Light detection and Image Processing

BIOENG-518
2025

École
polytechnique
fédérale
de Lausanne

What do you recall from the last lecture?

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Detection of photons

- How are photons detected
- Limitations
- Consequences for downstream image analysis and processing

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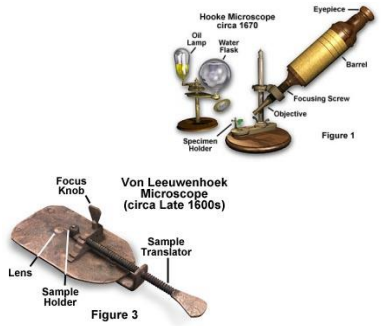
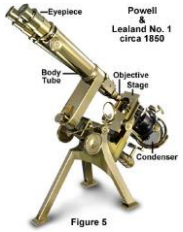
Digital imaging Outlook





1600 1700 1800 1900 2000

Digital Camera
(Kodak, 1975)

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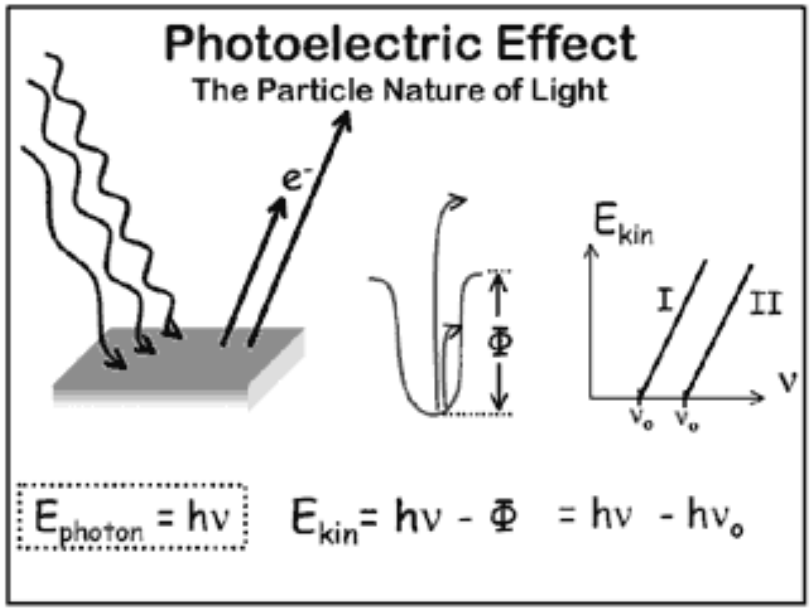
Photon detection

Physical Principles

Photoelectric effect

- Albert Einstein
  Nobel price 1921
- Experiments by Lennart 1902
  - Maximal energy of the photo-electrons depends on the intensity of the light
  - Slope is the same for different cathode materials
  - Light can be described as particles called photons or light quants.
- Detection of Photons



UC Berkeley's Digital Chem1A

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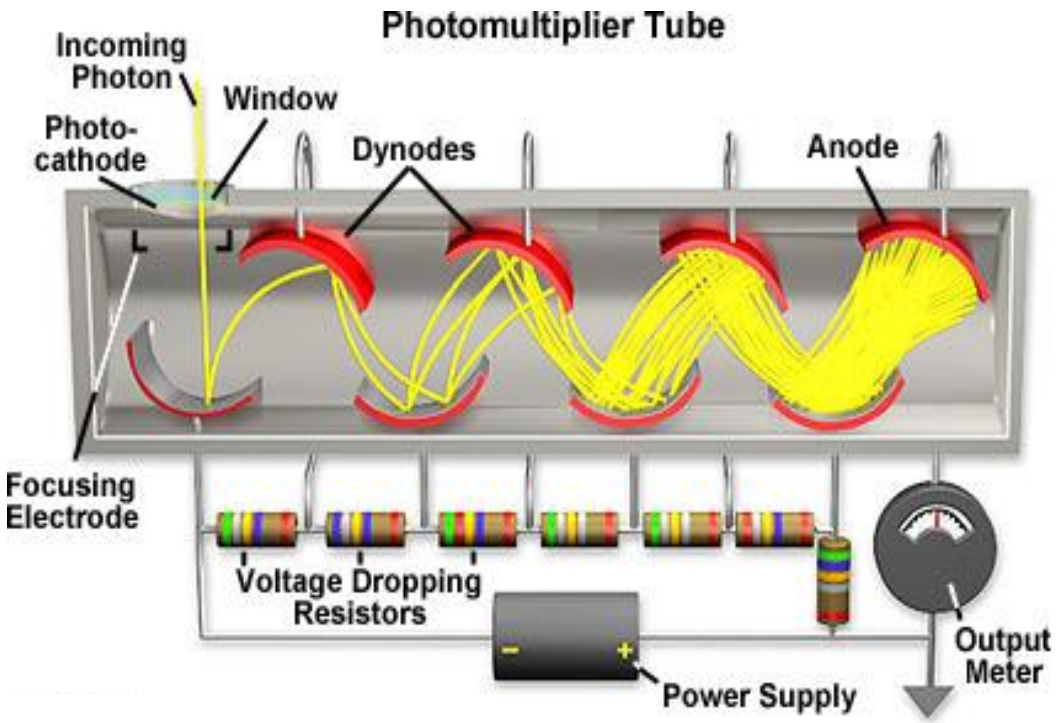
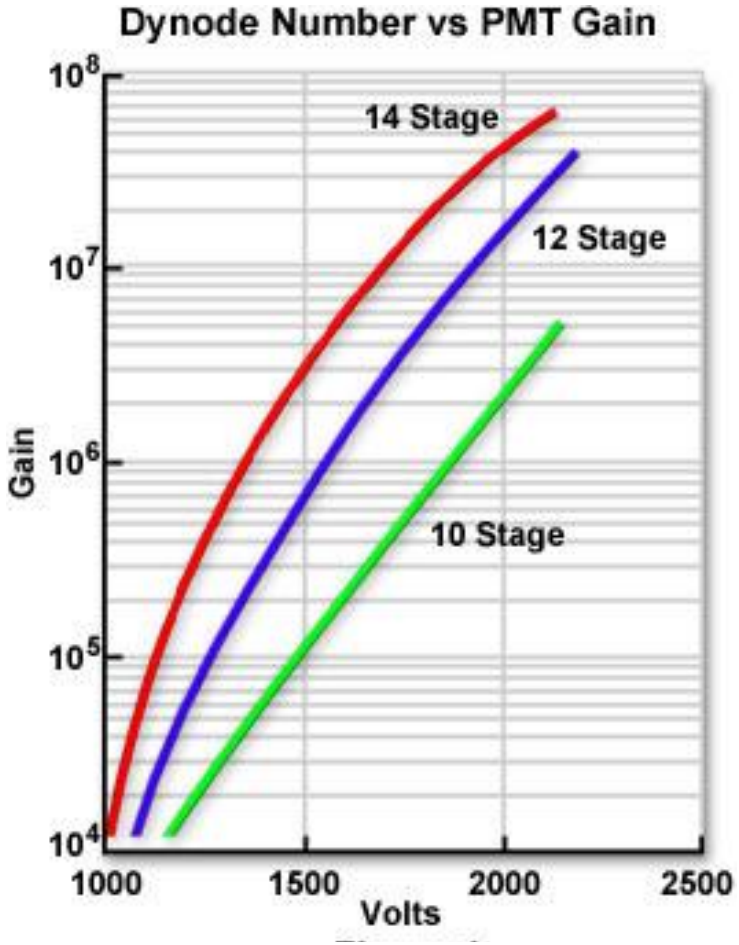
Photomultiplier





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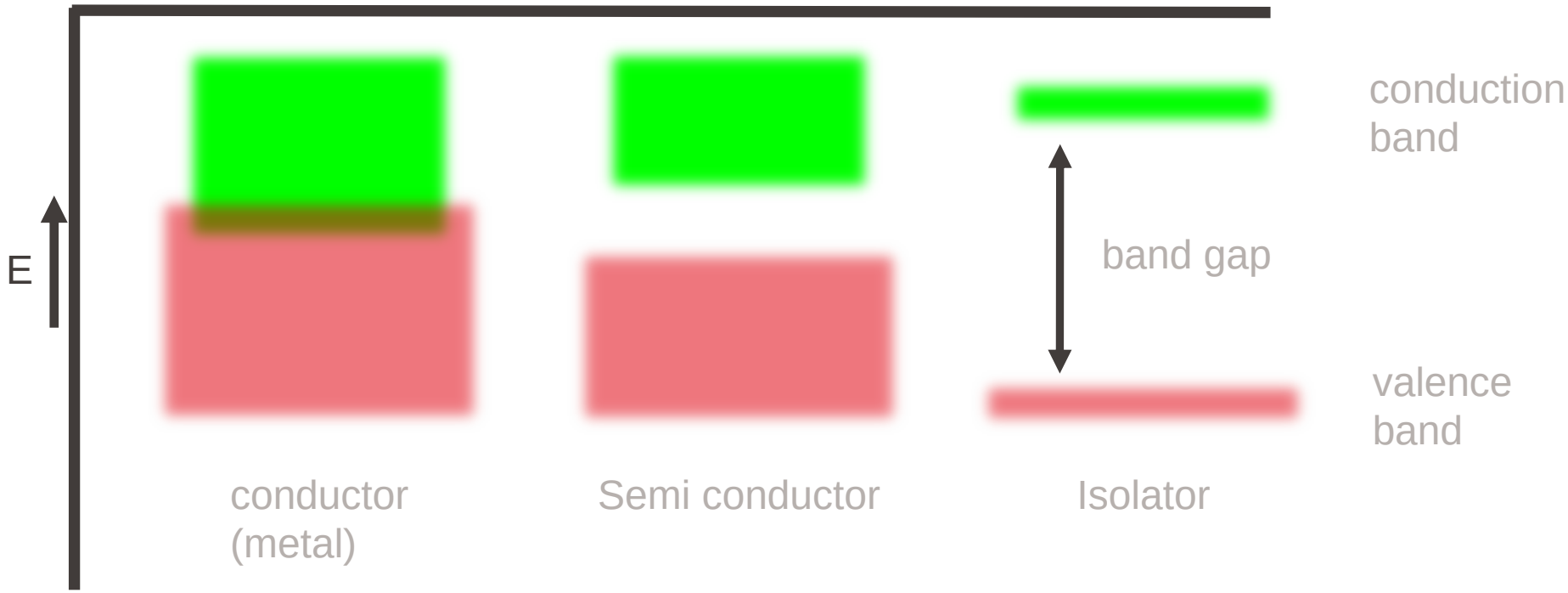
Photomultiplier

- Photons “produce” electrons which are multiplied by acceleration and detected afterwards.
- Gain (=multiplication factor) can be varied.
- Large linear range.
- Quantum efficiency of the photocathode up to 30% in the visible range.
- “no” spatial resolution, i.e. photomultipliers can only be used as point (scanning) detectors

Digital imaging

- Array of photosensitive elements
- Signal is dependent on the number of detected photons; ideally linearly dependent=high dynamic range
- Easy read-out procedure
- Reusable
- Appropriate size, comparable to an analogue film

CCD Cameras



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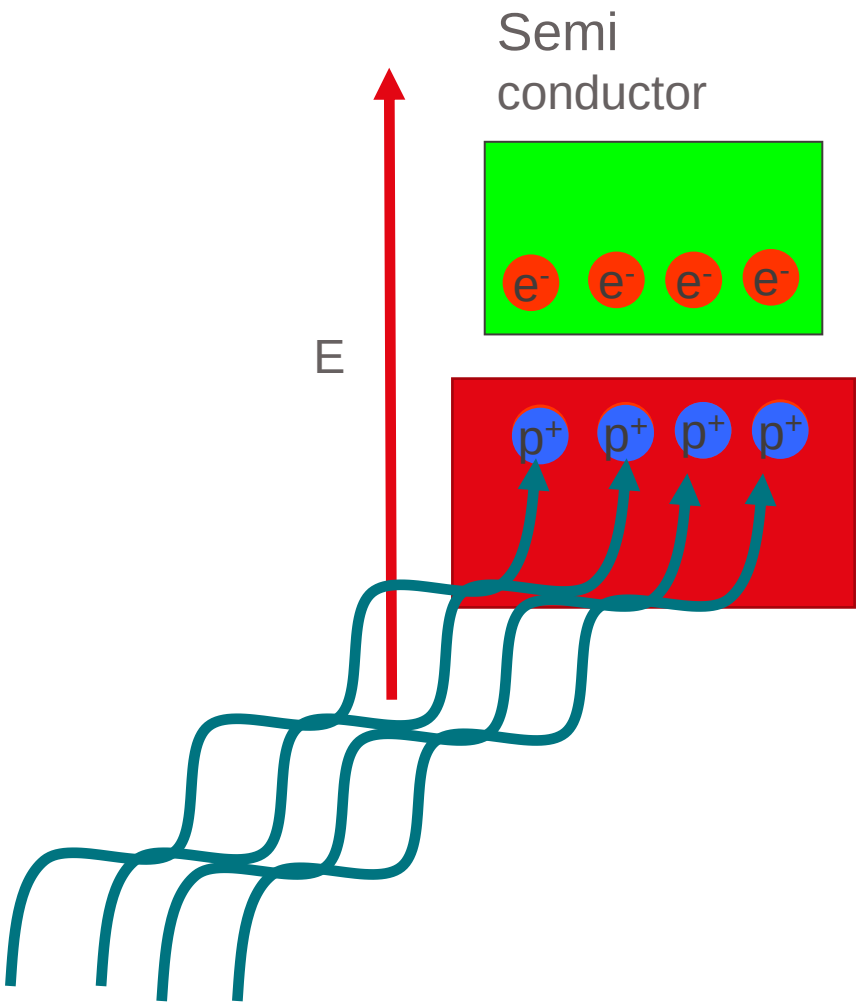
CCD Cameras

- Principle
  - Internal photoelectric effect.
  - Energy of the photon is used to transfer an electron from the valence band to the conduction band (semi conductors).
  - Photodiodes



