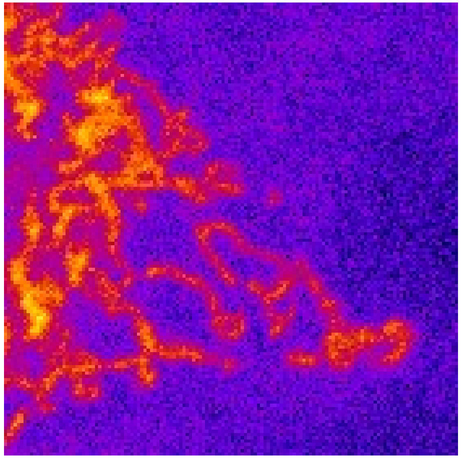
Difference between Background and Noise and Unspecific Staining



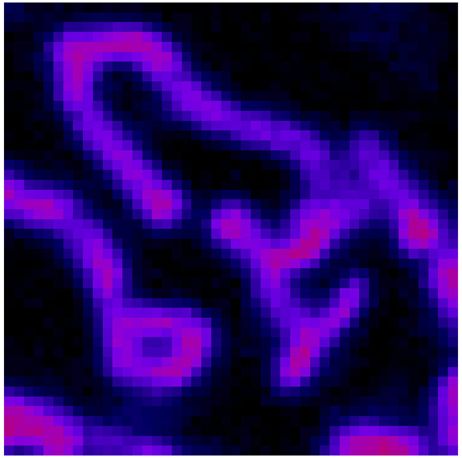
You can **Subtract** Background but not Noise

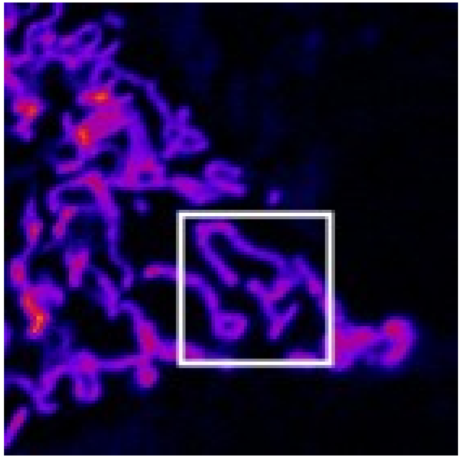
Background
(low noise)

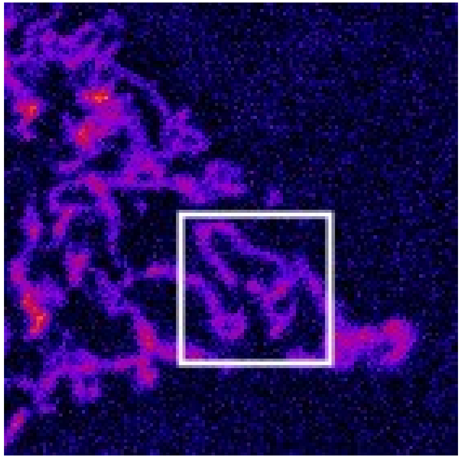


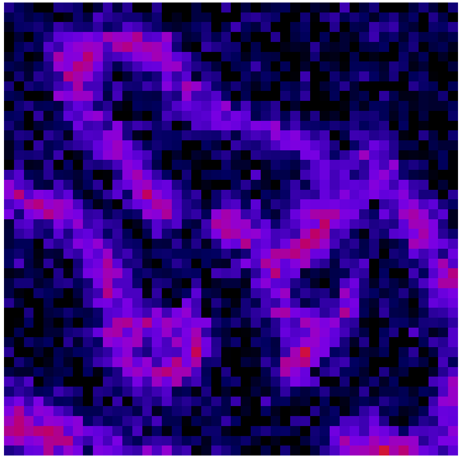


Strong Noise
(and background)