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Photon detection

Practical Aspects

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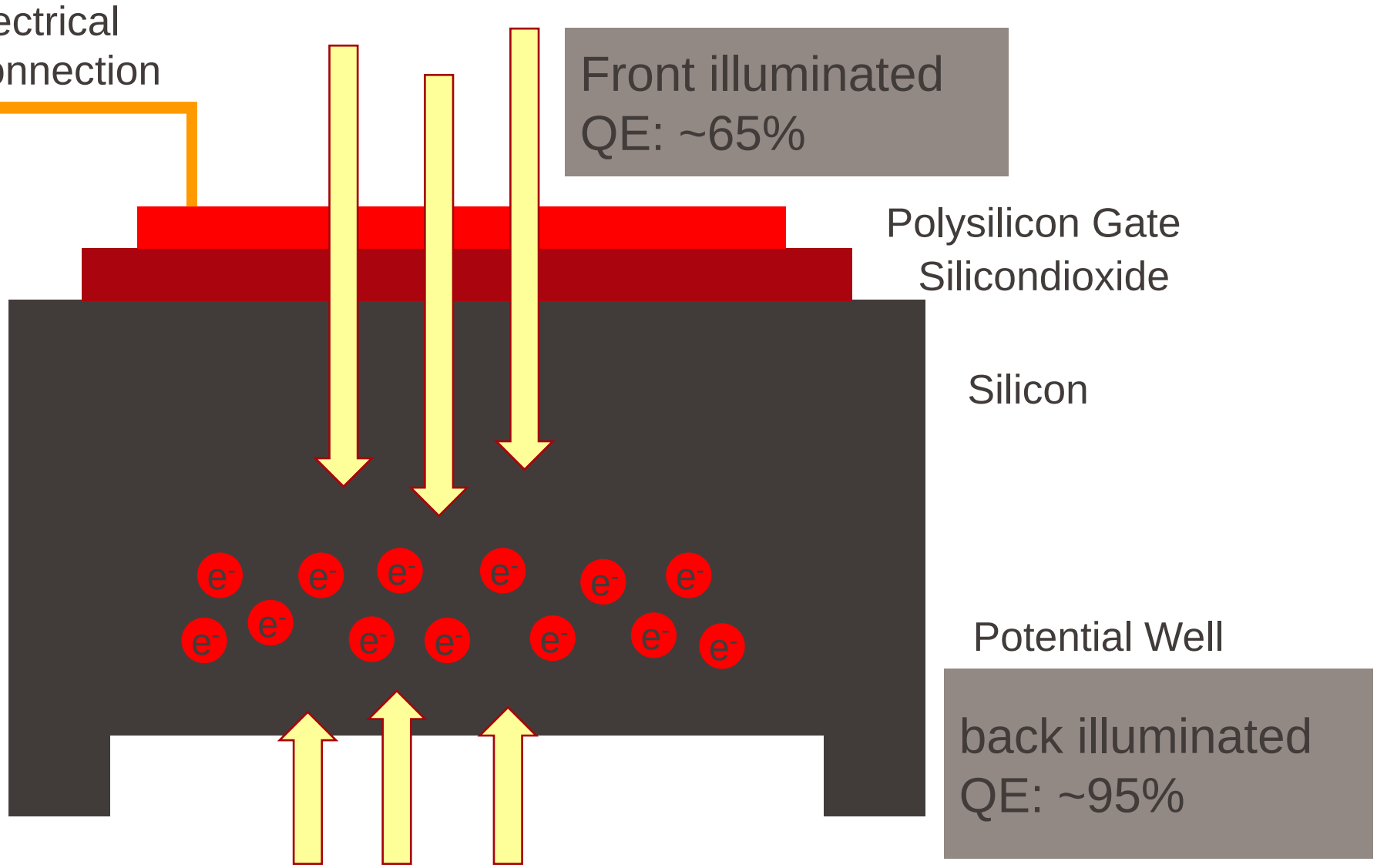
CCD Architecture



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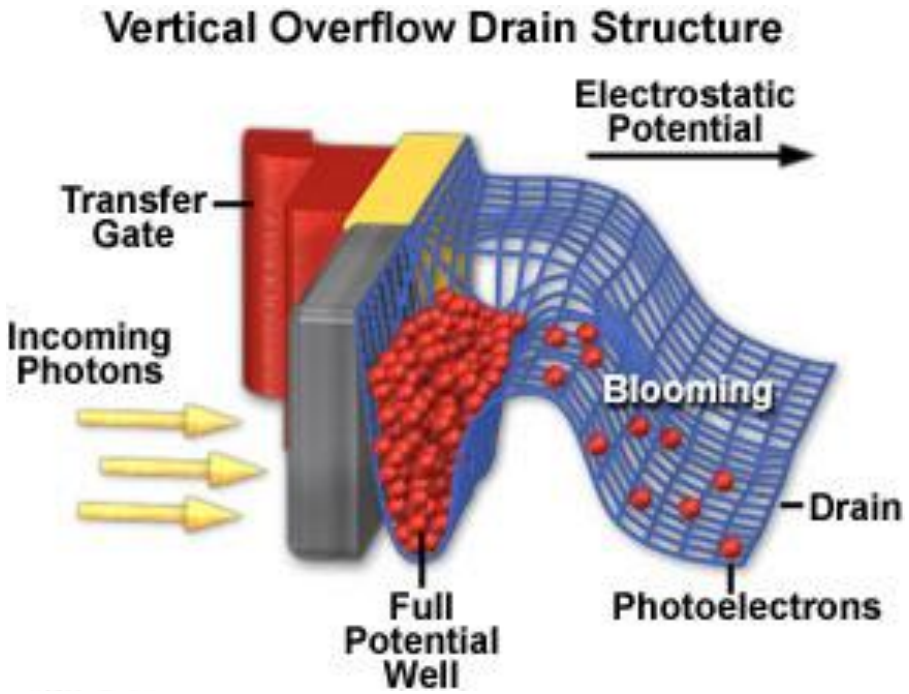
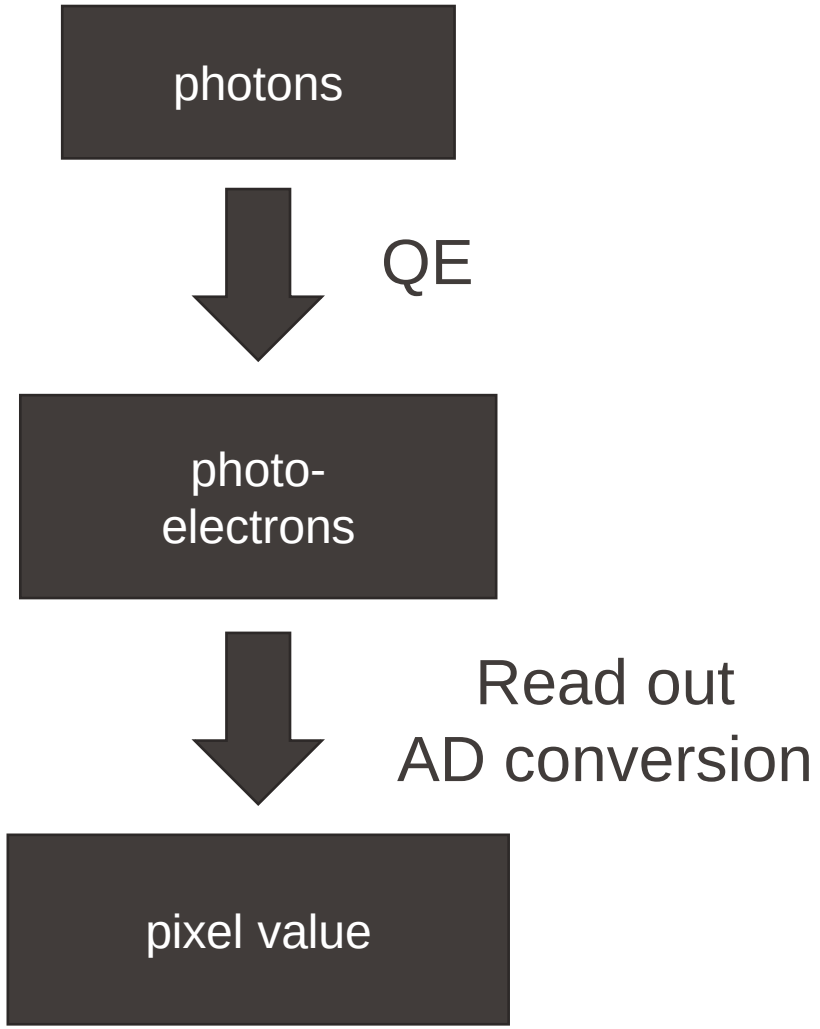
Full-Well capacity





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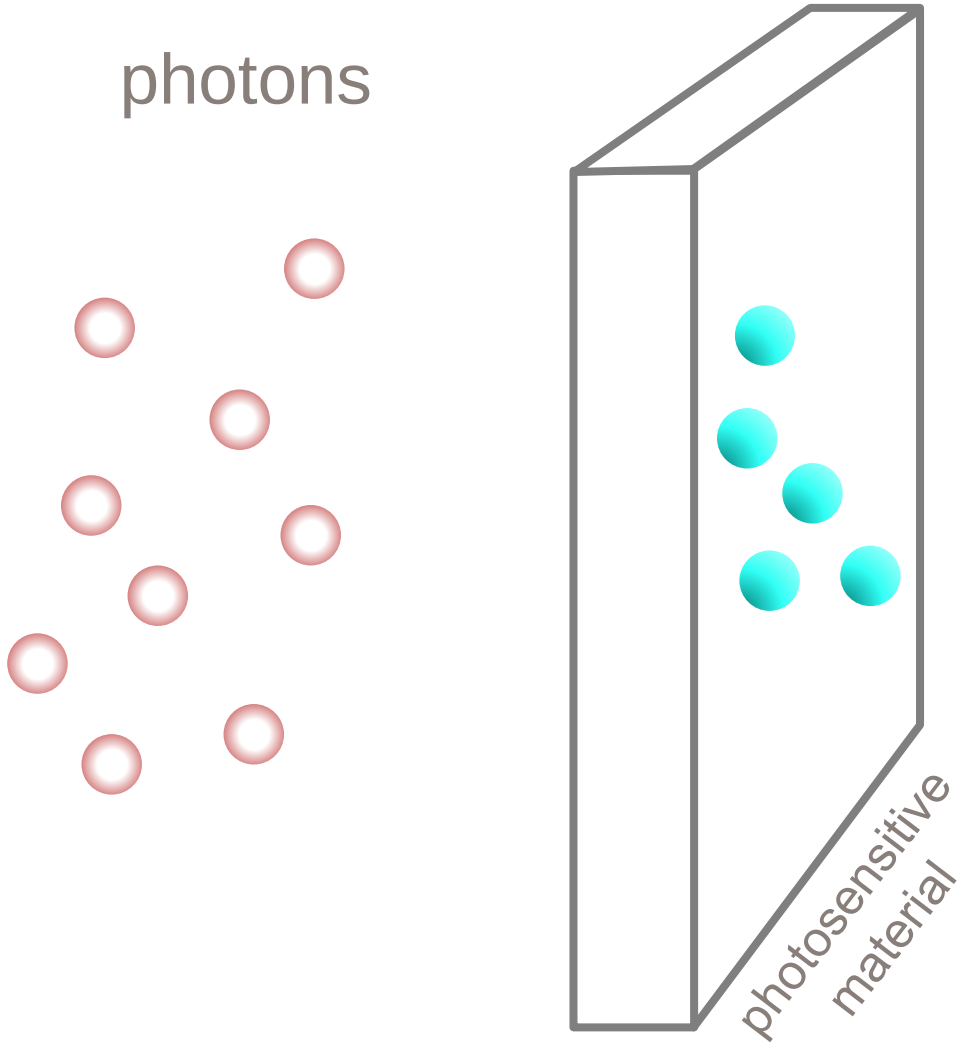
Photon detection



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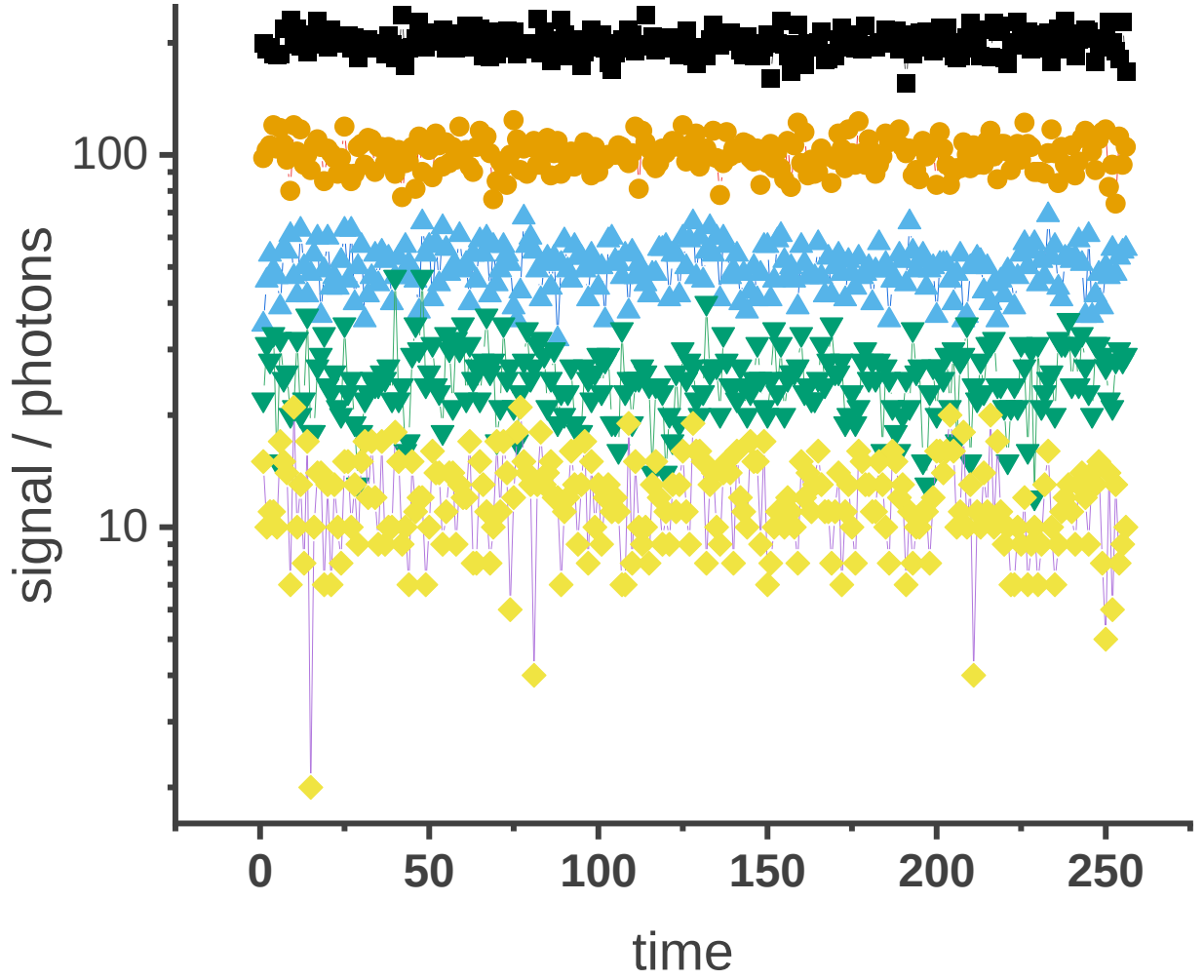
Photon detection

photons

photosensitive material

(photo)electrons



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Digital Detection

Usage in light microscopy

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Resolution/Pixel Size

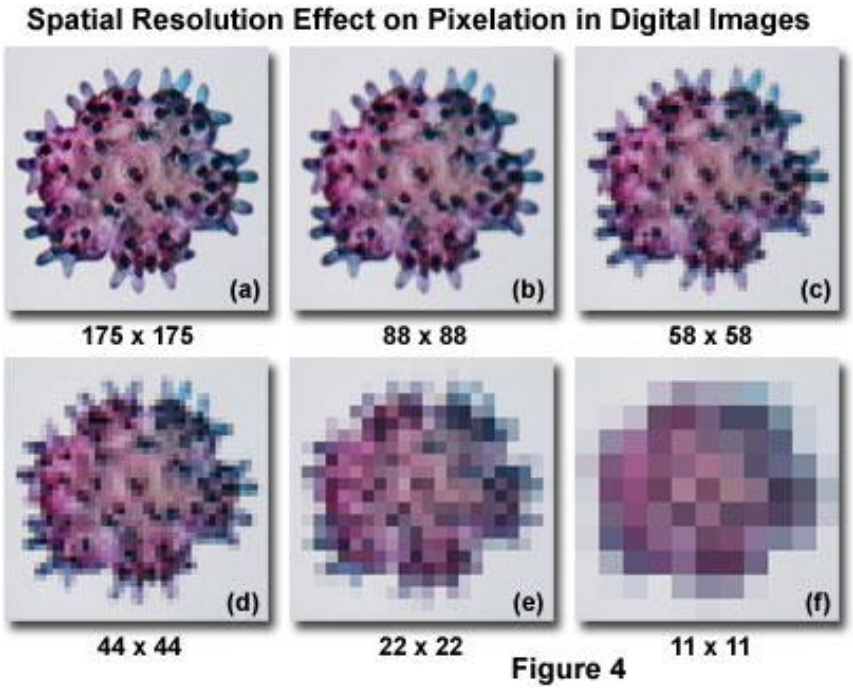

Figure 4

Same area
Less pixels
Larger pixel size
(in the image)
Less sampling frequency

Lateral resolution

$$\delta^R = 0.61\frac{\lambda}{\mathrm{NA}}$$

- Nyquist theorem
- Undersampling/oversampling

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Resolution/Pixel Size

Objective (numerical aperture)	Resolution Limit (microns)	Projected Size on CCD (microns)	Required Pixel Size (microns)
4x (0.20)	1.5	5.8	2.9
10x (0.45)	0.64	6.4	3.2
20x (0.75)	0.39	7.7	3.9
40x (0.85)	0.34	13.6	6.8
40x (1.30)	0.22	8.9	4.5
60x (0.95)	0.31	18.3	9.2
60x (1.40)	0.21	12.4	6.2
100x (0.90)	0.32	32.0	16.0
100x (1.25)	0.23	23.0	11.5
100x (1.40)	0.21	21.0	10.5

CCD camera Binning

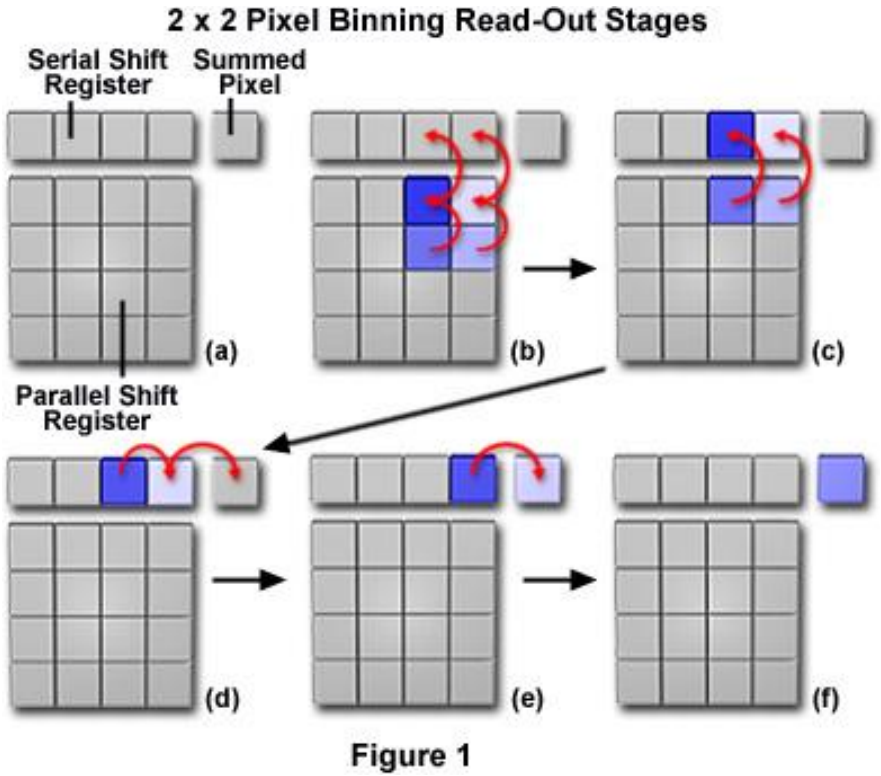

Figure 1

Binning:
- increases effective pixel size
-Decreases sampling frequency
-Increases read-out speed

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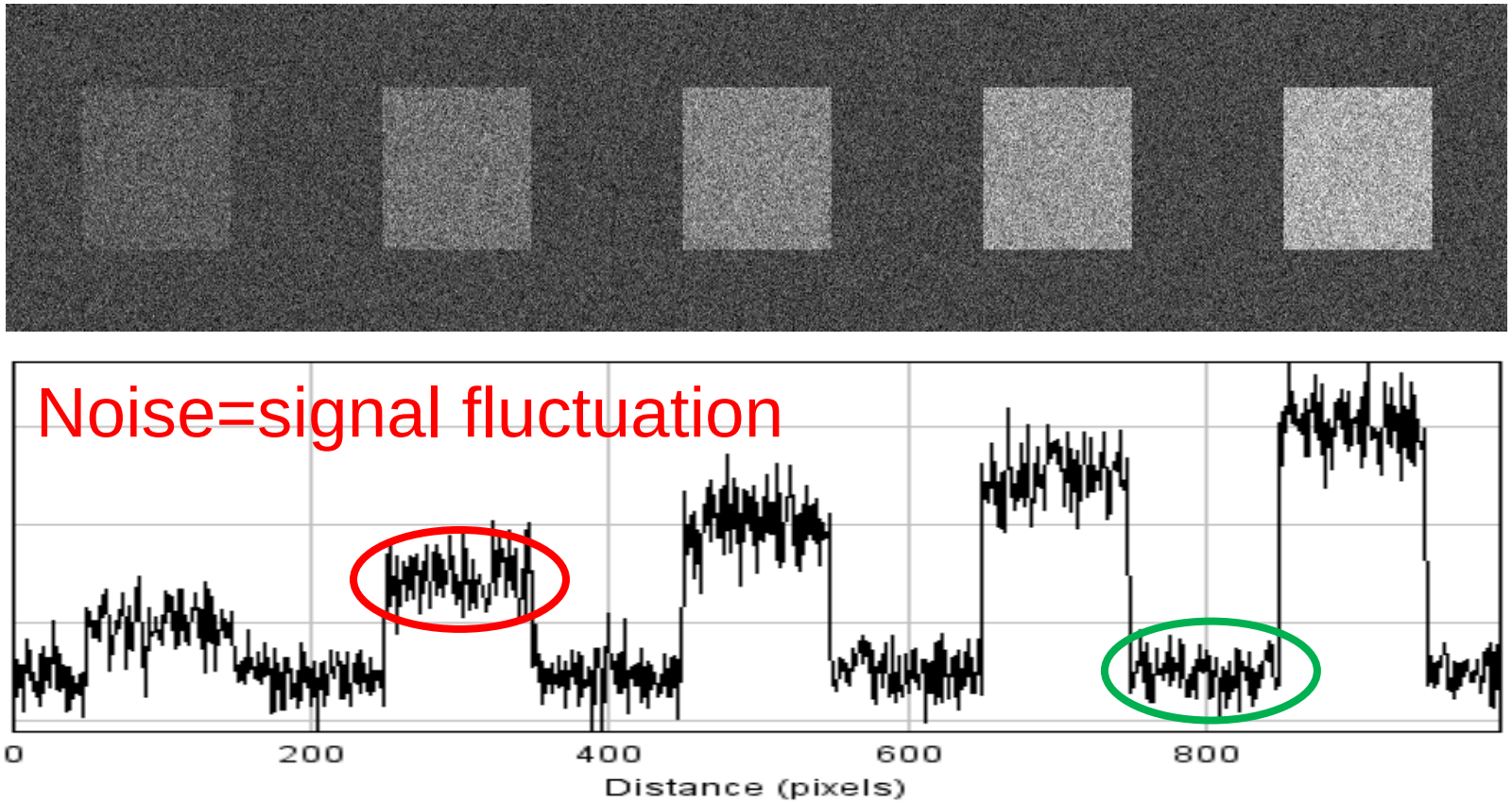
Noise/Background



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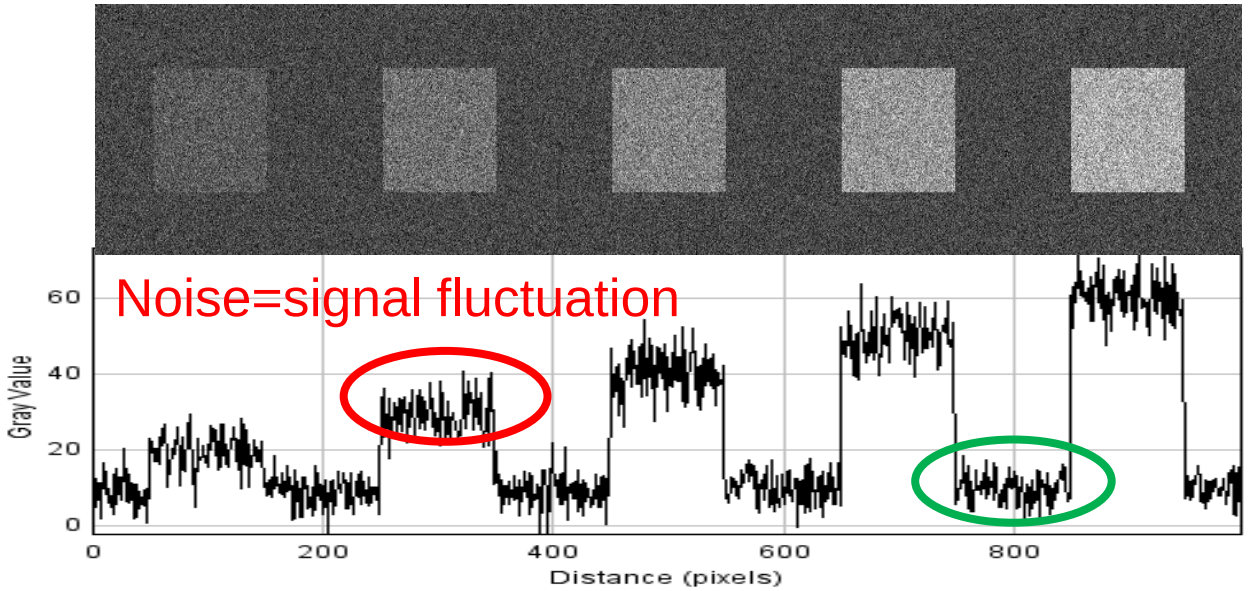
Noise/Background



background

$$N_{tot} = N_{Sig}^2 + N_{Cam}^2$$

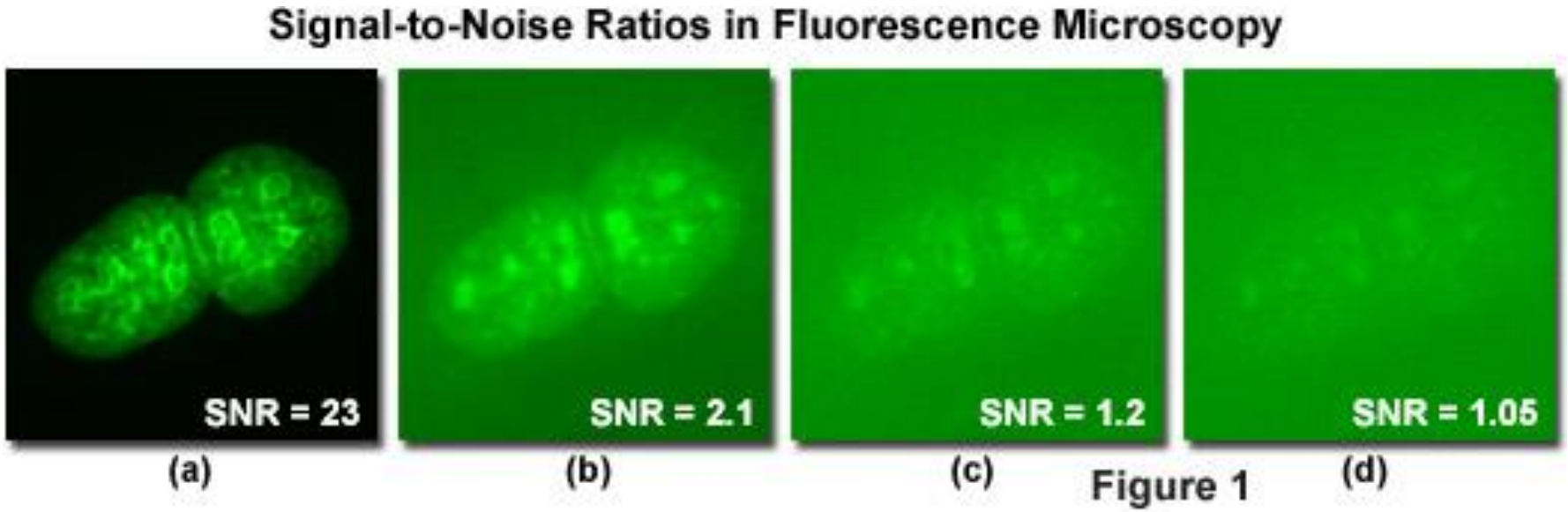
$$N_{Sig}^2 = QE * P$$

$$N_{Cam}^2 = N_{Dark}^2 + N_{\mathrm{Re}ad}^2$$

Sources of noise:
Detector noise
- Dark noise
- Read noise

Photon/shot noise

Signal to Noise Ratio



Photonflux: 10^4 photons/s
Exposure time: 0.1 s
SNR: ~ 5 for a typical CCD camera

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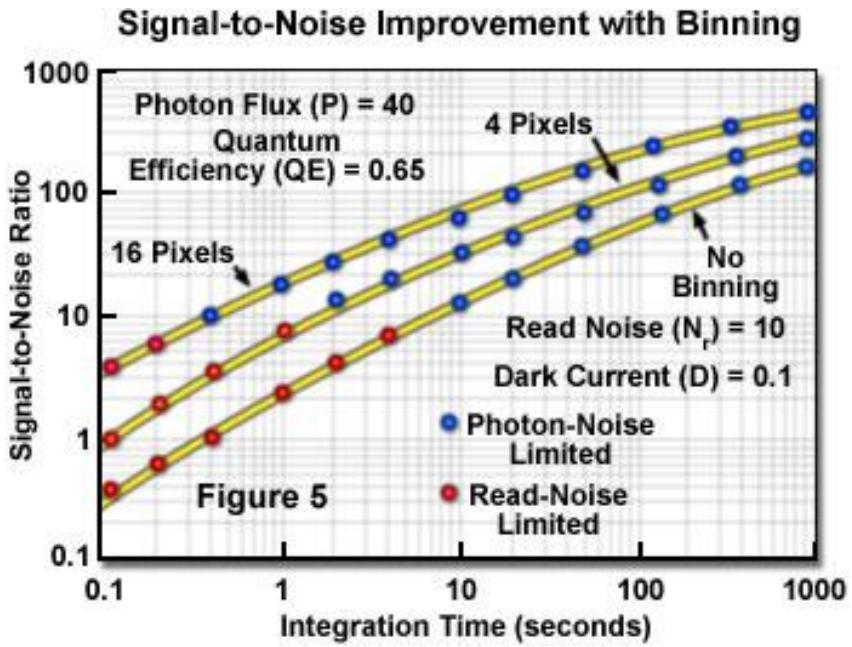
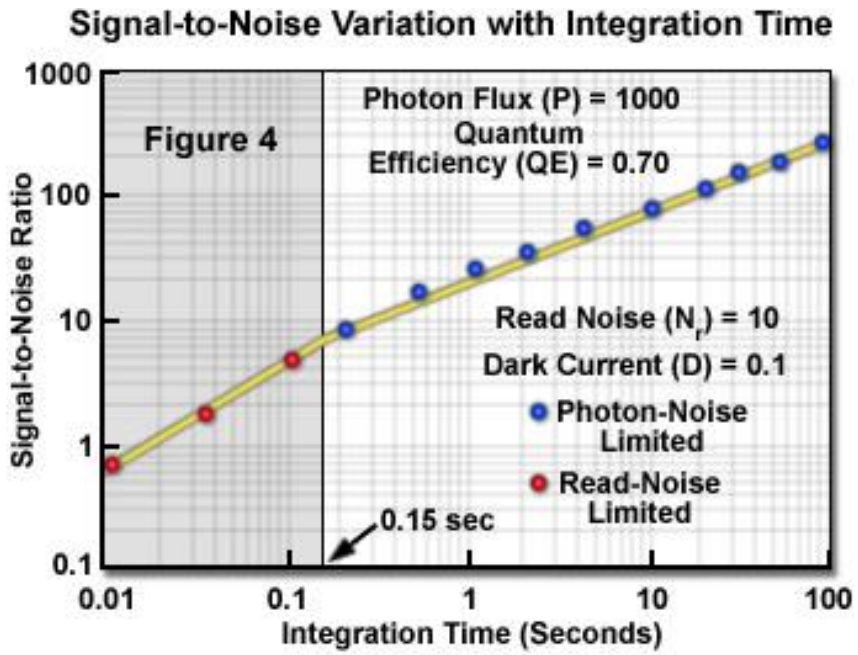
Signal to Noise Ratio