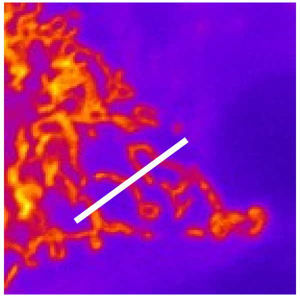


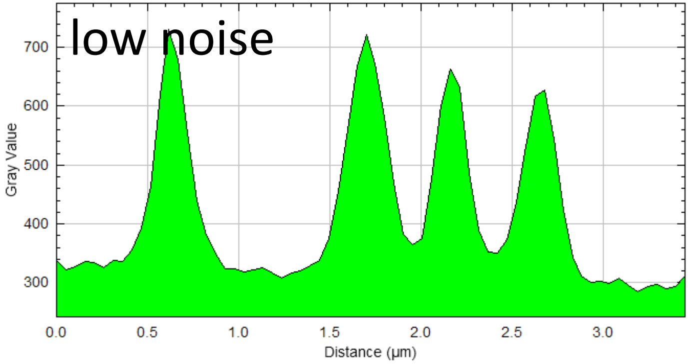


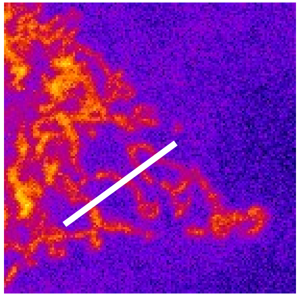


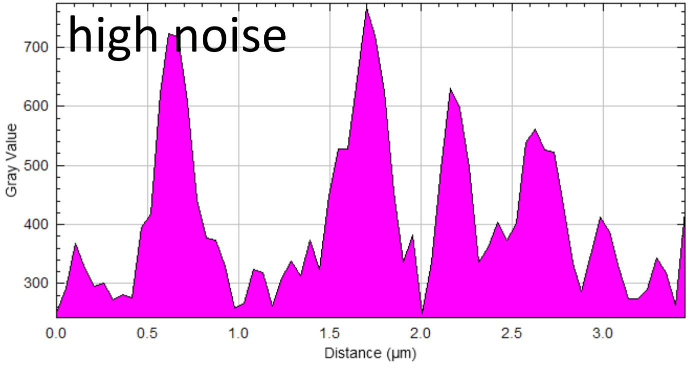


Noise Reduces the Precision of your Analysis

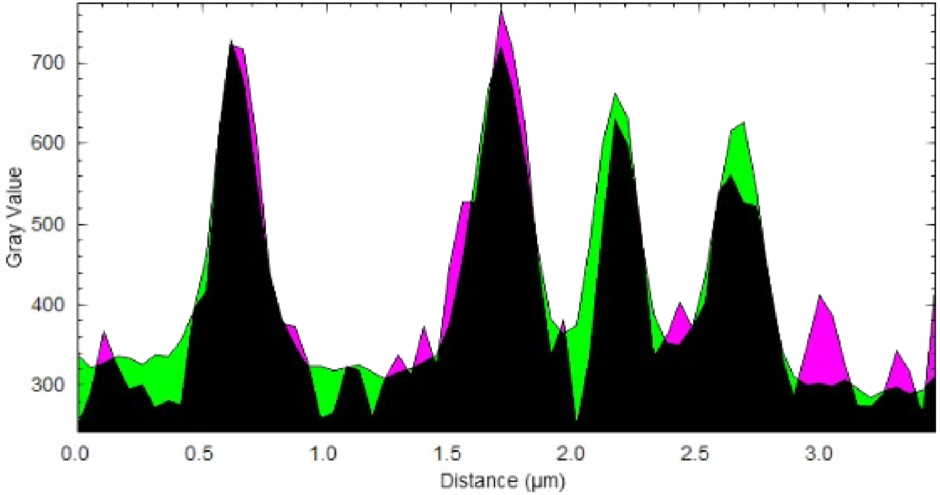






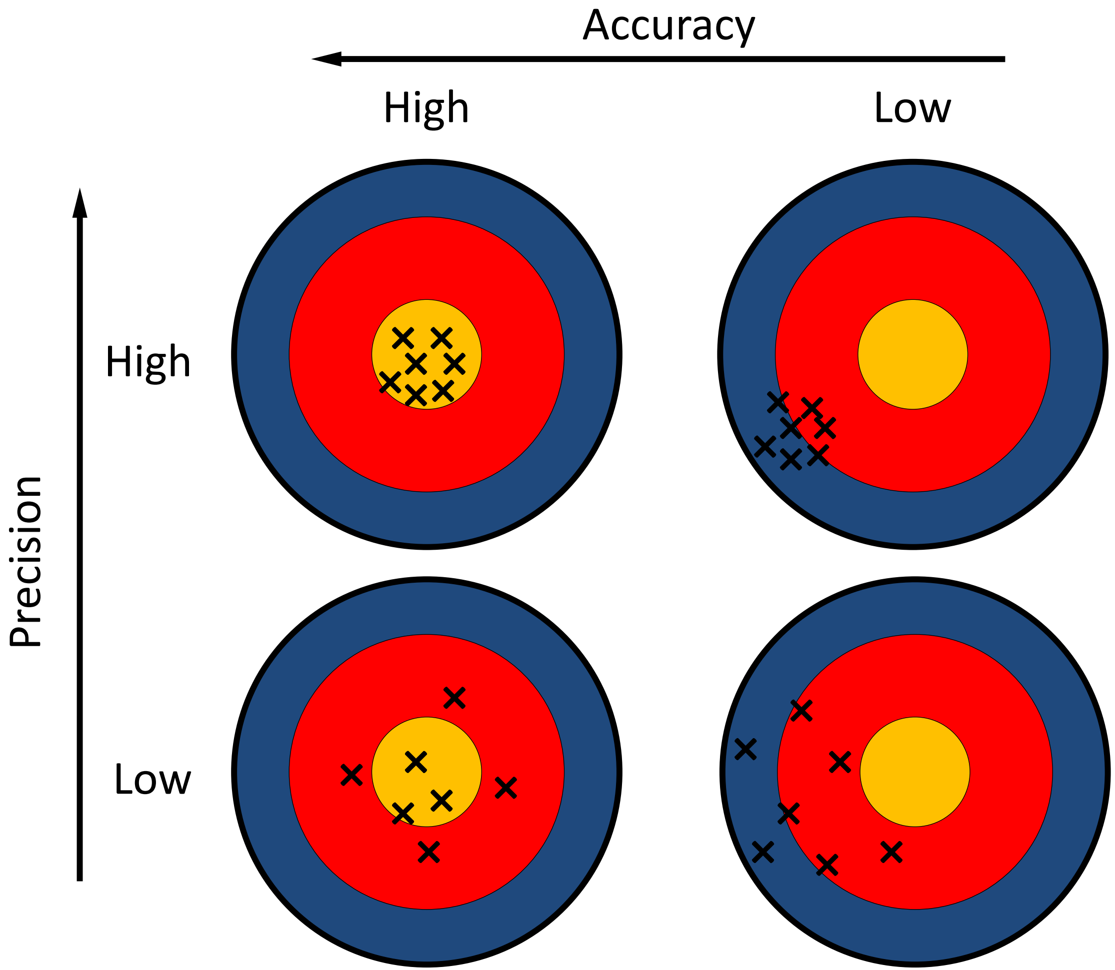


Overlap



BioVoxxel

Precision and Accuracy in Quantitative Measurements



Effect of Background and Noise on Analysis Precision and Accuracy

high noise

high background