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Colour Array detection devices

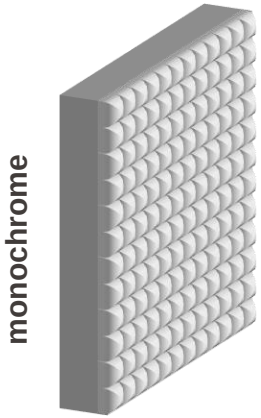
photons

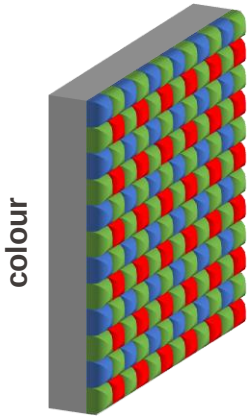
↓

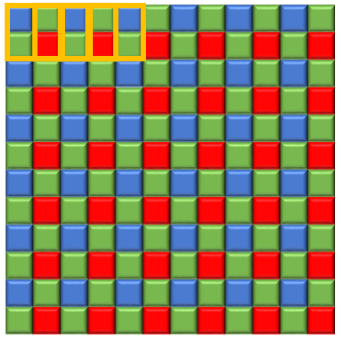
photo-electrons

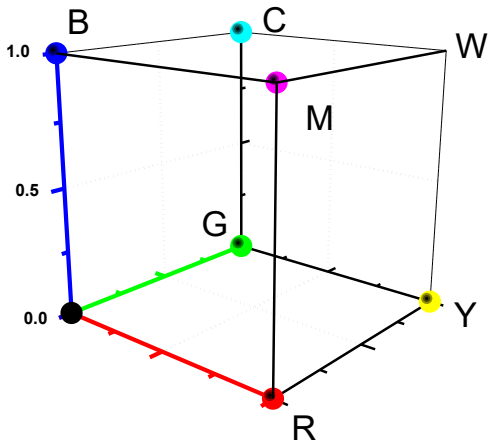
↓ Read out AD conversion

pixel value









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Object detection

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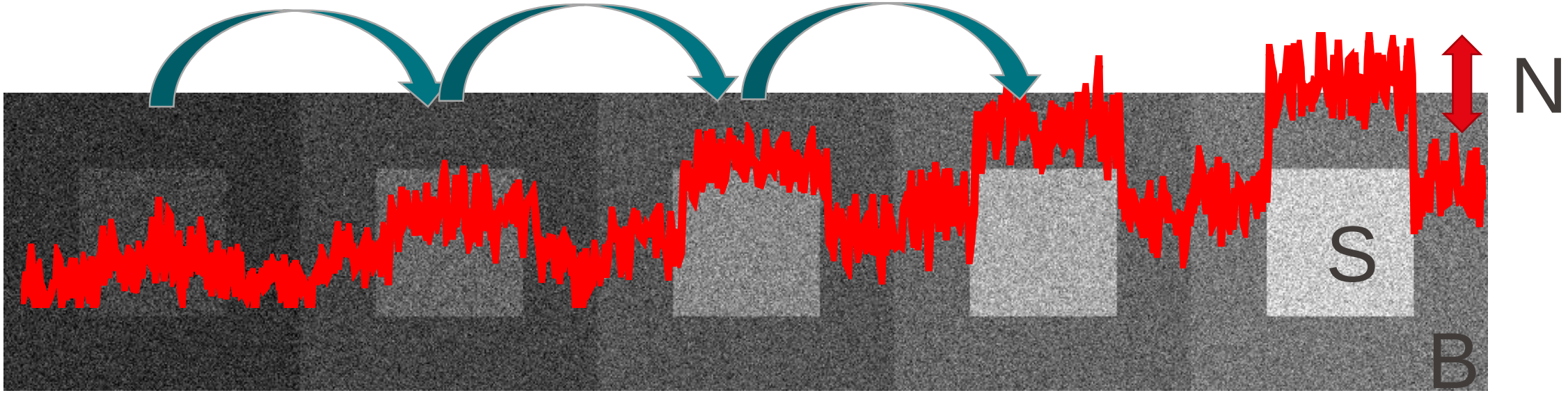
Signal and Noise



- Signal/Background →increase
- Signal/Noise →increases
- Contrast

→constant

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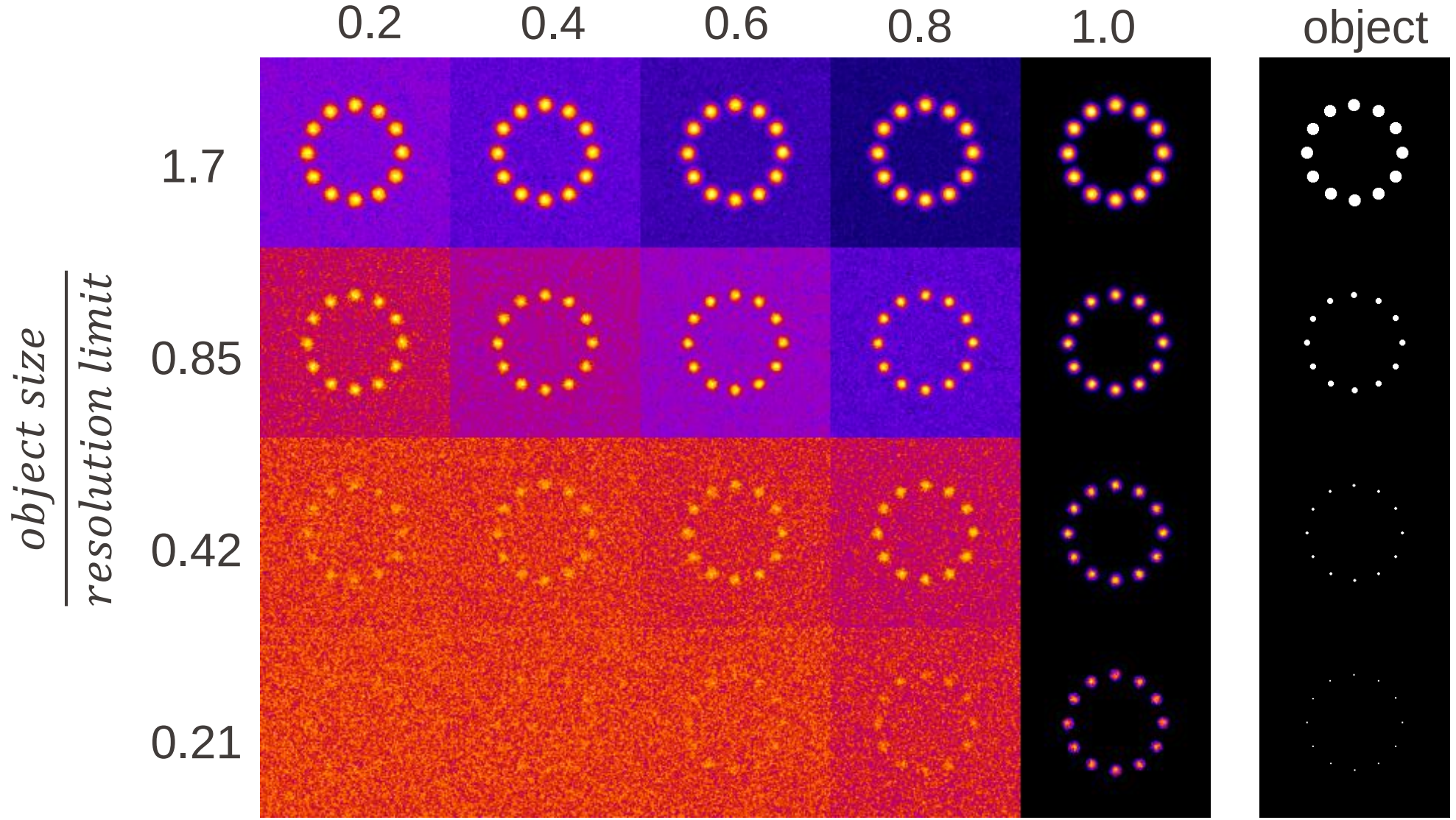
$$\text{contrast} = \frac{(signal - background)}{(signal + background)}$$



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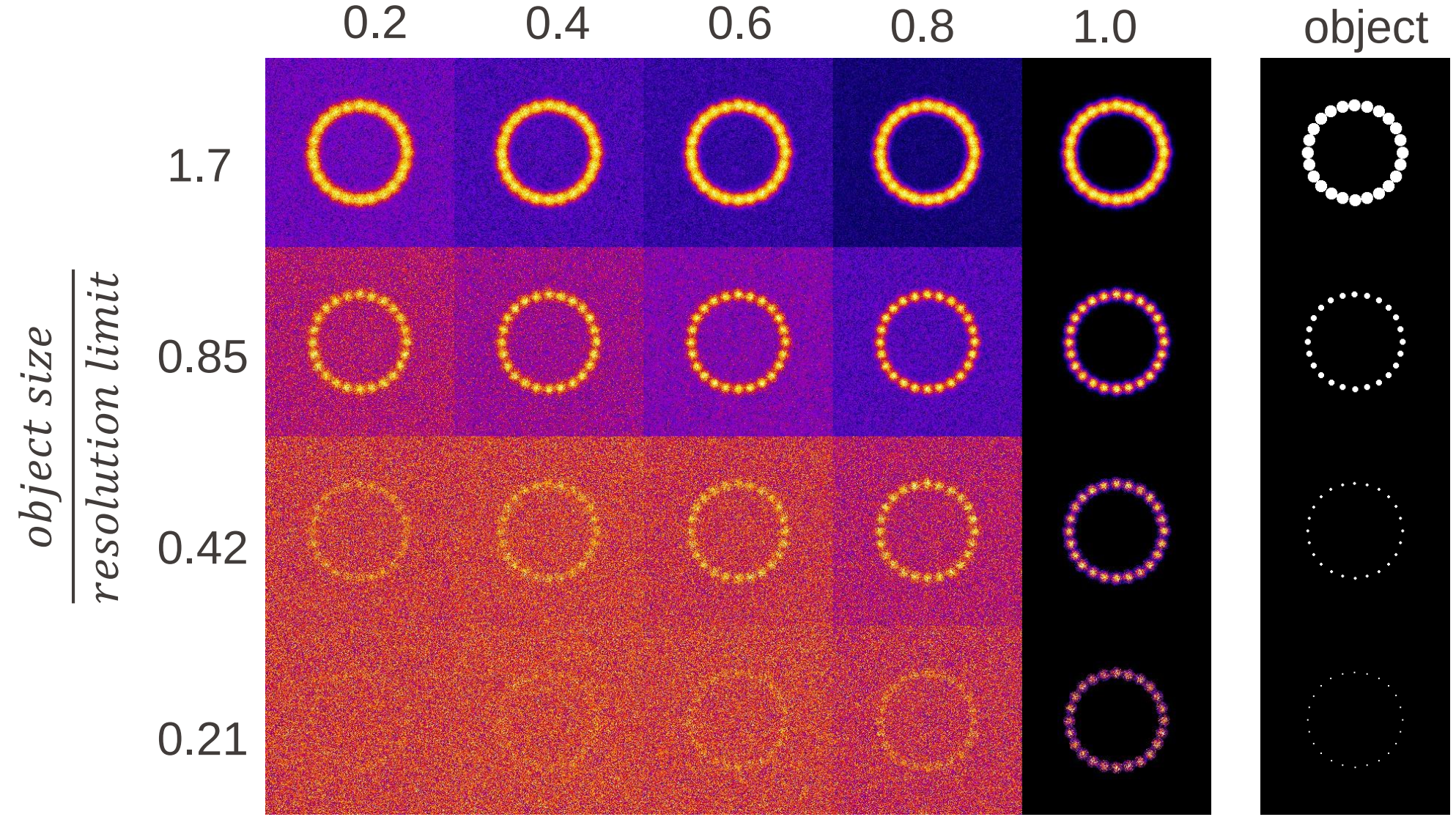
$$\text{contrast} = \frac{(signal - background)}{(signal + background)}$$



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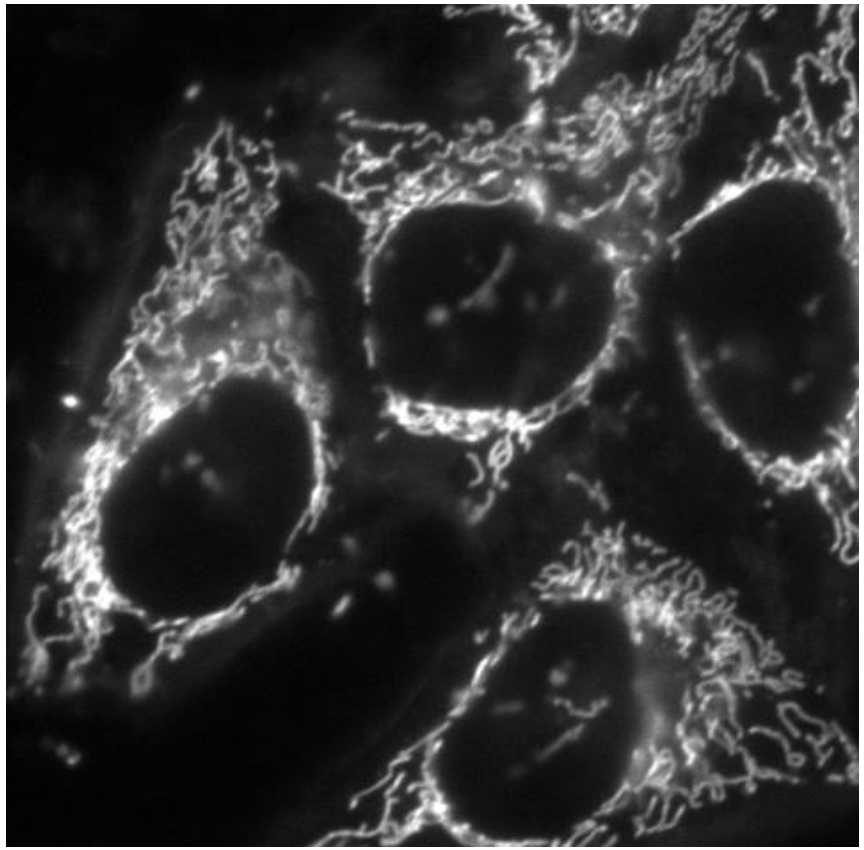
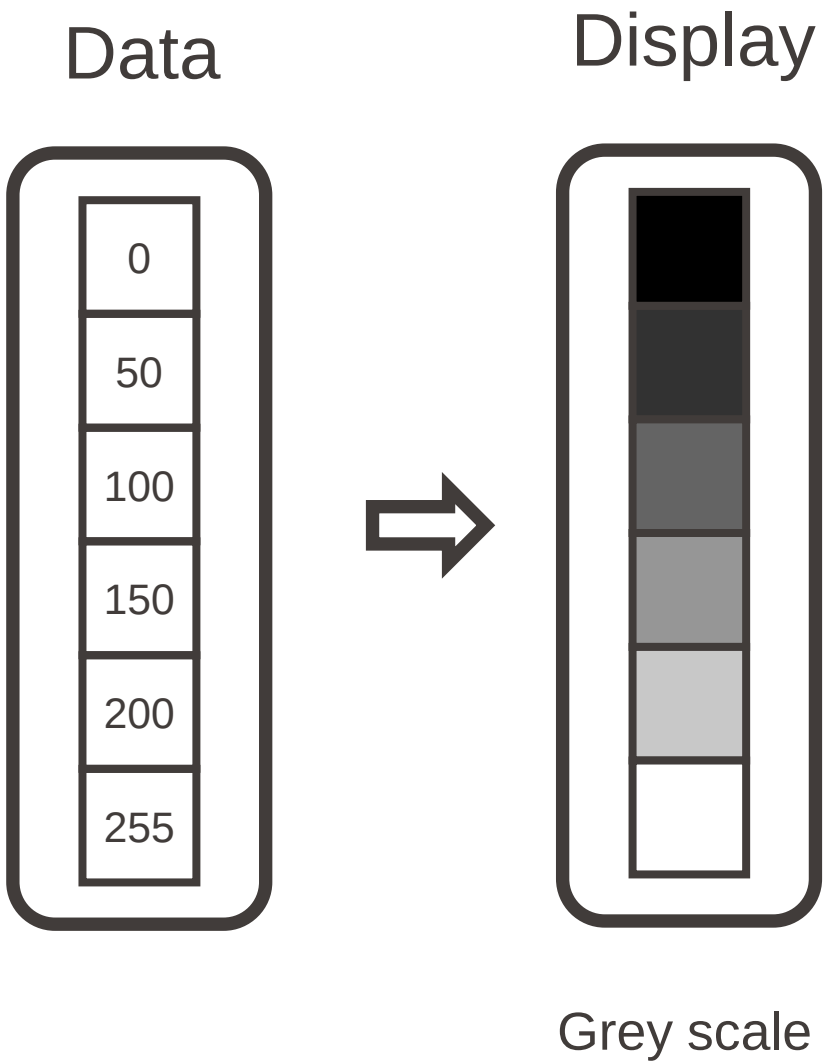
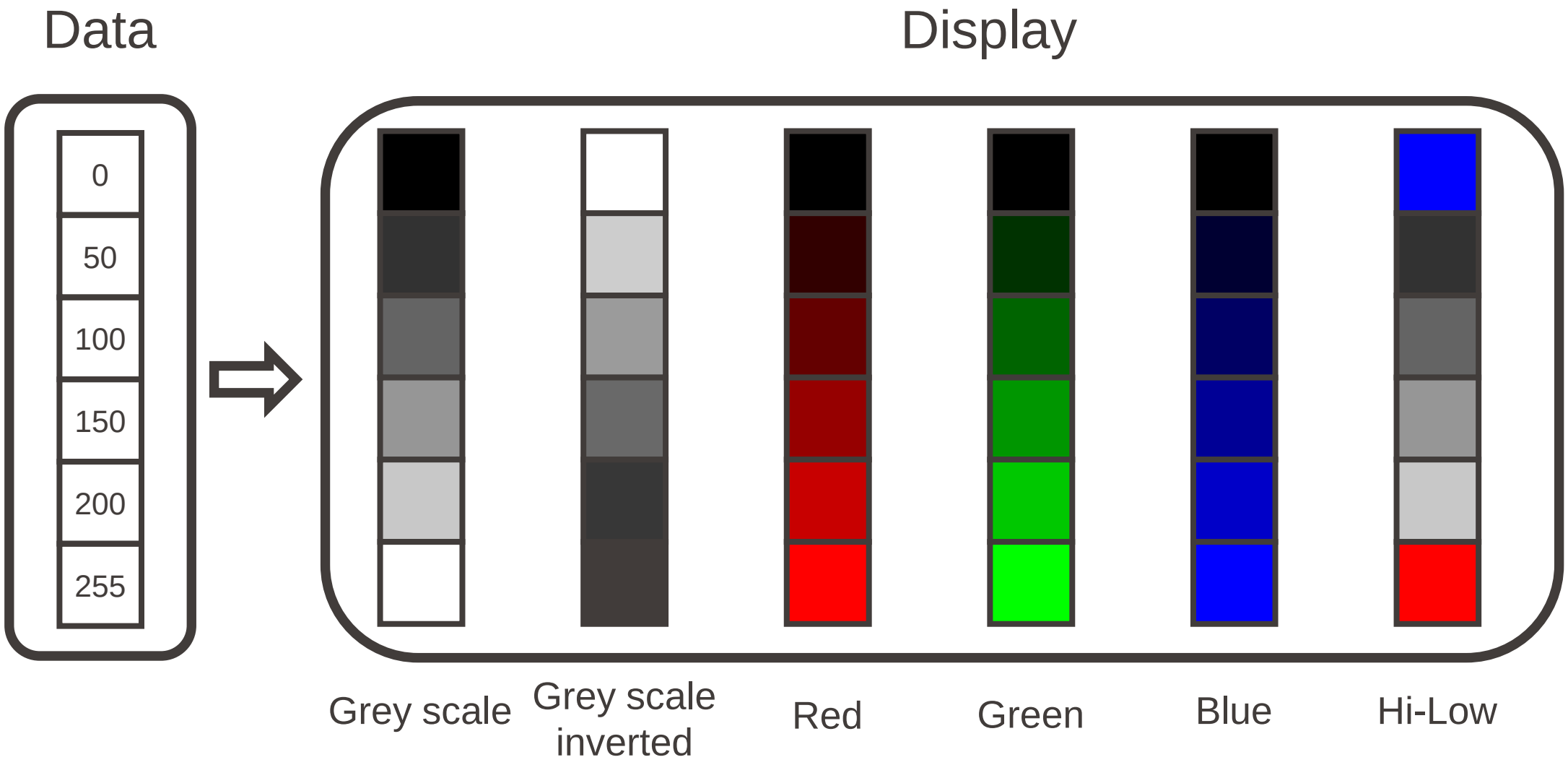
Quantification and display of Image data




Data
Display
0
50
100
150
200
255
Grey scale

Mapping function



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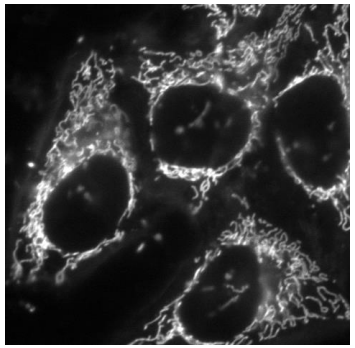
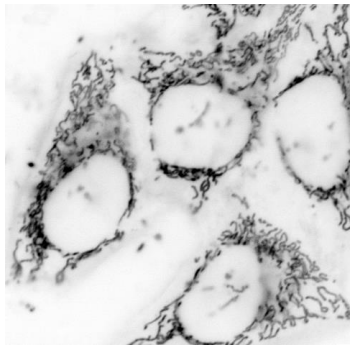
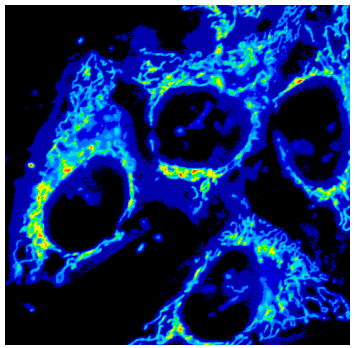
Look up tables



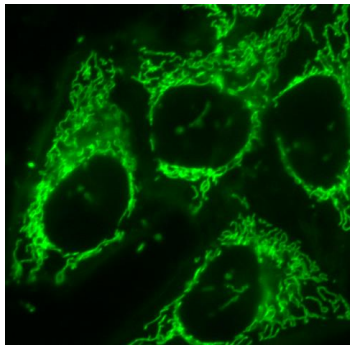
Grey scale



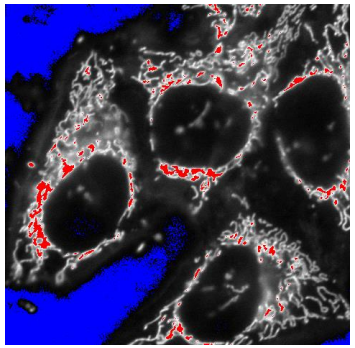
Grey scale (inverted)



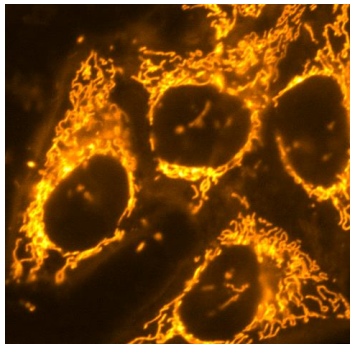
16 colours



Green



Hi-Lo



Orange-Hot

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Image data display

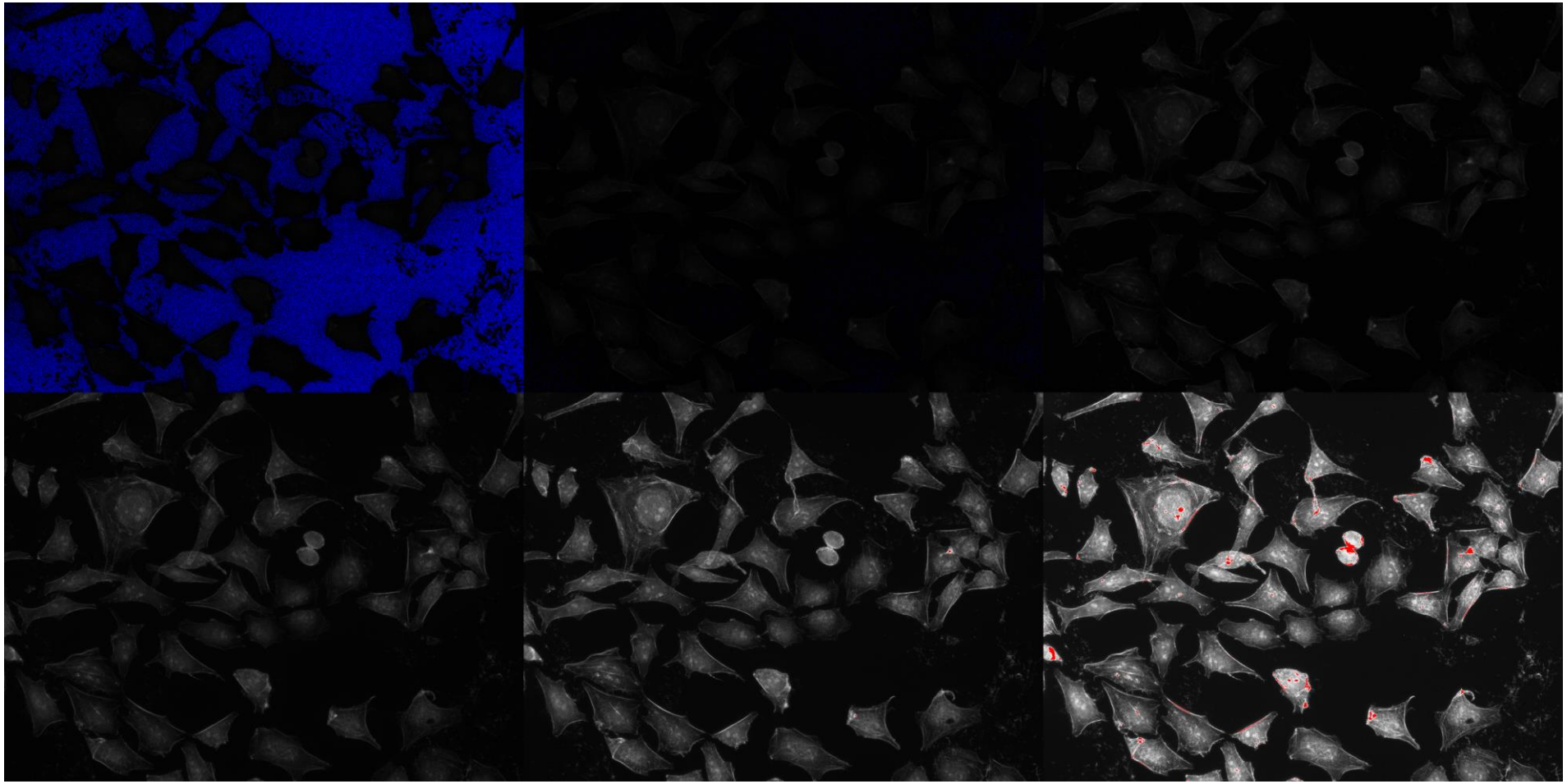
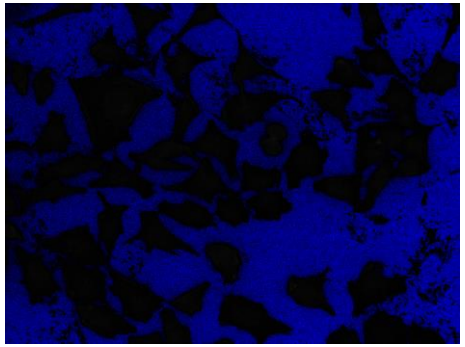
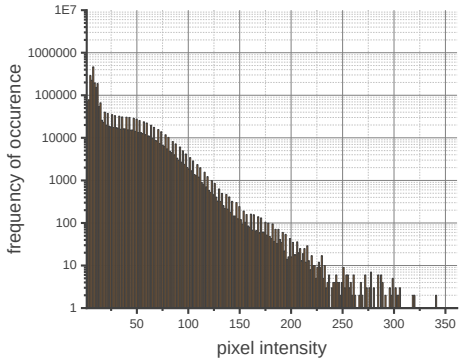
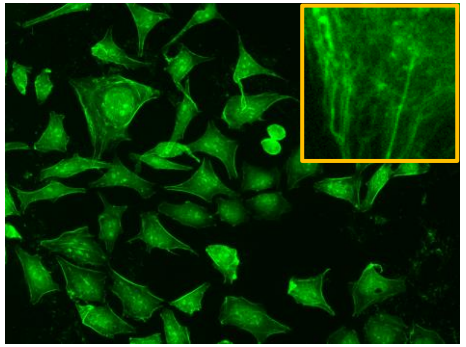
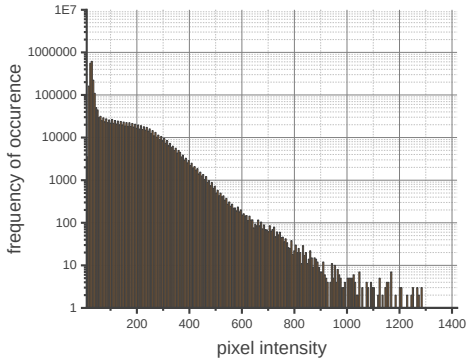


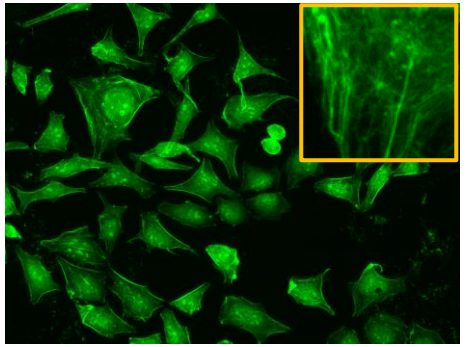
Image data display

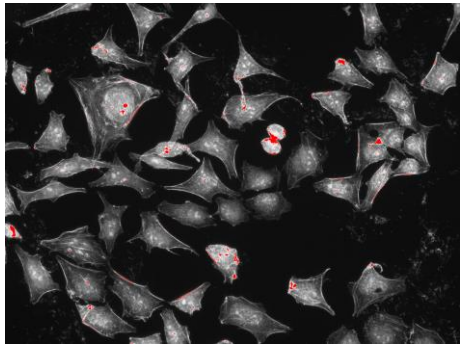


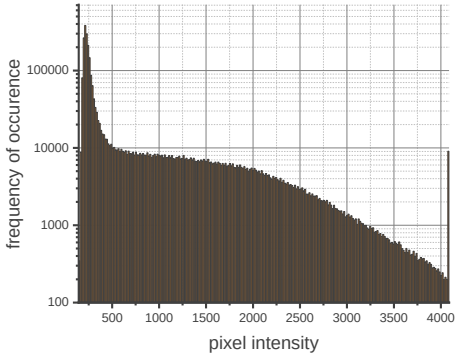


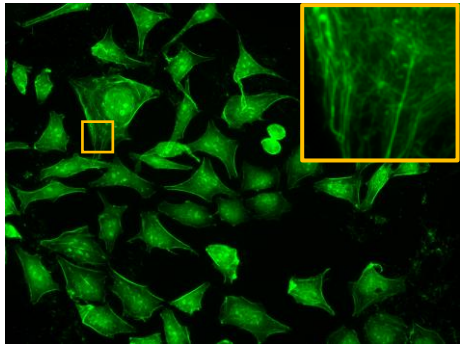












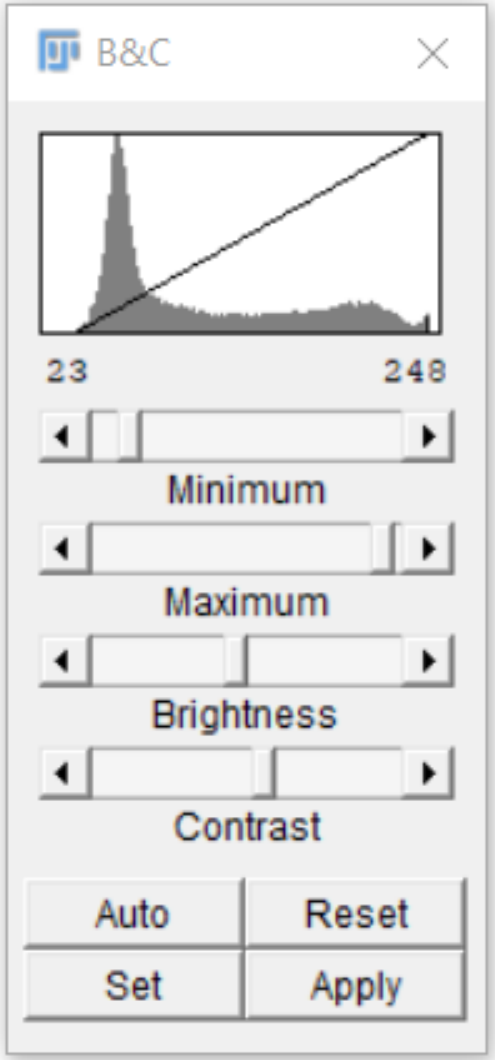
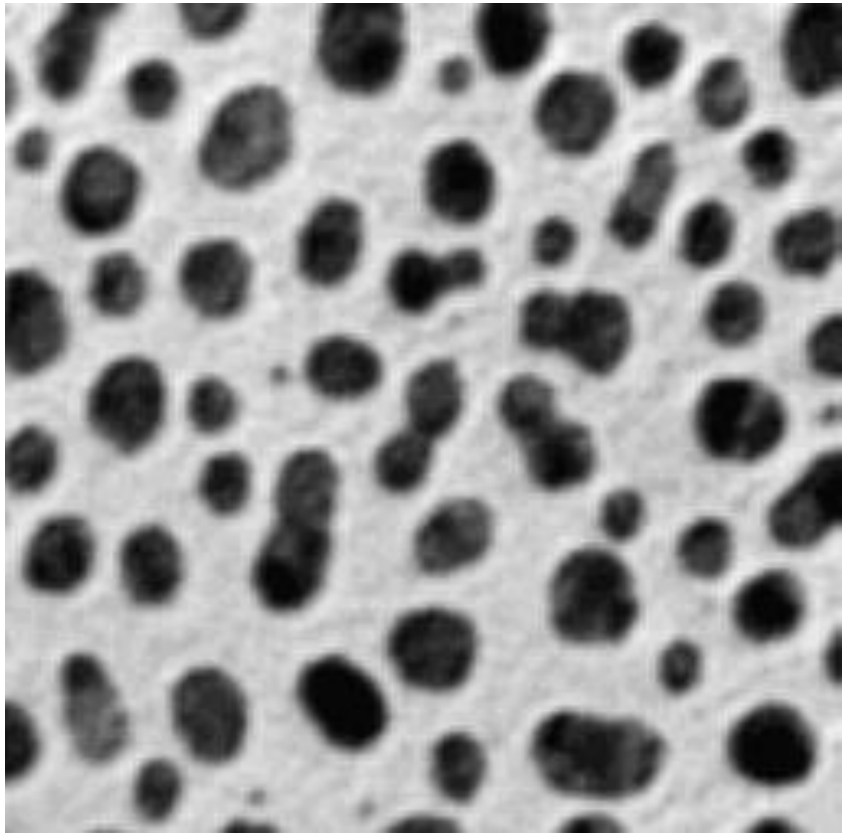
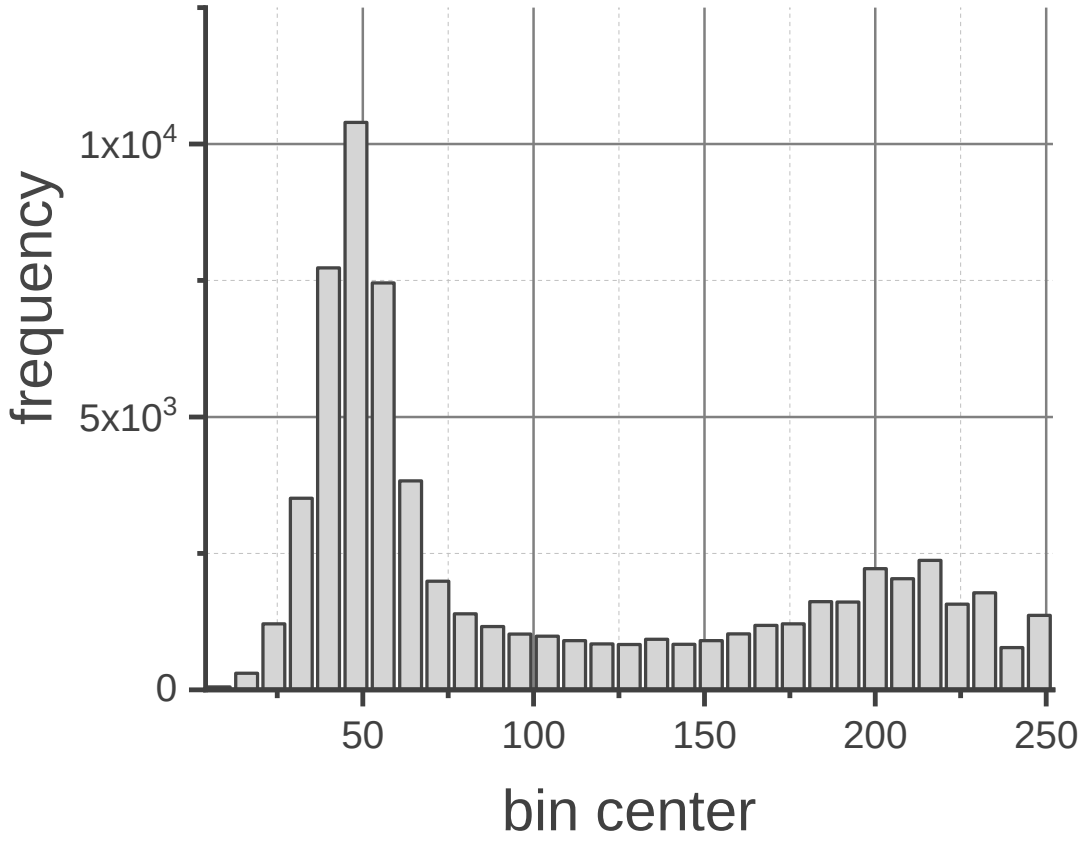


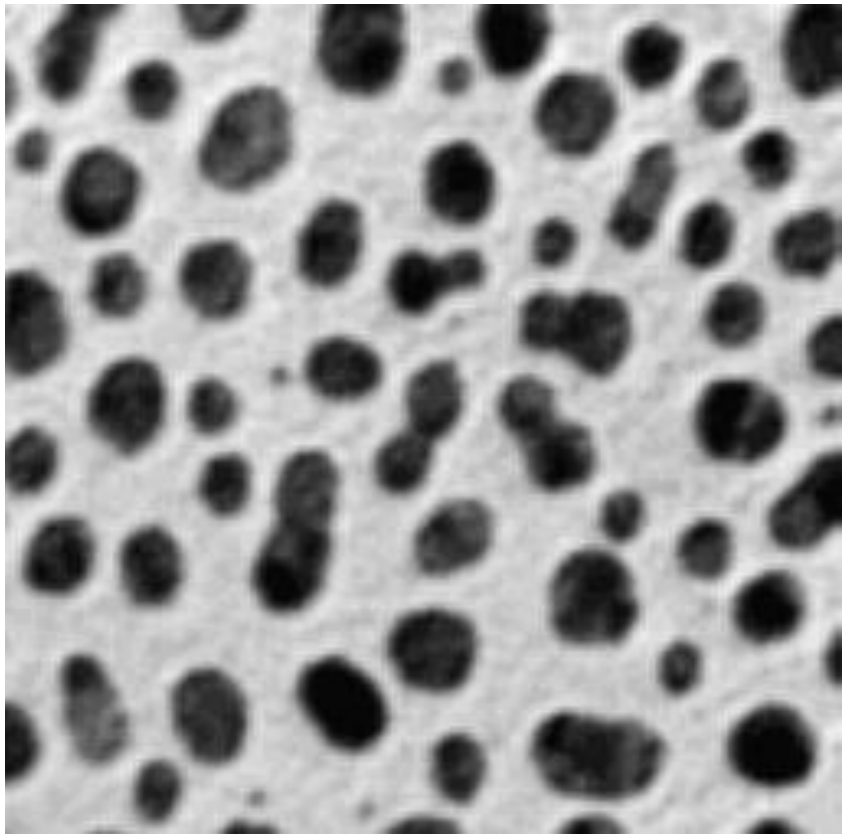
Image Segmentation

- Algorithms that are based on discontinuity and similarity
- Intensitiy based thresholding



Intensity Thresholding





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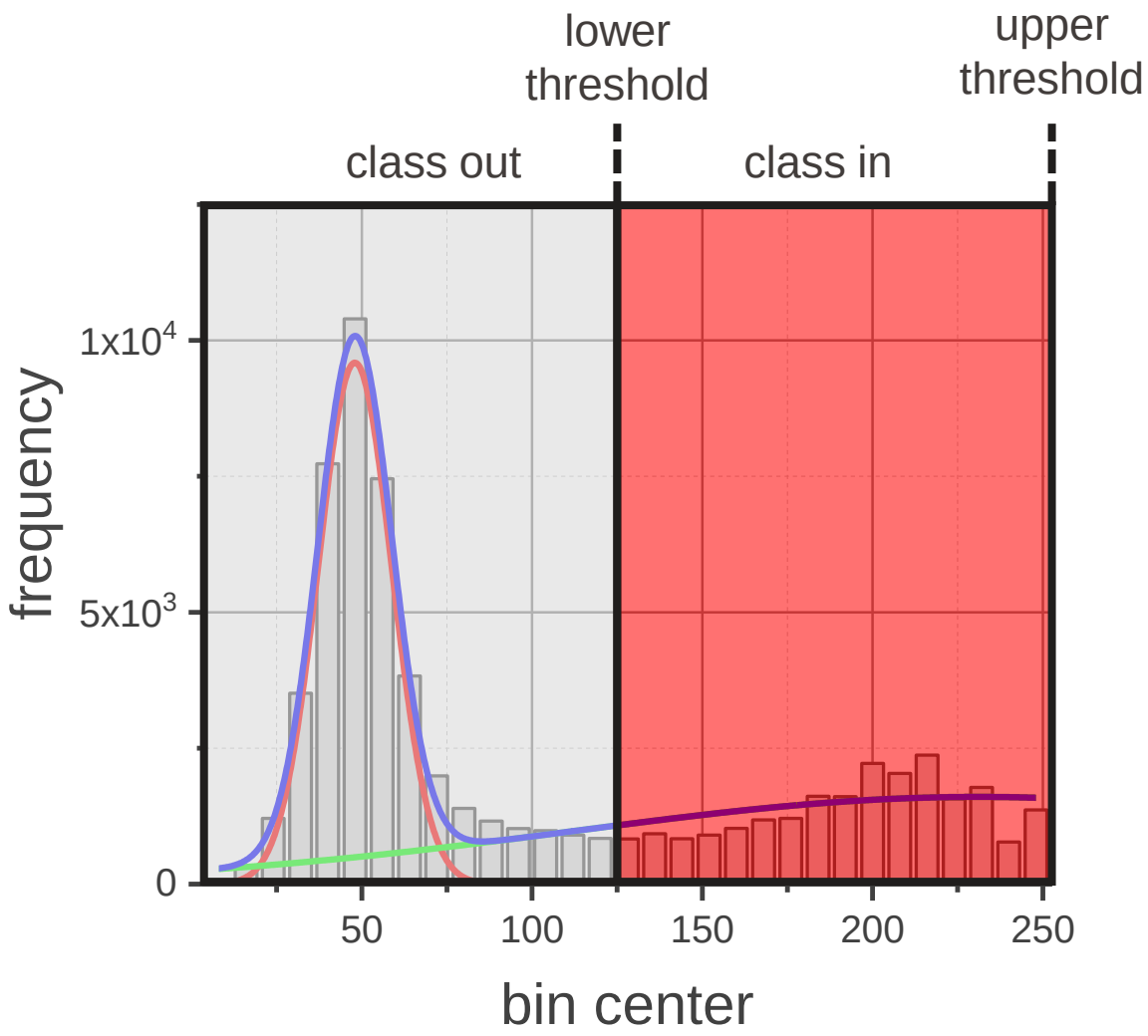
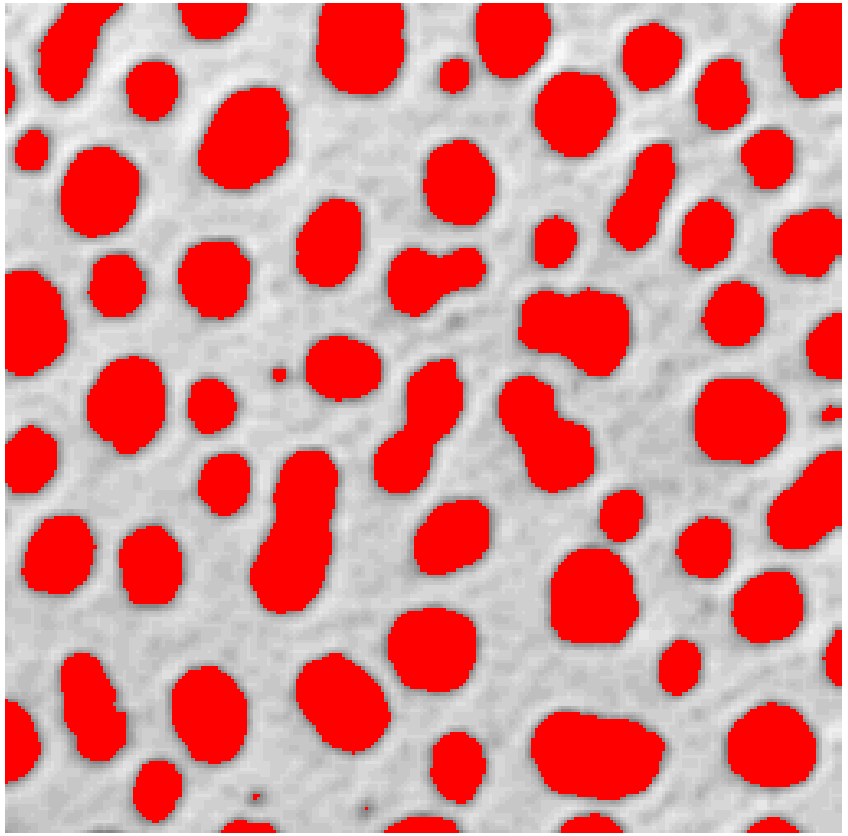
Intensity Thresholding





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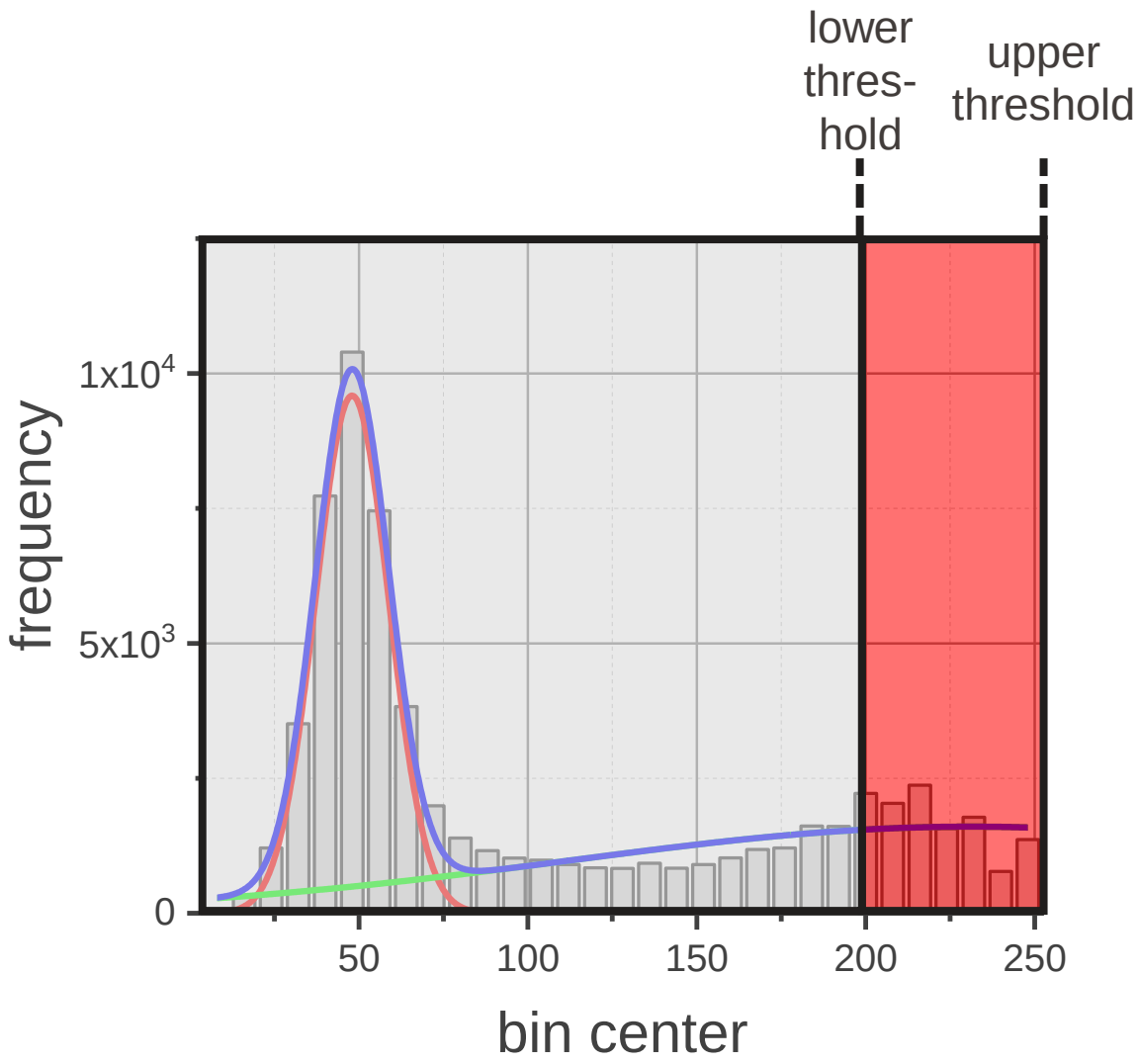
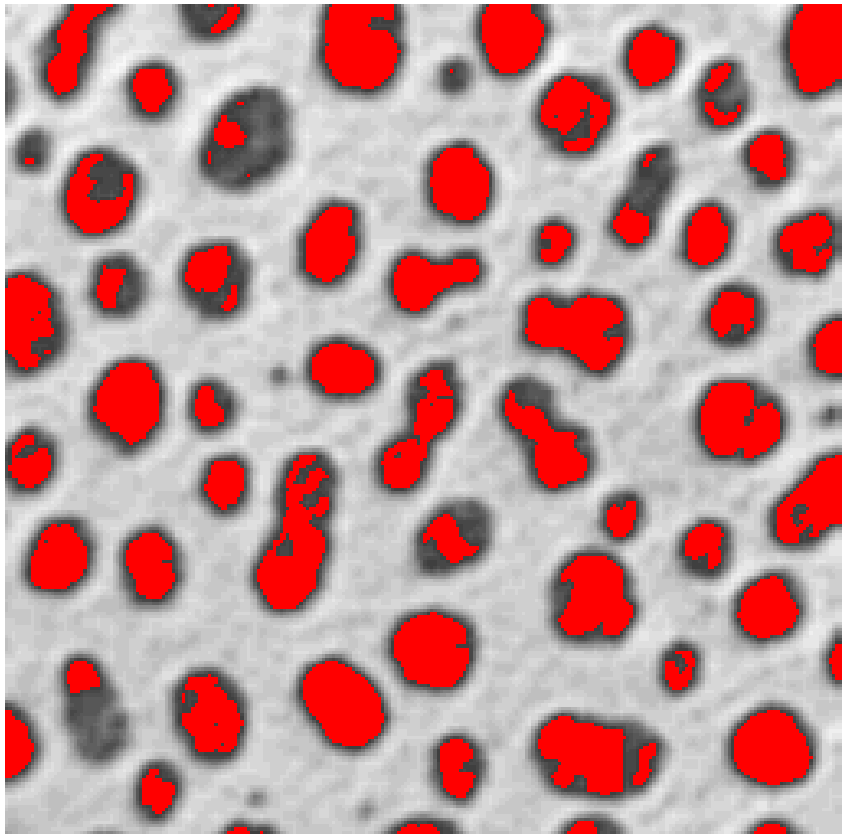
Intensity Thresholding





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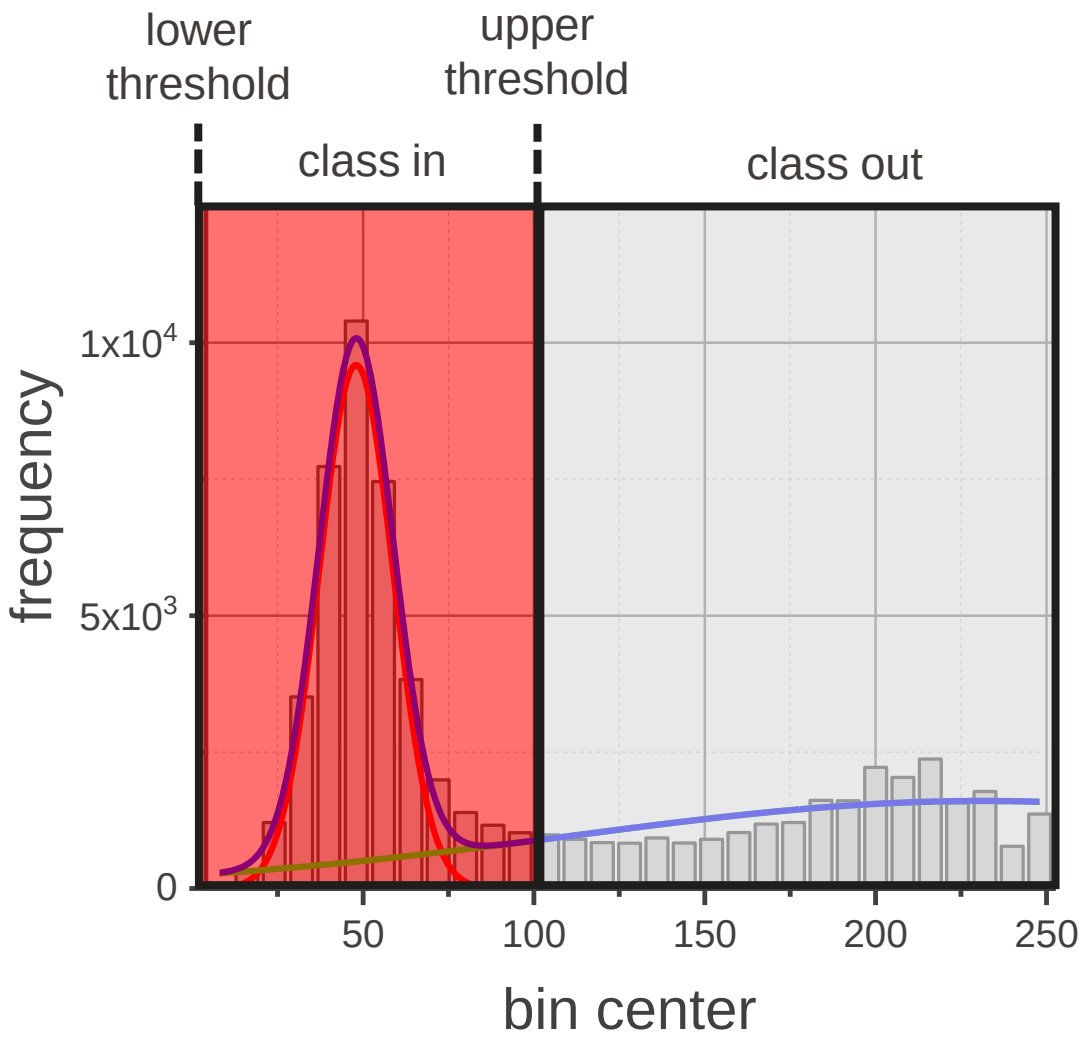
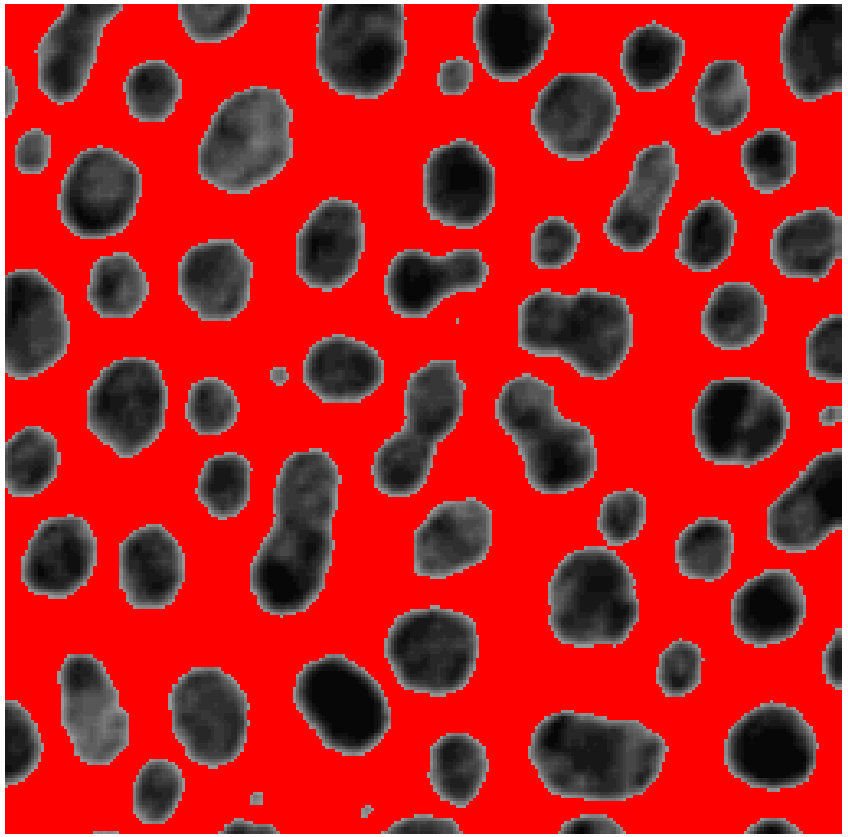
Intensity Thresholding





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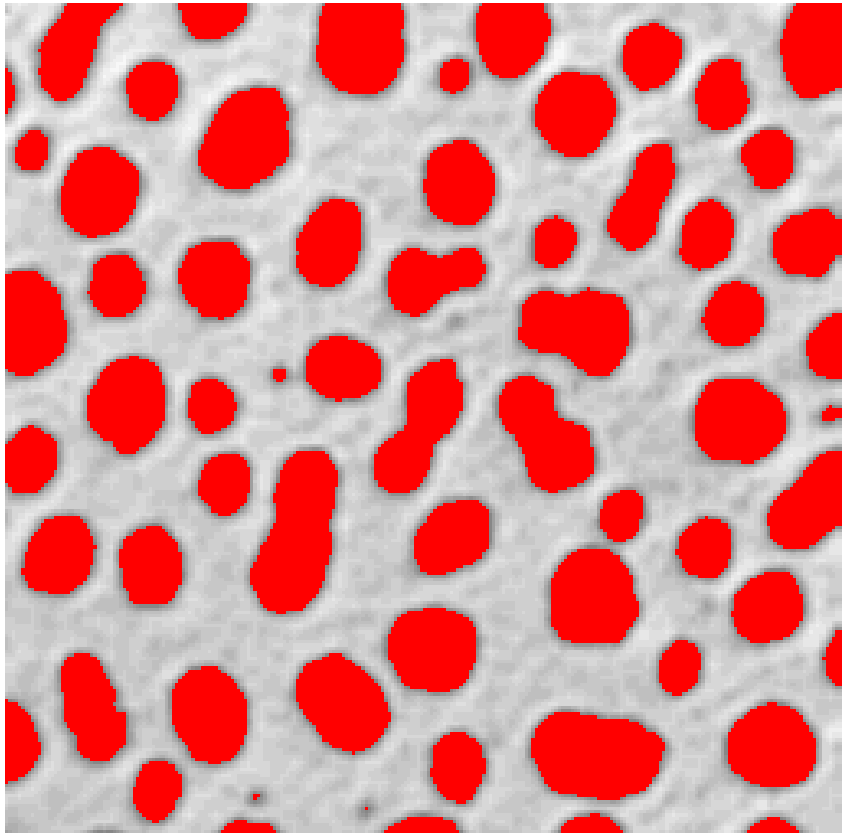
Intensity Thresholding

- Segmantic and global approach
- Simple and intuitive
- Requires user input or algorithm to set T

$$g(x,y) = \begin{cases} 1 & if\ f(x,y) > T \\ 0 & if\ f(x,y) \leq T \end{cases}$$

$$g(x,y) = \begin{cases} 0 & if\ f(x,y) \geq T_{up} \\ 1 & if\ \ T_{low} < f(x,y) < T_{up} \\ 0 & if\ f(x,y) \leq T_{low} \end{cases}$$



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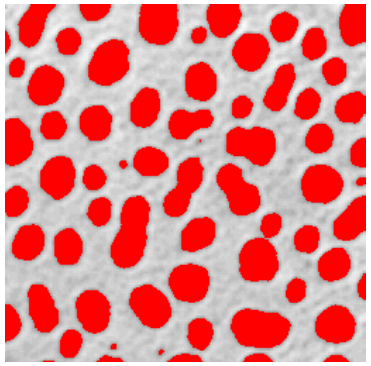
Basic global thresholding

- Select an estimate for the global threshold T
- Segment the image using T
- Calculate the mean for the two classes: m_1,m_2
- New threshold:

$$T = 0.5(m_1 + m_2)$$

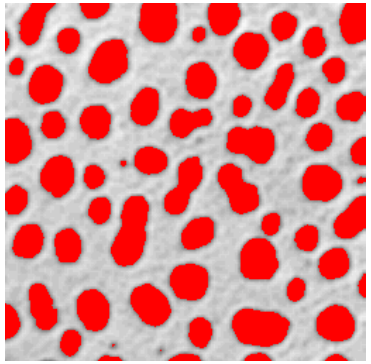
- Repeat until DT is smaller then a predefined value

T_{start}=103



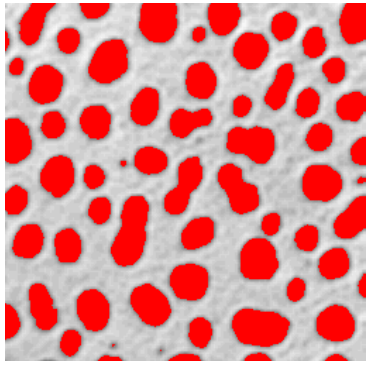
m_1=186
m_2= 52
T_1 = 121

T_{start}=121



m_1=195
m_2= 56
T_2 = 125

T_{start}=125



m_1=195
m_2= 56
T_3 = 125

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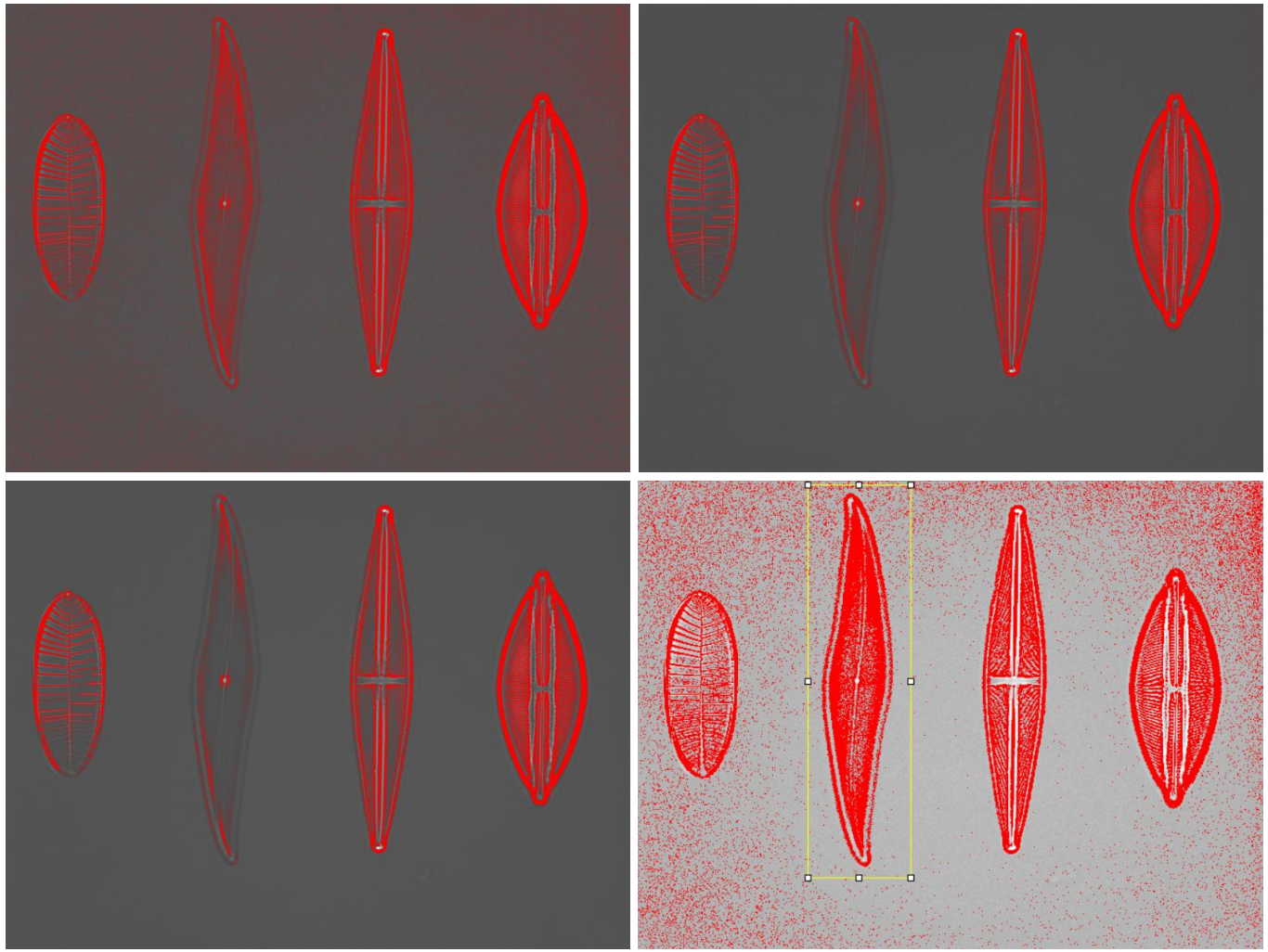
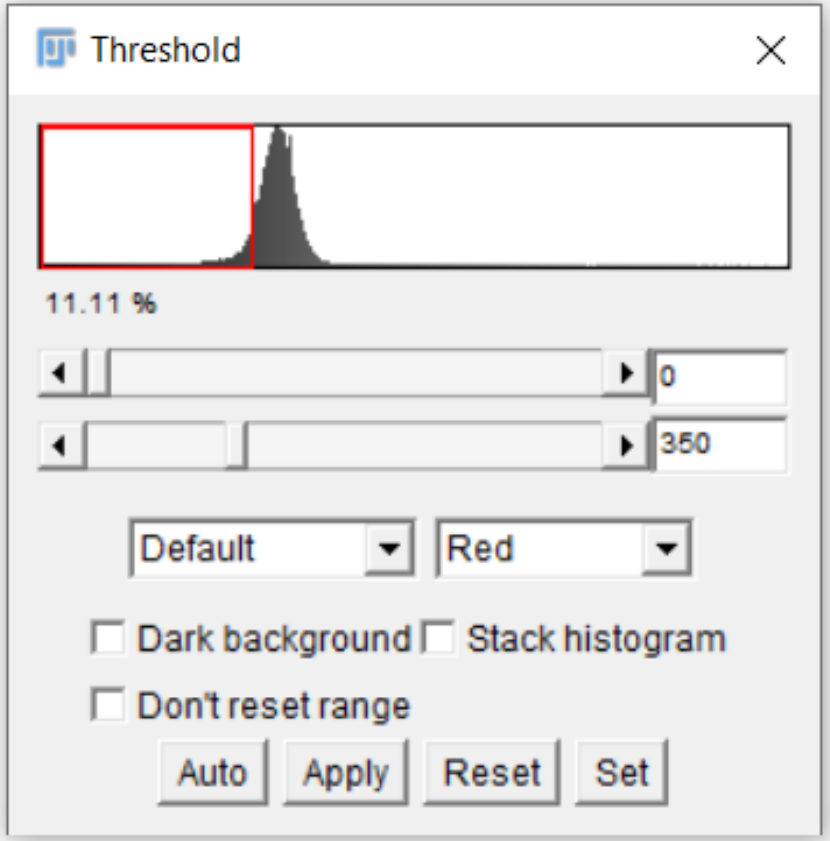
Segmentation variability





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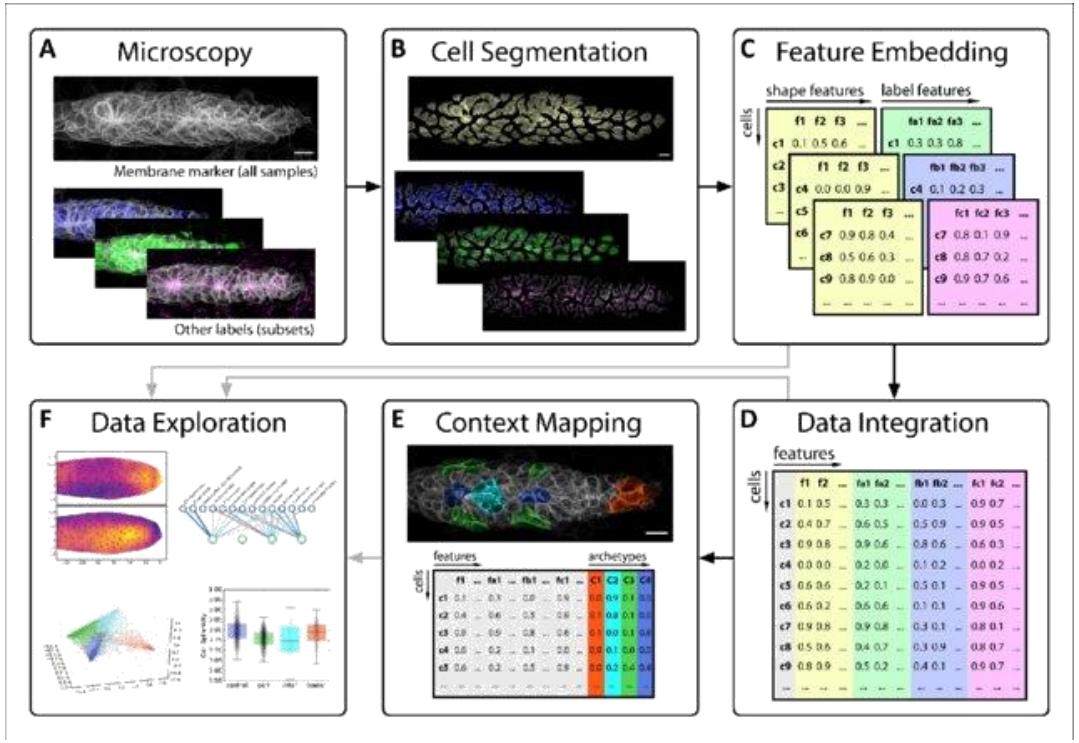
Objectives

- Rember the basic foundations of image formation
- Recall the parameters defining image quality
- Understand the interplay of parameters influencing image quality
- Understand how image quality affects downstream image analysis (segmentation)
- Develop the mindset of “critical thinking” for image analysis



Jonas Hartmann, Mie Wong, Elisa Gallo, Darren Gilmour **(2020)** An image-based data-driven analysis of cellular architecture in a developing tissue eLife 9:e55913

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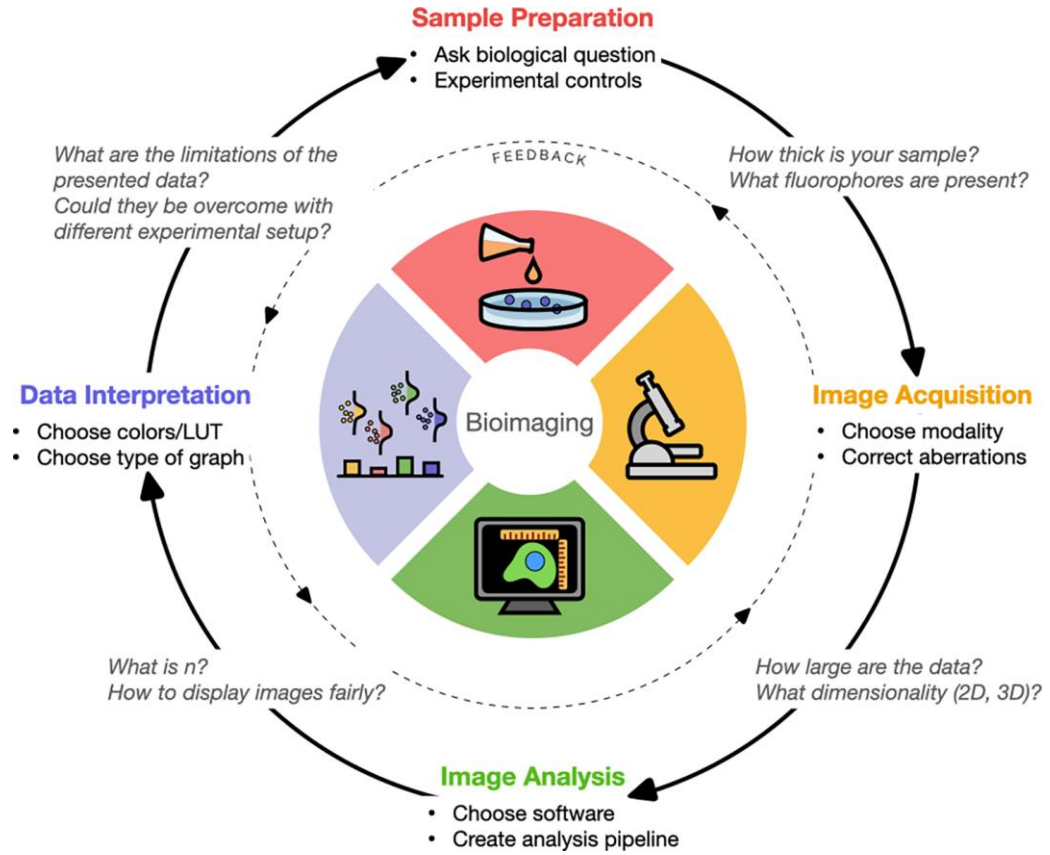
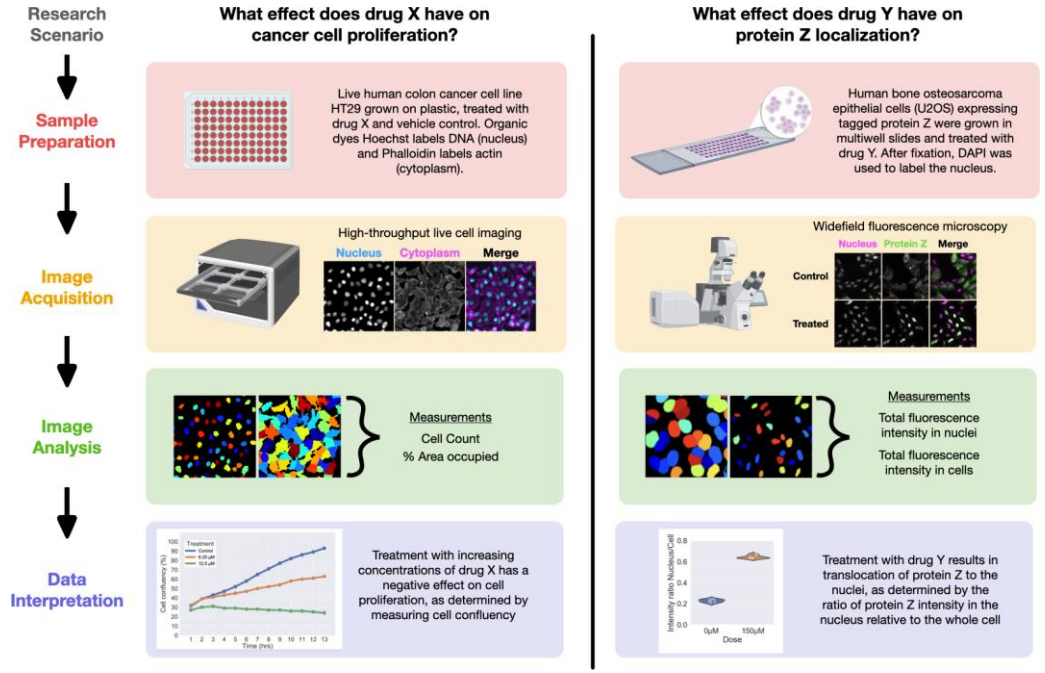
Quantitative bioimaging experiment





Senft RA, Diaz-Rohrer B, Colarusso P, Swift L, Jamali N, Jambor H, et al. (2023)
A biologist's guide to planning and performing quantitative bioimaging experiments.
PLoS Biol 21(6): e3002167. https://doi.org/10.1371/journal.pbio.3002167